CENTRAL MECHANISMS RESPONSIBLE FOR GENERATING RESPIRATORY-MODULATED SYMPATHETIC NERVE DISCHARGE

by

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ABSTRACT

The experimental work presented in this thesis explores, in anaesthetized and mid-collicular decerebrate, unanesthetized, cats, the properties of two components of the discharge of sympathetic nerves which are time-locked to the central respiratory cycle and are presumably generated within the central nervous system. One is the inspiration-synchronous burst, the other is a previously unknown late-expiratory burst. The properties of the inspiration-synchronous burst and its temporal relation to the phrenic nerve burst were studied under conditions in which the frequency of the latter was changed over a wide range by superior laryngeal nerve stimulation, by changes in ventilation frequency while the phrenic nerve burst was locked to the pump, and by hypocapnic hyperthermia. The data obtained are consistent with the hypothesis of a common rhythmic driver for phrenic motoneurons and sympathetic preganglionic neurons. Stimulation of low-threshold afferents in the superior laryngeal nerve selectively suppressed the phrenic burst together with the inspiration-synchronous sympathetic discharge and produced vasodilatation. The contribution of the inspiration-synchronous sympathetic discharge to neurogenic vasoconstriction was estimated, in the hindlimb of the cat, from the magnitude of the vasodilatation. A late-expiratory burst of sympathetic discharge was produced by systemic hypercapnia and by raising end-expiratory pressure to between 2 and 7 cmH_2O. Circumstantial evidence suggests this late-expiratory burst is due to input from

late-expiratory neurons to sympatnetic preganglionic neurons. As a background to the experimental data a survey is presented of present knowledge of the mechanisms providing mechanical and neural coupling between respiration and circulation. The functional significance of respiratory modulation of sympathetic activity is discussed.

RESUME

Cette thèse présente les résultats d'expériences effectuées chez des chats anesthesiés et chez les chats nonanesthesiés décérébrés au niveau mi-colliculaire, portant sur les propriétés de deux composantes de la décharge nerveuse sympathique chronologiquement liée au cycle respiratoire et que l'on suppose être engendrée dans le système nerveux central. La première composante est une rafale de décharges synchrone avec l'inspiration, la deuxième est une rafale expiratoire tardive, inconnue jusque-là. Nous avons étudié les propriétés de la première et sa relation à la rafale d'activités du nerf phrénique en variant largement la fréquence de ces dernières au moyen de stimulation du nerf laryngé supérieur, au moyen de changements de la fréquence ventilatoire alors que les impulsions phréniques étaient synchronisées avec la pompe respiratoire et au moyen d'une hyperthermie hypercapnéique. Les résultats obtenus sont en accord avec l'hypothèse d'un même générateur de rythme aux neurones moteurs phréniques et aux neurones préganglionnaires sympathiques. Lors de cette expérience, la stimulation de nerfs afférents à seuil bas dans le nerf laryngé supérieur a supprimé sélectivement la rafale de décharges sympathiques et la composante sympathique synchrone avec l'inspiration, tout en entraînant une vasodilatation. Nous avons évalué la contribution de la décharge sympathique synchrone à l'inspiration à la vasoconstriction neurogène dans le membre postérieur du chat à partir du degré de vasodilatation observé. Nous avons produit une rafale expiratoire tardive de décharges sympathiques au moyen d'une hypercapnie systémique

et d'une augmentation de la pression expiratoire terminale à 2 à 7 cm d'eau. Nous avons obtenu des preuves indirectes que la rafale de décharges tardives expiratoires était causée par l'action de neurones "expiratoires tardifs" sur les neurones préganglionnaires sympathiques. Afin de mettre nos résultats en perspective, nous présentons également une revue des connaissances actuelles des mécanismes de couplage neural entre la respiration et la circulation. Nous discutons de la signification fonctionnelle de la modulation de l'activité sympathique par la respiration.

ACKNOWLEDGEMENTS

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Although I have been unable to avoid the term "my work" in this thesis, I am reminded that my opinions and thoughts have been shaped by conversations with many members of the Department of Physiology. To them all, I owe a sincere debt of gratitude.

I am grateful to Joe Petrella for his expert technical assistance and his tireless attention to matters concerning the day to day running of this laboratory. I would like to thank all fellow graduate students on the 11th and 12th floors for their friendship; a special thanks to the "old guard" in whose company I spent many late evenings.

I would like to thank Mrs. Christine Pamplin for her care and effort in preparing this thesis. Special thanks to Dr. John Setrakian for the translation of the abstract into French at short notice. I owe a special debt of gratitude to my mother and father for it was their perseverance and sacrifice that put me in a position that allowed me to carry out the work described in this thesis. Finally, I would like to thank Elizabeth Maher, for all her help, patience and understanding during the final stages of completion of this thesis.

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PREFACE

I have chosen the option provided in section 7 of the Guidelines Concerning Thesis Preparation issued by the Faculty of Graduate Studies and Research, McGill University which allows me to include - as chapters of this thesis - the texts of manuscripts which I co-authored with my supervisor, Dr. Canio Polosa. Chapters 2, 3, 4 and 5 are presented in the form in which they have been published. Chapter 2 was published in Journal of Physiology (London) 385: 545-564, 1987. Chapter 3 was published in a monograph: Organisation of the Autonomic Nervous System: Central and Peripheral Mechanisms, pp. 187-202, 1987. Chapters 4 and 5 were published in the Journal of Physiology (London): 364: 183-198, 1985; 378: 375-390, 1986, respectively.

STATEMENT OF AUTHORSHIP

The four published papers which are part of the thesis were co-authored with C. Polosa. Both of us have contributed to various aspects of the work, from formulation of the questions and planning of the experimental strategy to interpretation and presentation of the results. All experiments were performed by myself with technical assistance from Joe Petrella.

CHAPTER 1 - GENERAL INTRODUCTION

INTRODUCTION

The history of research on the influence of respiration on circulation is now more than two centuries old. Brecher (1956) cites in his monograph the observation of Valsalva and of Morgagni, made in the 18th century, that the jugular vein of the dog collapses during inspiration and swells during expiration. Hales (1753), who is credited with the first direct measurement of systemic arterial pressure (SAP) in animals, noted a fluctuation of arterial pressure at the frequency of respiration. Ludwig (1847) undertook the first investigation of the mechanism of these respiratory fluctuations in SAP. He concluded that the respiratory oscillation in SAP is the result of mechanical and neural influences of respiration on the cardiovascular system. Later investigations have partially filled in some of the details of the neural and mechanical coupling between respiration and circulation. From the past work the view emerges that taking a breath, in terms of the number of mechanisms triggered by it, has as dramatic an effect on the cardiovascular system as does, for instance, going from the supine to the upright posture. Much is left that we still do not understand. Yet, knowledge of the cardiovascular mechanisms triggered by breathing is important not only for a full understanding of how the cardiovascular system normally works but also for predicting the cardiovascular effects of changes in

respiratory system function induced by disease.

This thesis takes up for study one aspect of the neural coupling between the respiratory and the cardiovascular system, namely that labelled in the earlier literature as due to "irradiation" or "spillover" of excitation from the respiratory center to the cardiovascular control centers (see Koepchen, 1981). This concept of spillover should be replaced, partly on the basis of the work to be presented here, with that of the existence of common neuron sets which are antecedent to both respiratory motoneurons and autonomic neurons controlling cardiovascular effectors.

The purpose of this chapter is to review the existing data concerning the effects of respiration on the circulation. This chapter is divided into two major parts. Part one deals with the mechanical coupling between the two systems. It describes the present state of knowledge about the ways in which the respiratory swings in intrathoracic pressure influence venous return and ventricular ejection. Since this is not the main focus of the thesis, however, this subject will be treated in less detail than the sections dealing with the neural coupling. In part two, the neural mechanisms by which respiration can affect the cardiovascular system will be dealt with. The review will present state of knowledge of how mechanisms operating within the CNS (i.e. central) and how reflexes triggered by the act of breathing and mediated by cardiopulmonary afferents and arterial baroreceptors

can influence the activity of the sympathetic and parasympathetic nervous systems.

PART 1. MECHANICAL EFFECTS OF RESPIRATION ON STROKE VOLUME

During a normal respiratory cycle there is an oscillation in SAP and cardiac output as a result of an oscillation in stroke volume. This oscillation in stroke volume is the consequence of the oscillation in intrathoracic pressure associated with respiration. The following is an overview of some of the relevant concepts. For a more extensive coverage of the subject see the reviews by Attinger, 1957; Brecher 1956; Bromberger-Barnea, 1981; Fishman, 1986; Guyton, 1973; Holt, 1969; Kalsmanson and Veyrat, 1978; McGregor, 1979; Moreno, 1969, 1978, 1982; Permutt and Caldini, 1978; Sharpey-Schafer, 1965.

1. Effects of Respiration on Venous Return

That respiration has an important influence on venous return to the heart has long been known. The monograph by Brecher (1956) describes in detail the historial evolution of concepts regarding the mechanisms by which changes in intrathoracic pressure affect venous return to the right atrium. The aspiration theory (Haller,

1760; Donders, 1859: cited in Brecher, 1956) was based on the assumption that veins behave like rigid tubes and that venous flow is proportional to the pressure gradient. Thus, blood flow towards the heart (venous return) must increase, and so must the filling of the heart, when intrathoracic pressure is lowered during inspiration. The 'collapse theory' (Holt, 1941, 1944), on the other hand, predicted that the extrathoracic veins would collapse, at points at which they enter into the thorax, due to a decrease in intrathoracic pressure. The term "collapse" refers to any state of the vein in which its cross-sectional area is not circular, thereby increasing resistance to flow, and does not necessarily mean a complete closure of the vein. Under these conditions, flow may be described with a reasonable degree of accuracy by a modification of the Hagen-Poiseuille law in which the radius (r) term of the Poiseuille equation (r^4) is replaced by a term describing the minor (a) and major (b) hemiaxes of an ellipse $\lceil (2a^2b^3/a^2+b^2) \rfloor$ (Brecher, 1956).

In both man and dog instantaneous blood flow in the inferior and superior vena cava during an inspiratory effort in which the pleural pressure is reduced from -5 cmH_2O to -10 cmH_2O (producing a normal tidal volume) may achieve peak values 2-3 times higher than at end-expiration (Brecher, 1952a; Nordenstrom and Norhagen, 1965). Under these conditions, venous return is directly proportional to the pressure gradient. However, when the

inspiratory effort is such that intrathoracic pressure is more negative than -10 cmH_2O there is an initial increase in venous return to the right atrium that is at least in part due to depletion of an extrathoracic venous compartment into the thoracic compartment. This initial stage is followed by collapse of venous vessels leading into the thorax, which limits flow. The degree of collapse is directly proportional to the negativity of the intrathoracic pressure (Holt, 1941, 1944; Brecher, 1952, Nordenstrom and Norhagen, 1965). Intrathoracic pressures more negative than -20 cmH_2O will produce complete collapse of veins entering the thorax. Under conditions of complete collapse of extrathoracic venous vessels right atrial stroke volume is maintained by the blood volume in the thoracic veins. Whether veins collapse at a sharp demarcation point at the thoracic inlet or whether it involves a longer segment of extrathoracic vessels is not clear. Evidence that collapse occurs when intrathoracic pressure is lowered below that required to produce normal tidal volume is provided by an earlier observation (Burton-Optiz, 1902). In the dog, the flow in the jugular vein increased during inspiration, but when inspiratory effort was increased, flow decreased and veins became distended. Thus, both views the "aspiration" and "collapse" theory apply for explaining the effect of intrathoracic pressure changes on venous return to the heart.

The forces acting on the venous wall to cause collapse during inspiration have been analyzed, Holt (1941, 1943), Duomarco and

Rimini (1954), Brecher (1956), Rodbard and Saiki (1955), Permut and Riley (1963). As a result, the general view has emerged that the pressure-flow characteristics of veins or any thin-walled collapsible tubing is drastically different from that in the more rigid arteries. The reason for this difference is that when transmural (extraluminal minus intraluminal) pressure approaches zero the cross-section of the veins does not remain cylindrical, like in the case of arteries, but flattens to an elliptical configuration, thereby increasing resistance and limiting venous return. In man and dog, in the supine position, at end-expiration, the pressure in the inferior and superior vena cava as they enter the right atrium is near atmospheric. The intrathoracic pressure at end-expiration (at FRC) is subatmospheric (-5 cmH_2O) while pressure in the abdominal cavity is slightly greater than atmospheric (2-3 cmH_2O); venous vessels of the head and neck and upper extremities are exposed to atmospheric pressure. During inspiration, as a consequence of the decreasing intrathoracic pressure which reaches values of -10 cmH_2O or more negative at end-inspiration, the intraluminal pressure of the thoracic veins is also reduced with respect to atmospheric pressure, although it remains positive with respect to intrathoracic pressure, while pressure in the abdominal compartment remains constant or may in fact show a modest increase (see the following section). At the boundary between the high and low pressure regions, the transmural pressure of the veins approaches

zero. As a result the veins begin to collapse at the points of entry into the thorax; the decrease in cross-sectional area increases resistance and thereby limits venous return. Further lowering of intrathoracic pressure increases further the negative transmural pressure gradient and thereby increases resistance further. Therefore, despite the increasing pressure gradient flow remains constant. One factor which is important in determining whether collapse will occur and if so at what intrathoracic pressure is the mean circulatory pressure. The role of venous filling is clearly illustrated by the observation that in congestive heart failure patients with high central venous pressure and in whom right atrial pressure does not decrease below zero during inspiration (-10 cmH_2O intrathoracic pressure) there is no sign of collapse of the inferior vena cava at the point of entry into the thorax (Brecher, 1956).

The venous pressure-flow relationship, when the pressure gradient is increased by lowering the downstream pressure, is graphically illustrated in Guyton's "venous-return" curves (Guyton, 1952). For a more comprehensive review of pressure-flow relationships in collapsible tubes the reader is referred to Conard (1969); Katz et al (1968); Holt (1969); Bower and Noordergraaf (1978); Kalsmanson and Veyrat (1978).

Increasing the pressure gradient for venous return by increasing inflow pressure (abdominal) while keeping intrathoracic pressure constant, produces an increase in venous flow (Conrad,

1969). However, increasing the pressure gradient for venous return by decreasing the downstream (intrathoracic) pressure, while keeping the other relevant variables constant, increases the resistance, so venous flow may increase or stay the same depending on whether the vessel remains open or begins to collapse as a result of the decreasing transmural pressure.

At the time during the respiratory cycle when venous return to the right heart increases, there is no equivalent increase of inflow to the left ventricle, since the pressure gradient from the pulmonary veins to the left atrium is unaffected by changes in intrathoracic pressure (heart and lungs are exposed to the same external pressure). In fact, for reasons described below, the inflow to the left ventricle actually decreases. Subsequently, of course, the increases in cardiac output of the right ventricle will increase flow through the pulmonary bed (see below) and eventually raise the left ventricular filling pressure.

2. Effects of Respiration on Blood Flow in the Abdominal Inferior Vena Cava

The effect of respiration on abdominal pressure has been the subject of debate. An earlier view (Emerson, 1911) was that intraabdominal pressure increases during inspiration as the diaphragm descends like a piston into the abdominal cavity, thus increasing the pressure gradient between abdominal and thoracic

segments of the IVC. Later experimental data (Moreno et al., 1967, 1977) show that intraabdominal pressure may increase, decrease or not change depending on the behaviour of the abdominal muscles. In reclining dogs, either awake or under light pentobarbital anesthesia, the abdominal muscles contract during expiration, raising intraabdominal pressure. At end-expiration, shortly before the onset of the diaphragmatic descent, the abdominal muscles relax. The resulting enlargement of the abdominal cavity leads to a fall in intra-abdominal pressure (Moreno et al., 1967). Under these conditions the value of abdominal pressure at any time during inspiration depends on the balance between the opposing effects of diaphragmatic contraction and of abdominal muscle relaxation. During a single inspiration, the abdominal muscle relaxation may dominate the initial part of inspiration, causing a decrease in abdominal pressure, while diaphragmatic contraction may dominate in the last part of inspiration, causing an increase in abdominal pressure. At deeper levels of anesthesia, the abdominal muscles are not recruited during the expiratory phase of the respiratory cycle while the intercostal muscles and the diaphragm maintain their activity (Moreno, 1977). Under these conditions the descent of the diaphragm is unopposed and intraabdominal pressure rises during inspiration, thereby increasing the gradient for venous return.

Concerning the question of how the increased abdominal pressure in inspiration influences abdominal IVC flow, one has to

consider separately the effects on the two constituent venous systems. The abdominal inferior vena cava channels venous blood from two vascular beds of approximately equal size, the 'systemic' and the 'splanchnic' system. The former channels the venous blood from the kidneys, pelvic organs and lower extremities directly into the IVC. The latter collects venous blood from the intestine and spleen into a single large vein, the portal vein. The portal vein enters the liver and subdivides extensively to feed the capillary bed (sinusoids) of the liver. The venous outflow from the liver flows through the hepatic venules and veins into the inferior vena cava. This anatomical arrangement introduces an additional resistance to the outflow from the splanchnic vascular bed. As a result the venous pressure in the splanchnic system is two to three times higher than in the systemic inferior caval system. As the diaphragm descends during inspiration, it compresses the liver and this results in collapse of the hepatic venules, which are devoid of supporting connective tissue (Moreno, 1977). As a result, hepatic venous outflow is reduced at the time the pressure gradient for venous return is greatest (i.e. end-inspiration).

Thus, during inspiration, flow in the inferior vena cava is largely the result of an increased component from the systemic inferior caval system while the component due to splanchnic flow declines. Conversely, during expiration flow in the supra-hepatic segment of the inferior vena cava is the result of an increased component from the splanchnic bed while the component from the

systemic caval system decreases.

The effect of respiration on venous return from the lower extremities varies in different species depending on the pattern of inspiratory muscle activity. This is markedly different in dogs and humans during quiet breathing. Anaesthetized dogs breathe mainly with the intercostal muscles (D'Angelo et al., 1988) while humans use mainly the diaphragm (Sharp et al., 1975). In human subjects, in the recumbent position, venous return from the legs, during inspiration is reduced to 35% of that at end-expiration (Willeput et al., 1984). This may be due to the fact that the preferential use of the diaphragm in humans results in an impairment of venous return from the legs during inspiration because the descent of the diaphragm and the resulting rise in abdominal pressure compress the iliac veins. As a consequence blood flow into the thorax is maintained by the blood volume contained in the abdominal vena cava which may be depleted. The magnitude of the fall in femoral venous blood flow during inspiration was related to the change in abdominal pressure which in turn depends on the contribution of the diaphragm to inspiration. If the intercostal muscles are used preferentially to lower intrathoracic pressure during inspirations, as occurs in dogs, abdominal pressure decreases and femoral venous flow will increase. Thus the pattern of venous return from the lower extremities during inspiration in the supine posture, in most animals, is largely determined by the pattern of inspiratory muscle

contraction, which in turn influences abdominal pressure. It must be pointed out, however, that this description only applies to the supine posture. In the upright posture, it is not clear whether the inspiratory-increase in intraabdominal pressure would be capable of collapsing the iliac veins because of the high intraluminal pressure resulting from the hydrostatic effect. Furthermore, changes in body position may modify the mechanics of the respiratory system (Navajas et al., 1988; Troyer and Ninane, 1987) and as a result modify the mechanical load the abdominal and thoracic compartments impose on the inferior vena cava.

3. Effects of Respiration on the Left Heart

A number of studies have simultaneously measured flow in the pulmonary artery and aorta (Robotham et al., 1979; Schrijen et al., 1975; Summer et al., 1979). During inspiration, right ventricular stroke volume increases while the left decreases. In expiration the reverse occurs. The explanation for the inspiratory decrease in left ventricular stroke volume was originally thought to be an increase in the capacity of the pulmonary vessels (see section 4b) resulting in pooling of blood in the lungs and consequently in a decreased venous return to the left atrium (Trimby et al., 1922; Ruskin et al., 1973). This hypothesis would predict that the Mueller manoeuver (inspiration against a closed glottis) which

results in very negative values of intrathoracic pressure but no change in lung volume, hence no change in pulmonary vascular volume), would eliminate the difference between right and left ventricular stroke volumes. In fact, the Mueller manoeuvre exaggerates the disparity between left and right stroke volume (Summer et al., 1979) compared to that caused by a normal breath. Therefore, an increase in pulmonary vascular capacity cannot be the only explanation for the fall in left ventricle stroke volume during inspiration. Two additional experimental findings are consistent with this conclusion. Firstly, pulmonary venous flow does not decrease during inspiration (Summer et al., 1979). Secondly, in right heart bypass preparations, in which pulmonary venous flow is kept constant, left ventricular stroke volume still falls during inspiration (Summer et al., 1979; Bromberger-Barnea et al., 1981).

A second hypothesis proposes that the fall in left ventricle stroke volume during inspiration may be a direct consequence of an increase in impedance to left ventricular emptying, as a result of the decrease in pleural pressure (Schrijen et al., 1975; Karim et al., 1984; Robotham et al., 1978; Summer et al., 1979). This increased impedance occurs because the fall in the pleural pressure surrounding the heart requires that the left ventricle generates a higher pressure before blood can leave the thorax (i.e. increased end-systolic volume). In other words, lowering the pressure around the heart has the same effect as raising the aortic

diastolic pressure. It is clear that during a Mueller manoeuver, when intrathoracic pressure can be as low as - 50 mmHg, the increased afterload may decrease stroke volume. It is not clear, however, whether the magnitude of the increased afterload that occurs during quiet breathing is sufficient to decrease left ventricular stroke volume to the extent observed (cf. Bromberger-Barnea, 1981), especially in light of the observation by Herndon and Sagawa (1969) that left ventricular stroke volume is not reduced by increases in aortic pressure up to 180 mmHg.

The decrease in intrathoracic pressure during inspiration also increases right ventricular impedance since the peripheral output bed of the right ventricle, the pulmonary circulation, is surrounded by alveolar pressure. Both the increased right ventricular filling (right ventricular prelaod) and the increased right ventricular impedance (afterload) raise pulmonary artery pressure and cause an increase in right ventricular end-diastolic volume.

A third explanation for the decreased left ventricular stroke volume in inspiration is that during inspiration the increased end-diastolic right ventricular volume limits left ventricular filling. The mechanism for this interventricular interaction appears to be that the increased right ventricular diastolic pressure shifts the interventricular septum towards the left ventricular cavity and causes a decrease in the free wall-to-septum diameter (Beyar et al., 1987; Brinker et al. 1985; Peters et al., 1988). This alteration in ventriculary geometry caused by the

increased right ventricular volume reduces the distensibility of the left ventricle. This is consistent with the finding of increase in left atrial filling pressure during inspiration (Olsen et al., 1985). The decrease in left ventricular distensibility produced by the enchroachment of the septum reduces left ventricular end-diastolic volume which would thereby limit left ventricular stroke volume.

A fourth possible mechanism of the inspiratory fall in left ventricular stroke volume is that distension of the right atrium and ventricle may increase pericardial pressure and hence limit the filling of the left ventricle. Lewis (1908) first raised the possibility that the pericardium may, under particular conditions, hinder atrial filling. An increase in the afterload of the left and right ventricles by constriction of the pulmonary artery or aorta produced a marked increase in right or left atrial pressure but failed to increase pericardial pressure (Kenner and Wood, 1966). When atrial and ventricular filling pressure was increased in dogs by rapid intravenous infusion of large volumes of Ringer solution which increased left ventricular end-diastolic pressures to around 20 mmHg, transpericardial (pericardial-pleural) pressure did not change (Tyson et al., 1984). These findings indicate that the pericardial "capacity" is not exceeded by large increases in atrial and ventricular diastolic volumes and therefore is unlikely to be exceeded by the increase in venous return during inspiration (cf. Glantz et al., 1978).

Under pathological conditions of pericardial effusion or pericarditis, however, the pericardial pressure is elevated. Under these conditions respiration can have a dramatic effect on cardiac output (Dornhorst, 1952b; Morgan et al., 1965; Assanelli et al., 1987), because the pericardium exerts a restraining force on ventricular filling, in particular on the thin-walled right ventricle. An inspiratory increase in the filling of the right ventricle can cause encroachment on the left ventricle through an increase in pericardial pressure and through a leftward displacement of the interventricular septum (i.e. decreased left ventricular compliance). The left ventricle is therefore compressed, left ventricular end-diastolic volume is reduced. This has been proposed as a possible mechanism for the marked decrease in pulse pressure due to a decrease in stroke volume (phenomenon of "pulsus paradoxus"), that is sometimes seen with constrictive pericarditis or pericardial effusion (Donhorst, 1952a; McGregor, 1979).

4. Effects of Lung Inflation on the Pulmonary Circulation

a) Resistance to flow

Pulmonary blood flow is influenced by the changes occurring in the pulmonary vascular bed during the respiratory cycle (see reviews by Culver and Butler, 1980; Fishman, 1986; Gil, 1980). The effect of a change in pleural pressure on the calibre of the

pulmonary vessels depends on the location of the vessels. The perivascular pressure of intra- and extra-pulmonary blood vessels is influenced directly by the pleural pressure. Alveolar vessels, on the other hand, are not affected by changes in pleural pressure since they are exposed to atmospheric pressure. Two forces act on pulmonary blood vessels during lung inflation. One acts in a direction parallel to the axis of the vessels and tends to lengthen and narrow the vessels as the lung expands, thereby increasing resistance. The other acts along the vessel radii due to the traction from the interstitium which tends to increase its diameter and thus decrease resistance.

Lung inflation by lowering pleural pressure while maintaining pulmonary artery and left atrial pressure constant has a biphasic effect on pulmonary vascular resistance (West, 1965). Starting from atmospheric pressure (residual volume), lung inflation initially produces a decrease in pulmonary vascular resistance, the maximum decrease occurring at a transpulmonary pressure of 10 to 17 cmH_2O. Further inflation approaching total lung capacity ($\sim$ 30 cmH_2O) increases resistance (Roos et al., 1961; Howell et al., 1961). This biphasic effect of pulmonary resistance is characteristic of lung inflation produced by lowering pleural pressure (i.e. negative pressure inflation). When pleural pressure is lowered, transmural pressure in the alveolar vessels remains constant relative to alveolar pressure, but the transmural pressure of extra-alveolar vessels increases. Positive pressure lung

inflation produces an increase in perivascular pressure (i.e. decreased transmural pressure) which effectively narrows the alveolar vessels and thus produces an increase in total pulmonary resistance. During positive pressure inflation, the pressure in the alveolar vessels decreases relative to alveolar pressure, whereas the pressure in the extraalveolar vessels remains constant relative to pleural pressure. Thus the net effect of lung inflation depends on how each segment of the circulation is influenced by alveolar and pleural pressures. Additionally, the magnitude of the change in pulmonary resistance produced by lung inflation depends on the existing intraluminal pressure. For example, Roos et al (1961) showed that the change in total pulmonary vascular resistance with lung inflation became much smaller when intravascular pressure was high. This can be explained by the fact that at high intraluminal pressure the vessel is close to its limit of distensibility, therefore a change in pleural pressure produces little change in vessel radius and consequently little change in pulmonary vascular resistance.

b) Pulmonary Blood Volume

Trimby and Nicholson (1924) originally reported that when lung inflation was produced by lowering the pressure on the external surfaces of the lungs, without altering the pressure on other thoracic structures, well marked respiratory waves in blood

pressure were observed, characterized by a fall during inspiration and a rise during expiration. Since the decrease in pleural pressure was restricted to the surface of the lung, this result clearly suggested that lung inflation by reducing pleural pressure results in an increase in pulmonary vascular bed capacity. This may in part be responsible for the inspiratory fall in SAP as well as for delaying the rise in pressure resulting from an increase in filling of the right heart during inspiration.

The effects of lung inflation of pulmonary capacitance were investigated by Permutt et al. (1961). In excised lungs of dogs, at low pulmonary arterial pressures (-4 to 0 cmH_2O) an increase in lung volume produced by lowering surrounding pressure (from 0-30 cmH_2O) caused an increase in pulmonary vascular volume. If pulmonary arterial pressure was higher; ($\geq$ 5 cmH_2O) lung inflation produced a decrease in vascular volume, at an intermediate value (0-5 cmH_2O), lung inflation produced a biphasic effect, an initial increase in vascular volume followed by a decrease. These results led to the hypothesis that the pulmonary vascular bed behaves as if it is composed of two compartments in parallel, responding oppositely to lung inflation. One compartment consists of the larger extraalveolar vessels which always increase in volume with inflation. The other compartment, thought to consist of the alveolar capillaries, decrease its vascular volume with lung inflation. The overall volume change in the vascular bed with inflation depends on the relative magnitude of these two effects.

The biphasic results described above could be explained by the hypothesis (Permutt et al., 1962) that when the vascular pressure is low, the compressed compartment contains a relatively small amount of blood, and the overall effect of inflation of the lungs is dominated by the expanded compartment. At higher vascular pressures the compressed compartment contains a relatively large amount of blood, which is forced out as transpulmonary pressure rises. At low vascular pressures ($\sim$ 5 cmH_2O) there is little change in pulmonary vascular volume, indicating that under these conditions the increase in volume of the expanded compartment equals the decrease in volume of the compressed compartment.

It may be expected that changes in pulmonary vascular volume during respiration will influence the inflow of blood to the left heart. Visscher et al (1924) conducted a series of experiments to determine how soon a change in venous return to the right atrium is reflected in a change in left ventricular output and hence in systemic arterial pressure. When the inferior vena cava was suddenly clamped near the heart in anesthetized dogs, in the absence of breathing movements, the arterial pressure did not change for 3-4 heart beats (2-3s) and then fell precipitously. When the clamp was released, arterial pressure did not change again for a period of 3-4 heart beats and then increased. The same results were obtained when the experiments were repeated on animals with denervated hearts to eliminate any possibility of reflex mechanisms obscuring the purely mechanical effects of interference

with the blood supply to the heart. These results suggest that the increased right ventricular stroke volume due to inspiration may not be reflected in an arterial pressure change until about 3-4 heart beats (2-3 s) after inspiration onset. Assuming 8-10 heart beats per respiratory cycle these data predict a fall in SAP in inspiration and a rise in expiration if right ventricular output increases in inspiration and decreases in expiration. In other words, the latency for a SAP change introduces a complete reversal in the phase relationship between intrathoracic pressure and the resulting change in blood pressure. The delay in left ventricular output following a change in right ventricular output is due to the high compliance of the pulmonary circulation and the increase in pulmonary blood volume during inspiration already described. The high compliance of the pulmonary vasculature not only delays the respiratory change in left ventricular stroke volume but also attenuates the amplitude of the resulting SAP oscillations (Maloney et al., 1968). Mean pulmonary transit time measured from the appearance of a test substance in the pulmonary vein following injection into the pulmonary artery is of the order of 3-4 seconds in both man and dog (Fishman, 1986). This transit time will vary in proportion to the fluctuation in pulmonary blood volume during the respiratory cycle.

From the data above that emphasize the delay imposed by properties of the pulmonary circulation on the left ventricular response to changes in right ventricular output it may be expected

that the oscillation of SAP caused by the mechanical effects of respiration will have a phase-relation to the respiratory cycle which depends on respiratory frequency. Lewis (1908) tabulated the data obtained in all investigations carried out on animals and human subjects between 1859 and 1906 of arterial pressure waves with the period of respiration. Of these investigations, 11 described an inspiratory rise in arterial pressure, 9 an inspiratory fall and 6 obtained either an inspiratory rise or an inspiratory fall in SAP depending on relative contribution to breathing of chest wall and abdominal muscles. From a synthesis of the data it is apparent that there is a relationship between the phase (inspiration or expiration) in which SAP would rise and the period of the respiratory cycle. At moderate rates of breathing (12-20 per minute) blood pressure fell during inspiration while at slower rates (10 per minute) inspiration was associated with a rise in SAP.

PART 2. NEURAL MECHANISMS COUPLING RESPIRATION AND CIRCULATION

1. Evidence of Neural Coupling Obtained From Effector Recording: Traube-Hering Waves of Systemic Arterial Pressure and Respiratory Sinus Arrhythmia

Traube (1865), observed that interruption of artificial ventilation for several consecutive pump cycles in vagotomized, curarized, dogs resulted in an increase in systemic arterial pressure (SAP), presumably due to asphyxia, superimposed upon which was a slow rhythmic oscillation with a period of 10 seconds. The oscillation period was comparable to the period of abortive respiratory movements observed, under the same conditions, in animals with incomplete paralysis due to insufficient curare. The oscillation period was also comparable to the period of the spontaneous respiratory activity prior to paralysis. Since the animals were paralysed and not ventilated, mechanical effects of ventilation were eliminated, while bilateral cervical vagotomy and lack of inflation eliminated vasomotor reflexes triggered by pulmonary sensory afferents as possible sources of these respiratory fluctuations in SAP. Traube (1865) proposed that this oscillation of SAP was due to an oscillation in vasoconstrictor tone consequent to an "irradiation" of excitation from the respiratory to the vasomotor centre. Similar findings of a "respiratory" oscillation of SAP in the absence of breathing

movements were later reported by Hering (1869, cited in Koepchen, 1984) who wrote that "we have adequately proved that the vascular system shows respiratory fluctuations associated with the respiratory movements which, as the latter, originate from the so-called respiratory centre" (quoted in Koepchen, 1984). Thus, the SAP waves described by Traube and by Hering can be considered as the first, indirect, demonstration of a central respiratory modulation of sympathetic activity since their observations were in vagotomized animals. This observation, and its insightful interpretation, preceded by at least half a century the first direct demonstration of respiratory modulation of sympathetic activity by electrical recording from sympathetic nerves (Adrian et al., 1932). These SAP waves at the respiratory frequency observed under the exceptional experimental conditions of absence of breathing movements have since been labelled Traube-Hering waves (Schweitzer, 1945). The term has later been extended to SAP oscillations of the same nature observed during spontaneous or artificial ventilation. The main criteria for defining a slow SAP oscillation as a Traube-Hering oscillation are that the SAP oscillation must (i) be neurogenic (ii) persist in the absence of mechanical ventilation, and (iii) have the same period as the simultaneously recorded phrenic nerve burst (Preiss and Polosa, 1974; Polosa, 1984). Joels and Samueloff (1955) improved on the experimental preparation for demonstrating Traube-Hering waves by using the paralysed, apneic, cat under "diffusion respiration".

The N_2 in the lungs is washed out and replaced with 100% O_2, thus there is a diffusion gradient for O_2 to ensure adequate oxygenation for a long time in the absence of ventilation. However, CO_2, cannot be washed out and respiratory acidosis develops. Hypercapnia is a potent stimulant of the respiratory centre activity via the central and peripheral chemoreceptors, and this enhances the probability of "irradiation" and hence of Traube-Hering waves production. Using this preparation Joels and Samueloff (1955) showed an important feature of Traube-Hering waves, namely that they disappear when the activity of the respiratory centres is depressed, e.g. by anesthetics. Further details about the peripheral mechanisms of Traube-Hering waves were added later. Koepchen and Thurau (1958) showed that Traube-Hering waves persist after cardiac denervation and therefore an oscillation in peripheral vascular resistance must be part of the mechanism of generation. Furthermore, Koepchen and Thurau (1958) demonstrated directly an oscillation in peripheral vascular resistance by showing, in the dog, a respiratory oscillation in flow through the popliteal artery perfused at constant pressure.

Traube-Hering waves have been at times confused with Mayer waves (Mayer, 1976) and vice versa (e.g. Guyton et al., 1952). Mayer waves are SAP waves reminiscent of Traube-Hering waves but can be distinguished from Traube-Hering waves mainly because they have a frequency lower than the respiratory frequency, typically 1-5 cycles/min, and persist when rhythmic central respiratory

neuron activity is abolished, e.g. by hypocapnia (Schweitzer, 1945). Mayer waves are observed in conditions of low SAP (Preiss and Polosa, 1974), impaired cerebral circulation (Guyton and Satterfield, 1952) or anoxia (Polosa, 1984). Mayer waves are due, like Traube-Hering waves, to a rhythmical fluctuation in sympathetic activity (Preiss and Polosa, 1974) and may appear in CNS-intact animals as well as in animals with cervical spinal cord transection (Kaminski et al., 1970). The properties of Mayer waves have been reviewed (Polosa, 1984).

In addition to a centrally-generated respiratory modulation of vascular resistance, there is evidence also for a centrally generated respiratory modulation of heart rate and ventricular contractility, mediated by a modulation of both sympathetic and parasympathetic cardiac nerve activity. A respiratory oscillation in heart rate, characterized by increase in inspiration and decrease in expiration, first described by Ludwig (1847), has been called respiratory sinus arrhythmia (RSA). RSA persists after bilateral stellectomy (Heymans, 1929). Thus RSA has been attributed to "irradiation" of respiratory centre activity resulting in inhibition during inspiration of cardiac parasympathetic neurons (Daly, 1983). A sympathetic component in RSA has been difficult to demonstrate, presumably because of the slow frequency-response characteristics of the sympathetic-cardiac effector system (see Introduction, Part II, section 7), when compared to the respiratory frequency. However, in vagotomized

dogs in which respiratory rate was lowered to less than 5 cycles per minute by hypothermia (32°C) Davis et al. (1977) were able to demonstrate a propranolol-sensitive RSA, which was absent at normal respiratory rates. A respiratory fluctuation in peak ventricular pressure was demonstrated in the isovolumic, paced, left ventricle of the vagotomized dog (Levy et al., 1966). The modulation persisted after muscle paralysis and was attributed to a central mechanism modulating the cardiac sympathetic innervation. There is no data on a possible role of the parasympathetic cardiac innervation in the respiratory modulation of ventricular contractility.

2. Respiration Modulates Sympathetic and Parasympathetic Discharge by Central and by Reflex Mechanisms

In addition to the central "irradiation" hypothesized by Traube (1865) as the generation mechanism of the respiratory modulation of vasoconstrictor tone, reflex mechanisms activated by sensory receptors which monitor physiological variables influenced by respiration (for instance lung volume, SAP) may produce a similar effect. Both sets of mechanisms, central and reflex, will be discussed in detail in separate sections to follow. Here some key experimental observations will be described which emphasise the range of mechanisms by which this single effect, the respiratory modulation of sympathetic and cardiac parasympathetic neuron

discharge, can be produced. As soon as the first electrical recordings of sympathetic nerve activity were made (Adrian et al., 1932) and with that came the demonstration of respiratory grouping in this activity, reflex mechanisms were proposed as the exclusive mechanism of generation of the respiratory grouping (Bronk et al., 1936). The role played by both central and reflex mechanisms was clearly demonstrated by Tang et al (1957) for the sympathetic activity and by Anrep et al (1936a,b) for the cardiac parasympathetic activity.

Tang et al (1957) showed that in bilaterally vagotomized, widely thoracotomized, sino-aortic denervated cats, a splanchnic nerve discharge coincided with the phrenic burst. When these animals were made hypocapnic both the central respiratory activity, as indicated by the absence of phrenic discharge, and the respiratory modulated pattern of sympathetic activity were abolished. This was consistent with the findings of Adrian et al (1932), that a respiratory-modulated pattern of sympathetic discharge could be produced by a central mechanism. In order to determine if reflex mechanisms activated by ventilation could influence sympathetic activity, the influence of the respiratory center was abolished by hyperventilation in air in cats with intact vagus and baroreceptor nerves. Under such conditions, splanchnic discharge was still modulated at the frequency of ventilation, thus indicating that the respiratory center was not the sole source of the respiratory modulation of sympathetic discharge. Two possible

sources of the ventilation-related modulation of sympathetic discharge were tested: the pulmonary afferents activated by lung inflation and baroreceptors activated by fluctuations in SAP due to the mechanical effects of ventilation (described in Section 1 of this thesis). In hypocapnic, bilaterally vagotomized cats, oscillations in sympathetic activity time-locked to ventilation persisted. Wide bilateral thoracotomy, which abolished the ventilation-related fluctuations in SAP, or bilateral carotid sinus nerve section eliminated the oscillation in sympathetic nerve discharge. To test the possibility that pulmonary afferents could reflexly produce a ventilation-related modulation of sympathetic discharge, hypocapnic cats were subjected to a bilateral pneumothorax which abolished fluctuations in SAP. Under these conditions, an oscillation in sympathetic discharge at the frequency of the ventilator persisted. Subsequent bilateral vagotomy abolished this oscillation. Thus, Tang et al (1957) showed that the respiratory influence on sympathetic discharge is mediated by a central mechanism involving respiratory neuron activity and by two reflex mechanisms, one triggered by arterial baroreceptors activated by ventilation-induced fluctuations in SAP, the other by pulmonary afferents activated by lung inflation.

In two classical papers, Anrep et al (1936a,b) demonstrated that central and reflex mechanisms can produce a respiratory modulation of parasympathetic cardiac motor tone, as inferred from the phenomenon of RSA. A cross-perfused preparation was used

whereby the head of the experimental dog was vascularly isolated from the systemic circulation and perfused with blood from a donor dog. This preparation ensures that the carotid sinus perfusion pressure is maintained at a stable, relatively high, level in order to maintain a constant, high level of background cardiac vagal tone. Hering (1930) had previously reported that RSA was critically dependent on the maintenance of a high carotid sinus pressure; RSA was abolished by carotid sinus nerve section or unloading of the baroreceptors by occlusion of the common carotid arteries. Furthermore, with such a preparation the level of central respiratory activity could be manipulated by varying the CO_2 tension of the blood perfusing the head without changes in systemic CO_2 in the experimental animals. The dogs were bilaterally stellectomized: thus the only mechanism for producing a RSA must be vagal. In order to study the central mechanism generating RSA, pulmonary reflexes were abolished by bilateral cervical vagotomy. Under normocapnic conditions, heart rate increased by 10-30 beats/min with a latency of 1 or 2 beats following onset of the phrenic burst. This level was maintained for the rest of the period of phrenic activity. Upon termination of the phrenic burst heart rate returned to the pre-inspiratory level. During cephalic perfusion with hypocapnic blood, the magnitude of the RSA decreased in proportion with central respiratory activity. RSA was abolished when phrenic activity ceased.

In order to study the reflex mechanism, the activity of the respiratory center was abolished by perfusing the head with hypocapnic blood. Lung inflation with pressures in the range from 2 to 15 cm H_2O caused a cardioacceleration the magnitude of which depended on the degree of lung inflation. Inflations with higher pressures, usually beyond 30-40 cm H_2O (approaching total lung capacity), produced a slowing of the heart. Both the cardioacceleration at moderate inflation pressures and the bradycardia at larger inflation pressures were reflex in nature. These effects were abolished by denervation of the lungs. Thus, Anrep et al (1936a,b) showed that the parasympathetic-mediated RSA is produced by a central mechanism, involving respiratory neuron activity, and by a reflex mechanism triggered by pulmonary afferents activated by lung inflation. An additional mechanism, triggered by baroreceptor afferents, is implied by their observation that parasympathetic cardiac tone is exquisitely sensitive to the level of carotid sinus pressure.

3. Centrogenic Respiratory Modulation of Sympathetic Discharge

A rhythmic discharge of sympathetic nerves of cats, synchronous with the phrenic nerve discharge, was first described by Adrian et al., 1932. The synchronism between phrenic and sympathetic burst persisted when depth and frequency of breathing were increased by asphyxia. Since then, this observation has been

repeatedly confirmed in recording from pre- and post- nerves in various species, in cats, dogs, rabbits and rats (Adrian et al., 1932; Okada and Fox, 1967; Tang et al., 1957; Cohen and Gootman, 1970; Koizumi et al., 1971; Seller, 1973; Preiss et al., 1975; Barman and Gebber, 1976; Preiss and Polosa, 1977; Gerber and Polosa, 1978, 1979; Janig et al., 1980; Bachoo and Polosa, 1985; Kubin et al., 1985; Bachoo and Polosa, 1986; Gilbey et al., 1986; Millhorn, 1986; Bachoo and Polosa, 1987; Czyzyk et al., 1987; McAllen, 1987). In whole nerve recordings, the rhythmic bursting of sympathetic nerves indicates a synchronized modulation of the probability of firing of populations of pre- and post-ganglionic neurons.

A few investigators have failed to detect an obvious centrally generated component of sympathetic discharge coincident with the phrenic burst (Bronk et al., 1936; Dontas, 1955). This may be due to a number of possible reasons: 1) Deep anesthesia, particularly with barbituates which markedly depresses sympathetic activity (Millar and Biscoe, 1965; Morita et al., 1987). The failure to detect an obvious respiration-synchronous sympathetic discharge may be symptomatic of a generally depressed sympathetic nervous system. Under such conditions a weak inspiration-related sympathetic discharge is only apparent after averaging, using the onset of the phrenic burst as a trigger (Cohen and Gootman, 1970, 1973). 2) Hypocapnia due to hyperventilation since in both the above mentioned studies the animals were paralyzed and ventilated with

positive pressure. In either case, no evidence of central respiratory activity (by recording phrenic nerve activity) was provided. 3) A reflex inhibition of sympathetic activity which may coincide with the phrenic burst thereby preventing the inspiratory burst of sympathetic discharge. A sympatho-inhibitory mechanism coincident with the phrenic burst is possibly mediated by arterial baroreceptors. During positive pressure ventilation, SAP increases during inflation (Tang et al., 1957). With intact vagus nerves pump-locking of the phrenic burst may occur (Petrillo et al., 1983). If the phase-relation of phrenic to pump was such that lung inflation coincided with the phrenic burst, the increase in SAP produced by inflation could cause an inhibition of sympathetic activity at a time when the central respiratory influences would otherwise have produced an increase in SPN activity. Another hypothetical mechanism may involve pulmonary afferents. With intact vagus nerves, lung inflation coincident with the phrenic burst can produce an inhibition of sympathetic activity (see section..) to an extent that a respiratory modulated pattern of sympathetic discharge is no longer evident even in averaged records of sympathetic activity. This possibility is clearly illustrated in Figure 1 of Gootman and Cohen (1980) where lung inflation produced by a positive pressure, phrenic triggered, ventilator abolished all evidence of facilitation of sympathetic discharge during the period of phrenic discharge. The inspiration-synchronous facilitatory influence was clearly apparent

when lung inflation was withheld.

Respiratory grouping of sympathetic discharge has been observed in animals with bilateral cervical vagotomy (see above): hence the grouping is not reflexly produced by cardiopulmonary vagal afferents activated, directly or indirectly, by lung inflation. Rather, enhancement of vagotomy of the respiratory modulation of sympathetic discharge has been reported by several investigators (Adrian et al., 1932; Okada and Fox, 1956; Cohen and Gootman, 1970).

The first detailed analysis was presented by Cohen and Gootman (1970) based on data obtained in decerebrate, unanesthetized or urethane-anesthetized cat. The electrical activity of the whole splanchnic or cervical nerve was averaged over several respiratory cycles. Characteristically, the level of activity was low in early inspiration (I), increased to reach the maximum in mid-I and thereafter remained approximately constant during the rest of I. When phrenic activity decreased abruptly, marking the end of I, so did the sympathetic activity. At the two phase transitions the change in sympathetic activity lagged behind the corresponding abrupt changes in phrenic nerve activity by 100-200 msec. During the early part of expiration (E) activity was at the minimum, after which it increased slightly to form a low plateau during mid-E and late-E. Procedures which increased or decreased phrenic nerve activity, like vagotomy, airway occlusion, changes in inspired

pCO_2, changes in anesthetic levels or hemorrhage, resulted in a proportional change in the amplitude of the I-component of sympathetic activity. These authors suggested that the observed pattern of modulation of sympathetic neurons activity during the respiratory cycle resulted from synaptic input to sympathetic preganglionic neurons, or to antecedent neurons, provided by a particular type of brainstem respiratory neuron, the I-E phase-spanning neuron (Cohen, 1979). This was the first specific neurophysiological nypothesis to be substituted to the prior undefined concept of "irradiation". Moreover, the complexity of the waveform described by Cohen and Gootman (1970), with two minima and two maxima for each respiratory cycle, suggests that the waveform may result from the superposition of several separate components. The components that have been characterized so far by further studies with both single unit and whole nerve recording will be discussed in the four sections that follow.

a) Inspiratory-related sympathetic discharge (IRSD)

Results of single unit analysis have contributed to understanding how this component of the respiratory modulation of sympathetic discharge is produced (Preiss et al., 1975). Two thirds of the spontaneously firing SPN's of the cervical sympathetic trunk fired during inspiration. One half of these units were silent during E and produced a burst of spikes, or a single spike, during I. The remainder of the units were active, at

the lowest frequency, during E, and generated a burst in I, during which they reached their peak firing frequency. The I-firing had a fixed onset delay from the start of the phrenic nerve burst. The smaller the number of spikes in the burst, the longer the delay from phrenic burst onset. During hypocapnia or hypercapnia the burst structure changed in a characteristic manner. With progressive hypocapnia, spikes were deleted from the burst according to their position within the burst: earlier spikes were deleted at higher pCO_2 than late spikes. With progressive hypercapnia, the instantaneous firing frequency within the burst increased. Earlier and earlier spikes appeared as pCO_2 increased, progressively reducing the delay of the sympathetic burst onset from the phrenic burst onset. Increasing the excitability of the neurons, e.g. by activating a somatic afferent input which facilitated, but did not fire, the units, produced effects on burst structure similar to those produced by hypercapnia. These observations suggest that during the early part of their I-burst SPNs have a level of excitation lower than during the later part of the burst, i.e. that their membrane may be depolarizing in a ramp-like manner. Since the burst firing in I of phrenic and external intercostal motoneurons is due to a ramp-shaped composite EPSP (Berger, 1979) it is possible that the burst firing of SPNs in I is driven by a similar input waveform to that which drives the burst firing of I-motoneurons. In addition, the observation of a parallelism between changes in sympathetic and phrenic I-burst

during changes in pCO_2 (e.g. Preiss and Polosa, 1977) suggests the possibility that the input waveform to both sets of neurons derives from a common source. A hypothesis of how the IRSD is generated, based on the just-described observations, is as follows (Preiss et al., 1975). The I-burst of SPN firing is the result of a ramp-depolarizing input, the same inspiratory drive potential (IDP) which drives the I-motoneurons. This IDP is superimposed, in different neurons and, for a given neuron, in different experimental conditions, on a membrane potential set by other synaptic input at various levels relative to firing threshold. In the case the membrane potential is well below threshold the neuron is silent in the absence of IDP, i.e. in E. The behaviour of the neuron in I will depend on whether the IDP is subthreshold (silence) or suprathreshold (firing). A suprathreshold IDP may generate a single spike, a doublet or a burst depending on by how much and for how long the membrane potential is above threshold. The delay between IDP onset and spike generation by the IDP depends on the level of membrane potential as well as the size and the steepness of IDP. Depolarized membrane potentials, large and steep IDPs will result in short delays, hyperpolarized membrane potentials, small and shallow IDPs in long delays, i.e. in the latter case threshold crossing, if it occurs, will only occur at the peak of the IDP. In the cases in which membrane potential is at firing threshold due to the action of respiration-unrelated excitatory synaptic input and the neuron fires tonically, the IDP

will modulate the firing frequency such that firing frequency will start increasing at the IDP onset and will continue to increase during the rising phase of the IDP, reaching peak at the peak of the IDP. In these cases, the delay between onset of IDP and onset of firing frequency acceleration will be minimal, since the membrane potential is at threshold. In these cases the neuron fires in E and accelerates its firing frequency in I. The previously described changes in burst structure during hypocapnia or hypercapnia (Preiss et al., 1975; Preiss and Polosa, 1977) can be explained, according to the just-proposed scheme, as follows. The number of spikes per burst is gradually reduced as arterial pCO_2 is progressively lowered. At some low pCO_2 levels the burst is suppressed. This occurs, presumably, because the size of the IDP is reduced by hypocapnia so that the IDP no longer can bring the membrane potential to threshold and/or because the membrane potential moves away from threshold due to a decrease in other excitatory inputs caused by hypocapnia. During hypercapnia the number and frequency of spikes per burst increase. The delay between onset of phrenic burst and first spike of the SPN burst shortens. This occurs, presumably, as a result of an increase size and slope of the IDP and/or because the membrane potential gets closer to threshold due to activation of non-respiratory excitatory inputs. The IDP pushes the membrane potential above threshold for a longer period of time and by larger amounts, hence the increased firing within the burst. The first spike in the burst also occurs

burst also occurs earlier, due to the earlier threshold crossing.

The above is, of course, hypothetical. Is there any evidence that the SPN shows a ramp depolarization in I? Intracellular recordings in SPN's of the cat revealed that such slow potentials can be observed in some neurons (Dembowski, personal communication). Very indirect evidence is the observation of a decrease, during inspiration, in the latency of the antidromic spike of the SPN, due to decrease in the initial segment spike-soma dendritic spike delay (Lipski et al., 1977). This observation was made on silent as well as tonically active SPN's and is consistent with depolarization of the SPN soma in I.

If the IRSD is generated by an IDP similar to that which drives the I-motoneurons, it is possible that the IDP of both SPNs and I-motoneurons results from the activity of bulbo-spinal I-neurons. Bulbospinal inspiratory neuron somata are concentrated in two main regions, the ventrolateral nucleus of the solitary tract, in the region referred to as the dorsal respiratory group (DRG), and in the region including the rostral portions of the nucleus ambiguous, nucleus retroambigualis, globally referred to as the rostral ventral respiratory group (VRG) (Euler et al., 1973; Berger, 1977). The axons of bulbospinal I-neurons in both the VRG and DRG project to the contralateral spinal cord. Their axons are concentrated in two separate tracts, which run ventral and lateral to the ventral horn. The axons cross the midline at the level of the obex and terminate either directly onto phrenic and intercostal

motoneurons or on to antecedent interneurons. There are three lines of evidence against the hypothesis that the IDP of SPNs is due to activity of bulbo-spinal I-neurons. The first is that the bulbospinal respiratory neurons have an axonal modal conduction velocity of 30 m/sec (Richter et al., 1975) which is considerably faster than that of 2-3 m/s established for descending sympatho-excitatory pathways (Coote and Macleod, 1984; Ciriello et al., 1986). The second is that electrical stimulation along the midline, in the region of the obex where axons of bulbospinal I neurons cross, evoked short-latency excitation of phrenic motoneurons but no response of SPNs (Kubin et al., 1985). The third is that localized lesions of the spinal cord white matter can eliminate the IDP of phrenic motoneurons without affecting the IRSD and vice versa (Connelly and Wurster, 1985b). In these experiments, the IDP of phrenic motoneurons was eliminated by lesions of the ventral white matter of cervical spinal cord segments C2-C3, while the IRSD recorded in the inferior cardiac nerve was eliminated by lesions of the dorsolateral funiculus in the same segments. Thus the IDP is transmitted to the SPN by a set of neurons other than the bulbospinal I neurons. A candidate set has been recently proposed (McAllen, 1987). Neurons of the subretrofacial nucleus of the ventrolateral medulla have properties suggesting that they provide a tonic facilitatory drive to the SPN. These neurons project to the intermediolateral column of the spinal cord. Chemical activation of neurons in this region with

microinjections of glutamate results in excitation of SPNs, while lesions of this region depress SPN activity. A sample of single units from this nucleus, recorded with extracellular microelectrodes, showed marked inspiratory acceleration of their discharge. It is possible, therefore, that the hypothetical IDP of SPNs is the result of this waveform being encoded in the discharge pattern of the bulbo-spinal, presumed sympatho-excitatory, subretrofacial neurons.

b) Post Inspiratory Depression (PID)

This component of the respiratory modulation of sympathetic discharge was first identified by Cohen and Gootman (1970) as a period of minimum activity in the phrenic triggered averages of sympathetic discharges. A number of later studies have confirmed this finding (Seller and Richter, 1971; Preiss et al., 1975; Gerber and Polosa, 1978; Bainton et al., 1985; Gilbey et al., 1986). The abrupt onset of the fast decay of the phrenic nerve burst, which marks the I-E transition, is followed by a tail of decrementing, low level phrenic nerve activity (postinspiratory activity) which terminates about half way through E. With whole nerve recording (Seller and Richter, 1970; Bainton et al., 1985) sympathetic discharge is also seen to decay although less steeply than the phrenic burst, at the I-E transition. However, during the time the phrenic nerve shows the tail of low level activity in early E, sympathetic activity is practically shut off. In other words, at the end of the I burst, sympathetic activity does not go

back to the level immediately preceding the I burst onset, but undershoots this level and approaches zero. In whole nerve recording the suppression of sympathetic discharge lasts 1-2 s (Seller and Richter, 1971). Some of the published data (Janig et al., 1980; Gootman et al., 1980; Bainton et al., 1985) show that under some experimental conditions there is a very modest increase in sympathetic activity coincident with the phrenic burst, and yet there is an obvious depression of sympathetic discharge during post-inspiration. This suggests that the absence of sympathetic activity during this phase may not be due to a post-excitatory depression resulting from the previous firing in inspiration but rather due to an inhibitory synaptic mechanism. Also, with single unit recording, at times the magnitude of the PID is out of proportion with the magnitude of the I burst which precedes it. Single units can fire at low frequency during I, e.g. 3-4 Hz, and yet display a PID (Preiss et al., 1975). Further along this line of reasoning is the observation (presented in chapter 2, figure 13) that high intensity SLN stimulation, which evoked a marked SPN discharge in hypocapnia, failed to evoke a similar response under normocapnic conditions during early E. In order to explain this result as post-firing depression, one would have to hypothesize that all the neurons recruited by SLN stimulation in hypocapnia had a strong inspiratory firing pattern in normocapnia and therefore were refractory to SLN stimulation in early E. The alternative

hypothesis is, of course, that a large fraction of the SPNs are synaptically inhibited during this phase.

If PID is due to synaptic inhibition, a possible source of this inhibition is the activity of early E or (post-inspiratory) neurons. These neurons fire a burst of spikes with onset 20-50 msec after the abrupt termination of the phrenic burst. These neurons fire with a decrementing frequency and terminate firing one-third to one-half through E. The properties of these neurons and their possible role in influencing sympathetic activity are considered in the General Discussion (Chapter 6, section 3b).

With single unit recording (Preiss et al., 1975; Preiss and Polosa, 1977; Gerber and Polosa, 1978) the post-inspiratory silent period ranges in duration from 0.2 seconds to 1.0 seconds. The units with the lowest firing rates in E tend to show the longest duration of silent period. During the post-I period SPNs are unresponsive to synaptic input as well as to direct chemical excitation of their membrane. For instance, the burst of sympathetic firing synchronous with the phrenic nerve burst is missing when a phrenic burst occurs at a short time interval after the immediately preceding one. This observation was made for phrenic interburst intervals of the order of 300-500 msec (Bachoo and Polosa, 1987). Iontophoretic application, directly onto the SPN membrane, of the excitant amino acid glutamate failed to evoke firing during the PI phase in SPNs with inspiration-related discharge (Gilbey et al., 1986).

Concerning the possibility that the depression of SPN responsiveness during the PI phase is due to relative refractoriness, the SPN spike is followed by a long lasting afterhyperpolarization (AHP) due to a calcium activated potassium conductance, which may be up to 2-3 seconds in duration. At the peak of AHP the membrane potential may be 10-15 mV negative to resting potential. During the time course of the AHP responses to synaptic or direct stimulation are depressed. The AHP may summate during repetitive activity (Yoshimura et al., 1986). It has been shown previously that an antidromically-evoked burst of SPN firing at frequencies as low as 5 Hz is followed by prolonged suppression of the background (orthodromic) firing of the neuron (Polosa, 1968; Mannard et al., 1977). Since some of the neurons exceed this firing during the I burst (Preiss et al., 1975; Bachoo and Polosa, 1987), it is possible that PID is due to the summation of relative refractoriness resulting from the preceding burst firing.

c. Expiration-related Sympathetic Discharge (ERSD)

The question of whether any component of sympathetic discharge occurring during E is locked to the respiratory cycle under the usual conditions of absence of sensory feedback, thus suggesting that it is driven by input from brainstem E-neurons, has not been addressed systematically prior to work by the author reported in chapter 5 of this thesis. The main difficulty with an experimental

approach to this question is the fact that the level of activity of brainstem E-neurons, under normal experimental conditions, is much lower than that of I-neurons. This is demonstrated by the absence of activity in E-motoneurons during eupnea (Bishop, 1967; Bainton et al., 1978; Bainton and Kirkwood, 1979; Sears et al., 1982), a condition in which expiration is passive. On this premise it must be expected that even if synaptic connections existed between brainstem E-neurons and SPNs their effect on SPN firing would be, at best, weak and, therefore, difficult to detect. Thus, a conclusive demonstration of the presence or absence in E of a respiration-synchronous component of sympathetic discharge may be difficult to obtain unless something is done to increase the level of activity of brainstem E-neurons. The latter is the approach taken in the experiments to be described in chapter 5 of this thesis.

In these experiments, the activity of brainstem E neurons was stepped up by applying a threshold load to deflation (positive end-expiratory pressure, PEEP) or by increasing chemoreceptor drive by systemic hypercapnia. As will be shown in that chapter, once brainstem E-neurons are turned on, the answer is unequivocal: there is a late expiratory component of sympathetic discharge, the magnitude of which is graded in relation to the level of reflex stimulation of E-neuron activity and which is locked to the respiratory cycle. Under particular experimental conditions, this E-component may become the most prominent respiration-synchronous

component of sympathetic discharge. Thus, when the E-component is present, sympathetic activity shows two bursts of firing during the respiratory cycle, one synchronous with inspiration and the other synchronous with late expiration. Two bursts of sympathetic discharge for respiratory cycle reach the cardiovascular effector cells under these conditions. Due to filtering resulting from the frequency-response relation of the sympathetic neuro-effector transmission system (see Introduction, Part II, section 7) SAP will not show two Traube-Hering waves per respiratory cycle at respiratory frequencies within the normal range. In particular experimental conditions, however, when respiratory frequency is abnormally low, it is possible to observe this phenomenon. An example is the observation we made in a vagotomized, paralyzed, artificially ventilated cat with a phrenic burst frequency of 4 bursts per minute. The cat was moderately asphyxic due to a partial obstruction of the airway, detected only after this particular phenomenon was observed. The SAP of the cat presented a regular oscillation which persisted when the ventilation pump was temporarily stopped and had the unusual feature of showing two oscillation cycles for each phrenic burst cycle. This pattern was stable thus showing synchronization of the SAP to the phrenic burst. Administration of a ganglionic blocker showed that the SAP oscillation was neurogenic. Although a detailed analysis of this phenomenon was not made, it is likely that this was a case of a late-E burst of sympathetic discharge intense enough to produce a

marked response of the peripheral cardiovascular effectors and, because of the long duration of the central respiratory cycle, a pressure wave distinguishable from that produced by the I-synchronous burst. Thus, during one cycle of phrenic nerve activity, presumably one SAP wave was driven by the I-synchronous burst, the other by the E-synchronous burst.

The main conclusion from the data to be presented in chapter 5 of this thesis, in terms of the brainstem respiratory neurons which are likely responsible for generating the late-E component of sympathetic discharge, is that the late E neurons have the activity pattern best suited for providing the drive that generates this component of sympathetic discharge. Other published descriptions of sympathetic discharges in E have not provided clues as to the possible relation of the discharge to the activity of brainstem E-neurons. Thus, in the case of phrenic burst-triggered averages of sympathetic discharge, activity in expiration is described as increasing from a low in early E to a high in late E and being at a lower level than reached during I (Gootman and Cohen, 1970; Bainton et al., 1985). A transient decline in activity was described at the E-I transition (Bainton et al., 1985). Some authors have reported the occasional observation of a wave-like discharge in late E (Koizumi and Kollai, 1987). These trajectories of augmenting sympathetic activity during E may be the sign of an augmenting facilitatory input from brainstem E neurons but may also represent a slow recovery of the background activity unrelated to

respiratory drive after the post-I depression.

There have been, in the past, reports that sympathetic activity is greater in E than is I (Bronk et al., 1936; Okada and Fox, 1967; Hagbarth and Vallbo, 1968; Eckberg et al., 1985). However, all these observations were made under conditions of intact sensory feedback loops and therefore these patterns of E-dominance may have been created reflexly rather than by central mechanisms.

The data obtained in sino-aortic denervated, vagotomized rats (Czyzyk et al., 1987; Gilbey et al., 1986) reveal another aspect of the process of respiratory modulation of sympathetic discharge which may result in greater activity in E than in I. Czyzyk et al (1987) reported that sympathetic activity in E, recorded from a whole nerve, is of the augmenting type and at a higher level than in I. A single unit study (Gilbey et al., 1986), in the same species and under the same conditions of open sensory feedback loops, showed that almost all SPNs studied had respiratory-modulated firing patterns. Three-fifths of the units fired in E, the remainder in I. Thus, these results are consistent with the results of Czyzyk et al (1987). Concerning the mechanism of these E-firing patterns Gilbey et al (1986) made the interesting observation that in hypocapnia, while the I neurons became silent, the E-neurons maintained their firing which became continuous, i.e. lost the respiratory modulation. Thus, the E-pattern of discharge of these units seems to be due to periodic suppression of their

activity during I, rather than to facilitation during E. A type of unit with similar behaviour had been previously described in the cat by Preiss and Polosa (1977).

d) Early Inspiratory Depression

There is some evidence that during the transition from expiration to inspiration there is a brief (50-100 ms) depression of sympathetic discharge. In comparison to the post-inspiratory depression, the early inspiratory depression is much smaller and not always apparent. Cohen and Gootman (1970) described a distinct depression of splanchnic activity during the early inspiratory phase when averaging sympathetic activity using the onset of the phrenic burst as trigger. A similar observation has been described by Bainton et al (1985). This early inspiratory depression of sympathetic activity may be due to the SPNs experiencing disfacilitation of input from late expiratory neurons before the inspiratory input starts.

4. Centrogenic Respiratory Modulation of Cardiac Parasympathetic Discharge

An oscillation in heart rate synchronous with respiration, characterized by an increase in heart rate during inspiration and a slowing of heart rate in expiration was apparently first described

in the dog by Ludwig (1847). This respiratory oscillation in heart rate was later called respiratory sinus arrhythmia (RSA, Hering, 1930). Analysis of this phenomenon showed that it persists in the absence of lung inflation in paralysed, artificially ventilated, dogs (Traube, 1865) and after selective denervation of the lungs (Heymans, 1929). Therefore RSA is not generated reflexly by sensory input related to lung inflation. Neither is it causally related to the oscillation in SAP produced by the mechanical effects of ventilation via the baroreceptor afferents, since it is eliminated by hypocapnia despite persistence of the oscillation in SAP (Heymans, 1929). By exclusion, the mechanism of generation is likely to be central. RSA persists after stellectomy (Heymans, 1929) therefore it is not mediated by the sympathetic neurons. RSA is abolished by muscarinic antagonists or by section of the cervical vagus nerve during absence of lung inflation in the paralysed, artificially ventilated animals (Anrep et al., 1936; Kunze, 1972). Thus, RSA is likely to be produced by a waxing and waning, synchronized to the central respiratory cycle, of the discharge of the parasympathetic preganglionic neurons which innervate the sinus node of the heart. These neurons are generally referred to in the literature as cardiac vagal motoneurons (CVM) and this denomination has been adopted in this thesis as well.

Electrical recording from single axons of CVMs in the cervical vagus nerve (Jewett, 1964; Iruchijima and Kumada, 1964; Katona et al., 1970; Kunze, 1972; Davidson et al., 1976) or from the

somadrendritic region of CVMs in the medulla (with either extracellular or intracellular microelectrodes) (McAllen and Spyer, 1978; Gilbey et al., 1984) have clarified the mechanism of RSA. In anaesthetized cats or dogs, in normocapnia, CVMs fire at the highest frequency in expiration and at a much lower level, or are silent, in inspiration. This respiratory pattern of CVM discharge is generated by mechanisms in the CNS. It persists following bilateral cervical vagotomy and wide thoracotomy which eliminates mechanical respiratory oscillations in SAP. In hypocapnia, when the activity of brainstem respiratory neurons is decreased, the discharge of the CVMs loses its respiratory modulation and becomes tonic; the level of activity in I increases with respect to the normocapnic level while the level in E stays more or less unchanged (Katona et al., 1970; Kunze, 1972). The change in pattern from normocapnia to hypercapnia suggests that the normocapnic pattern of respiratory modulation of these neurons results from a depression in I rather than from facilitation in E of the background firing which mostly depends on excitation from arterial baroreceptors (Spyer, 1982). Intracellular recording (Gilbey et al., 1984) show that CVMs receive a burst of chloride-mediated IPSP's during inspiration. The amplitude and frequency of the IPSP's increases progressively during the augmenting phase of the phrenic burst. At the termination of the phrenic burst the burst of IPSPs ends abruptly. During the post-inspiratory phase these neurons receive EPSPs predominantly of baroreceptor origin, while during late-E a

weak inhibition appears which is time-locked to the firing of early-I neurons (Feldman, 1986). During I, EPSPs (presumably from baroreceptor afferents) are present but are dramatically reduced in amplitude, by comparison with their size during E, by the decreased neuron input resistance produced by the IPSPs. On the basis of these observations and other data Gilbey et al (1984) suggested that the two synaptic inputs most significant in shaping the firing pattern of the CVMs are the phasic inhibitory input during I from brainstem I neurons which modulates at the respiratory frequency the tonic excitatory infact from baroreceptor afferents.

5. Reflexogenic Respiratory Modulation of Sympathetic Discharge

a) Lung Inflation

Lung inflation can activate various sets of pulmonary receptors, which in turn can excite or inhibit sympathetic neurons and CVM's thus producing a reflex respiratory modulation of their activity. The properties of various sets of pulmonary afferents that can be activated by lung inflation, including (a) threshold pressures (b) conduction velocities of the afferents (c) blocking temperatures (d) and the nerves carrying afferents; have been reviewed by Paintal (1973); Sant'Ambrogio (1982); Coleridge and Coleridge (1984). The chest wall and diaphragm, which are also periodically deformed during the act of breathing also contain sets

of mechano-receptors that have the respiratory rhythm encoded in their activity (Shannon, 1980; Jammes et al., 1983: Duran, 1986). There is no evidence at present, however, that non-pulmonary afferents activated by lung inflation can influence sympathetic neurons or CVM's activity.

Lung inflation with large volumes produce a SAP fall due to a decrease in peripheral vascular resistance (Daly et al., 1967). This response is reflex and is mediated by the sympathetic system since it is abolished by bilateral cervical vagotomy and alpha-adrenergic block (Daly, 1967, 1968). This response is unrelated to the mechanical effects of lung inflation on cardiac output, since it is observed after barodenervation (Daly, 1968) and in preparations in which these mechanical effects are eliminated by a bilateral thoracotomy, and carotid nerve section (Daly, 1968). The decrease in peripheral vascular resistance is graded with extent of lung inflation in the range of airway pressures from 10 to 40 cm H_2O (Daly and Robinson, 1968). The range of airway pressures below 10 cmH_2O has only been studied by Hainsworth (1974) in open chest dogs. Lung inflation within this range produce an increase in the vascular resistance of the hindlimb. This is to date, the only report in the literature of an excitatory effect of lung inflation on vascular resistance. This effect was also reflex and mediated by vagal afferents and sympathetic efferents. This

effect is probably the peripheral effect of the excitation by small positive end-expiratory pressures of sympathetic neurons in late-E to be described in chapter 5 of this thesis. Recordings of electrical activity of sympathetic activity of sympathetic nerves have shown depression during lung inflation (Bronk et al., 1936; Gootman and Cohen, 1980; Bachoo and Polosa, 1986).

It has been tacitly assumed that the reflex inhibition of sympathetic discharge by large lung inflations is produced by activation of pulmonary stretch receptor afferents, the same afferents which produce the Hering-Breuer inspiration-inhibiting, expiration-excitatory reflex (Gootman and Cohen, 1970). However, the fact that the lung inflation pressures used by most investigators to produce a sympatho-inhibitory effect are at least twice as large as the pressures generated during normal breathing, and the fact that cooling the cervical vagus nerve to a 7°C temperature at which conduction is blocked in myelinated PSR afferents mediating the Hering-Breuer reflex failed to abolish the sympatho-inhibition suggests that this assumption is probably incorrect. It is more likely that the sympatho-inhibition produced by large lung inflations, is due to unmyelinated pulmonary vagal afferents which are activated by large lung volumes (threshold pressures 7-10 cmH_2O; Sant Ambrogio, 1982).

On the other hand, several studies have demonstrated a close parallelism between level of phrenic motoneuron activity and IRSD. It may be expected, therefore that the afferents producing the

Hering-Breuer reflex will (Trippenbach and Milic-Emili, 1977) will depress the IRSD as a secondary consequence of their inspiration-inhibitory action (Gerber and Polosa, 1978). This disfacilitatory action will be concurrent with sympatho-excitation in late E as a secondary consequence of the expiratory facilitatory action of these afferents. These two opposite and simultaneous actions of small lung inflations on the respiratory-modulated sympathetic discharge are well illustrated in Fig. 3 of the study presented in Chapter 5 of this thesis. This figure shows the simultaneous progressive decrease of the IRSD and progressive increase of the late E burst of the cat cervical trunk during application of PEEP between 2.5 and 7.5 cmH_2O.

Based on all the above evidence it is likely that reflexes elicited by lung inflation over a wide range of lung volumes can influence sympathetic discharge. This seems to be the result of the activation of at least two sets of reflex mechanisms. At the low end of the lung volume range, where only the myelinated afferents which produce the Hering-Breuer reflex are activated, it is possible that the resulting sympathetic effects are mostly indirectly mediated by brainstem respiratory neurons. Thus, as a counterpart to the Hering-Breuer expiratory promoting reflex, there is excitation of sympathetic neurons in late-E (Chapter 5 of this thesis) and, presumably, the peripheral vasoconstrictor described by Hainsworth (1974) in the dog. As a counterpart of the Hering-Breuer inspiration inhibiting reflex there is the selective

depression of IRSD, described by Gerber and Polosa (1978). At higher lung volumes the reflex effects of lung inflation on sympathetic activity seems to be independent of effects on respiration. Thus, Gootman et al (1980) showed depression by large lung inflations of sympathetic discharge in E, unrelated to the respiratory cycle. Lipski et al (1977) showed a slowing in the progagation of the antidromic action potential of SPN from the initial segment to the somadendritic membrane by large inflation applied during E, suggesting a hyperpolarization of the membrane.

It seems possible to conclude that the lungs can provide afferent signals capable of modulating the sympathetic discharge at the frequency of lung inflation. Since modulation by the Hering-Breuer reflex afferents seems to act via brainstem respiratory neurons, modifying their activity level and hence the drive they provide to the SPN, this mechanism cannot be considered as separate from the central mechanism by which I and E brainstem neurons influence sympathetic discharge. Modulation of sympathetic discharge by afferents other than those of the Hering-Breuer reflex seems to be independent of the respiratory drive (Gootman et al., 1980; Lipski et al., 1977) and constitute a real reflexogenic mechanism by which lung inflation can modulate sympathetic discharge.

b) Arterial Baroreceptors

Reflex baroreceptor modulation of sympathetic activity is an

extensively studied feature of autonomic cardiovascular control (Kirchheim, 1976). Several studies have shown a reciprocal relation between arterial pressure and level of sympathetic outflow to kidney, abdominal organs, muscle and the heart (Ninomiya et al., 1971; Gootman and Cohen, 1970). More recent studies have emphasized regional differences in the extent of baroreceptor control. For example, baroreceptor activation may reduce sympathetic outflow to some vascular beds (e.g. muscle) more than to others (e.g. skin); see Janig (1985).

A modulation of sympathetic discharge at the respiratory frequency of ventilation can be produced by the effects of ventilation induced blood pressure fluctuations on the arterial baroreceptors. Tang et al (1957) observed that in artificially ventilated cats, in the absence of central respiratory activity due to systemic hypocapnia, section of the vagus nerves reduced the amplitude of the fluctuations in sympathetic discharge but failed to eliminate them. A wide bilateral, pneumothorax which markedly reduced the arterial pressure fluctuations also abolished the fluctuations in splanchnic nerve activity at the frequency of the respiratory pump. A similar result was achieved by carotid sinus nerve section. Tang et al (1957) suggested, therefore, that the ventilation synchronous pattern of sympathetic discharge was the result of the ventilation induced blood pressure fluctuations which modulated sympathetic activity through the reflex mechanism. There is evidence that the role of the baroreceptor reflex in producing a

respiratory modulation of sympathetic discharge may vary under different experimental conditions, presumably as a result of changes in the sensitivity of set point of the reflex. Thus, Wurster and Connelly (1987) have reported that in cats with lesions of the pneumotaxic centre the component of the respiratory oscillation in cervical sympathetic trunk discharge due to the baroreflex was larger than the centrogenic component.

c) Atrial Reflexes

During inspiration blood flow to the right atrium increases, stretching the atrium. This may activate a reflex originating from atrial receptors activated by atrial stretch. Stretch receptors have been described in the atria of a variety of species (Donald and Sheperd, 1980). Two types of atrial receptors whose afferent fibres travel in the cervical vagus have been identified by Paintal (1973) and termed type A and type B on the basis of the timing of their discharge in relation to the cardiac cycle. Type A units firing during the a-wave of the atrial pressure pulse and presumably monitor active tension developed during atrial systole. Type B receptors fire during the atrial V-wave and presumably monitor the change in atrial volume. It is not known whether the discharge pattern of these receptors is modulated by the fluctuations in venous return which occurs during the breathing cycle.

The atrial receptors have been implicated in reflexes

affecting heart rate (Ledsome and Linden, 1964, 1967; Linden, 1976), systemic vascular resistance (Lloyd, 1972; Mancia et al., 1975) and control of fluid and electrolyte balance (Henry et al., 1956; Ledsome and Linden, 1967). Distension of the venoatrial junction of left or right atrium causes a reflex tachycardia (Linden et al., 1964). The afferent limb of this reflex involves sensory axons which travel in the vagus nerve and course along spinal nerves (Malliani et al., 1975). The efferent limb of this reflex involves an increase in sympathetic activity and not a decrease in cardiac vagal tone. This reflex can also be evoked in C-1 spinal animals suggesting that participation of the brainstem is not a prerequisite. In regards to the sensitivity of this reflex, Barnes et al (1979) have shown in conscious dogs, that a rise in mean left atrial pressure of one mmHg - produced by a saline load - can produce up to a 10% increase in heart rate. Due to this high sensitivity, these atrial reflexes could be activated by the respiratory oscillations in cardiac filling pressure and thus influence cardiac output (i.e. Bainbridge reflex). To date, no experimental demonstration of such a mechanism has been published.

Activation of atrial receptors produces little effect on peripheral vascular resistance although it has been reported to produce a pronounced inhibition of renal vasomotor activity (Mancia et al., 1975). Thus, if receptors generate a reflex respiratory modulation of sympathetic activity, this effect is likely to be mainly exerted on the population of sympathetic neurons that

control heart rate.

6. Reflexogenic Respiratory Modulation of Parasympathetic Cardiac Discharge

Anrep (1936a,b) studied in dogs the reflex effects of lung inflation on heart rate in a sympathectomized preparation in which the head of the experimental animal was perfused with blood from a donor animal. When perfusion was made with hypocapnic blood, moderate lung inflation (10-15 cm H_2O) produced a weak, inconsistent tachycardia. This response was pronounced when higher inflation pressures were used (30 cm H_2O). In hypocapnia the activity of brainstem respiratory neurons would presumably be depressed and any effect of lung inflation on heart rate would likely be due to a reflex connection directly between lung afferent and cardiac vagal motoneurons. When the head was perfused with normocapnic blood and central respiratory activity was evident, lung inflation with moderate volumes (10-15 cm H_2O) produced a pronounced bradycardia. The pulmonary afferents responsible for this reflexly evoked bradycardia are likely to be those connected to pulmonary stretched receptors. This reflex is evoked at normal tidal volumes and PSR's are known to be excited at low lung volumes. The reflex is well maintained during a static increase in lung volume and PSRs are known to be non-adapting. Cooling the vagus nerve at temperatures which block conduction in myelinated

PSR afferents abolishes the response. Since lung inflation did not produce a direct inhibition of CVMs (in hypocapnia) the mechanism of this bradycardia under normocapnic conditions is likely a secondary consequence of disinhibition of CVMs due to the inhibition of central inspiratory activity by the pulmonary afferents. Thus lung inflation affects the CVM's in opposite ways, by a direct inhibitory action and by a disinhibitory action resulting from the inspiration-inhibiting Hering-Breuer reflex. At moderate volumes the effect mediated by the Hering-Breuer reflex dominates over the direct inhibitory action and the net effect is bradycardia.

Thus, as already discussed for sympathetic neurons, the lungs can provide afferent signals which can modulate the discharge rate of CVMs at the frequency of lung inflation. The modulation mediated by Hering-Breuer reflex afferents is indirect, resulting from modulation of brainstem I neuron activity and of the inhibitory input they provide to CVMs. Thus this mechanism is really part of the centrogenic modulation provided by the brainstem inspiratory neurons. The modulation mediated by afferents activated by large lung volumes, presumably different from those mediating the Hering-Breuer reflex, seems independent of the respiratory drive, being present in hypocapnia, and represents a true reflexogenic mechanism of inflation related modulation of CVM activity.

7. Limitations of the Cardiovascular System Response to the Modulation of Sympathetic and Cardiac Parasympathetic Discharge by the Frequency Dependence of the System Response to Neural Input.

The previous sections described the mechanisms which may produce a respiratory rhythm in the discharge of autonomic neurons. This rhythmic discharge of sympathetic and parasympathetic preganglionic neurons may produce a rhythmic modulation in the calibre of resistance vessels, in the capacitance of venous vessels, in cardiac contractility and in heart rate. Each of these effects will contribute to a rhythmic modulation of SAP. The size of the modulation of these effector systems, and hence of SAP, depends mainly in the ability of these systems to faithfully translate the oscillating neural input into an oscillating response. Typically, the frequency response properties of cardiovascular effector cells are such that the response of these cells is slow (they behave like low pass filters). This property determines whether or not a rhythm present in the neural signal will produce an oscillation in effector cell activity and SAP. And, if it does, it determines the extent of attenuation of the effector response oscillation compared to the neural signal input oscillation.

a) Resistance Vessels

The resistance vessels' response to neural stimulation is also slow. In response to a "step" increase in the frequency of electrical stimulation of the lumbar sympathetic trunk in anaesthetized cats, hindlimb vascular resistance started to rise with a delay of 0.2 - 2.0 s from stimulus onset, rose during stimulation with an exponential time course which had a time constant of 3-12 s and when the stimulus was turned off, fell after a delay of 2-5 s at a rate slower than the rate of rise (Rosenbaum and Race, 1968). Onset delay was shorter, and rate of rise faster, while off-delay was longer, and rate of decay slower, with higher stimulus frequencies. Data more immediately applicable to the case of the respiratory modulation of sympathetic activity are those obtained in the same investigation using sinusoidal analysis. A stimulus of a given mean frequency (2 Hz) was frequency modulated by a slower sine wave of variable frequency. This stimulus pattern is reminiscent of the pattern of respiratory modulated activity sympathetic nerves, in which action potential frequency waxes and wanes during the respiratory cycle. This stimulus pattern does not, however, stimulate the waxing and waning of the number of firing neurons which occurs with respiratory modulation. This feature, i.e. recruitment of respiratory modulated units at different times in the cycle could also be mimicked by modulating the intensity of the stimulus; this would provide a more accurate approximation of the natural pattern of activity. At any rate, two relevant pieces of information provided by this analysis are

'attenuation' and 'phase-relation' as a function of modulation frequency. Attenuation is described as the ratio of the amplitude of the respiratory oscillation at a given frequency to the amplitude at the same mean frequency during a step function test. Phase lag is defined as the delay between a given point of the response oscillation and the corresponding point of the stimulus oscillation. In terms of respiratory modulation of sympathetic activity these plots permit prediction of the approximate extent of attenuation of the effector response oscillation at various respiratory frequencies as well as of the phase of the respiratory cycle at which the response occurs. This analysis shows a "corner" frequency (the highest frequency transmitted without attenuation) of 0.016 Hz (approximately, one cycle per minute). At 10 cycles/min, a typical respiratory frequency, the response oscillation was reduced to approximately 5% of its original amplitude. The phase-lag increased as the modulation frequency increased: at only 3 cycles/min the oscillation in perfusion pressure is 140° out of phase with the sympathetic signal. These two features of a sine wave sympathetic signal are reminiscent of experimental data presented in chapter 4, Fig. 4. This figure shows an oscillation in perfusion pressure of the cat hindlimb, perfused at a constant flow. Each cycle of increase in perfusion pressure is preceded by a burst of sympathetic activity time-locked to the phrenic burst. Therefore this oscillation is presumably mediated by that component of sympathetic activity produced by

the inspiratory drive. The amplitude of these oscillations is 10-15 mmHg; however SLN stimulation which 'selectively' eliminates the inspiratory synchronous component of sympathetic discharge for the duration of the stimulus (30 s) which produces a decrease in perfusion pressure of ~ 40 mmHg. This data suggests that: (1) because of slow frequency response characteristics of the resistance vessels the oscillatory component of the signal is reduced to less than half its steady state value; (2) there is a 180° phase delay between the signal (the IRSD) inspiratory synchronous sympathetic component and the response (rise in perfusion pressure); the delay is sufficient for the increase in perfusion pressure to coincide with expiration.

b) Capacitance Vessels

The properties of capacitance vessels play a critical role in determining the right atrial filling pressure and hence cardiac output. The capacitance of venous vessels, particularly of the splanchnic circulation, is reduced by activation of the sympathetic nervous system (Rothe, 1983).

In dogs, the maximum amount of blood that can be expressed from the systemic venous system as the system goes from full relaxation to full constriction has been estimated using sympathetic blockade by hexamethonium to cause relaxation of the venous smooth muscle and norepinephrine to cause vasoconstriction

(Rothe, 1976). At a mean circulatory filling pressure of 10 mmHg, this averaged 15 ml/kg body weight. Stimulation of the splanchnic nerves in the vascularly isolated abdominal circulation of the dog reduced capacitance by 7.2 ml/kg. An equivalent increase in preload produced by an i.v. saline volume load produced up to two fold increase in cardiac output. The following section describes the dynamics of the venous vessels in response to sympathetic activation.

Mellander (1960) has shown that the frequency response curve of the capacitance vessels to sympathetic nerve stimulation, in the anaesthetized cat, is sigmoid and displaced to the left of the frequency response curve of resistance vessels, which has a similar shape. Recently, Nilsson (1985) reported similar frequency response relationship to field stimulation in isolated venous segments from various parts of the circulation. In all venous segments the half maximal response was between 3-4 Hz. The half-maximal response of isolated arterial segments was at 6-8 Hz (Nilsson, 1985). Thus in spite of a less dense innervation of the veins by comparison with the arteries (Nilsson, 1985) the sympathetic postganglionic fibres can fully activate the veins at comparatively low rates of firing. A demonstration of respiratory modulation of capacitance vessels tone, presumably due to a neural mechanism, is provided by the observation of Browse et al (1967). Using the occluded limb technique in anesthetized dogs, fluctuations in venous pressure in the occluded limb and in SAP at

the respiratory frequency were described. A brief arrest of the ventilation markedly diminished the amplitude of the fluctuation in SAP, or suppressed it while the frequency and amplitude of the venous pressure oscillation in the occluded limb remained unaffected. These venous pressure waves could not be explained as a mechanical effect of ventilation since they were not abolished by cessation of artificial ventilation but were likely due to a respiratory modulation of sympathetic activity since they were abolished by extirpation of the lumbar sympathetic chain.

The difference in the frequency response relation between resistance and capacitance vessels may be of functional significance in relation to the change in response that can be expected in response to changes in sympathetic neuron firing rate. The tonic discharge rate is slow i.e. 1-2 Hz (Polosa, 1968). In the case of the capacitance vessels such frequencies are on the linear part of the frequency response relation, whereas in the case of the resistance vessels they are on the low non-linear foot of the frequency response curve (Folkow, 1955). Therefore changes in SPN firing frequency as they may occur during respiratory modulation of the firing may produce larger responses in capacitance than in resistance vessels.

Another factor which may be significant in determining the effect of a respiratory-modulated sympathetic neuron firing on the capacitance vessels is the usually slower venoconstrictor response to a reflexly induced change in the level of sympathetic nerve

activity by comparison with the resistance vessel response. Guyton et al (1970) have reported a delay of 7-10 sec between a decrease in carotid sinus pressure and a measurable constriction of the veins detected as an increase in mean circulatory filling pressure. By comparison, the delay for a detectable increase in peripheral resistance following a drop in carotid sinus pressure was approximately 1 sec. Similar results were obtained by Rolewicz et al (1969) who examined the vasoconstrictor response obtained during 'selective' activation of the arterial and venous sympathetic nerves in the perfused hindpaw of the dog. The increase in arterial resistance reached 68% of the maximal response 0.5 min after the onset of arterial nerve activation while at the same time venous nerve stimulation produced a response which was only 19% of maximum.

The consequence of these properties of the veins, as far as the respiratory modulation of sympathetic activity is concerned is that at normal respiratory rates (10-15 breaths per min) the oscillating activity may not produce a corresponding oscillation of venoconstrictor tone and as a consequence, of cardiac preload. Factors which may contribute to the more slowly developing response of venous smooth muscle include the time for delivery of the transmitter to the smooth muscle following release from nerve varicosities, the rate of delivery of calcium to the contraction mechanism and the viscoelastic properties of the venous smooth muscle (Rothe, 1984).

c) Heart Rate

The frequency-response characteristics of the heart rate response to a sinusoidally modulated frequency of cardiac sympathetic nerve stimulation have not been determined experimentally (Chess et al., 1975). If one neglects the nonlinearities in the onset and decay characteristics of the cardioaccelerator response to sympathetic nerve stimulation then the corner frequency, f, can be predicted from the step function response using the formula $f = 1/(2\pi t)$, where t is the time constant of the onset response (Chess and Calaresu, 1973). The accuracy of this formula has been demonstrated experimentally on the response of heart rate to vagal stimulation (Chess and Calaresu, 1973). From data of heart rate responses to step function stimulation of the inferior cardiac nerve in acute spinal cats (Bachoo et al., 1988) the predicted corner frequency is 0.5 cycles/min (0.008 Hz): If this 'derived' characteristic corner frequency could be verified experimentally it may go some way in explaining the almost total absence of a sympathetically mediated respiratory modulation of heart rate despite the numerous observations demonstrating a respiratory modulated discharge pattern of inferior cardiac nerve (Kollai and Koizumi, 1977, 1979). The vagal mechanism of heart rate control is characterized by a fast frequency response. There is little attenuation of the oscillatory power of the signal at normal respiratory rates, the

corner frequency is ~ 5 cycles/min (0.7 Hz) and 90% attenuation only occurs at 24 cycles/min (0.4 Hz); in addition there is little evidence of a phase lag at normal respiratory rates. The fast frequency-response characteristics of this neuro-effector system allows a more faithful translation of the temporal components of the incoming neural signal.

REFERENCES

Adrian, G.V., D.W. Bronk, G. Phillips (1932). Discharges in mammalian sympathetic nerves. J. Physiol. 74: 115-138.

Angell-James, J.E., M. de B. Daly (1978). The effects of artificial lung inflation on reflexly induced bradycardia associated with apnea in the dog. J. Physiol. 274: 349-366.

Angelone, A., N.A. Coulter (1964). Respiratory sinus arrhythmia: a frequency dependent phenomenon. J. Appl. Physiol. 19: 479-482.

Anrep, G.V., W. Pascual, R. Rossler (1936a). Respiratory variations of heart rate I. Proc. Roy. Soc. Lond. Biol. B. 119: 191-217.

Anrep, G.V., W. Pascual, R. Rossler (1936b). Respiratory variations of heart rate II. The central mechanism of respiratory arrhythmia and their inter-relationships between central and reflex mechanisms. Proc. Roy. Soc. Lond. Biol. 119: 218-230.

Assanelli, D., W.Y. Lew, R. Shabetai, M.M. LeWinter (1987). Influence of the pericardium on right and left ventricular filling in the dog. J. Appl. Physiol. 63: 1025-1032.

Attinger, E.O. (1957). Some interrelations between ventilation and circulation. Am. Heart J. 54: 837-850.

Bachoo, M., C. Polosa (1985). Properties of a sympatho-inhibitory and vasodilator reflex evoked by superior laryngeal nerve

afferents in the cat. J. Physiol. 364: 184-198.

Bachoo, M., C. Polosa (1987). Properties of the inspiration-related activity of sympathetic preganglionic neurons of the cervical trunk in the cat. J. Physiol. 385: 545-564.

Bachoo, M., E. Isacoff, C. Polosa (1988). Slow cardioacceleration mediated by noncholinergic transmission in the stellate ganglion of the cat. Can. J. Physiol. Pharmacol. 66: 1066-1974.

Bainton, C.R., P.A. Kirkwood, T.A. Sears (1978). On the transmission of the stimulation effect of carbon dioxide to the muscles of respiration. J. Physiol. 280: 249-272.

Bainton, C.R., P.A. Kirkwood (1979). The effects of carbon dioxide on the tonic and rhythmic discharges of respiratory bulbospinal neurones. J. Physiol. 296: 291-314.

Bainton, C.R., D.W. Richter, H. Seller, D. Ballantyne, J.P. Klein (1985). Respiratory modulation of sympathetic activity. J. Auto. Nerv. Syst. 12: 77-90.

Barman, S.M., G. Gebber (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. Am. J. Physiol. 231: 1601-1607.

Barnes, G.E., B.C. Chevis, M.J. Granger (1979). Regulation of cardiac output during rapid volume loading. Am. J. Physiol. 237: R197-R202.

Berger, A.J. (1977). Dorsal respiratory group neurons in the

medulla of cat: spinal projections, responses to lung inflation and superior laryngeal nerve stimulation. Brain Res. 135: 231-254.

Berger, A.J. (1979). Phrenic motoneurons in the cat: subpopulations and nature of respiratory drive potentials. J. Neurophysiol. 42: 76-90.

Berger, A.J. (1987). Spinal respiratory motoneuronal organization and recruitment during breathing: an overview. In: Respiratory Muscles and their Neuromotor Control, p. 129-134.

Beyar, R., M.J. Hansknecht, H.R. Halperin, F.C.P. Yin, M.L. Weisfeldt (1987). Interactions between cardiac chambers and thoracic pressure in intact circulation. Am. J. Physiol. 253: H1240-H1252.

Bishop, B. (1908). Diaphragm and abdominal muscle activity during induced hypotension. J. Appl. Physiol. 25: 73-79.

Bishop, B. (1967). Diaphragm and abdominal muscle responses to elevated airway pressures in the cat. J. Appl. Physiol. 22: 959-965.

Blumberg, M., W. Janig (1983). Enhancement of resting activity in postganglionic vasoconstrictor neurones following short-lasting repetitive activation of preganglionic axons. Pflügers Arch. 396: 89-94.

Boutellier, U., L.E. Farhi (1986). Influence of breathing frequency and tidal volume on cardiac output. Resp. Physiol. 66: 123-133.

Bower, R.W., Noordergraaf, A. (1973). Pressure-flow characteristics of collapsible tubes: A reconsiliation of seemingly contradictory results. Ann. Biomed. Engineering 1: 333-355.

Bower, R.W., A. Noordergraaf (1978). Theory of steady flow in collapsible tubes and veins. In: Cardiovascular System Dynamics. Ed. J. Baan, A. Noordergraaf, J. Raines. MIT Press, Cambridge, Mass., p. 256-266.

Brecher, G.A. (1952). Mechanisms of venous flow under different degrees of aspiration. Am. J. Physiol. 169: 423-433.

Brecher, G.A., A. Mixter, L. Shane (1952). Dynamics of venous collapse in the superior vena cava system. Am. J. Physiol. 171: 194-203.

Brecher, G.A., G. Mister (1953). Effects of respiratory movements on superior cava flow under normal and abnormal conditions. Am. J. Physiol. 172: 457-461.

Brecher, G.L. (1956). Venous Return. New York: Grune and Stratton.

Brinker, J.A., J.L. Weiss, D.L. Lappe, J.L. Rabson, W.R. Summer, S. Permutt, M.L. Weisteldt (1985). Leftward septal displacement during right ventricular loading in man. Circulation 61: 626-633.

Bromberger-Barnea, B. (1981). Mechanical effect of inspiration on heart function: a review. Fed. Proc. 40: 2172-2177.

Bronk, D.W., L.K. Ferguson, R. Margaria, D.Y. Solandt (1936). The

activity of cardiac sympathetic centers. Am. J. Physiol. 117: 237-249.

Browse, N.L., J.T. Shepherd, D. Donald (1966). Differences in response of veins and resistance vessels in limbs to same stimulus. Am. J. Physiol. 211: 1241-1247.

Burton-Opitz, R. (1902). The flow of blood in the external jugular veins. Am. J. Physiol. 7: 435-450.

Campbell, E.J.M., J.H. Green (1953). The variation in intra-abdominal pressure and the activity of the abdominal muscles during breathing; a study in man. J. Physiol. 122: 282-290.

Chartier, A.A., P.M. Jaumin, H. Pouleur (1974). Circulatory effects of deep inspirations, blocked expirations and positive pressure inflations at equal transpulmonary pressures in conscious dogs. J. Physiol. 241: 589-605.

Chess, G.F., F.R. Calaresu (1973). Frequency response model of vagal control of heart rate in the cat. Am. J. Physiol. 220: 554-557.

Chess, G.F., D.W. Varsey, J.L. Henry, F.R. Calaresu (1975). A mathematical model of the sympathetic heart period system in the cat. Ann. Biomed. Eng. 3: 189-198.

Ciriello, J., M.M. Caverson, C. Polosa (1986). Function of the ventrolateral medulla in the control of the circulation. Brain. Res. Rev. 11: 359-391.

Cohen, M.I., P.M. Gootman (1970). Periodicities in efferent

discharge of splanchnic nerve of the cat. Am. J. Physiol. 218: 1092-1101.

Cohen, M.I. (1979). Neurogenesis of respiratory rhythm in the mammal. Physiol. Rev. 59: 1105-1173.

Coleridge, J.C.A., H.M. Coleridge (1984). Afferent vagal innervation of the end airways and its functional significance. Rev. Physiol. Biochem. Pharmacol. 99: 1-110.

Connelly, C., R.O. Wurster (1985). Sympathetic rhythms during hyperventilation induced apnea. Am. J. Physiol. 249: R424-R431.

Connely, L.A., R.D. Wurster (1985b). Spinal pathways mediating respiratory influence on sympathetic nerves. Am. J. Physiol. 249: R91-R99.

Conrad, W.A. (1969). Pressure-flow relationships in collapsible tubes. In: Symposium on the Venous System: Characteristics and Functions. A Biomedical Engineering Approach. Ed. A. Noordergraaf, E. Kresch. IEEE Transactions on Biomedical Engineering, 16: 284-295.

Coote, J.H., V.H. Macleod (1984). Estimation of conduction velocity in bulbospinal excitatory pathways to sympathetic outflows in the cat spinal cord. Brain Res. 311: 97-107.

Culver, B.M., J. Butler (1980). Mechanical influences on the pulmonary microcirculation. Ann. Rev. Physiol. 42: 187-198.

Czyzyk, M.F., L. Fedorko, A. Trezebski (1987). Pattern of the respiratory modulation of the sympathetic activity is species

dependent: synchronization of the symathetic outflow over the respiratory cycle in the rat. In: Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms. Ed. J. Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa, pp. 143-152, Alan Liss Inc.

D. Angelo, E., N. Garzaniti, F. Bellemare (1988). Inspiratory muscle activity during unloaded and obstructed rebreathing in dogs. J. Appl. Physiol. 64: 90-101.

Daly, M. de B (1983). Peripheral arterial chemoreceptors and the cardiovascular system. In: Physiology of Peripheral Arterial Chemoreceptors. Ed. Acker, H., O'Regan, R.G., Amsterdam: Elsevier/North-Holland, p. 325-393.

Daly, M. de B., J.L. Hazzledine, A. Ungar (1967). The reflex effects of alterations in lung volume in systemic vascular resistance in the dog. J. Physiol. 118: 331-351.

Daly, M. de B., B.H. Robinson (1968). An analysis of the reflex systemic vasodilator response elicited by lung inflation in the dog. J. Physiol. 144: 148-166.

Davis, A.L., D.I. McCloskey, E.K. Potter (1977). Respiratory modulation of baroreceptors and chemoreceptor reflexes affecting heart rate through the sympathetic nervous system. J. Physiol. 272: 691-703.

Davidson, N.S., S. Goldner, D.I. McCloskey (1976). Respiratory modulation of baroreceptor and chemoreceptor reflexes affection heart rate and cardiac vagal efferent nerve

activity. J. Physiol. 259: 523-530.

DeBono, E.F., C.G. Caro (1963). Effect of lung inflation pressure on pulmonary blood pressure and flow. Am. J. Physiol. 205: 1178-1186.

DeGeest, H., M.N. Levy, H. Zieske, R.I. Lipman (1965). Depression of ventricular contractility by stimulation of the vagus nerves. Circ. Res. 17: 222-229.

DeGroat, W.C., P.M. Lalley (1974). Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. Brain Res. 80: 17-40.

Donald, D.E., J.T. Shepherd (1980). Autonomic regulation of peripheral circulation. Ann. Rev. Physiol. 42: 429-439.

Donders, F.C. (1859). Physiologies des Menchen. cited in G.A. Brecher, Venous Return. New York, Grune and Stratton, 1956.

Dontas, A.S. (1955). Effect of protoveratine, serotonin and ATP on afferent and splanchnic nerve activity. Circ. Res. 3: 363-373.

Dornhorst, A.C., P. Howard, G.L. Leathart (1952). Respiratory variations in blood pressure. Circulation 6: 553-558.

Dornhorst, A.C., P. Howard, G.L. Leathart (1952). Pulsus Paradoxus. Lancet 1: 746-748.

Duomarco, J.C., R. Rimini (1954). Energy and hydraulic gradients along systemic veins. Am. J. Physiol. 178: 215-220.

Duran, B. (1986). Intercostal and diaphragmatic muscle endings and afferents. In: Regulation of Breathing. Ed. I. Hornbien,

F. Thomas. Marcel Dekker Inc., New York.

Eckberg, D.L., C. Nerhed, B.G. Wollin (1985). Respiratory modulation of muscle sympathetic and vagal cardiac outflow in man. J. Physiol. 365: 181-196.

Emerson, M. (1911). Intra-abdominal pressure. Arch. Intern. Med. 7: 754-784.

Euler, C. von, J.N. Hayward, I. Marttila, R.J. Wyman (1973). The spinal connections of the inspiratory neurons of the ventrolateral nucleus of the cats tractus solitarius. Brain Res. 61: 23-33.

Feldman, J.L. (1986). Neurophysiology of respiration in mammals. In: Handbook of Physiology, section Nervous system, vol. V, ed. F.E. Bloom, p. 463-524. Am. Physiol. Soc., Bethesda, Maryland.

Fishman, A.P. (1986). Dynamics of pulmonary circulation. In: Handbook of Physiology, section II, Respiratory System, p. 1667-1743.

Folkow, B. (1955). Nervous control of blood vessels. Physiol. Rev. 35: 629-663.

Frederiq, L. (1887). Was soll man under "Traube-Hering-schen wellen" verstechen. Arch. Anat. Physiol. :351-354.

Freyschuss, U., A. Melcher (1975). Sinus arrhythmia in man: influence on tidal volume and esophageal pressure. Scand. J. Clin. Lab. Invest. 35: 487-496.

Gebber, G. (1980). Central oscillators responsible for sympathetic

nerve discharge. Am. J. Physiol. 239: H143-H155.

Gerber, U., C. Polosa (1978). Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neuron firing. Can. J. Physiol. Pharmacol. 56: 191-198.

Gerber, U., C. Polosa (1979). Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neuron firing. Can. J. Physiol. Pharmacol. 57: 1073-1081.

Gil, J. (1980). Organization of microcirculation of the lung. Ann. Rev. Physiol. 42: 177-186.

Gilbey, M.P., D. Jordan, P.W. Richter, K.M. Spyer (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. J. Physiol. 356: 65-78.

Gilbey, M.P., Y. Numao, K.M. Spyer (1986). Discharge patterns of cervical sympathetic preganglionic neurons related to central respiratory drive in the rat. J. Physiol. 378: 253-265.

Glantz, S.A., G.A. Misbach, W.Y. Moores, D.G. Mathey, J. Lekven, D.F. Stowe, W.W. Parmley, J.V. Tyberg (1978). The pericardium substantially affects the left ventricular diastolic pressure volume relationship in the dog. Circ. Res. 42: 433-441.

Gootman, P.M., M.I. Cohen (1970). Efferent splanchnic activity and systemic arterial pressure. Am. J. Physiol. 219: 897-903.

Gootman, P.M., M.I. Cohen (1970). Efferent splanchnic activity and systemic arterial pressure. Am. J. Physiol. 219: 987-903.

Gootman, P.M., M.I. Cohen (1973). Periodic modulation (cardiac and respiratory) of spontaneous and evoked sympathetic discharge. Acta Physiol. Polon 24: 97-109.

Gootman, P.M., I.M. Cohen (1974). Interrelationships between sympathetic discharge and central respiratory drive. In: Central Rhythm and Regulation. Eds. W. Umbach, H.P. Koepchen. Hyppocrates, Stuttgart, p. 195-209.

Gootman, P.M., J.L. Feldman, M.I. Cohen (1980). Pulmonary afferent influences on respiratory modulation of sympathetic discharge. In: Central Interaction Between Respiratory and Cardiovascular Control Systems. ed. H.P. Koepchen, S.M. Hilton, A. Trezebski, Springer-Verlag, Heidelberg.

Guyton, A.C., L.H. Adkin (1954). Quantitative aspects of the collapse factor in relation to venous return. Am. J. Physiol. 177: 523-527.

Guyton, A.C., J.H. Satterfield (1952). Vasomotor waves possibly resulting from CNS ischemic reflex oscillation. Am. J. Physiol. 170: 601-605.

Guyton, A.C., C.E. Jones, T.G. Coleman (1973). Circulatory physiology: Cardiac output and its regulation. Philadelphia: W.B. Saunders.

Guyton, A.C., A.W. Lindsey, B. Abernathy, T. Richardson (1957).

Venous return at various right atrial pressures and the normal venous return curve. Am. J. Physiol. 189: 609-615.

Guz, A., A. Innes, K. Murphy (1987). Respiratory modulation of left ventricular stroke volume in man measured using pulsed doppler ultrasound. J. Physiol. 393: 499-512.

Hagbthra, K.E., A.B. Valbo (1968). Pulse and respiratory grouping of sympathetic impulses and human muscle nerves. Acta Physiol. Scand. 74: 96-108.

Hainsworth, R. (1974). Circulatory responses to lung inflation in anesthetized dogs. J. Physiol. 220: 247-255.

J. Physiol. 334: 409-419.

Hales, S. (1733). Statistical Essays, vol. 2: London, W. Innys.

Haymet, B.T., D.I. McCloskey (1975). Baroreceptor and chemoreceptor influences on heart rate during the respiratory cycle in the dog. J. Physiol. 245: 699-712.

Hoffman, J.I., A. Guz, A.A. Charlies, D.E. Wilken (1965). Stroke volume in conscious dogs, effects of respiration posture and vascular occlusion. J. Appl. Physiol. 20: 865-877.

Hering, H.E. (1930). Uber die Abhangigkeit der bei lungenaufblasung und bei der respiratorischen arrhythmie auftretenden frequenzänderungen des Herzens von den blutdruckzuglern. Z. Kreislaufforschung 22: 593-598.

Herndon, C.W., K. Sagawa (1969). Combined effects of aortic and right atrial pressure on aortic flow. Am. J. Physiol. 217: 65-72.

Henry, J.P., O.H. Gauer, R.L. Reedes (1956). Evidence of the atrial location of receptors influencing urine flow. Circ. Res. 4: 85-90.

Heymans, C. (1929). Der mechanismus der Herzarhythmie respiratorischen ursprungs. Ergebn. Physiol. 28: 292-296.

Holt, J.P. (1941). The collapse factor in the measurement of venous pressure. Am. J. Physiol. 134: 292-299.

Holt, J.P. (1944). The effect of positive and negative intrathoracic pressure on cardiac output and venous pressure in the dog. Am. J. Physiol. 142: 594-603.

Holt, J.P. (1969). Flow through collapsible tubes and through in situ veins. IEEE Transactions on Biomedical Engineering 16: 274-283.

Howell, J.B.L., S. Permutt, D.F. Proctor, R.L. Riley (1961). Effect of inflation of the lung on different parts of the vascular bed. J. Appl. Physiol. 16: 71-76.

Hukumaki, M.O.K. (1987). Seventy years of the Bainbridge reflex. Acta Physiol. Scand. 130: 177-185.

Iriuchijima, J., M. Kumada (1964). Activity of single vagal fibers efferent to the heart. Jap. J. Physiol. 14: 479-487.

Jammes, Y., B. Buchler, S. Delpierre, A. Rasidakis, C. Grimaud, C. Roussos (1983). Phrenic afferents and their role in inspiratory control. J. Appl. Physiol. 60: 854-860.

Joels, N., M. Samueloff (1956). The activity of the medullary centers in diffusion respiration. J. Physiol. 133: 360-372.

Jewett, D.L. (1964). Activity of single efferent fibers in the cervical vagus nerve in the dog, with special reference to possible cardio-inhibitory fibres. J. Physiol. 175: 321-357.

Janig, W., M. Kummel, L. Wiprich (1980). Respiratory rhythmicities in vasoconstrictor and sudomotor neurones supplying the cats hindlimb. In: Central Interaction Between Respiratory and Cardiovascular Control Systems. ed. H.P. Koepchen, S.M. Hilton, A. Trzebski, Springer-Verlag, Heidelberg.

Janig, W., A. Sundlof, B.A. Wallin (1983). Discharge patterns of sympathetic neurons supplying skeletal muscle and skin in man and cat. J. Auto. Nerv. Syst. 7: 239-256.

Janig, W. (1985). Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. Rev. Physiol. Biochem. Pharmacol. 102: 119-213.

Kahn, N., E. Mills (1967). Centrally evoked sympathetic discharges: A functional study of medullary vasomotor areas. J. Physiol. 191: 339-352.

Kalsmanson, D., C. Veyrat (1978). Clinical aspect of venous return: A velocimetric approach to a new system dynamics concept. In: Cardiovascular System Dynamics. Ed. J. Baan, A. Nordergraaf, J. Raines. MIT Press, Cambridge, Mass., p. 297-305.

Kaminski, R.J., G.A. Meyer, D.L. Winter (1970). Sympathetic unit activity associated with Mayer waves in the spinal dog. Am. J. Physiol. 219: 1768-1771.

Karan, M., R.A. Wise, T.K. Natrajan, S. Permutt, H.N. Wagner (1984). Mechanism of decreased left stroke volume during inspiration in man. Circulation 69: 866-873.

Katona, P.G., J.W. Poitras, G.O. Barnett, B.S. Terry (1970). Cardiac vagal efferent activity and heart period in the carotid sinus reflex. Am. J. Physiol. 218: 1030-1037.

Katz, A.I., Y. Chen, A.H. Moreno (1968). Flow through a collapsible tube. Experimental analysis and mathematical model. Biophysical J. 9: 1261-1279.

Kenner, U.M., E.H. Wood (1966). Intrapericardial intrapleural and intracardiac pressures during acute heart failure in dogs studied without thoractomy. Circ. Res. 19: 1071-1079.

Kirchheim, H.R. (1976). Systemic arterial baroreceptor reflexes. Physiol. Rev. 56: 100-176.

Koepchen, H.P., K. Thurau (1957). Zur entstehung der respiratorischen arrhythmie. Pflügers Arch. 266: 22-23.

Koepchen, M.P. (1983). Respiratory and cardiovascular centers: Functional entity or separate structures? In: Central Neuron Environment. Ed. M.E. Schläfke, H.P. Koepchen, W.R. See. Springer-Verlag, Berlin, Heidelberg, p. 221-237.

Koepchen, H.P., D. Klussendorf, D. Sommer (1981). Neurophysiological background of central neural cardiovascular respiratory coordination: basic remarks and experimental approach. J. Auto. Nerv. Syst. 3: 335-368.

Koepchen, H.P., K. Thurau (1958). Untersuchungen über

Karan, M., R.A. Wise, T.K. Natrajan, S. Permutt, H.N. Wagner (1984). Mechanism of decreased left stroke volume during inspiration in man. Circulation 69: 866-873.

Katona, P.G., J.W. Poitras, G.O. Barnett, B.S. Terry (1970). Cardiac vagal efferent activity and heart period in the carotid sinus reflex. Am. J. Physiol. 218: 1030-1037.

Katz, A.I., Y. Chen, A.H. Moreno (1968). Flow through a collapsible tube. Experimental analysis and mathematical model. Biophysical J. 9: 1261-1279.

Kenner, U.M., E.H. Wood (1966). Intrapericardial intrapleural and intracardiac pressures during acute heart failure in dogs studied without thoractomy. Circ. Res. 19: 1071-1079.

Kirchheim, H.R. (1976). Systemic arterial baroreceptor reflexes. Physiol. Rev. 56: 100-176.

Koepchen, H.P., K. Thurau (1957). Zur entstehung der respiratorischen arrhythmie. Pflügers Arch. 266: 22-23.

Koepchen, M.P. (1983). Respiratory and cardiovascular centers: Functional entity or separate structures? In: Central Neuron Environment. Ed. M.E. Schläfke, H.P. Koepchen, W.R. See. Springer-Verlag, Berlin, Heidelberg, p. 221-237.

Koepchen, H.P., D. Klussendorf, D. Sommer (1981). Neurophysiological background of central neural cardiovascular respiratory coordination: basic remarks and experimental approach. J. Auto. Nerv. Syst. 3: 335-368.

Koepchen, H.P., K. Thurau (1958). Untersuchungen über

zussamenhänge zwischen Blutdruckwellen und ateminnervation. Pflügers Arch. ges. Physiol. 267: 10-26.

Koepchen, H.P., H.D. Lux, P.H. Wagner (1961). Untersuchungen über zeitbedarf und zentrale verarbeitug des pressor-receptorischen Herzeieflexes. Pflügers Arch. ges. Physiol. 273: 413-430.

Kirkwood, P.A., J.B. Munson, T.A. Sears, R.M. Westfall (1988). Respiratory interneurons in the thoracic spinal cord of the cat. J. Physiol. 395: 161-192.

Herzeieflexes. Pflügers Arch. ges. Physiol. 273: 413-430.

Koizumi, K., T. Ishikawa, H. Nishino, C.M. Brooks (1975). Cardiac and autonomic system reactions to stretch of the atria. Brain Res. 87: 247-261.

Koizumi, K., M. Kollai (1980). Patterns of single unit activity in sympathetic postganglionic nerves. J. Auto. Nerv. Syst. 1: 305-312.

Koizumi, K., M. Kollai (1987). Cardiorespiratory relationships; correlation between the activity of cardiac vagus, sympathetic and phrenic nerves. In: Organization of the Autonomic Nervous System. Central and Peripheral Mechanisms. Ed. J. Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa. p. 153-167, Allan R. Liss Inc.

Koizumi, K., H. Seller, A. Kaufman, C.M. Brooks (1971). Pattern of sympathetic discharges and their relation to baroreceptor and respiratory activities. Brain Res. 27: 281-294.

Kollai, M., K. Koizumi (1977). Differential responses in

sympathetic outflow by chemoreceptor activation. Brain Res. 138: 159-165.

Kollai, M., K. Koizumi (1979). Reciprocal and non-reciprocal action of the vagal and sympathetic nerves innervating heart. J. Auto. Nerv. Syst. 1: 33-52.

Konno, K., J. Mead (1967). Measurements of separate volume changes of rib cage and abdomen during breathing. J. Appl. Physiol. 22: 407-422.

Kunze, D.L. (1972). Reflex discharge patterns of cardiac vagal efferent fibers. J. Physiol. 222: 1-15.

Kubin, L., A. Trezebski, J. Lipski (1985). Split medulla preparation in the cat arterial chemoreceptor reflex and respiratory modulation of the renal sympathetic nerve activity. J. Auto. Nerv. Syst. 12: 211-225.

Lahiri, S., R.A. Delaney (1975). Stimulus interaction in che response of carotid body chemoreceptor single afferent fibers. Resp. Physiol. 24: 249-266.

Ledsome, J.R., R.J. Linden (1964). A reflex increase in heart rate from distension of the pulmonary vein atrial junction. J. Physiol. 170: 456-473.

Ledsome, J.R., R.J. Linden (1967). The effect of distending a pouch of the left atrium on heart rate. J. Physiol. 193: 121-129.

Ledsome, J., R. Linden (1968). The role of left atrial receptors in the diuretic response to left atrial distension. J.

Physiol. 198: 487-503.

Levy, M.N., M. DeGeest, H. Zieske (1966). Effects of respiratory center activity on the heart. Circ. Res. 18: 67-78.

Lewis, R. (1908). Studies of the relationship between respiration and blood pressure. J. Physiol. 32: 233-243.

Linden, R. (1976). Reflexes from receptors in the heart. Cardiology. 61: suppl. 1: 7-30.

Lipski, J., J. Coote, A. Trzebski (1977). Temporal patterns of antidromic invasion latencies of sympathetic preganglionic neurons related to central inspiratory activity and the pulmonary stretch receptor reflex. Brain Res. 135: 162-166.

Lloyd, T. (1972). Control of systemic vascular resistance by pulmonary and left heart baroreflexes. Am. J. Physiol. 22: 1511-1517.

Ludwig, C. (1847). Beiträge zur kenntniss des einflusses der respirations bewegungen auf den blutlauf im aortensysteme. Arch Anat. Physiol. Wiss. Med. 6: 242-302:

Maloney, J.E., D. Bergel, J.B. Glazier, J.M. Hughes, J.B. West (1968). Transmission of pulsatile blood pressure and flow through the isolated lung. Circ. Res. 23: 11-24.

Malliani, A., F. Lombardi, M. Pagani, A. Recordati, P.J. Schwartz (1975). Spinal cardiovascular reflexes. Brain Res. 87: 239-246.

Mancia, G.J., J.T. Shepherd, D.E. Donald (1975). Role of cardiac,

pulmonary and cardiac mechanoreceptors in the control of hindlimb and renal circulation in dogs. Circ. Res. 37: 200-208.

Mannard, A., C. Polosa (1973). Analysis of background firing of single sympathetic preganglionic neurons of cat cervical nerve. J. Neurophysiol. 36: 398-408.

Mannard, A., P. Rajchgot, C. Polosa (1977). Effect of post-impulse depression on background firing of sympathetic preganglionic neurons. Brain Res. 126: 243-261.

Mayer, S. (1876). Studied zur physiologies des herzens und der blutgefasse. V. Ubes spontane blut druckschwankungen. Saech. Akad. Wiss. Sitz. Math. Naturw. CI 74: 281-307.

McAllen, R.M., K.M. Spyer (1978). The baroreceptor input to cardiac vagal motoneurons. J. Physiol. 282: 365-374.

McAllen, R.M. (1986). Action and specificity of ventral medullary vasopressor neurones in the cat. Neuroscience 18: 51-59.

McAllen, R.M. (1986). Location of neurons with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. Neuroscience 18: 43-49.

McAllen, R.M. (1987). Central respiratory modulation of subretrofacial bulbospinal neurones in the cat. J. Physiol. 388: 533-545.

McGregor, M. (1979). Pulsus Paradoxus. New Eng. J. Med. 301: 480-482.

Melcher, A. (1976). Respiratory sinus arrhythmia in man. A study

in heart rate regulating mechanisms. Acta Physiol. Scand. (Suppl.) 435: 1-31.

Mellander, S. (1960). Comparative studies on adrenergic neurohumoral control of resistance and capacitance vessels in the cat. Acta. Physiol. Scand. 50 (suppl. 176) 1-86.

Millar, R.A., T.J. Biscoe (1965). Preganglionic sympathetic activity and the effects of anesthetics. Br. J. Anesth. 37: 804-832.

Millhorn, D.E. (1988). Neural respiratory and circulatory interaction during chemoreceptor stimulation and cooling of ventral medulla in cats. J. Physiol. 370: 217-231.

Mirgorodsky, V.N., V.I. Skok (1969). Intracellular potentials recorded from a tonically active mammalian sympathetic ganglion. Brain Res. 15: 570-572.

Moreno, A.M., A.R. Burchell, R. Van Der Woude, J.H. Burke (1967). Respiratory regulation of splanchnic and systemic venous return. Am. J. Physiol. 213: 455-465.

Moreno, A.H., L.D. Gold, J. Negrin (1977). Respiratory changes in intrapleural and intraperitoneal pressures and their effects upon pressure in the central veins. J. Surg. Res. 22: 149-160.

Moreno, A.H., A.I. Katz, L.D. Gold (1969). An integrated approach to the study of the venous system with steps towards a detailed model of dynamics of venous return to the right heart. IEEE Transactions on Biomedical Engineering 16:

308-324.

Moreno, A.H., A.R. Burchell (1982). Respiratory regulation of splanchnic and systemic venous return in normal subjects and in patients with hepatic cirrhosis. Surg. Gynecol. Obstet. 154: 257-267.

Morgan, B.C., A. Guntheroth, D.M. Dillard (1965). Relationship of pericardial to pleural pressure during quiet respiration and cardiac tamponade. Circ. Res. 16: 493-498.

Morita, H., Y. Nishida, N. Uemura, H. Hosomi (1987). Effect of pentobarbital anesthesia on renal sympathetic nerve activity in the rabbit. J. Auto. Nerv. Syst. 20: 57-64.

Navajas, D., R. Farre, M. Rotger, J. Milic-Emili, J. Sanchis (1988). Effect of body posture on respiratory impedance. J. Appl. Physiol. 64: 194-199.

Nilsson, M. (1985). Adrenergic nervous control of resistence and capacitance vessels: studies on isolated blood vessels from the rat. Acta. Physiol. Scand. (suppl. 541), p. 1-33.

Ninomiya, I., N. Nisimaru, H. Irisawa (1971). Sympathetic nerve activity to the spleen, kidney and heart in response to baroreceptor input. Am. J. Physiol. 221: 1346-1351.

Nordenstrom, B., A. Norhagen (1965). Effect of respiration on venous return to the heart. Am. J. Roentgerol 65: 655-661.

Okada, H., I.J. Fox (1967). Respiratory grouping of abdominal sympathetic activity in the dog. Am. J. Physiol. 213: 48-56.

Olsen, C.O., G.S. Tyson, G.W. Maier, J.M. Davis, J.S. Rankin

(1985). Diminished stroke volume during inspiration: a reverse thoracic pump. Circulation 72: 668-679.

Pagani, M., P.J. Schwartz, R. Banks, F. Lombardi, A. Malliani (1974). Reflex responses of sympathetic preganglionic neurons initiated by different cardiovascular receptors in spinal animals. Brain Res. 68: 215-225.

Paintal, A.S. (1973). Vagal sensory receptors and their reflex effects. Physiol. Rev. 53: 159-227.

Peiper, U., .. Genese (1963). Traube-Heringscher blutdruck-schwakungen. Arch. Kreislaufforsch 40: 97-116.

Penaz, J. (1978). Mayer waves: History and methodology. Automedia 2: 135-141.

Permutt, S., B. Bromberger-Barnea, H.N. Bane (1962). Alveolar pressure, pulmonary venous pressure and the vascular waterfall. Med. Thoracalis. 19: 239-260.

Permutt, S., J.B.L. Howell, D.F. Procter, R.L. Riley (1962). Effect of lung inflation on static pressure volume characteristics of pulmonary vessels. J. Appl. Physiol. 16: 64-70.

Permutt, S., R.L. Riley (1963). Hemodynamics of collapsible vessels with tone; the vascular waterfall. J. Appl. Physiol. 18: 924-932.

Permutt, S., P. Caldini (1978). Regulation of cardiac output by the circuit: Venous return. In: Cardiovascular System Dynamics. Ed. J. Baan, A. Noordergraaf, J. Raines. MIT

Press, Cambridge, Mass. pp 465-479.

Peters, J., M.K. Kindred, J.L. Robotham (1988). Transient analysis of cardiopulmonary interactions I. Diastolic events. J. Appl. Physiol. 64: 1506-1517.

Petrillo, G.A., L. Glass, T. Trippenbach (1983). Phase-locking of the respiratory rhythm in cats to a mechanical ventilator. Can. J. Physiol. Pharmacol. 61: 599-607.

Polosa, C. (1968). Spontaneous activity of sympathetic preganglionic neurons., Can. J. Physiol. Pharmacol. 46: 887-896.

Polosa, C. (1984). Rhythms in the activity of the autonomic nervous system: their role in the generation of systemic arterial pressure waves. In: Mechanisms of Blood Pressure Waves. Ed. K. Miyakawa. Japan Sci. Soc. Press, Tokyo. Springer-Verlag, Berlin, pp 27-41.

Polosa, C., U. Gerber, R. Schondorf (1980). Central mechanisms of interaction between sympathetic preganglionic neurons and the respiratory oscillator. In: Central Interactions Between Respiratory and Cardiovascular Control Systems. Ed. H.P. Koepchen, S.M. Hilton, A. Trezebski, Springer-Verlag, Berlin, pp 137-143.

Potter, E.K. (1981). Inspiratory inhibition of vagal responses to baroreceptor and chemoreceptor stimulation in the dog. J. Physiol. 316: 177-190.

Preiss, G., C. Polosa (1974). Pattern of sympathetic neuron

activity associated with mayer waves. Am. J. Physiol. 226: 724-730.

Preiss, A., S. Iscoe, C. Polosa (1975). Analysis of a periodic breathing pattern associated with Mayer waves. Am. J. Physiol. 228: 768-774.

Preiss, G., C. Polosa (1977). The relation between end-tidal CO_2 and discharge patterns of sympathetic preganglionic neurons. Brain Res. 122: 255-267.

Preiss, G., F. Kirchner, C. Polosa (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. Brain Res. 87: 363-374.

Quebbeman, E.J., C. Dawson (1977). Effects of lung inflation and hypoxia on pulmonary arterial blood volume. J. Appl. Physiol. 43: 3-13.

Rabinovici, N., N. Navot (1980). The relationship between respiration and flow distribution in the vena cava and portal and hepatic veins. Surg. Gynecol. Obstet. 151: 753-763.

Richter, D.W., F. Heyde, M. Gabriel (1975). Intracellular recording from different types of medullary respiratory neurons in the cat. J. Neurophysiol. 38: 1162-1171.

Robotham, J.L., J. Robson, S. Permutt, B. Bromberger-Barnea (1979). Left ventricular hemodynamics during respiration. J. Appl. Physiol. 47: 1295-1301.

Rodbard, S., M. Saiki (1963). Flow through collapsible tubes. Am. Heart J. 65: 648-655.

Rolewicz, T.F., L. Whitmore, B.G. Zimmerman (1969). Transmitter release and response of arteries and veins during nerve stimulation. Am. J. Physiol. 217: 1459-1463.

Roos, A., L.J. Thomas, E.L. Nagel, D.C. Prommas (1961). Pulmonary vascular resistance as determined by lung inflation and vascular pressures. J. Appl. Physiol. 16: 77-84.

Rosenbaum, M., D. Race (1968). Frequency-response characteristics of vascular resistance vessels. Am. J. Physiol. 215: 1397-1402.

Rothe, C.F. (1976). Reflex vascular capacity reduction in the dog. Circ. Res. 39: 705-710.

Rothe, C.F. (1983). Venous System: physiology of the capacitance vessels. In: Handbook of Physiology, section 2: The Cardiovascular System, vol. III, part 1. Ed. J.T. Shephard, F.M. Abboud, p. 397-453. Bethesda, U.S.A. Am. Physiol. Soc.

Ruskin, J., R. Backe, J.C. Rembert, J.C. Greenfield (1973). Pressure-flow studies in man: effect of respiration on left ventricle stroke volume. Circulation 48: 79-85.

Sant'Ambrogio, G. (1982). Information arising from the tracheobronchial tree of mammals. Physiol. Reviews 62: 531-569.

Schrigen, F., W. Ehslich, S. Permutt (1975). Cardiovascular changes in conscious dogs during spontaneous deep breaths. Pflügers Arch. 355: 205-211.

Schwietzer, A. (1945). Rhythmical fluctuations in arterial pressure. J. Physiol. 104: 25P.

Sears, T.A., A.J. Berger, E.A. Phillipson (1982). Reciprocal tonic activation of inspiratory and expiratory motoneurons by chemical drives. Nature 299: 728-730.

Sellden, H., H. Sjorall, S.E. Ricksten (1986). Sympathetic nerve activity and central hemodynamics during mechanical ventilation with positive end-expiratory pressure in rats. Acta Physiol. Scand. 127: 51-60.

Seller, H., D.W. Richter (1971). Some quantitative aspects of the central transmission of the baroreceptor activity. In: Research in Physiology, Eds. F.F. Kao, K. Koizumi, M. Vassalle. Honor. Professor C. McC Brooks, Auto Caggi, Bologna, pp 541-549.

Seller, H. (1973). The discharge pattern of single units in thoracic and lumbar white rami in relation to cardiovascular events. Pflügers Arch. 343: 317-330.

Shannon, R. (1979). Involvement of thoracic nerve afferents in the respiratory response to chest compression. Respir. Physiol. 36: 65-79.

Sharp, J.T., N.B. Goldberg, W.S. Druz, J. Danon (1975). Relative contributions of rib cage and abdomen to breathing in normal subjects. J. Appl. Physiol. 39: 608-618.

Sharpey-Schafer, E.P. (1965). Effect of respiratory acts on the circulation. In: Handbook of Physiology, section 2:

Circulation, vol. III. Ed. J. Hamilton, P. Dow, pp. 1875-1886. Washington, D.C. Am. Physiol. Soc.

Shoukas, A., K. Sagawa (1978). Baroreceptor reflex control of the veins. In: Cardiovascular System Dynamics. Ed. J. Baan, A. Nordergraaf, J. Raines. MIT Press, Cambridge, Mass. pp 291-297.

1875-1886. Washington, D.C. Am. Physiol. Soc.

Spyer, K.M., R.M. McAllen (1980). The interaction of central and peripheral inputs onto vagal cardiomotor neurons. In 'Central Interactions between Respiratory and Cardiovascular Control Systems', ed. H.P. Koepchen, S.M. Hilton, A. Trezbski; Springer-Verlag, Berlin p. 8-14.

Spyer, K.M. (1979). The baroreceptor control of vagal preganglionic activity. In: Integrative functions of the autonomic Nervous System. Ed. C. McC. Brooks, K. Koizumi, A. Sato, p. 283-292. Tokyo.

Summer, W.R., S. Permutt, K. Sagawa, A. Shoukas, B. Bromberger-Barnea (1979). Effects of respiration on spontaneous respiration on canine left ventricular function. Circ. Res. 45: 719-725.

Tang, P.C., T.W. Maire, V.E. Amassian (1957). Respiratory influence on the vasomotor center. Am. J. Physiol. 191: 218-224.

Traube, L. (1865). Uber periodische Tatigkeitsausserungen des vasomotorischen und Hemmungs-Nervenzentrums. Cbe Med. Wiss

56: 881.

Thorvaldson, J., A. Ilebekk, S. Leraand, F. Kiil (1984). Determinants of pulmonary blood volume. Effects of acute change in pulmonary vascular pressure and flow. Acta Physiol. Scand. 121: 45-56.

Trippenbach, T., J. Milic-Emili (1977). Vagal contribution to the inspiratory off-switch mechanism. Fed. Proc. 36: 2395-2399.

Trimby, R.M., H.C. Nicholson (1924). Some observations on the nature of the respiratory waves in arterial blood pressure. Am. J. Physiol. 70: 289-291.

Am. J. Physiol. 70: 289-291.

Sharp, J.T., N.B. Goldberg, W.S. Druz, J. Danon (1975). Relative contributions of rib cage and abdomen to breathing in normal subjects. J. Appl. Physiol. 39: 608-618.

Spyer, K.M., R.M. McAllen (1980). The interaction of central and peripheral inputs onto vagal cardiomotor neurons. In 'Central Interactions between Respiratory and Cardiovascular Control Systems'. Ed. H.P. Koepchen, J.M. Hilton, A. Trezebski, Springer-Verlag, Berling, p. 8-14.

Summer, W.R., J. Permutt, K. Sagawa, A. Shoukas, B. Bromberger-Barnea (1979). Effects of respiration on spontaneous respiration on canine left ventricular function. Circ. Res. 45: 719-725.

Tang, P.C., T.W. Maire, V.E. Amassian (1957). Respiratory influence on the vasomotor center. Am. J. Physiol. 191:

218-224.

Traube, L. (1865). Uber periodische thätigkeits aeusserungen des vasomotorischen und hemmungs-nervenzentrums. Cbe Med. Wiss. 56: 881-885.

Trippenbach, T., J. Milic-Emili (1977). Vagal contribution to the inspiratory off-switch mechanism. Fed. Proc. 36: 2395-2399.

Troyer, A., V. Ninane (1987). Effect of posture on expiratory muscle use during breathing in the dog. Resp. Physiol. 67: 311-322.

Tyson, A.S., A.W. Mayer, C.O. Olsen, J.W. Davis, S.J. Rankin (1984). Pericardial influences on ventricular filling in the conscious dog: an analysis based on pericardial pressure. Circ. Res. 54: 173-184.

Visscher, M.B., A. Rupp, F.M. Scott (1924). The respiratory wave in arterial blood pressure. Am. J. Physiol. 70: 586-606.

Wallin, B.A., J.A. Fagius (1986). The sympathetic nervous system in man - aspects derived from microelectrode recordings. T.I.N.S. 6: 63-67.

Weidinger, H., V. Leschhorn (1964). Sympathetic tenscerung and rhythmische blutdruckschwankungen. Z. Krislangforsch. 53: 985-1002.

West, J.B., C.T. Dollery (1965). Distribution of blood flow in isolated lungs, relation to vascular and alveolar pressures. J. Appl. Physiol. 19: 713-724.

Willeput, R., C. Rondeux, A. De Troyer (1984). Breathing affects

venous return from legs in humans. J. Appl. Physiol. 57: 971-976.

Wurster, R.D., C.A. Connelly (1987). Phase changes of sympathetic activity with respiration before and after pontine lesions. In: Organization of the Autonomic Nervous System. Central and Peripheral Mechanisms, ed. J. Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa, Allan Liss Inc., pp. 169-178.

Yoshimura, S.M., C. Polosa, S. Nishi (1986). Afterhyperpolarization mechanisms in cat sympathetic preganglionic neurons in vitro. J. Neurophysiol. 55: 1234-1246.

CHAPTER II

PROPERTIES OF THE INSPIRATION-RELATED ACTIVITY OF SYMPATHETIC PREGANGLIONIC NEURONES OF THE CERVICAL TRUNK IN THE CAT

INTRODUCTION

In normal experimental conditions a large fraction of sympathetic preganglionic and postganglionic neurones of the cat fire in rhythmic bursts, synchronous with inspiration (e.g. Cohen & Gootman, 1970; Preiss, Kirchner & Polosa, 1975; Barman & Gebber, 1976). The rhythmic bursting persists in the absence of respiratory movements and following sino-aortic denervation and vagotomy. Therefore this rhythm is thought to be generated by central mechanisms (Tang, Maire & Amassian, 1957). Two hypotheses have been proposed to explain the mechanism of generation of this inspiration-related component of sympathetic discharge. One attributes this firing pattern to facilitatory input from brainstem inspiratory neurones to sympathetic preganglionic, or to antecedent, neurones. This hypothesis receives support from observations of analogies in the responses to a variety of stimuli of inspiratory motoneurones and of sympathetic preganglionic neurones with inspiration- related firing pattern (Preiss, Kirchner & Polosa, 1975; Preiss & Polosa, 1977; Gerber & Polosa, 1978, 1979; Connelly & Wurster, 1985). The other hypothesis proposes that the inspiration- related activity of sympathetic neurones is driven by a hypothetical neural oscillator, independent of, but coupled to, the brainstem respiratory oscillator, and entrained to the latter at normal respiratory frequencies (Koepchen, 1962; Barman & Gebber, 1976).

In the present study an analysis was made of the temporal structure of the inspiration-related sympathetic burst and experiments were performed to clarify its generation mechanism. Specifically, these experiments were aimed at the question of whether or not the relation of sympathetic to phrenic nerve activity showed behaviour expected of a system of two coupled oscillators (Pavlidis, 1973). Therefore, the phase-delay between the two bursting rhythms was examined under conditions in which the respiratory oscillator was forced to assume frequencies different from its natural frequency, both in the transient and in the steady-state. To this end respiratory frequency was modified by various means, which include changes in body temperature and stimulation of vagal or superior laryngeal nerve afferents.

METHODS

Cats of either sex were used (2.5 to 4.5 kg). Anaesthesia was obtained with pentobarbitone (35 mg/kg i.p., followed by i.v. supplements of 9 mg/kg every 3 h). With this dosage the withdrawal reflex on pinching forepaw or hindpaw was suppressed for the duration of the experiment. After cannulation of the trachea, all animals were artificially ventilated, while continuously monitoring tidal CO_2 concentration with an infrared gas analyser and tracheal pressure with a strain gauge, and paralysed with pancuronium bromide (initial dose 200 μg/kg followed by maintenance doses of

100 μg/kg given every 2-3 h, when the effect of the previous dose had worn off, as evidenced by the appearance of spontaneous breathing movements, and after testing for adequancy of the level of anaesthesia). Artificial ventilation was adjusted to obtain, in control conditions and during ventilation with room air or with 100% O_2, end-tidal P_{CO_2} of between 30 and 45 mm Hg. A respiratory pump rate of 15 to 18 cpm and peak tracheal pressure of 4-6 cmH_2O were used in control conditions. In some experiments, in which high respiratory pump frequencies were used which resulted in hyperventilation, CO_2 mixtures in O_2 were used to maintain end-tidal P_{CO_2} close to control values. In these cases in control conditions ventilation was with 100% O_2. An artery and vein were cannulated for continuous recording of systemic arterial pressure and for injection of drugs, respectively. Rectal temperature was maintained at 37°C by means of a servo-controlled infrared heat lamp.

The electrical activity of the whole phrenic nerve and cervical sympathetic trunk was recorded monophasically, after desheathing, with silver hook electrodes, amplified (bandpass 30 Hz to 10 KHz), displayed on an oscilloscope and stored on magnetic tape. After half-wave rectification and low-pass filtering (RC circuit with 100 ms time constant), the electrical activity was also displayed on a pen recorder. These rectified, low-pass filtered, records of neural activity are usually referred to in the literature as "integrated" activity. The level of "zero" activity

for the recording of the cervical sympathetic trunk was obtained by applying procaine to the nerve or by crushing the nerve proximal to the recording electrode. Thin filaments were dissected from the cervical sympathetic trunk, under a dissection microscope, for single unit recording. All units studied were spontaneously firing in control conditions. In 7 cats the central end of the cut internal branch of the superior laryngeal nerve was desheathed and mounted on a pair of silver hook electrodes for stimulation. Monophasic square wave pulses (0.2 ms duration) from a stimulator were delivered through a stimulus isolation unit. Trains of stimuli were delivered at selected times during the respiratory cycle, by triggering the stimulator at variable delays from phrenic burst onset with a square-wave pulse obtained at the onset of the burst. All nerves were kept under mineral oil in a pool made with the skin flaps.

In all animals the aortic nerves were bilaterally identified, separated from the vagus nerves and cut. The vagus nerves were also cut bilaterally in the neck except in the experiments of entrainment of the respiratory oscillator to the respiratory pump. The carotid sinus nerves were also bilaterally cut in all experiments, except in those in which the effects of baroreceptor activation on the inspiration-related sympathetic discharge were studied. In the text, the term inspiration is used to define the phase of phrenic nerve activity from onset to beginning of rapid decline, while the interval between end of one inspiration and

onset of the next is defined as expiration. Lung inflation is indicated by the increase in tracheal pressure caused by the respiratory pump. The phase-relation of the respiratory pump cycle to the central respiratory cycle is defined by the delay (in ms) between onset of phrenic nerve burst and onset of rise in tracheal pressure. Similarly, the phase-relation of the sympathetic burst cycle to the central respiratory cycle is defined as the delay (in ms) between phrenic burst onset and sympathetic cervical burst onset. This measurement is also referred to in the text as phase-delay. Entrainment of the phrenic nerve burst cycle to the respiratory pump cycle refers to the condition in which the periodic changes in lung volume, caused by the respiratory pump, affect the rhythm of phrenic nerve activity such that lung inflation occurs with a fixed delay, which is a function of pump frequency, following phrenic burst onset. Intrinsic frequency of an oscillator is defined as the frequency in the absence of input.

RESULTS

a) Pattern of Sympathetic Discharge In Inspiration

When the electrical activity of the whole cervical sympathetic trunk was recorded under normal experimental conditions, a burst of spikes was observed in close temporal relation with the phrenic nerve burst (Fig. 1A and B). The peak amplitude of the sympathetic burst increased or decreased when phrenic burst amplitude increased

FIGURE LEGENDS

FIG. 1 The inspiration-related sympathetic discharge. A: recording of the electrical activity of the phrenic nerve (upper trace) and cervical sympathetic trunk. B: same records as in A after half-wave rectification and low-pass filtering (time constant 100 ms).

FIG. 1

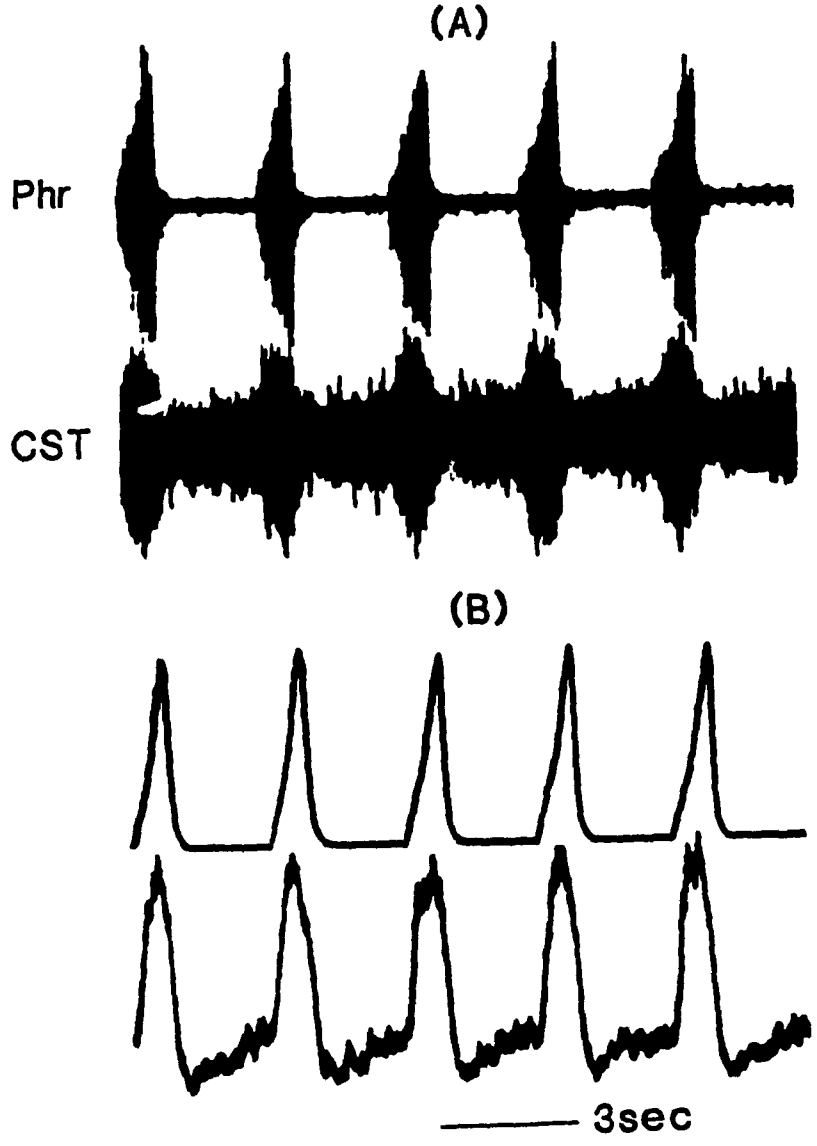
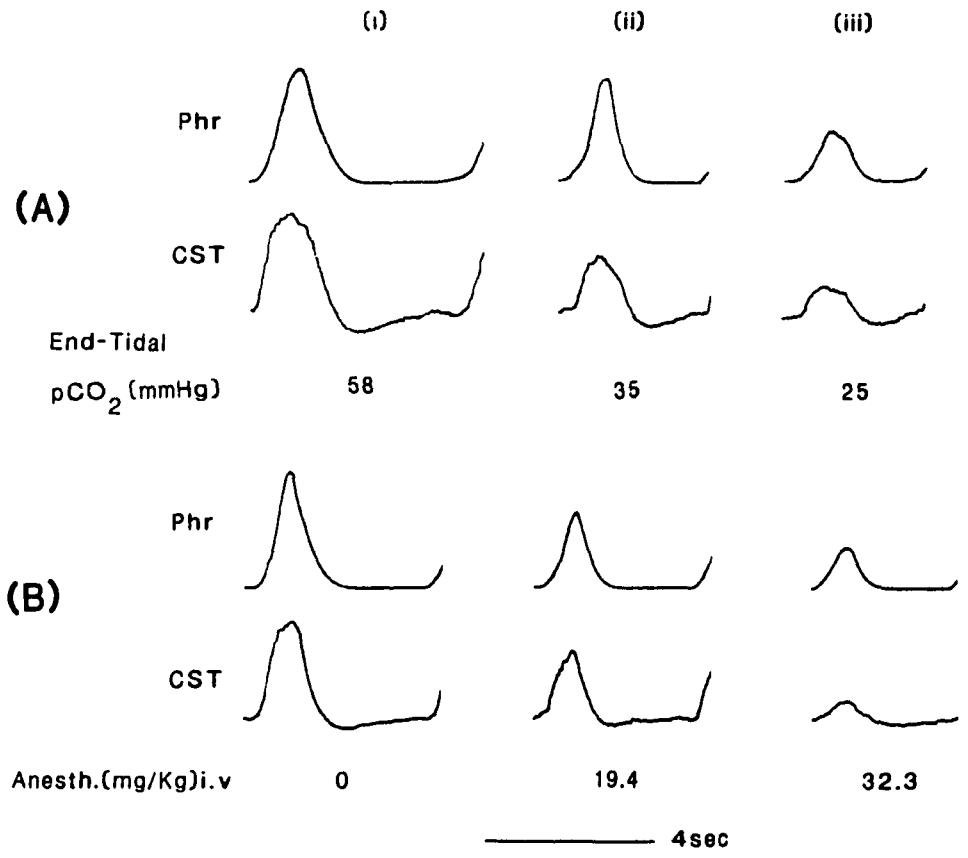


FIG. 2 Relation between phrenic (Phr) and cervical sympathetic trunk (CST) burst discharge at various levels of central respiratory drive. Each trace is the average of 20 sweeps. (A) A decrease in central chemical respiratory drive by hyperventilation in air has equivalent effects on the phrenic and sympathetic bursts. Even at the lowest levels of PCO_2 which produced rhythmic phrenic nerve activity (iii) there was an associated sympathetic discharge. (B) Increasing levels of pentobarbitone anesthesia cause an equivalent depression of phrenic and sympathetic bursts.

FIG. 2


(i)
(ii)
(iii)
(A)
Phr
CST
End-Tidal
pCO2 (mmHg)
58
35
25
(B)
Phr
CST
Anesth.(mg/Kg)i.v
0
19.4
32.3
4sec

or decreased with changes in CO_2 or anesthetic level (Fig. 2). Onset and termination of the sympathetic burst occurred in a fixed time-relation to onset and termination of the phrenic nerve burst, for a given set of experimental conditions, and were delayed with respect to the latter. A measure of the constancy of time relation, the standard deviation of the phrenic to sympathetic burst onset delay, is shown in Fig. 9. The onset delay of the sympathetic burst varied inversely with end-tidal PCO_2 (Fig. 3) as would be expected if the output of brainstem inspiratory neurones, which is also known to vary inversely with end-tidal PCO_2 (Cohen, 1968), was providing the synaptic drive for the burst. The delay varied also with the level of anesthesia and was longest at the highest anesthetic doses (not shown). The values for onset delay shown in the figure are typical for the group of animals studies. After the onset, the contour of the low-pass filtered inspiration-related sympathetic wave showed one of two trajectories. The most frequent trajectory (22 cases out of 26) was characterized by an initial progressive increase in amplitude, reaching its maximum during the initial part of the phrenic nerve burst (approximately during the first 30% - 40% of the phrenic nerve burst duration); afterwards, amplitude stayed approximately constant for the remainder of the duration of the phrenic nerve burst (Fig. 4A). This waveshape will be referred to as square wave-like. In the remaining 4 cases the trajectory was a replica of the

FIG. 3 Effect on the phrenic (Phr) to sympathetic (CST) burst onset delay of various levels of end-tidal PCO_2. Each trace is the average of 25 sweeps, times (ms) indicate the delay at the respective end-tidal PCO_2. Increasing pCO_2 from a relatively hypocapnic (i) to a normocapnic (ii) level decreases the delay by 20 ms. Hypercapnia (iii) & (iv) produces further decrease in delay. Arrows indicate onset times. End-tidal pCO_2 values in (ii), (iii) and (iv) were obtained by ventilation with CO_2-containing gas mixtures.

FIG. 3

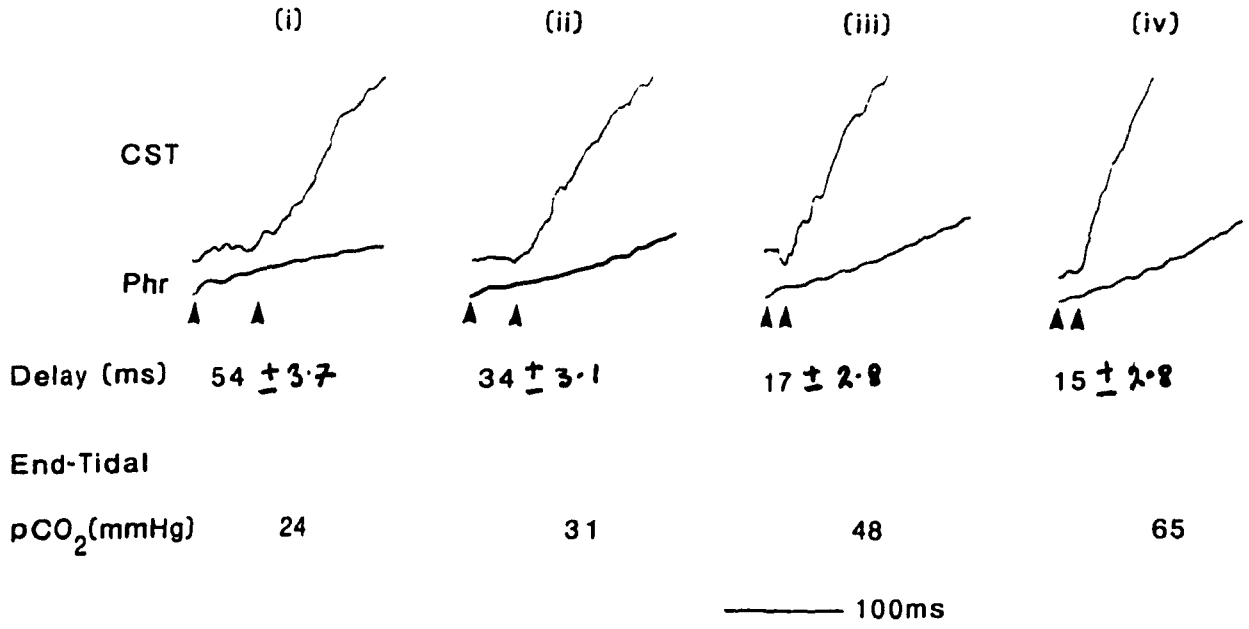
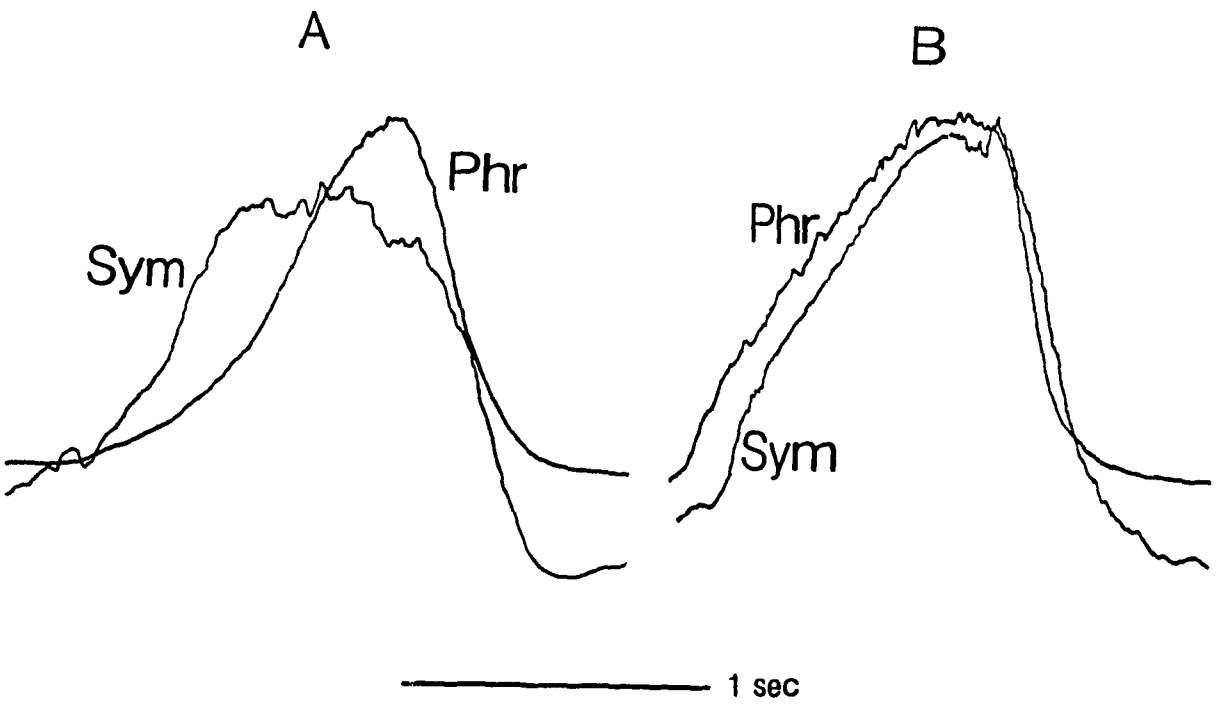

(i)
(ii)
(iii)
(iv)
CST
Phr
Delay (ms) 54 ± 3·7
34 ± 3·1
17 ± 2·8
15 ± 2·8
End-Tidal
pCO2(mmHg) 24
31
48
65
100ms

FIG. 4 Comparison of phrenic bursts (Phr) and inspiration-related bursts (recorded in the cervical sympathetic trunk, CST). Rectified, low-pass filtered whole nerve recordings. A and B are from two different cats. Each trace is the average of 25 sweeps. A: the most commonly observed sympathetic waveform has a square-wave shape. B: exceptionally, the sympathetic waveform has the same ramp-shape as the phrenic waveform.

FIG. 4



trajectory of the low-pass filtered phrenic nerve burst, i.e. amplitude increased progressively reaching its peak in approximate coincidence with the peak of the phrenic nerve wave (Fig. 4B). This waveshape will be referred to as ramp-like. After the occurrence of the peak of the phrenic wave, both types of trajectories decayed to the pre-burst activity level (3 of 26), or to below it (23 out of 26), at a rate similar to, or slower than, that at which phrenic nerve activity decayed. Inspiration-related sympathetic discharge was present, in control conditions, in all the animals studied. The discharge persisted without attenuation after i.v. administration of up to 20 mg/kg hexamethonium bromide, which suggests that it was recorded from preganglionic axons.

The temporal characteristics of the inspiration-synchronous sympathetic discharge were examined in 20 units of the cervical sympathetic trunk. These units were silent during expiration and discharged a burst of spikes during inspiration. The inspiratory burst, in various units, was made of from 3 to 32 spikes (Fig. 5). For a given unit and for a given set of experimental conditions the number of spikes in the burst was remarkably constant. The times, during the phrenic nerve burst, at which these units were first recruited are shown in Fig. 6. The majority (16 out of 20) was recruited during the first 30% of inspiration. Once recruited, the majority of units fired at a frequency similar to the onset frequency. Thus, the ratio of the last to the first interspike interval of the burst was, on average, 1.15 ± .42 (n=20, p 0.01).

FIG. 5 Firing patterns of sympathetic preganglionic units during their inspiratory burst. Four different units are shown, generating bursts with different numbers of spikes. Top trace in each panel is low-pass filtered phrenic nerve burst. Calibrations under B and D apply also to A and C, respectively.

FIG. 5

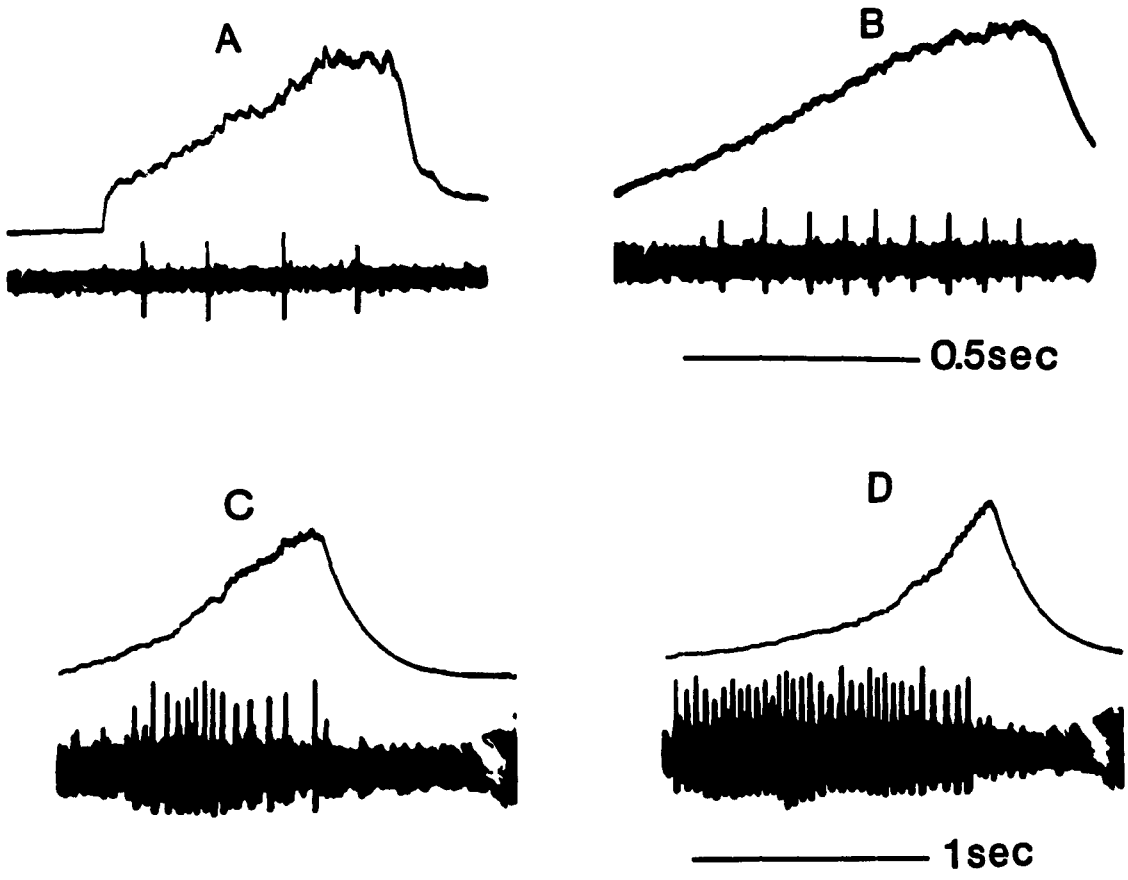
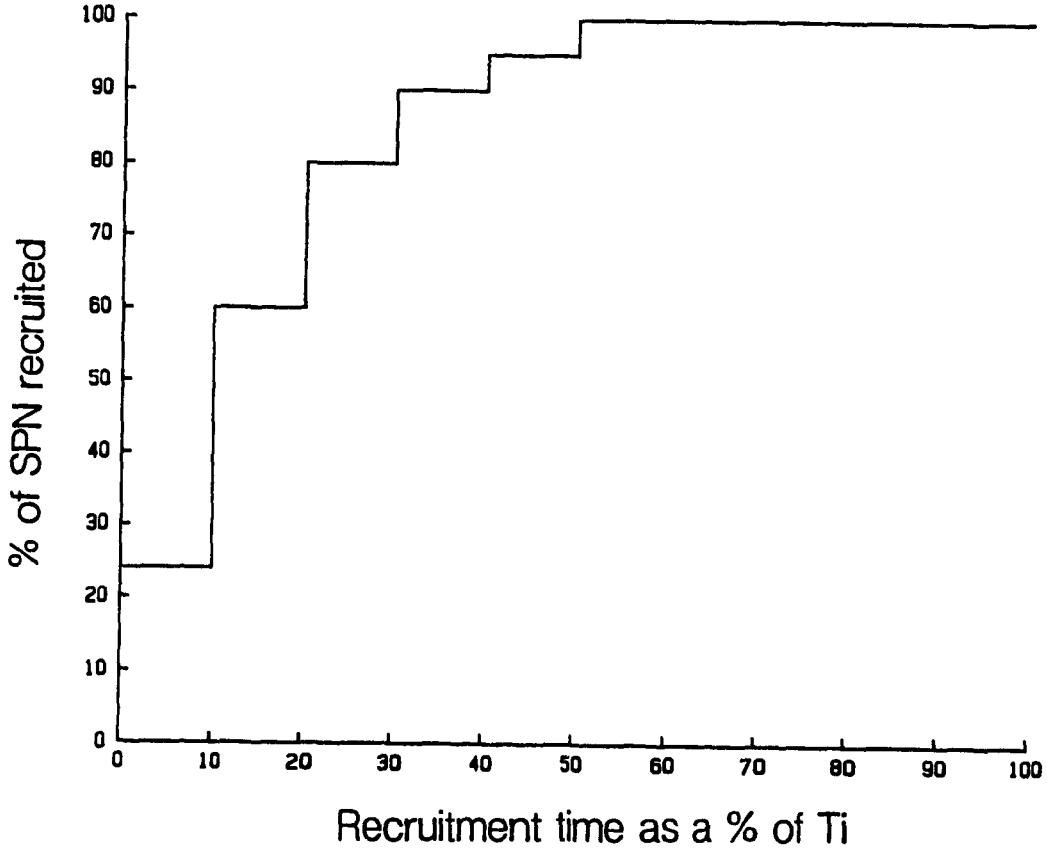


FIG. 6 Cumulative histogram of recruitment times of sympathetic units during inspiration. Recruitment time is time between phrenic burst and sympathetic unit burst onset. This time is expressed as fraction of inspiration duration (Ti). N = 20.

FIG. 6


% of SPN recruited
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Recruitment time as a % of Ti

For the same 20 units, 65 % of the intraburst interspike intervals following the first were within ± 20% of the first interval. This is in contrast with the intraburst firing pattern of phrenic motoneurones which is characterized by a progressive acceleration of frequency during the burst (Iscoe, Dankoff, Migicovsky & Polosa, 1976).

b. Experimental Tests of the Coupled Oscillator Hypothesis

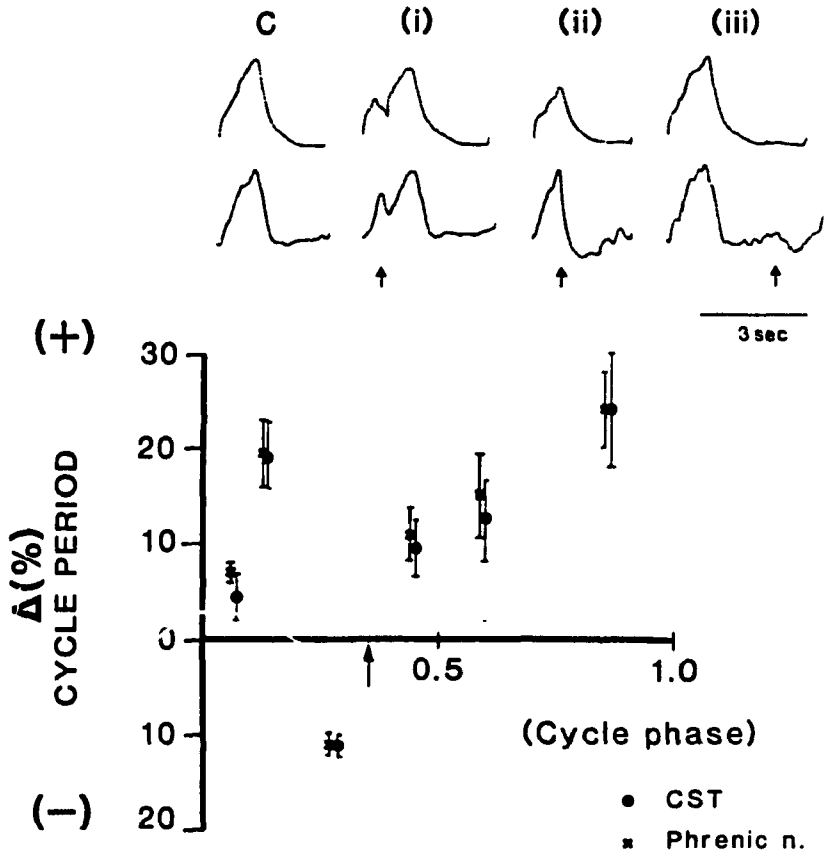
i) Superior Laryngeal Nerve Stimulation: Phase-Response Curves

The properties of a biological oscillator can be characterized by the effect of a stimulus, presented at different times (or phases) of the cycle, on the duration of the same cycle, i.e. its phase-response curve (Pavlidis, 1973). It is known that superior laryngeal nerve afferents can reset the rhythm of the phrenic motoneurones' burst (Larrabee & Hodes, 1948). On the basis of the coupled oscillator hypothesis, mentioned in the introduction, the premise for this experiment (performed on 7 cats) is that any phase-advance or phase-delay, caused in the cycle of the respiratory oscillator by the stimulus, would result in the output of this oscillator reaching the hypothetical sympathetic oscillator during a different phase of its cycle. Hence, it is predicted that the phase delay between the outputs of the two oscillators should be different from that observed in the unstimulated condition (Pavlidis, 1973; Jalife, 1984; Delmar, Jalife & Michaels, 1985).

Electrical stimuli (trains of 4 stimuli at 200 Hz, 0.2 ms, 0.2

FIG. 7 Identity of the resetting effect of a train of stimuli to the superior laryngeal nerve (4 stimuli at 200 Hz, 0.2 ms, 0.2 mV) on the rhythm of the phrenic and of the inspiration-related sympathetic discharge. This effect is displayed in the graph as the change in cycle duration on the ordinate (expressed as a fraction of control) caused by stimuli presented at various times of the cycle (shown on the abscissa and expressed as cycle phase, i.e. fraction of cycle duration). Plus and minus signs indicate prolongation and shortening, respectively. Arrow on abscissa indicates end of the inspiration phase of the cycle. Inspiration onset is at the origin. Superior laryngeal nerve stimulation prolongs the period of the phrenic and of the inspiration-related sympathetic discharge except when delivered during the last part of inspiration. Insets show phrenic nerve (top) and sympathetic waveform in control conditions (C) and for stimulation (arrow) in middle (i) and late (ii) inspiration and late expiration (iii). Notice that the two waveforms have similar shapes and maintain the same temporal relation to each other during curtailed or prolonged cycles as in the control, unstimulated, cycles. Each point in the graph is average ± S.D. of 20 cycles. Each waveform in insets is average of 10 sweeps. Notice similarity of phrenic and sympathetic waveforms.

FIG. 7


C
(i)
(ii)
(iii)
3 sec
(+)
(−)
30
20
10
0
10
20
Δ(%) CYCLE PERIOD
0.5
1.0
(Cycle phase)
CST
Phrenic n.

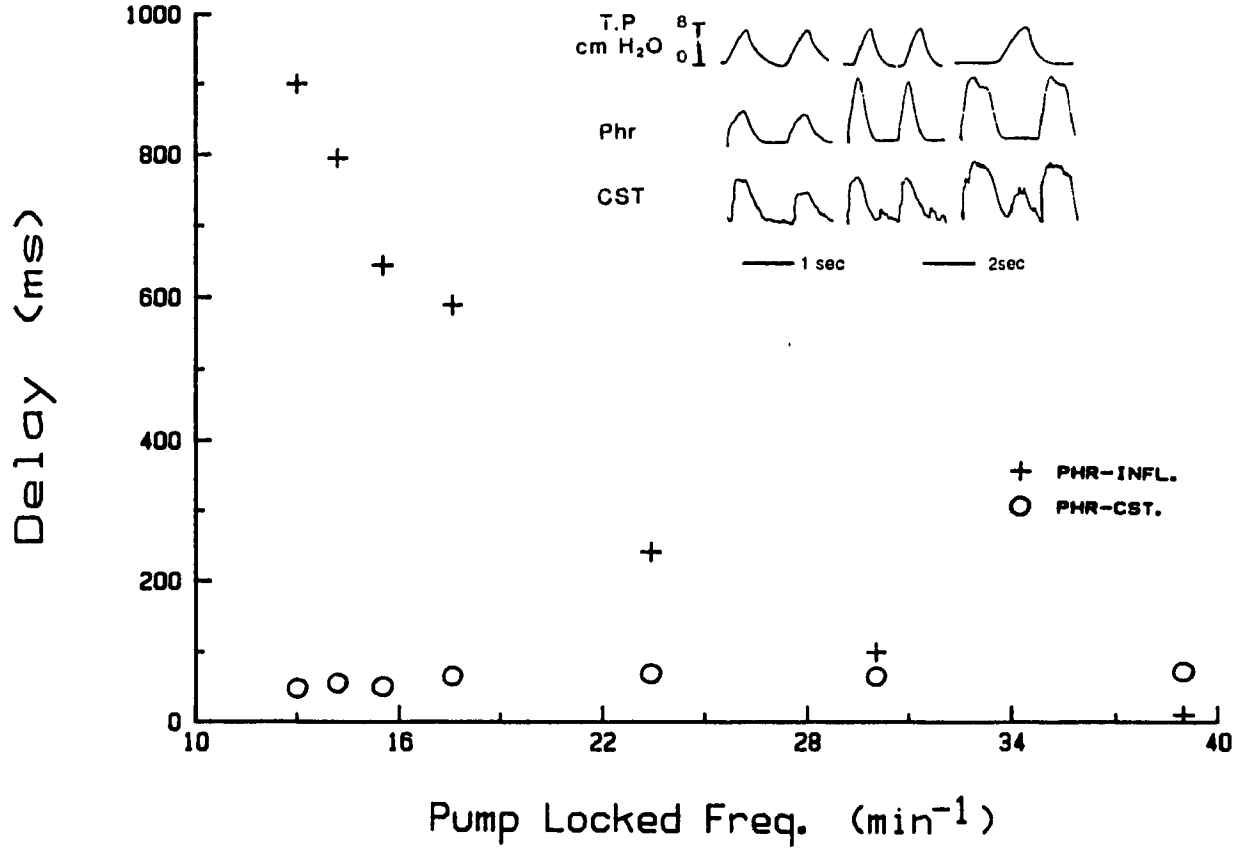
mV) were delivered to the central cut end of the superior laryngeal nerve every fifth respiratory cycle, at various delays from the onset of the phrenic nerve burst. Fig. 7 shows the resulting phase-response curve for phrenic nerve and inspiration-related sympathetic discharge, i.e., the changes in cycle duration (or phase shift, expressed as % of control) as a function of the time of the cycle (expressed as a fraction of the normalized cycle duration) at which the stimulus was delivered. Except for the shortening of the cycle caused by stimuli delivered during the last part of inspiration (Fig. 7ii), at all other times of the cycle the effect of stimulation was a prolongation, which varied in magnitude in a characteristic manner with stimulus timing. The significant finding, in relation to the hypothesis being tested, was that the phase-shift caused by the stimulus was the same for both phrenic and inspiration-related sympathetic discharge, i.e. the two waveforms maintained the same temporal relation to each other during the curtailed or prolonged cycles as in the unstimulated cycles. An additional observation was that stimuli delivered during inspiration caused characteristic changes in the phrenic waveform which were always associated with equivalent changes in the waveform of the inspiration-related sympathetic discharge (e.g. Fig. 7 (i)) as described earlier (Bachoo & Polosa, 1985).

ii) Entrainment of the Phrenic Nerve Burst to Changing Respiratory Pump Rates

In another experiment (performed on 6 cats) the periodic stimulus provided by phasic lung inflation was used to modify in a stable manner, i.e. entrain, the cycle duration of the brainstem respiratory oscillator. For a system of two coupled oscillators, entrainment is produced by the periodic input from the driving oscillator modifying the period of the driven oscillator on a cycle by cycle basis (von Holst, 1939). Stable entrainment in a one-to-one ratio between driving and driven oscillator frequencies is limited in the case of described neural oscillators, to a narrow range of frequencies bordering the intrinsic frequency of the driven oscillator (Pinsker, 1977b; Ayers & Selverston 1979). Within the limits of stable one-to-one entrainment, a change in frequency of the driving input will shift the phase at which the input reaches the driven oscillator, accordingly modifying the period of the driven oscillator such that equality with the period of the rhythmic input is achieved. When the period of the driving oscillator is too different from that of the driven oscillator, entrainment ratios different from one will occur (Pavlidis, 1973). If the bursting rhythms of the phrenic nerve and of the cervical sympathetic trunk were the result of the activity of such coupled oscillators, a varying phase-delay between the respective waveforms at different frequencies would be expected. In addition, the frequencies at which stable one-to-one entrainment of the two

FIG. 8 Relation between phrenic and sympathetic burst discharge during entrainment of the phrenic nerve burst to changing respiratory pump rates. Cat with intact vagus nerves. The plot shows on the ordinate the delay in ms between phrenic and sympathetic burst onsets (open circles) at various phrenic burst frequencies (abscissa) obtained by changing respiratory pump frequency in a range within which there is a 1:1 ratio of pump to phrenic burst frequency. Crosses indicate delays in ms between onsets of phrenic burst and of lung inflation (measured as tracheal pressure) at the various phrenic burst frequencies shown on the abscissa. Notice relative constancy of the delay between phrenic and sympathetic burst. In contrast, notice large variation in the delay between phrenic burst and lung inflation, which is typical of biological oscillators driven by a periodic synaptic input of varying frequency, as may be generated by another oscillator (Pavlidis, 1973; Pinsker, 1977; Ayers & Selverston, 1979). Insets show sample records of tracheal pressure (T.P.), phrenic (Phr) and sympathetic (CST) discharge from the same experiment plotted in the graph. At the lowest respiratory pump frequency (right most records) inflation occurs in expiration and is associated with a burst of sympathetic discharge as described previously (Bachoo & Polosa, submitted). Each point on graph is average of 20 cycles. Each waveform in insets is average of 10 sweeps.

FIG. 8


T.P
cm H2O
8
0
Phr
CST
1 sec
2sec
1000
800
600
400
200
0
Delay (ms)
10
16
22
28
34
40
PHR-INFL.
PHR-CST.
Pump Locked Freq. (min-1)

waveforms could be maintained would be limited to a range bordering the intrinsic frequency of the hypothetical, driven, sympathetic oscillator. Alternatively, if the inspiration-related sympathetic waveform is the result of the sympathetic preganglionic neurones sharing with phrenic motoneurones input from the respiratory oscillator, the two waveforms may be expected to maintain a constant time delay at all frequencies.

Sensory input, presumably from pulmonary stretch receptor afferents, generated by lung inflation at various frequencies, was used to entrain the respiratory oscillator to the respiration pump (Cohen, 1969; Vibert, Caille & Segundo, 1981; Petrillo, Glass & Trippenbach, 1983) and thereby change the respiratory oscillator frequency, within limits, by changing ventilation pump frequency. The results of such an experiment are shown in Fig. 8. Within the ventilation pump frequencies of 13 and 39 cycles/min, at constant tidal volume, the ratio of phrenic nerve burst rate to pump rate was one. The phase-delay between the phrenic nerve burst and the tracheal pressure trace (the latter giving an indication of the time course and amplitude of the input) changed in a characteristically smooth manner over this range of frequencies (Fig. 8 crosses) from 900 ms to near zero. This is a behaviour typical of biological oscillators driven by periodic synaptic input of variable frequency, as may be generated by another oscillator (Pavlidis, 1973; Pinsker, 1977a; Ayers & Selverston, 1979). In

contrast, over the same range of frequencies the phase-delay between phrenic nerve burst and inspiration-related sympathetic discharge remained approximately constant (Fig. 8, open circles). A small increase (by 8 ms) at the higher frequencies was within the magnitude of change that can be observed as a result of changes in excitability of the sympathetic preganglionic neurone or in the magnitude of the input. This trend can be accounted for by the changes in end-tidal P_{CO_2} which occurred with changes in respiratory pump frequency between 13 and 39 cpm. In a typical experiment, end-tidal P_{CO_2} varied from 48 mmHg at the lowest frequency to 35 mmHg at the highest frequency. At frequencies greater than 18 cpm CO_2 was administered to the animal to prevent marked hypocapnia. At constant, high respiratory pump frequency, an increase in delay of comparable magnitude was observed when end-tidal P_{CO_2} was changed within this range (see Results section 1) by switching from ventilation with various CO_2-containing gas mixtures to ventilation with room air. Thus, Fig. 8 shows an absence of changes in the phase-delay between phrenic and inspiration-related sympathetic discharge in the frequency range in which a one-to-one relation between respiratory pump and phrenic nerve burst frequencies exist. Analogous results were obtained in the other 5 cats in which a similar experiment was performed. It should be mentioned that outside the range of frequencies in which one-to-one phase-locking between phrenic burst and respiratory pump frequency occurs, entrainment ratios of phrenic to pump frequency

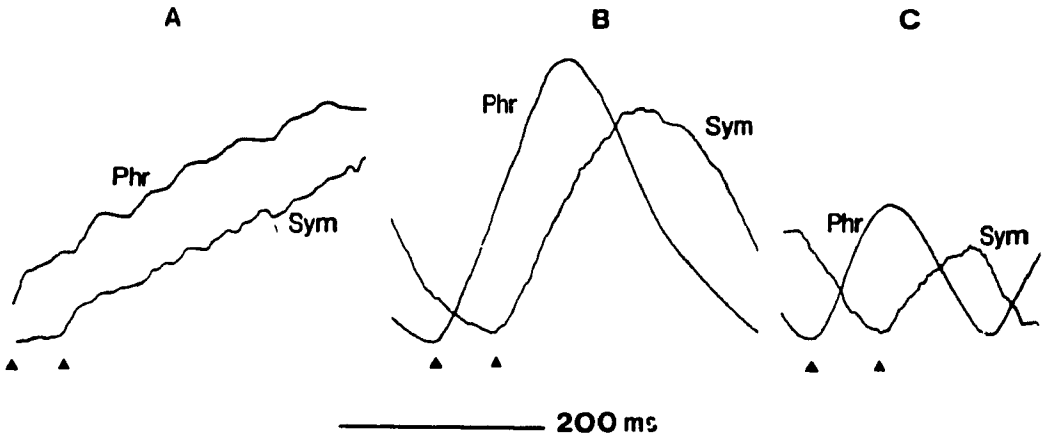
greater or smaller than one occurred, as described in Petrillo, Glass and Trippenbach (1983). The inspiration-related sympathetic discharge always duplicated the phrenic behaviour and maintained a constant phase delay relative to the phrenic nerve burst. Thus, constancy of the phase delay between the inspiration-related sympathetic discharge and the phrenic nerve burst was observed under all relations of phrenic frequency to pump frequency studied.

iii) Hyperthermic Hypocapnic Polypnea

The phase-delay of the inspiration-related sympathetic discharge to phrenic nerve burst was studied under conditions in which changes in phrenic nerve burst frequency greater than those caused by superior laryngeal nerve stimulation or by lung inflation were produced. In two vagotomized, sino-aortic denervated, paralysed, artificially ventilated cats hyperthermia (42°C rectal T) produced by radiant heat was used to induce polypnea. In one cat, in normocapnia, the frequency of the phrenic nerve burst increased from 15 bursts/min at 37°C to 36 bursts/min at 42°C. A further increase in frequency, to 324 bursts/min, was obtained when, at a temperature of 42°C, the animals wsd made hypocapnic by hyperventilation in air (end-tidal P_{CO_2} 10 mmHg; Cohen 1964, Monteau, Hilaire & Ouedraogo, 1974). Similar results were obtained in the other cat. The inspiration-related sympathetic discharge frequency maintained a one-to-one relation to the phrenic burst frequency over the whole range of frequencies. Fig. 9 shows that

FIG. 9 Phase-relation between phrenic and sympathetic burst discharge during hyperthermic-hypocapnic polypnea in a vagotomized cat. The table shows that the delay between phrenic and sympathetic waveforms varies little within the wide range of respiratory cycle durations obtained in this experiment. Each value is the mean of 40 measurements. See text for possible explanations of the increase in delay at the highest frequency. A, B and C show the average waveform (40 sweeps) recorded at cycle duration values of 3800, 450 and 185 ms. Arrow heads indicate delay. Phr: phrenic. CST: sympathetic cervical trunk.

FIG. 9



PHRENIC TO SYMPATHETIC BURST DELAY AT VARIOUS RESPIRATORY CYCLE DURATIONS

CYCLE DURATION (ms)	3800	1060	760	300	185
DELAY (ms) MEAN ± S.D.	43 ± 2.7	41 ± 2.6	42 ± 2.7	47 ± 3.8	60 ± 4.3

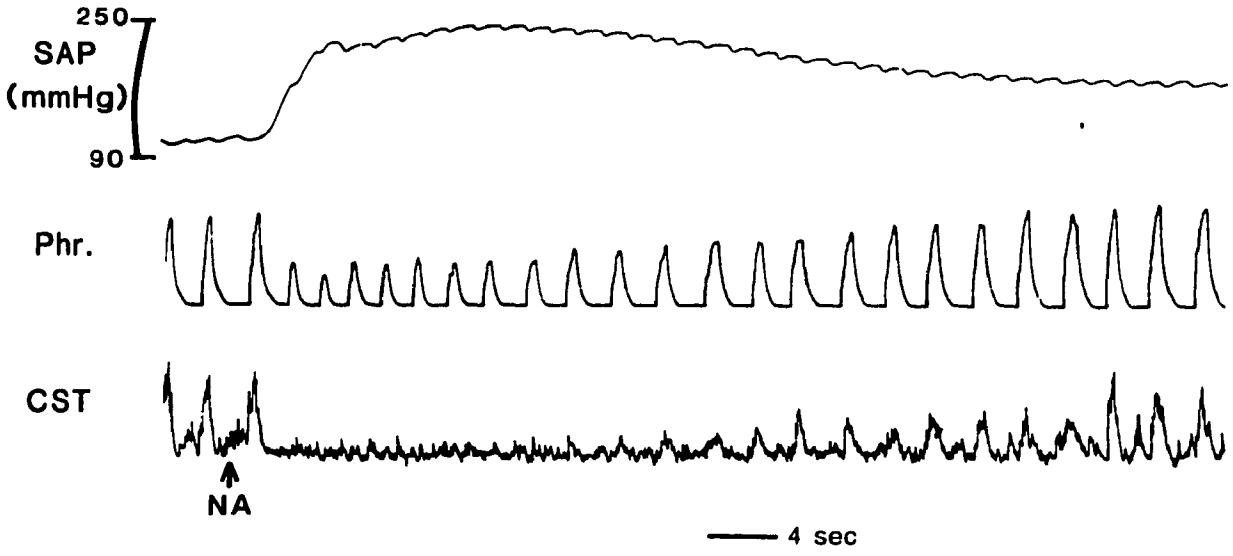
the phase delay between the two waveforms varied relatively little in spite of a 20-fold change in frequency, being 43 ms in control conditions and 60 ms at the highest respiratory frequency. The increased delay at the highest frequencies (an increase of less than 1% of control cycle period) can be attributed to changes in the excitability of the preganglionic neurone as well as in the properties of the synaptic drive, resulting from factors such as hypocapnia (see Fig. 3), hyperthermia and high bursting rates. An additional feature to be noted in Fig. 9 is that the relative constancy of the phrenic burst onset - sympathetic burst onset delay over the whole range of frequencies causes the greatest part of the sympathetic burst to occur during the expiratory phase of the respiratory cycle at the highest respiratory frequencies.

c. Cases of Absence of Inspiration-Related Sympathetic Discharge in the Presence of Central Inspiratory Activity

The picture emerging from the experiments described in the previous section is one of a tight relationship between phrenic and sympathetic burst activity. This picture is consistent with results of previous experiments demonstrating parallel changes of the two bursts in a number of different experimental situations (changes in systemic PCO_2, Preiss & Polosa, 1977, Connelly & Wurster, 1985; lung inflation, Gerber & Polosa, 1978; superior laryngeal nerve stimulation, Gerber & Polosa, 1979, Bachoo &

FIG. 10 Disappearance of inspiration-related sympathetic discharge during the arterial pressure increase caused by injection of noradrenaline (5 μg/kg i.v.). From top: mean systemic arterial pressure, low-pass filtered phrenic and cervical trunk recording. The disappearance is presumably due to baroreceptor-evoked depression of i) sympathetic neurone excitability and ii) input from brainstem inspiratory neurons (as suggested by the depression in peak phrenic amplitude). After sino-aortic denervation, the same increase in arterial pressure had no effect on either sympathetic or phrenic nerve activity (not shown). Time of NA injection indicated by arrow.

FIG. 10

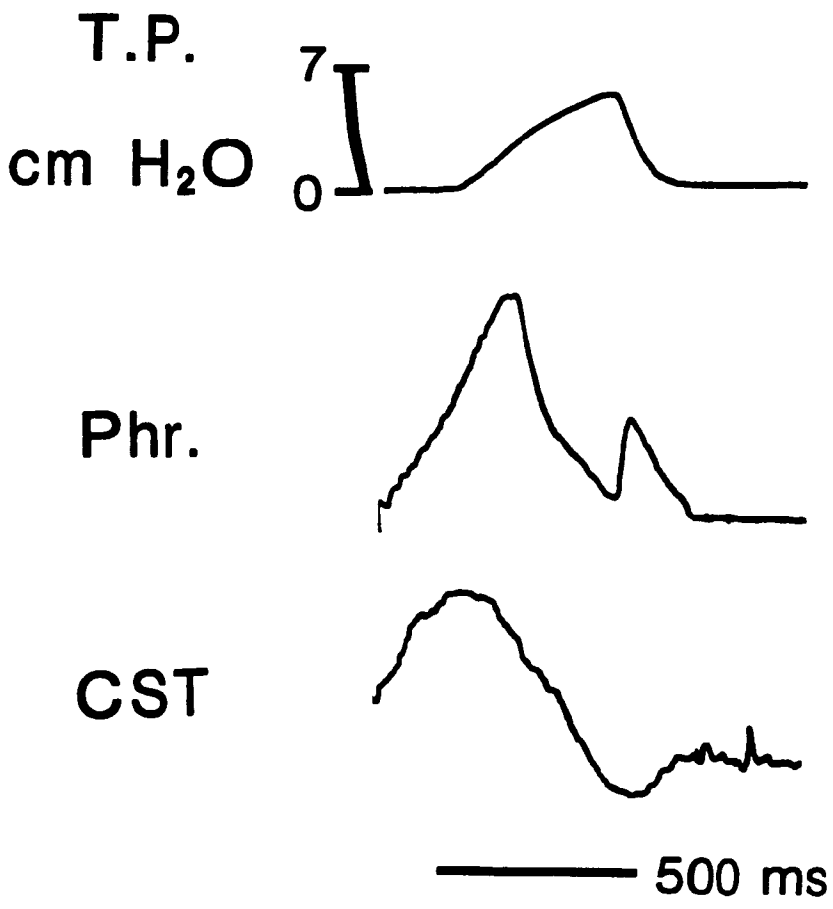


Polosa, 1985). However, in some experimental conditions the inspiration-related sympathetic discharge was seen to disappear although the phrenic nerve burst was present. One such condition is during the depression of sympathetic preganglionic neurone activity, presumably of baroreceptor origin, associated with the pressor response caused by i.v. injection of noradrenaline (Iggo & Vogt 1962). In these conditions (Fig. 10) the peak amplitude of the phrenic nerve burst was markedly attenuated (by approximately one half with respect to control) and the inspiration-related sympathetic discharge disappeared. The reduction in the level of central inspiratory activity, caused by the baroreceptor reflex (Nishino & Honda, 1982) is unlikely to be the entire cause of the disappearance of the inspiration-related sympathetic discharge because reductions of comparable magnitude in the level of central inspiratory activity, caused by lowering end-tidal P_{CO_2} or by increasing the level of anesthesia, resulted in a roughly proportional reduction in this discharge as in the amplitude of phrenic nerve activity (Fig. 2). Therefore, the absence of the inspiration-related sympathetic discharge is likely due to a block of the input from brainstem inspiratory neurones along its transmission path to the sympathetic preganglionic neurone by the powerful inhibitory action of the arterial baroreceptors.

Another condition in which the sympathetic discharge corresponding to a phrenic burst was absent was the occurrence of central inspiratory activity (in rather unusual experimental

FIG. 11 Absence of inspiration-related sympathetic discharge when a phrenic burst occurs with a short delay after the end of the preceding phrenic burst. This condition occurred during phase-locking of the phrenic nerve burst to the ventilation pump frequency, with two phrenic bursts occurring for each pump cycle. Notice presence of sympathetic burst when large phrenic burst occurs, absence when small phrenic burst occurs. T.P.: tracheal pressure. Phr: phrenic nerve activity. CST: cervical sympathetic trunk activity. Averages of 2-4 sweeps.

FIG. 11



situations) during the phase of the respiratory cycle immediately following the end of inspiration. An example is shown in Fig. 11. This record was obtained in an animal with intact vagus nerves in which entrainment between phrenic nerve burst and respiratory pump frequency occurred in a 2:1 ratio. There was, for each pump cycle, a phrenic nerve burst of normal amplitude and duration, leading the inflation by 150 ms and followed, at 310 ms from the peak, by another burst of much smaller amplitude and shorter duration. The smaller burst coincided with the deflation phase of the respiratory pump cycle. This burst occurred at the time of the respiratory cycle at which the level of sympathetic discharge was at its minimum. As the figure shows, there was no sympathetic burst corresponding to the small phrenic burst. Another example is shown in Fig. 12. These records are from a cat in which phrenic nerve activity spontaneously became of the gasping type (St. John & Knuth, 1981), i.e. was characterized by bursts of short duration, fast rise time and irregular occurrence (Fig. 12A, B & C). Some of the phrenic bursts occurred in close succession. When the onset of a burst occurred at an interval of 130 ms or less after the peak of the preceding one, no corresponding sympathetic discharge occurred (B). At an interval of 170 ms (C) the inspiration-related sympathetic discharge was present but was much attenuated in amplitude (compare with A). The observations shown in Fig. 13 and 14 could be explained with the hypothesis of a post-inspiratory phase of sympathetic preganglionic neurone depression, during which

FIG. 12 Absence of inspiration-related sympathetic discharge when a phrenic burst occurs with a short delay after the end of the preceding phrenic burst. Phr: phrenic nerve-activity. CST: cervical sympathetic trunk activity. A, B, and C: during spontaneous "gasping" respiratory cycle duration is very irregular. Notice absence of second sympathetic burst in B and its presence, but with great attenuation, in C.

FIG. 12

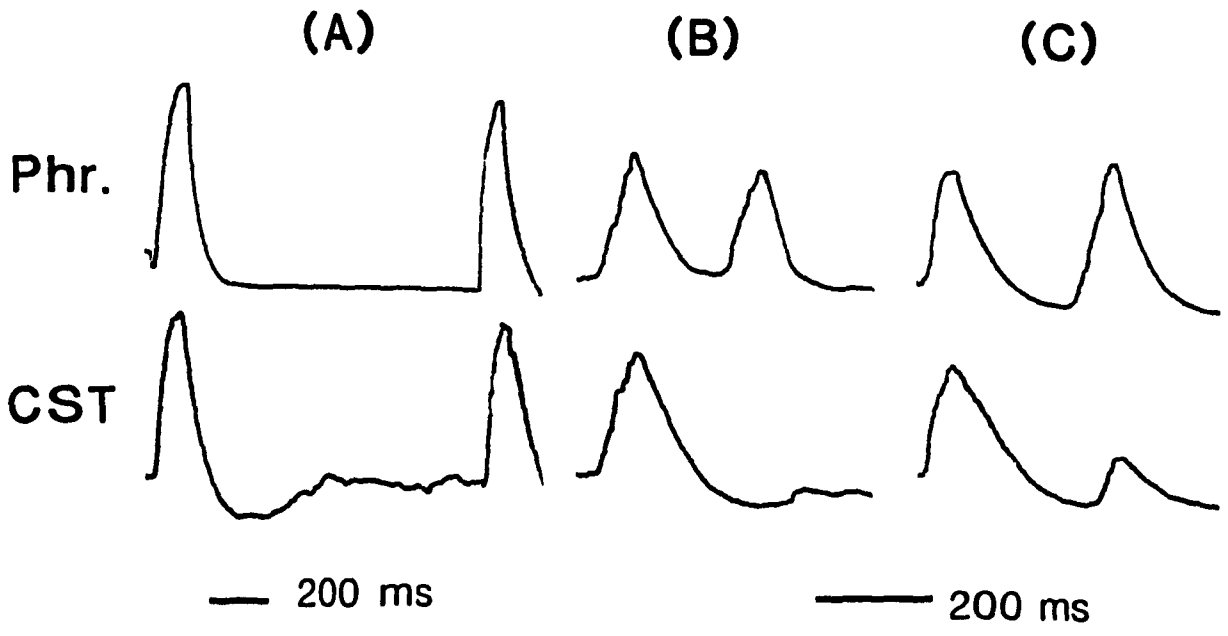
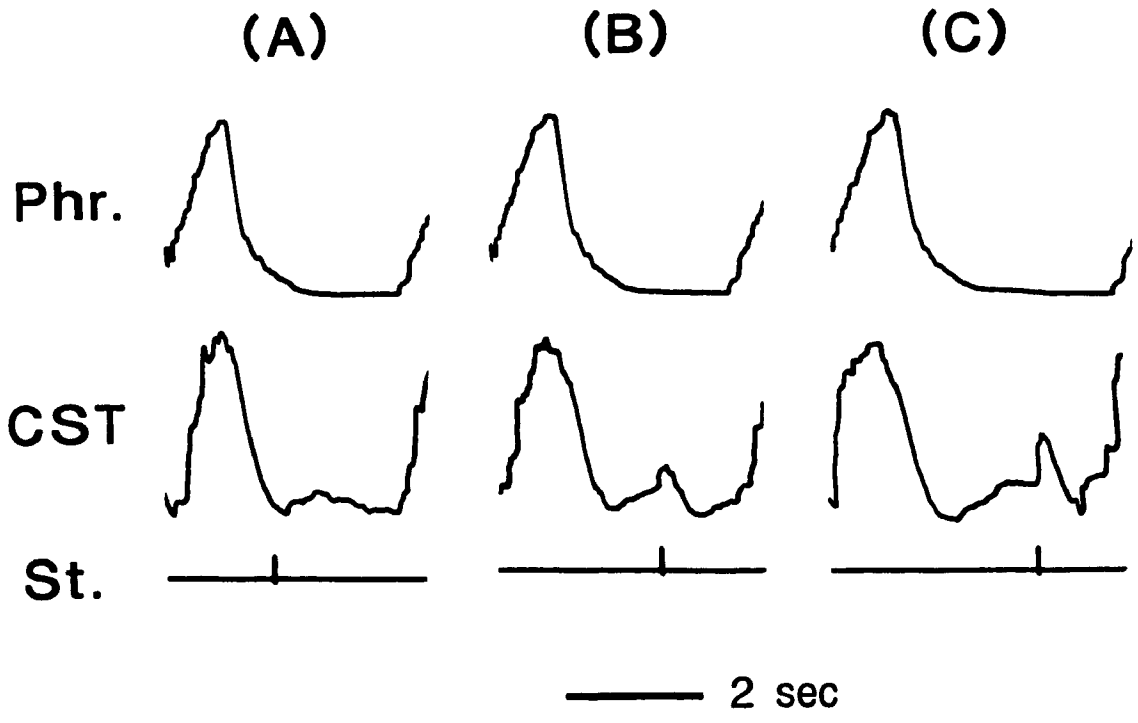


FIG. 13 The sympatho-excitatory effect of a train of stimuli to the superior laryngeal nerve (8 pulses at 40 Hz, 2V, 0.2 ms) varies with time in expiration. The excitatory effect is absent in early expiration (A). It appears later in expiration and increases with time in expiration (B & C). Phr & CST: phrenic and cervical sympathetic trunk activity. St: stimulus. Each trace is average of 10 sweeps.

FIG. 13



the input from brainstem inspiratory neurones could become less effective in producing a discharge. Results consistent with this hypothesis were obtained by stimulation of superior laryngeal nerve afferents, which have been shown to have an excitatory action on the sympathetic preganglionic neurone, independent of the central respiratory activity (Bachoo & Polosa, 1985). A train of stimuli was given at various times of the expiratory phase (Fig. 13). A stimulus train, given 640 ms after the end of inspiration did not cause a discharge (A) of sympathetic preganglionic neurones. A discharge first appeared when the stimulus train was given 1.6 s after the end of inspiration (B) and the discharge increased in amplitude as the stimulus was given later in expiration 2 s after inspiration end (C). Since in hypocapnia, resulting in abolition of rhythmic phrenic nerve activity, the response was similar in amplitude to that recorded in C, these data are interpreted as showing that after the end of inspiration the sympathetic preganglionic neurone undergoes a period of depression, which wanes with time in expiration.

DISCUSSION

The experiments just reported have provided a description of several properties of the inspiration related sympathetic discharge. Some of the experiments have studied the phase-delay between phrenic and inspiration- related sympathetic waveforms

either in response to a discrete stimulus (to the superior laryngeal nerve) presented at various phases of the respiratory cycle or, in the steady state, when the phrenic nerve frequency was changed over a wide range of values by an external rhythmic input (the periodic lung inflation by the respiratory pump) or by altering physiological variables (temperature, end-tidal P_{CO_2}). These observations are relevant to the question of whether or not the inspiration-related sympathetic discharge results from the activity of a hypothetical neural oscillator, synaptically coupled and entrained to the respiratory oscillator (Koepchen, 1962; Barman & Gebber, 1976).

The most obvious feature of the pattern of resetting of the phrenic and sympathetic activity cycle by superior laryngeal nerve stimulation is their close similarity, resulting in the absence of relative phase-shifts. On the assumption that the superior laryngeal nerve afferent input acts independently on the respiratory oscillator and on the postulated sympathetic oscillator, the observed similarity implies (Pavlidis, 1973) that the two neuron systems undergo an identical cycle of varying sensitivity to this input. Although the details of the physiological mechanisms which generate the phrenic and inspiration-related sympathetic waveforms are incompletely understood, it seems unlikely that the properties of the two systems should be identical. Hence, the above assumption of an action of the laryngeal afferents independently on two oscillators

can also be considered unlikely. In view of the well known, apnea-promoting, superior laryngeal nerve reflex (Larrabee & Hodes, 1948) a more likely assumption is that the superior laryngeal nerve input acts primarily to reset the central respiratory cycle and that the cycle of the putative sympathetic oscillator is perturbed secondarily by synaptic coupling between the two. This hypothesis too is unlikely, however, since for a system of coupled oscillators it is predicted that during perturbation of either oscillator the phase delay between the two output waveforms will change (Pinsker, 1977b), and yet no change in the phase delay between phrenic burst and inspiration-related sympathetic discharge was found during the perturbed cycle or the succeeding cycles. The similarity of the pattern of response of phrenic and inspiration-related sympathetic waveforms to superior laryngeal nerve stimulation, together with the absence of response features expected from a system of coupled oscillator, suggests that the superior laryngeal nerve input acts on a common oscillator which drives both the phrenic and the sympathetic discharges.

It is well known (Vibert, Caille & Segundo, 1981; Petrillo, Glass & Trippenbach, 1983) that in cats with intact vagus nerves the phrenic nerve discharge becomes time-locked to lung inflation by the respiratory pump, via the Hering-Breuer reflex circuit, in a manner which complies with theoretical predictions (Pham Dinh, Demongest, Baconnier & Benchetrit, 1983; Petrillo & Glass, 1984) and experimental observations (Pinsker, 1977a) of the behaviour

of a neural oscillator driven by a periodic input. Namely, i) the range of frequencies over which stable one-to-one entrainment can be maintained is limited to a set which borders the intrinsic frequency of the respiratory oscillator, (i.e. the frequency in the absence of pulmonary stretch receptor input) and ii) at each respiratory pump frequency, within this set, the forcing input (inflation) assumes a unique position in the phrenic activity cycle. For frequencies higher than the intrinsic frequency of the respiratory oscillator, inflation occurs with a short delay from the phrenic burst onset, resulting in a shortened phrenic nerve discharge and subsequent expiratory duration (Clark & von Euler, 1972). For frequencies lower than the intrinsic frequency of the respiratory oscillator, inflation occurs in expiration and results in a prolongation of expiration and of the subsequent phrenic nerve discharge (Zuperku & Hopp, 1985). Thus, within the range of stable one-to-one entrainment of the respiratory oscillator frequency to the respiratory pump frequency there is a continuous change in the phase delay between inflation and onset of phrenic nerve discharge as a function of frequency (Fig. 9, see also Petrillo, Glass & Trippenbach, 1983, Fig. 10a). In marked contrast, the absence of any comparable change in the phase delay between phrenic and sympathetic waveforms (same Fig. 8) over the same range of frequencies suggests that the equality of period of phrenic and inspiration-related sympathetic discharge is not due to mechanisms comparable to those which maintain equality of the respiratory pump

and phrenic period. Thus, these findings make it unlikely that the frequency of the inspiration-related sympathetic discharge results from the activity of an autonomous neural oscillator coupled to the respiratory oscillator. Instead, these results are compatible with the hypothesis of a common neural oscillator which drives both the phrenic and the sympathetic discharges.

Although, as stated above, experimental observations (Wendler, 1974; Pinsker, 1977a; Ayers & Selverston, 1979; Peterson & Calabrese, 1982) uphold the theoretical predictions (Pavlidis, 1973; Winfree, 1980) that a stable one-to-one entrainment of coupled oscillators is limited to within a narrow range of frequencies bordering the free-run frequency for the coupling conditions prevailing in neural systems, the possibility may be considered that the absence of a frequency-dependent change in the delay between inspiration-related sympathetic discharge and phrenic discharge in the pump-locking experiments was due to the too limited range of frequencies tested. In the experiments of hyperthermic, hypocapnic polypnea there was an approximately 20-fold increase in phrenic burst and inspiration-related sympathetic discharge frequency, again without apparent frequency-dependent phase shifts between the two waveforms.

The entrainment of oscillators is a phenomenon which is ubiquitous through the animal kingdom (von Holst, 1939) extending from oscillators with free run periods of milliseconds (Winfree,

1980) to circadian oscillators (Moore-Ede, Sulzman & Fuller, 1982). The present experiments have focused on two testable features of the phrenic and inspiration-related sympathetic discharge which would help identify the existence of the postulated sympathetic oscillator, namely the range of frequencies over which a stable one-to-one relation is maintained and the phase delay between the respective waveforms. The general applicability of these testable features is demonstrated by the published examples of known synaptically coupled neural oscillators, or of known biological oscillators which can be entrained by rhythmic synaptic inputs, (Perkel, Schulman, Bullock, Moore & Segundo, 1964; Levy, Iano & Zieske, 1972; Stein, 1976; Pinsker, 1977a; Ayers & Selverston, 1979; Peterson & Calabrese, 1982; for comprehensive reviews, see Winfree, 1980; Pinsker & Ayers, 1983; Selverston & Moulins, 1985).

Although the mechanisms underlying entrainment in the examples cited above are likely to be different, all these studies illustrate the generality of the features of coupled oscillators, upon which the present experiments have focussed. The qualifying limitation must be made, in extrapolating from the data just reviewed to the experimental situation of the present experiments, that the dynamic behaviour of entrained oscillators in invertebrate pacemaker cells or in non-neural mammalian systems may not be applicable to complex neural oscillators. However the experimental evidence appears to be to the contrary (Pinsker & Ayers, 1983).

Since the hypothesis of coupled oscillators has been shown to be unlikely by the results discussed previously, the fixed temporal relation between phrenic nerve discharge and the inspiration-related sympathetic discharge may be explained with the hypothesis that the phrenic motoneurones and the sympathetic preganglionic neurones are driven by a common rhythmic input. Although onset and offset of the inspiration-related sympathetic discharge have fixed temporal relation to the onset and offset of phrenic nerve activity, in the majority of cases the shapes of the two waveforms were different. The difference may result from differences in the properties of the pathways delivering the oscillator output to the two neurones. For instance, the sympathetic preganglionic neurones may be receiving the same periodic, ramp-shaped excitatory post-synaptic potential as the phrenic motoneurones (Berger, 1979), and the recruitment and firing properties of the two neurones may account for the difference in wave shape. In normocapnia, phrenic motoneurones are recruited throughout inspiration (St. John & Bartlett, 1979) and their firing frequency increases with time during inspiration, reaching a maximum at end-inspiration (Iscoe, Dankoff, Migikovsky & Polosa, 1976). The shape of the inspiration-related sympathetic discharge could be explained with the hypothesis that the majority of sympathetic preganglionic neurones are recruited early in inspiration and thereafter their firing frequency stays more or less constant. The data presented on the temporal structure of the inspiration-synchronous bursts of

single sympathetic preganglionic neurones are consistent with this hypothesis. Interestingly, the inspiration-synchronous discharge of the whole hypoglossal nerve has a square-wave like shape, similar to that of sympathetic nerves (Hwang, Bartlett and St. John, 1983). Hypoglossal motoneurones are recruited mostly at inspiration onset and, once recruited, fire at a relatively constant rate during inspiration (Hwang, Bartlett & St. John, 1983). A property of the sympathetic preganglionic neurone which may be contributing to the maintenance of a relatively constant firing frequency during inspiration is a large amplitude, long duration, summating, afterhyperpolarization (Yoshimura & Nishi, 1982).

Finally, the hypothesis of a common oscillator driving both phrenic motoneurones and sympathetic preganglionic neurones does not require that both populations of driven neurones be always simultaneously active. Differences in excitability of the neurones themselves or of the connecting pathways to the driving oscillator may be expected to result in the possible absence of rhythmic activity in one and persistence in the other, as shown by some of the present observations (Fig. 10-13).

The conclusions reached in the present study may appear to be in contrast with some of the conclusions reached by Barman and Gebber (1976). It must be realized, however, that the data on which these two studies are based are different. The observations of Barman and Gebber (1976) concern a slow amplitude modulation

(with period similar to that of the phrenic bursting rhythm) of a 3-4 Hz repetitive waveform recorded in sympathetic postganglionic nerves. In several of their records this slow modulation appears to be independent of, i.e., not phase-locked to, the phrenic nerve burst. The present study, in contrast, concerns the periodic burst of sympathetic preganglionic neuron firing which previous work has defined as inspiration-related on the basis of constant phase-relation to, and similarity of properties with, the phrenic nerve burst (Preiss, Kirchner and Polosa, 1975; Gerber and Polosa, 1979). The data presented in the study by Barman and Gebber are mostly non-stationary and therefore information concerning the statistical properties of the slow modulating signal and its phase-relation to the phrenic nerve cycle is not available for some of their most intriguing observations (e.g. their Fig. 4 and 5). Thus, in the absence of insight into the relation, if any, between the periodic, inspiration-related, sympathetic burst, studied here, and the slow modulation, studied by Barman and Gebber, it cannot be stated whether the discrepancy between these two sets of conclusions is real or is the result of different phenomena being studied in the two investigations.

ACKNOWLEDGEMENTS

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SUMMARY

1. The experiments reported here have examined some temporal characteristics of the inspiration-related sympathetic discharge of the cat in control conditions and during forcing of the respiratory oscillator into marked deviations from its natural frequency. Purpose of these experiments was to establish whether or not the relation of sympathetic to phrenic nerve activity shows properties consistent with the hypothesis that the inspiration-related sympathetic discharge is driven by a neural oscillator, independent of, but coupled and stably entrained to, the brainstem respiratory oscillator.
2. The electrical activity of the whole cervical sympathetic trunk (n = 26) or of small strands of the cervical trunk containing single units (n = 20) and of the phrenic nerve was recorded in pentobarbitone anaesthetized, paralysed, artificially ventilated, sino-aortic denervated cats. Most of the cats were bilaterally vagotomized.
3. The onset of the inspiratory burst of the sympathetic preganglionic neurones had a fixed delay from the onset of the phrenic nerve burst. The level of activity within the burst, in whole cervical trunk recording, reached a maximum in early inspiration and then was maintained at approximately this level for the rest of inspiration (22/26 cats). In four cats the activity level increased throughout the burst. Individual

sympathetic preganglionic neurones displaying inspiration-related burst firing were characteristically recruited in early inspiration and thereafter maintained an approximately constant firing frequency for the rest of inspiration.

4. Electrical stimulation of afferents in the superior laryngeal nerve during various phases of the respiratory cycle caused equivalent, phase-dependent, resetting patterns of both phrenic nerve and inspiration-related sympathetic discharge.
5. In cats with intact vagus nerves, entrainment of the brainstem respiratory oscillator to the frequency of the respiratory pump was used to change the frequency of the former, within limits, by changing the frequency of the latter. Over the range of frequencies tested, the pump-to-phrenic delay varied as a function of frequency, while the delay between phrenic and sympathetic burst onset was essentially independent of frequency.
6. In hyperthermic-hypocapnic cats phrenic nerve burst frequency increased up to about 300 bursts/min from a value of 15 bursts/min in normothermia-normocapnia. At all frequencies within this range the sympathetic burst maintained a delay, with respect to the phrenic burst, which was essentially independent of frequency.

7. The fact that phrenic nerve and sympathetic burst maintained a one-to-one relation with essentially constant delay over all frequencies tested is inconsistent with the known behaviour of coupled neural oscillators. Therefore, the equality of period of phrenic nerve burst and inspiration-related sympathetic discharge is unlikely to result from the activity of an autonomous sympathetic oscillator coupled to the brainstem respiratory oscillator. Instead, these results are compatible with the hypothesis of a common oscillator which drives both the phrenic and the sympathetic discharge.

8. In some experimental conditions phrenic nerve activity without a corresponding sympathetic burst was observed. This dissociation was seen i) during baroreceptor activation caused by the increased systemic arterial pressure resulting from injection of a pressor drug, and ii) when, during abnormal respiratory rhythms, a phrenic nerve burst occurred at a short interval after a preceding burst. This dissociation is likely due to depression of the sympathetic neurone and/or to depression of the input from the brainstem respiratory oscillator. Hence these observations are not inconsistent with the hypothesis above.

REFERENCES

Ayers, A.L. and Selverston, A.I. (1979). Monosynaptic entrainment of an endogenous pacemaker network: a cellular mechanism for von Holst's magnet effect. Journal of Comparative Physiology 129, 5-17.

Bachoo, M. and Polosa, C. (1985). Properties of a sympatho-inhibitory and vasodilator reflex evoked by superior laryngeal nerve afferents in the cat. Journal of Physiology 364, 183-198.

Barman, S.M. and Gebber, G.L. (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. American Journal of Physiology 231, 1601-1607.

Berger, A.J. (1979). Phrenic motoneurons in the cat: Subpopulations and nature of respiratory drive potentials. Journal of Neurophysiology 42: 76-90.

Clark, F.J. and von Euler, C. (1972). On the regulation of depth and rate of breathing. Journal of Physiology 222, 267-295.

Cohen, M.I. (1964). Resiratory periodicity in the paralyzed vagotomized cat: hypocapnic polypnea. American Journal of Physiology 206, 847-864.

Cohen, M.I. (1968). Discharge patterns of brainstem respiratory neurons in relation to carbon dioxide tension. Journal of Neurophysiology 31, 142-165.

Cohen, M.I. (1969). Discharge patterns of brainstem respiratory

neurons during Hering-Breuer reflex evoked by lung inflation. Journal of Neurophysiology 32, 356-374.

Conen, M.I. and Gootman, P.M. (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. American Journal of Physiology 218, 1092-1101.

Connelly, C.A. & Wurster, R.D. (1985). Sympathetic rhythms during hyperventilation-induced apnea. American Journal of Physiology 249, R424-R431.

Delmar, M., Jalife, J. and Michaels, D.C. (1985). Effects of changes in excitability and intercellular coupling on synchronization in the rabbit sino-atrial node. Journal of Physiology 370, 127-150.

Gerber, U. and Polosa, C. (1978). Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 56, 191-198.

Gerber, U. and Polosa, C. (1979). Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 57, 1073-1081.

Holst, E. von (1939). Die relative Koordination als Phanomen und als Methode zentral-nervoser Funktionanalyse. Ergebnisse der Physiologie, Biologischen Chemie und experimentelle Pharmakologie 42, 228-306.

Hwang, J.C., Bartlett, D. and St. John, W.M. (1983). Characterization of respiratory-modulated activities of hypoglossal motoneurons. Journal of Applied Physiology 55: 793-798.

Iggo, A. and Vogt, M. (1962). The mechanism of adrenaline-induced inhibition of sympathetic preganglionic activity. Journal of Physiology 161, 62-72.

Iscoe, S., Dankoff, J., Migicovsky, R., and Polosa, C. (1976). Recruitment and discharge frequency of phrenic motoneurons during inspiration. Respiration Physiology 26: 113-128.

Jalife, J. (1984). Mutual entrainment and electrical coupling as mechanisms for synchronous firing of rabbit sino-atrial pace-maker cells. Journal of Physiology 356: 221-243.

Koepchen, H.P. (1962). Die Blutdruckrhythmik, Darmstadt. Dietrich-Steinkopff.

Larrabee, M.G. and Hodes, R. (1948). Cyclic changes in the respiratory centers, revealed by the effects of afferent impulses. American Journal of Physiology 155, 147-164.

Levy, M.N., Iano, T. and Zieske, H. (1972). Effects of repetitive bursts of vagal stimulation on heart rate. Circulation Research 30: 186-195.

Monteau, R., Hilaire, G. and Ouedraogo, C. (1974). Contribution à l'étude de la fonction ventilatoire au cours de la polypnée thermique ou hypocapnique. Journal de Physiologie 68, 97-120.

Moore-Ede, M.C., Sulzman, F.M. and Fuller, C.A. (1982). The clocks that time us. Physiology of the circadian timing system. Harvard Univ. Press. Massachusetts - London - England.

Nishino, T. & Honda, Y. (1982). Changes in pattern of breathing following baroreceptor stimulation in cats. Japanese Journal of Physiology 32, 183-195.

Pavlidis, T. (1973). Biological oscillators: their mathematical analysis, New York, Academic.

Perkel, D.M., Schulman, J., Bullock, T.H., Moore, G.P. and Segundo, J.P. (1964). Pacemaker neurons: effects of regularly spaced synaptic input. Science 145: 61-63.

Peterson, E.L. and Calabrese, R.L. (1982). Dynamic analysis of a rhythmic neural circuit in the leech Hirudo medicinalis. Journal of Neurophysiology 47: 256-271.

Petrillo, G.A., Glass, L. and Trippenbach, T. (1983). Phase-locking of the respiratory rhythm in cats to a mechanical ventilator. Canadian Journal of Physiology and Pharmacology 61, 599-607.

Petrillo, G.A. and Glass, L. (1984). A theory for phase locking of respiration in cats to a mechanical ventilator. American Journal of Physiology 246, R311-R320.

Pham Dinh, T., Demongest, J., Baconnier, P. and Benchetrit, G. (1983). Simulation of a biological oscillator: the respiratory system. Journal of Theoretical Biology 103,

113-132.

Pinsker, H.M. (1977a). Aplysia bursting neurons as endogenous oscillators. II. Synchronization and entrainment by pulsed inhibitory synaptic input. Journal of Neurophysiology 40, 544-556.

Pinsker, H.M. (1977b). Synaptic modulation of endogenous neuronal oscillators. Federation Proceedings 36, 2045-2049.

Pinsker, H.M. and Ayers, J. (1983). Neuronal oscillators. In The Clinical Neurosciences. Vol. 5: Neurobiology, ed. Rosenberg, R.N., pp. 203-266. New York: Churchill Livingstone.

Preiss, G., Kirchner, F. and Polosa, C. (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. Brain Research 87, 363-374.

Preiss, G. and Polosa, C. (1977). The relation between end-tidal CO_2 and discharge patterns of sympathetic preganglionic neurons. Brain Research 122, 255-267.

Selverston, A.I. and Moulins, M. (1985). Oscillatory neural networks. Annual Review of Physiology 47, 29-48.

Stein, P.S.G. (1976). Mechanisms of interlimb phase control. In R. Herman, S. Grillner, P.S.G. Stein and D.G. Stuart (Eds.). Neural Control of Locomotion, New York, Plenum Press, pp. 465-498.

St. John, W.M. and Bartlett, D. Jr. (1979). Comparison of phrenic motoneuron responses to hypercapnia and isocapnic hypoxia.

Journal of Applied Physiology 46, 1096-1102.

Tang, P.C., Maire, T.W. and Amassian, V.E. (1957). Respiratory influence on the vasomotor center. American Journal of Physiology 191, 218-224.

Vibert, J.F., Caille, D. and Segundo, J.P. (1981). Respiratory oscillator entrained by periodic vagal afferents: An experimental test of a model. Biological Cybernetics 41, 119-130.

Wendler, G. (1974). The influence of proprioceptive feedback on locust flight coordination. Journal of Comparative Physiology 88, 173-200.

Winfree, A.T. (1980). The Geometry of Biological Time. Springer-Verlag, New York, Berlin.

Yoshimura, M. and Nishi, S. (1982). Intracellular recordings from lateral horn cells of the spinal cord in vitro. Journal of the Autonomic Nervous System 6: 5-11.

Zuperku, E.J. and Hopp, F.A. (1985). On the relation between expiratory duration and subsequent inspiratory duration. Journal of Applied Physiology 58, 419-430.

CHAPTER III

LACK OF EVIDENCE OF COUPLED OSCILLATOR MECHANISMS IN THE GENERATION OF SYMPATHETIC RHYTHMS

1. INTRODUCTION

Under usual experimental conditions, the background discharge of sympathetic preganglionic or postganglionic neurons, in preparations with intact CNS, shows periodicities time-locked to the central respiratory cycle or to the cardiac cycle. One possible explanation of these periodicities is that sympathetic preganglionic neurons (SPN) activity is modulated by the rhythmic output of brainstem respiratory neurons (respiratory periodicity) or by rhythmic inhibition from arterial baroreceptor afferents (cardiac periodicity). This explanation implicitly excludes the possibility that the SPN themselves, or the associated antecedent circuitry, have intrinsic rhythmicity, i.e. rhythmicity independent of that resulting from these two periodic inputs. It has been reported, however, that in particular experimental conditions sympathetic discharge can show periodicities similar to, but independent of, the central respiratory (Koepchen, 1962; Barman & Gebber, 1976) and cardiac (Gebber, 1980) rhythms. These observations have raised the possibility that these sympathetic periodicities result from an intrinsic rhythmicity of neuron networks antecedent to the SPN (i.e. of "sympathetic" oscillators, Gebber, 1980). It has been proposed that under most experimental conditions such intrinsic rhythms may become entrained to the rhythm of activity of arterial baroreceptor afferents or of brainstem respiratory neurons. Thus, the SPN and antecedent

circuitry may be viewed as a system made of an oscillator which receives rhythmic input from external sources. Such a system is referred to as a coupled oscillator system.

There is ample theoretical (Pavlidis, 1973; Stein, 1977; Segundo & Kohn, 1981) and experimental (Pinsker & Ayers, 1983) literature describing the behaviour of systems of coupled oscillators in biology. A comparative survey of a variety of such systems involving nerve or cardiac cells will be presented to illustrate their main features. Experimental tests will be described of whether or not the respiratory rhythm of sympathetic discharge conforms with these features. In addition, published experimental evidence, which has been used as basis for the hypothesis that an independent sympathetic oscillator generates the cardiac modulation of sympathetic discharge, will be examined in search of the essential features of oscillator behaviour.

2. GLOSSARY

An attempt has been made to use in this paper terminology consistent with that used in oscillator literature (Pavlidis, 1973; Pinsker, 1977b). Some terms and concepts are defined here. A neural oscillator is a single neuron, or a network of neurons, which generates a rhythmic output in the absence of rhythmic input. An oscillation is a cyclical change in a variable (e.g. neural activity) with relatively constant waveform and period. The

free-run or intrinsic frequency of an oscillator is the frequency in the absence of periodic input. When considering two oscillators, coupling between the two exists if perturbing the rhythm of one resets the rhythm of the other. For neural oscillators coupling is provided by synaptic connections. Coupling can be weak or strong, depending on the strength of the synaptic connection. The relationship between two oscillators can be described by the temporal relationship of their respective waveforms, expressed in real time as delay or normalized to cycle duration as phase angle. Entrainment refers to a condition in which an oscillator (the follower) is driven at a frequency different from its free-run frequency by a rhythmic input originating from another oscillator (the driver) to which it is coupled or from an external periodic input, e.g. from a sensory source: a constant phase angle between oscillator input and output is evidence of entrainment.

3. COUPLED OSCILLATOR PROPERTIES

Any input which produces a transient perturbation of an oscillator free-run period (i.e. a phase-shift) can entrain that oscillator (which becomes a follower) at frequencies higher or lower than its free-run frequency. The input produces the entrainment by either phase-advancing or phase-delaying, on a cycle by cycle basis, the follower oscillator (a phase-advance occurs

when the onset of the cycle occurs earlier than predicted from the free-running frequency, a phase-delay when the onset of the cycle occurs later than predicted). A phase response curve (PRC) describes the just mentioned phenomenon, i.e. the change in oscillator cycle duration produced by a single discrete input, as a function of the time of the cycle at which the input occurs. The maximum phase advance or phase delay caused by the input defines the limits within which the frequency of an oscillator can be modified by that input. For known neural oscillators these limits define a narrow range of frequencies around the free-run frequency (see below).

In the steady-state, as a consequence of the properties disclosed by the PRC: (1) an oscillator coupled to a rhythmic synaptic input can be stably entrained to the frequency of the perturbing external source in a one to one ratio only over a narrow range of frequencies; (2) at each frequency in the one-to-one range the equality of the period of the driven oscillator with the input period is achieved by the input occurring at a unique phase of the cycle, such that the input-output phase difference may span the entire free run cycle duration of the driven oscillator; (3) when the frequency of the driving input exceeds the limits of the one-to-one range, different entrainment patterns involving small integer ratios are established between the frequency of the input and the frequency of the driven oscillator. For instance, beyond the boundaries of the 1:1 entrainment ratio, the entrainment

pattern assumes a 2:3, 1:2 etc. ratio, as the input frequency is reduced and 3:2, 2:1 etc. ratio as the input frequency is increased. Under these conditions the phase relation varies on a cycle to cycle basis in an orderly, repeating manner. The range of ratios outside the 1:1 range is also described as relative coordination (von Holst, 1939). At frequencies of the input which are at the boundary between ranges giving stable entrainment, the driven oscillator may exhibit transient irregular dynamics with no fixed phase relation to the input.

4. BEHAVIOUR OF COUPLED BIOLOGICAL OSCILLATORS

The above mentioned features of coupled oscillators, namely limited range of frequencies over which a stable one-to-one relation is maintained and different phase-relation between respective waveforms at different frequencies within the one-to-one range, are derived from both theoretical considerations (Pavlidis, 1973; Stein, 1977; Glass & Mackey, 1979; Winfree, 1980; Segundo & Kohn, 1981) and experimental observations (see below). They apply to oscillators with free run periods from milliseconds (Perkel et al., 1964; Guevara et al., 1981) to circadian (Enright, 1965; Moore-Ede et al., 1982; Turek, 1985). The generality of these features is illustrated by examples of coupled oscillators drawn from single pacemakers neurons (Perkel et al., 1964; Pinsker, 1977a), simple neural network oscillators in invertebrates (Stein,

1976; Ayers & Selverston, 1979; Peterson & Calabrese, 1982), complex neural network oscillators in vertebrates (von Holst, 1939; Petrillo et al., 1983) and cardiac pacemaker cells (Levy et al., 1972; Guevara et al., 1981; Jalife, 1984). Although this list of examples is by no means exhaustive (for comprehensive reviews see Pinsker and Ayers 1983; Selverston and Moulins, 1985; Winfree, 1980) it illustrates the principle that, at least qualitatively, the dynamics of coupled oscillator behaviour is predictable and applies to a wide variety of biological oscillators.

Entrainment of single pacemaker neurons by rhythmic electrical stimulation of an excitatory or inhibitory synaptic input has been well characterized in a number of preparations. Pinsker (1977a) demonstrated the range of stable 1:1 entrainment of an endogenously bursting neuron of Aplysia by an inhibitory input to be limited to 6% above and 30% below its free-run frequency. At each frequency the burst of the pacemaker neuron assumed a unique phase-relation to the rhythmic driving stimulus. Similar findings have been described by Ayers and Selverston (1979) in the stomatogastric neural network of the lobster in response to stimulation of inhibitory or excitatory interneurons. Peterson and Calabrese (1982) reported that the neural network generating the rhythm of the heart beat in the leech could be synaptically driven by rhythmic electrical stimulation of an antecedent neuron, at rates different from its free-run frequency. They noted stable locking of the cardiac neural oscillator rhythm to the driving frequency

within well defined limits (10% above and 40% below the free-run frequency). Stein (1976) has shown a similar phenomenon for neural oscillators which govern limb movement in crayfish, while v. Holst (1939) reported similar findings for central locomotor pattern generators in the spinal cord of the dogfish. An example of this phenomenon in vertebrates is provided by the effects of vagus nerve stimulation on the period of the cardiac sinus rhythm. Over a restricted range of frequencies, both above and below the intrinsic sinus node frequency, the sinus rhythm can be entrained to the period of the vagal stimulus, with a unique phase relation of the stimulus to the cardiac cycle at each frequency (Levy et al., 1972; Slenter et al., 1984). A more detailed analysis of the behaviour of cardiac pacemaker cells entrained by rhythmic intracellular current pulses (Guevara et al., 1981) or iontophoretic pulses of acetylcholine (Michaels et al., 1984) has been reported, again with very similar results to those described above. Jalife (1984) developed an in vitro cardiac preparation which is instructive in defining the essential elements involved in the behaviour of coupled oscillators. His data shows that the range of frequencies over which two independent pacemaker centers in the rabbit sino-atrial node can be stably entrained is a function of the strength of the coupling between the two centers. The coupling strength was manipulated by a variable shunt resistance connecting the two autonomous pacemaker populations. With infinitely high shunt resistance (zero coupling) each pacemaker had its intrinsic

frequency and was not affected by changes in the frequency of the other. With intermediate values of shunt resistance, mutual 1:1 entrainment occurred which was limited to a range of frequencies bordering the intrinsic frequency of the pacemakers and was characterized by a unique phase- relation at each frequency. At frequencies beyond those characterizing the 1:1 range, mutual entrainment involved integer ratios. With zero shunt resistance, i.e. with infinitely tight coupling, the two pacemaker centers assumed an identical frequency and behaved as a single pacemaker. In vertebrates, the brainstem neural network generating the rhythmic central respiratory drive for motoneurons of the respiratory muscles (which will be referred to as the respiratory oscillator) can be entrained by rhythmic sensory input, presumably from pulmonary stretch receptors, associated with lung inflation produced by a mechanical ventilator. Thus, the situation is analogous to that of two coupled oscillators, i.e. there is a rhythmic synaptic input acting on an intrinsically rhythmic neural network. A number of investigators (Vibert et al., 1981; Pham Dihn et al., 1983; Petrillo & Glass, 1984) have described the entrainment behaviour of the respiratory oscillator to the mechanical ventilator. One of the most detailed accounts of this behaviour is by Petrillo et al (1983). Their data show that it is possible to entrain the frequency of the respiratory oscillator to that of the mechanical ventilator such that it may be speeded up or slowed down with respect to its free-run frequency. The 1:1

entrainment is limited to a narrow range of frequencies and within this range each frequency is obtained by the lung inflation occurring at a specific phase of the respiratory cycle. The described dynamic characteristics of coupled biological oscillators are observed in experimental situations, with the exception of the data by Jalife (1984), in which entrainment is unidirectional. However, two or more oscillators may be mutually entrained such that their phase-relation is the result of the interplay of their coupling signals. The dynamic characteristics of unidirectionally entrained oscillators also apply to mutually entrained oscillators (Buno & Fuentes, 1984; Jalife, 1984; Pearce & Friesen, 1985).

5. IS THE INSPIRATION-RELATED SYMPATHETIC DISCHARGE GENERATED BY AN INDEPENDENT SYMPATHETIC OSCILLATOR?

We have examined the periodic burst of SPN firing which previous work has defined as inspiration-related on the basis of its timing in the respiratory cycle and of similarities of properties with the phrenic nerve burst (Preiss et al., 1975; Gerber & Polosa, 1978, 1979). The purpose of the study was to see whether the relation of this component of sympathetic discharge to the phrenic nerve burst would show the properties expected of a system of coupled oscillators. A detailed description of these experiments is presented elsewhere (Bachoo & Polosa, in press). A brief summary of the relevant experiments is presented here.

i) Phase-Response Curve to Superior Laryngeal Nerve Stimulation

One way of characterizing the properties of an oscillator is by describing the phase-dependent effects (phase response curve described above) produced by brief stimulation of an input. Stimulation of low threshold afferents in the superior laryngeal nerve (SLN) was used to perturb the cycle of the respiratory oscillator. Its output, measured as the phrenic nerve burst, could be phase-advanced or delayed, with respect to its free-run period, by a stimulus train applied to the SLN (Bachoo & Polosa, 1985). The phase response curve was identical for the rhythm of both phrenic and sympathetic burst, suggesting that the stimulus was acting on a common oscillator mechanism. If sympathetic bursting activity was independently rhythmic, as the respiratory oscillator cycle was phase-advanced or phase-delayed by the stimulus, the respiratory oscillator input would occur at a different phase of the hypothetical sympathetic oscillator cycle and therefore, according to the behaviour of coupled oscillators, the two waveforms would have to change their time relation to each other. The assumption here is that the SLN stimulus perturbs the respiratory oscillator and that the latter then perturbs the sympathetic oscillator. Thus, the SLN stimulus would not be expected to have the same effect on the respiratory and sympathetic oscillator cycles.

b) Entrainment of the Phrenic and Inspiration Related Sympathetic Burst to the Mechanical Ventilator

We modified the respiratory frequency, in cats with intact cervical vagus, by changing the ventilation pump frequency. The prediction here was that if the sympathetic, inspiration-related, burst was generated by an independent sympathetic oscillator which was entrained to the respiratory oscillator, the relation between phrenic and inspiration-related sympathetic burst would be expected to display the behaviour observed in coupled oscillator systems when the frequency of the driver oscillator is changed. Namely, a limited 1:1 range and, within this range, a different phase-relation at each frequency. The results show instead an unlimited 1:1 range, i.e. whatever frequency the respiratory oscillator was forced to adopt by the mechanical ventilator, so did the sympathetic burst. Over the entire frequency range studied (10 to 40 cycles per min) the phase relation between the phrenic and inspiration-related sympathetic burst remained essentially constant. This constrasts with the phase relation of the phrenic burst to the inflation cycle, which was different at each frequency, as expected of an oscillator driven by a rhythmic input (Petrillo et al., 1983).

c) Hypocapnic-Hyperthermic Polypnea and the Inspiration-Related Sympathetic Burst

In vagotomized cats, raising the core temperature from 37 to 42°C by means of radiant heat increased the frequency of the phrenic and inspiration-related sympathetic burst from 15 to 36 bursts/min. Subsequent hyperventilation (lowering end-tidal pCO_2 to 10 mm Hg from a control of 35 mmHg) produced a very marked increase in the frequency of both bursts, which reached a maximum value of approximately 300 bursts/min (Cohen, 1964; Monteau et al., 1974). Over this entire range of frequencies, from 15 to 300 cycles/min, phrenic and sympathetic bursts maintained a 1:1 relation and a phase-relation which was essentially constant.

The fact that the phrenic and inspiration-related sympathetic burst maintained a one-to-one relation with essentially constant delay at all frequencies tested is inconsistent with the behaviour expected of coupled neural oscillators. Therefore, the equality of period of phrenic nerve burst and inspiration-related sympathetic burst is unlikely to result from the activity of an autonomous sympathetic oscillator coupled to the brainstem respiratory oscillator. Instead, these results are compatible with the hypothesis of a common oscillator which drives both the phrenic and the sympathetic discharges.

6. THE CARDIAC MODULATION OF SYMPATHETIC DISCHARGES

Modulation of sympathetic discharge at the frequency of the heart beat has been described by a number of investigators (Adrian et al., 1932; Downing & Siegel, 1963; Cohen & Gootman, 1970). The first detailed analysis was presented by Green and Heffron (1968). The cardiac modulation of sypathetic discharge was considered to be the consequence of the sympatho-inhibitory baroreceptor reflex: the arterial pressure increment associated with systolic ejection produces an increment in the level of sympathetic inhibition above that which exists at diastolic levels of arterial pressure. Thus, the records show bursts of spikes occurring between the phases of depression produced by consecutive heart beats (Green & Heffron, 1968). The bursting, however, persists, at a mean frequency similar to that of the heart beat, in barodenervated cats (Taylor & Gebber, 1975; Gebber 1976). In low-pass filtered records these bursts appear as a series of irregular slow waves. Based on these observations on barodenervated animals, the hypothesis proposed by Gebber (1976) is that the slow wave activity is the result of the activity of an independent sympathetic oscillator which, in the presence of arterial baroreceptor input, is entrained to the rhythm of the heart beat. The published records (Gebber, 1976) show that this slow wave activity is characterized, in barodenervated cats, by waves of variable shape occurring at irregular intervals. Statistical analysis of this aperiodic burst discharge reveals a broad power spectrum (2-6 Hz) without preferred frequencies and a

flat autocorrelogram. We are unaware of any previous description of a biological oscillator with such aperiodic characteristics. It is possible for an oscillator to show aperiodic (chaotic) behaviour in particular conditions associated with a forcing input (Guevara & Glass, 1982; Olsen & Degn, 1985) and this could be a possible explanation for lack of periodicity of the 2-6 Hz wave activity. However, the lack of periodicity of this signal, whether resulting from absence of intrinsic rhythmicity in the generator or from chaotic behaviour of an intrinsically rhythmic generator, precludes a search for properties of coupled oscillator systems because such an analysis presupposes a constant period of the signals as a starting condition. Published records of this activity in real time (Gebber, 1976) permit the additional observation that in the presence of arterial baroreceptor input the phase-relation between peaks of individual waves and the cardiac cycle, at constant heart rate, is variable. Averaged records are not useful in this context because they give no information on the variability of the signal and hence of the phase-relation. Since entrainment of oscillators (sc above) requires fixed phase-relation, this observation suggests that there is no entrainment of the 2-6 Hz waves to the cardiac cycle when the baroreceptor input is intact. Thus, the lack of evidence that the 2-6 Hz wave activity is rhythmic in barodenervated animals and the lack of evidence that the 2-6 Hz wave activity is locked to the cardiac cycle when baroreceptor input is intact precludes the possibility of applying the coupled

oscillator hypothesis to the cardiac rhythm of sympathetic discharge. As a corollary, described phenomenology concerning the interaction of baroreceptor inhibition with the 2-6 Hz wave activity is not interpretable in terms of the properties of coupled oscillators.

CONCLUSION

This paper has attempted to identify the criteria that can be used to define a biological system as a system of two coupled oscillators. The approach is empirical, using criteria based on common features which underly the behaviour of known cases of coupled biological oscillators. When the respiratory and cardiac periodicities of sympathetic discharge are tested with such criteria, neither periodicity shows properties consistent with the hypothesis that it is generated by a system of coupled oscillators.

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REFERENCES

Adrian ED, Bronk DW, Phillips G (1932). Discharges in mammalian sympathetic nerves. J Physiol 74: 115-133.

Ayers AL, Selverston AI (1979). Monosynaptic entrainment of an endogenous pacemaker network: a cellular mechanism for von Holst's magnet effect. J Comp Physiol 129: 5-17.

Bachoo M, Polosa C (1985). Properties of a sympatho-inhibitory and vasodilator reflex evoked by superior laryngeal nerve afferents in the cat. J Physiol 364: 183-198.

Bachoo M, Polosa C. Properties of the inspiration-related activity of sympathetic preganglionic neurones of the cervical trunk in the cat. J Physiol. In press.

Barman SM, Gebber GL (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. Am J Physiol 231: 1601-1607.

Buno W, Fuentes J (1984). Coupled oscillators in an isolated pacemaker neuron? Brain Res 303: 101-107.

Cohen MI (1964). Respiratory periodicity in the paralyzed vagotomized cat: hypocapnic polypnea. Am J Physiol 206: 847-864.

Cohen MI, Gootman PM (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. Am J Physiol 218: 1092-1101.

Downing SE, Siegel H (1963). Baroreceptor and chemoreceptor influences on sympathetic discharge to the heart. Am J

Physiol 204: 471-479.

Enright JT (1965). Synchronization and ranges of entrainment. In Aschoff J (ed): "Circadian Clocks", Amsterdam: North-Holland, pp. 112-124.

Gebber GL (1976). Basis for phase relations between baroreceptor and sympathetic nerve discharge. Am J Physiol 230: 263-270.

Gebber GL (1980). Central oscillators responsible for sympathetic nerve discharge. Am J Physiol 239: H143-H155.

Gerber U, Polosa C (1978). Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neuron firing. Can J Physiol Pharmacol 56: 191-198.

Gerber U, Polosa C (1979). Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neuron firing. Can J Physiol Pharmacol 57: 1073-1081.

Glass L, Mackey M (1979). A simple model for phase-locking of biological oscillators. J Math Biol. 7: 339-352.

Green JH, Heffron PF (1968). Studies upon the relationship between baroreceptor and sympathetic activity. Quart J Exp Physiol 53: 23-32.

Guevara M, Glass L, Shrier A (1981). Phase locking, period-doubling bifurcations and irregular dynamics in periodically stimulated cardiac cells. Science 214: 1350-1353.

Guevara M, Glass L (1982). Phase locking, period doubling bifurcations and chaos in a mathematical model of a periodically driven oscillator: A theory for entrainment of

biological oscillators and the generation of cardiac dysrhythmias. J Math Biol 14: 1-23.

Holst E. von (1939). Die relative koordination als phänomen und als methode zentral-nervose funktionanalyse. Erg Physiol Biol Chem u exp Pharmakol 42: 228-306.

Jalife J (1984). Mutual entrainment and electrical coupling as mechanisms for synchronous firing of rabbit sino-atrial pace-maker cells. J Physiol 356: 221-243.

Koepchen HP (1962). "Die Blutdruckrhythmik". Darmstadt, D. Steinkopff Verlag.

Levy MN, Iano T, Zieske H (1972). Effects of repetitive bursts of vagal stimulation on heart rate. Circ Res 30: 186-195.

Michaels DC, Matyas EP, Jalife J (1984). A mathematical model of the effects of acetylcholine pulses on sinoatrial pacemaker activity. Circ Res 55: 89-101. Monteau R, Hilaire G, Ouedraogo C (1974). Contribution à l'étude de la fonction ventilatoire au cours de la polypnée thermique ou hypocapnique. J de Physiol 68: 97-120.

Moore-Ede MC, Sulzman FM, Fuller CA (1982). "The clocks that time us: Physiology of the circadian timing system". Cambridge Ma Harvard U Press.

Olsen LF, Degn M (1985). Chaos in biological systems. Quart Rev Biophys 18: 165-225.

Pavlidis T (1973). "Biological oscillators. Their mathematical analysis". New York Academic.

Pearce RA, Friesen WO (1985). Intersegmental coordination of the leech swimming rhythm. I. Role of cycle period gradient and coupling strength. J Neurophysiol 54: 1444-1459.

Perkel DM, Schulman J, Bullock TH, Moore GP, Segundo JP (1964). Pacemaker neurons: effects of regularly spaced synaptic input. Science 145: 61-63.

Petrillo GA, Glass L, Trippenbach T (1983). Phase-locking of the respiratory rhythm in cats to a mechanical ventilator. Can J Physiol Pharmacol 61: 599-607.

Petrillo GA, Glass L (1984). A theory for phase locking of respiration in cats to a mechanical ventilator. Am J Physiol 246: R311-R320.

Pham Dinh T, Demongest J, Baconnier P, Benchetrit G (1983). Simulation of a biological oscillator: the respiratory system. J Theor Biol 103: 113-132.

Pinsker HM (1977a). Aplysia bursting neurons as endogenous oscillators. II. Synchronization and entrainment by pulsed inhibitory synaptic input. J Neurophysiol 40: 544-556.

Pinsker HM (1977b). Synaptic modulation of endogenous oscillators. Fed Proc 36: 2045-2049.

Pinsker HM, Ayers J (1983). Neuronal oscillators. In Rosenberg RN (ed.): "The Clinical Neurosciences", New York: Churchill Livingstone, vol. 5 pp 203-266.

Preiss G, Kirchner F, Polosa C (1975). Patterning of preganglionic neuron firing by the central respiratory drive. Brain Res 87:

363-374.

Segundo JP, Kohn AF (1981). A model of excitatory synaptic interactions between pacemakers. Its reality, its generality and the principles involved. Biol Cybern 40: 113-126.

Selverston AI, Moulins M (1985). Oscillatory neural networks. Ann Rev Physiol 47: 29-48.

Slenter VAJ, Salata JJ, Jalife J (1984). Vagal control of pacemaker periodicity and intranodal conduction in the rabbit sinoatrial node. Circ Res 54: 436-446.

Stein PSG (1976). Mechanisms of interlimb phase control. In Herman R, Grillner S, Stein PSG, Stuart DG (eds): "Neural Control of Locomotion", New York, Plenum pp 465-498.

Stein PSG (1977). Application of the mathematics of coupled oscillator systems to the analysis of the neural control of locomotion. Fed Proc 36: 2056-2059.

Taylor DG, Gebber GL (1975). Baroreceptor mechanisms controlling sympathetic nervous rhythms of central origin. Am J Physiol 228: 1002-1013.

Turek FW (1985). Circadian neural rhythms in mammals. Ann Rev Physiol 47: 49-64.

Vibert JF, Caille D, Segundo JP (1981). Respiratory oscillator entrained by periodic vagal afferents: An experimental test of a model. Biol Cybern 41: 119-130.

Winfree AT (1980). "The geometry of biological time". New York, Springer Verlag.

CHAPTER IV

PROPERTIES OF A SYMPATHO-INHIBITORY AND VASODILATOR REFLEX EVOKED BY SUPERIOR LARYNGEAL NERVE AFFERENTS IN THE CAT

1. INTRODUCTION

The background discharge of sympathetic nerves in the cat shows a slow rhythmic modulation related to the respiratory cycle (Adrian, Bronk & Phillips, 1932). A similar observation has been made in man (Wallin, 1981). A conspicuous feature of this modulation is an increase in firing rate in synchrony with inspiration. This inspiratory component of sympathetic activity persists under experimental conditions in which sensory afferents providing reflex routes for coupling respiration to sympathetic activity have been eliminated (Tang, Maire & Amassian, 1957) and can therefore be assumed to originate from within the central nervous system. Experimental procedures, such as changing arterial PCO_2 and activation of pulmonary stretch receptor or superior laryngeal nerve afferents, which modify the activity of brainstem inspiratory neurones in a predictable way, cause parallel modifications of the inspiration-synchronous firing of sympathetic preganglionic neurones, suggesting that this pattern of firing is the result of excitatory synaptic input from brainstem inspiratory neurones (Preiss & Polosa, 1977; Gerber & Polosa, 1978 & 1979). This input appears to be relayed to the sympathetic preganglionic neurones through a descending pathway which is independent of the bulbo-spinal projection to phrenic motoneurones (Kubin, Trzebski & Lipski, 1984) and runs in the dorsal half of the spinal cord (Connelly & Wurster, 1982).

Whereas it has been established that the inspiration-synchronous fraction of total sympathetic nerve activity is large (Cohen & Gootman, 1970; Preiss, Kirchner & Polosa, 1975; Barman & Gebber, 1976) the extent of the contribution of this phasic component of sympathetic activity to the maintenance of vasoconstrictor tone is unknown. This information is important for a description of cardiovascular regulation in terms of the extent of control exerted by various synaptic inputs on the level of sympathetic preganglionic neuronal activity.

In a previous study (Gerber & Polosa, 1979) it was shown that the burst firing in inspiration of sympathetic preganglionic units was suppressed by electrical stimulation of an afferent fibre group in the superior laryngeal nerve which causes inspiratory suppression (Hillenbrand & Boyd, 1936; Larrabee & Hodes, 1948). That observation is the starting point for the present study, which describes changes in vascular resistance in the hindlimb of cats, perfused at constant flow, during stimulation of the same inspiration-inhibitory afferents. These afferents evoke a vasodilatation, the properties of which suggest that it is caused by suppression of the inspiration-synchronous activity of vasoconstrictor neurones. The data indicate that this component of sympathetic discharge makes a significant contribution to the neurogenic vasoconstrictor tone of the hindlimb.

2. METHODS

The experiments have been conducted on 20 adult cats of either sex (2.5 to 4.5 kg body weight) under sodium pentobarbital anaesthesia (35 mg/kg i.p. followed by i.v. supplements of 9 mg/kg every 3 hours). With this dosage the withdrawal reflex on pinching forepaw or hindpaw was suppressed for the duration of the experiment. An artery and vein were cannulated for monitoring systemic arterial pressure and for injection of drugs or measurement of central venous pressure, respectively. All cats were paralyzed with pancuronium bromide (initial dose 200 µg/kg followed by maintenance doses of 100 µg/kg which were given every 2-3 hours, when the effect of the previous dose had worn off, as evidenced by the appearance of spontaneous breathing movements, and after testing for adequacy of the level of anaesthesia). Artificial ventilation was adjusted to obtain an end-tidal PCO_2 of between 25 and 35 mmHg. Hypocapnia (end-tidal PCO_2 of 10-15 mmHg) was produced in some animals by increasing the frequency of the respiratory pump from the control value of 15 to 25 cycles/min. Rectal temperature was monitored and maintained at 37°C by means of a feedback controlled infra-red lamp. A cardiotachometer, triggered by the blood pressure signal, was used to measure heart rate in some of the cats. Tidal PCO_2 was continuously monitored with an infra-red gas analyzer.

The electrical activity of the phrenic nerve and cervical

sympathetic trunk was recorded monophasically with silver hook electrodes, amplified (1/2 amplitude bandpass 30-3000 Hz), displayed on a storage oscilloscope and stored on magnetic tape. The amplified phrenic and cervical trunk signals were also low-pass filtered (RC circuit with 100 ms time constant) after half-wave rectification and displayed on a pen recorder together with other variables. The phase of phrenic nerve activity from onset to the beginning of rapid decline is defined as inspiration, while the phase of phrenic nerve silence is defined as expiration. Sympathetic discharge was characterized, in control conditions, by two components: a burst of action potentials, synchronous with the phrenic nerve burst, and a relatively steady level of discharge during expiration, on which the burst was superimposed. The former component will be referred to as inspiration-synchronous sympathetic discharge, the latter as tonic discharge. The level of zero activity in the cervical sympathetic trunk was determined by applying procaine to the nerve proximal to the recording electrode, or by crushing it. The cut central end of the internal branch of the superior laryngeal nerve was desheathed and mounted on a pair of silver hook electrodes for stimulation. Monophasic square wave pulses (0.2 ms duration and 50 Hz) were delivered to the nerve with a stimulator and isolation unit. In those experiments in which trains of stimuli were delivered at selected times during the respiratory cycle, a square wave pulse was obtained at the onset of the phrenic nerve burst and used to trigger the stimulator with

variable delays. All nerves were kept under mineral oil in a pool made with the skin flaps.

In all animals the vagus and aortic nerves were sectioned in the neck. In 13 animals the carotid sinus nerve was also cut bilaterally. The purpose of these denervations was to eliminate reflex effects on sympathetic activity evoked by pulmonary stretch receptor and arterial baroreceptor afferents. Baroreceptor denervation was necessary because, as described under 'Results', systemic arterial pressure changes occurred during superior laryngeal nerve stimulation. Denervation also eliminated the effects of peripheral chemo-receptors on brainstem respiratory neurones and caused a decreased output of these neurones (Miserocchi, 1976). In these cats, peak amplitude of the integrated phrenic nerve burst decreased after sino-aortic denervation as did the amplitude of the inspiration-synchronous sympathetic discharge. The peak amplitude of both signals was brought back to control by adding CO_2 to the inspired gas mixture. Thus, experiments in these peripherally chemoreceptor-denervated cats were performed at end-tidal PCO_2 values of 35 to 45 mmHg. In this group of moderately hypercapnic animals results were qualitatively similar to those obtained in a normocapnic group (n = 5) with intact carotid sinus nerves (end-tidal PCO_2 25-35 mmHg).

The effects of superior laryngeal nerve stimulation on neurogenic vasoconstrictor tone was studied by measuring perfusion pressure during constant flow perfusion of the innervated hindlimb.

One external iliac artery was exposed by a laparotomy, isolated and cannulated. The abdominal aorta was ligated just rostral to the bifurcation. With a precalibrated roller pump, blood was pumped at constant flow (9 to 13 ml/min) from the common carotid artery of the same cat (autoperfusion, 13 experiments) or of a donor cat (cross-perfusion, 5 experiments) into the cannulated external iliac artery. Heparin (500 units i.v.) was given before onset of perfusion and every 90 min thereafter. At the beginning of each experiment the flow rate was adjusted so as to obtain a hindlimb perfusion pressure equal to systemic arterial pressure (of the recipient, in the case of cross-perfusion). Perfusion pressure of the hindlimb was measured less than 5 cm anterior to the point of cannulation of the external iliac artery. Central venous pressure was monitored through a catheter advanced approximately 20 cm from the femoral vein into the thoracic inferior vena cava. An effective degree of isolation of the arterial supply to the hindlimb was indicated by the observation that when the pump was stopped for approximately 30 s hindlimb perfusion pressure fell from 187 ± 40 to 28 ± 6 mmHg (mean ± SD) under control conditions and to a level of less than 10 mmHg after ganglionic block with hexamethonium (10 mg/kg i.v.). Absence of any significant collateral circulation was also shown by cross-perfusion experiments ($n = 3$) in which the systemic arterial pressure of the recipient was raised by i.v. administration of noradrenaline. No

changes in hindlimb perfusion pressure were observed for at least 20 s following increases of the recipient's systemic arterial pressure by as much as 125 mmHg. In the cross-perfusion experiments blood was returned from a common carotid artery of the recipient to a jugular vein of the donor at the same rate as it was withdrawn from the donor to perfuse the limb. The absence of shifts in blood volume between the two cats was demonstrated by the lack of weight changes of the donor cat, the weight of which was continuously monitored with an accuracy of 10 g. With constant-flow perfusion, vasoconstriction and vasodilatation were indicated by an increase or a decrease in hindlimb perfusion pressure, since central venous pressure did not change during these experiments. Hexamethonium (10 mg/kg i.v.) caused hindlimb perfusion pressure to fall by 51 ± 15%, from 187 ± 40 to 92 ± 13 mmHg (n = 18, P .001): this fall in perfusion pressure was taken as a measure of the neurogenic component of vasoconstrictor tone.

The following drugs were used: hexamethonium bromide (Sigma), phentolamine mesylate (CIBA), propranolol (Ayerst), atropine sulphate (Squibb), noradrenaline (Winthrop).

Numerical results are given in the text as mean ± standard deviation. A paired t-test was used to evaluate the significance of differences between means.

3. RESULTS

a) Depressant Effect of Superior Laryngeal Nerve Stimulation (SLN) on Sympathetic activity

Repetitive stimulation of the superior laryngeal nerve (50 Hz, 0.2 ms pulses) at an intensity just sufficient to abolish phrenic nerve bursts (typically 100-200 mV) also abolished the inspiration-synchronous wave of sympathetic activity (Fig. 1a). This component of sympathetic discharge was suppressed for as long as the phrenic nerve burst was inhibited, usually for the whole duration of stimulation. In cases in which phrenic nerve activity resumed in spite of the continuing stimulation, the inspiration-synchronous sympathetic discharge always resembled the phrenic nerve response (e.g. Fig. 6a). At these low stimulus intensities depression of this phasic component of sympathetic discharge was the only observable effect of superior laryngeal nerve stimulation on sympathetic nerve activity. Stimulation at higher intensities also caused an increase in the level of the tonic component of the sympathetic discharge (just noticeable in Fig. 1b and obvious in Fig. 1c). The properties of this excitatory effect of superior laryngeal nerve stimulation are described in a later section.

When cats were made hypocapnic by hyperventilation with air until both phrenic nerve burst activity and inspiration-synchronous sympathetic discharge had disappeared, superior laryngeal nerve stimulation of intensity just sufficient to produce inspiration-suppression either had no effect on sympathetic activity (Fig. 2) or caused weak excitation. Depression of sympathetic activity was

LEGENDS

Fig. 1 Effects of superior laryngeal nerve stimulation on phrenic and sympathetic neurone discharge. From above: integrated phrenic nerve activity (Phr), integrated cervical sympathetic trunk activity (CST), stimulus signal (SLN). Stimulation was repetitive at 50 Hz, 0.2 ms and the intensity indicated. Threshold intensity for phrenic nerve activity suppression was 0.1 V. In this, and all subsequent figures, an upward deflection indicates increased phrenic or cervical sympathetic trunk discharge. Notice disappearance of inspiration-synchronous component of sympathetic activity during superior laryngeal nerve stimulation and increase in tonic sympathetic firing at the highest stimulus intensity (panel c).

FIG. 1

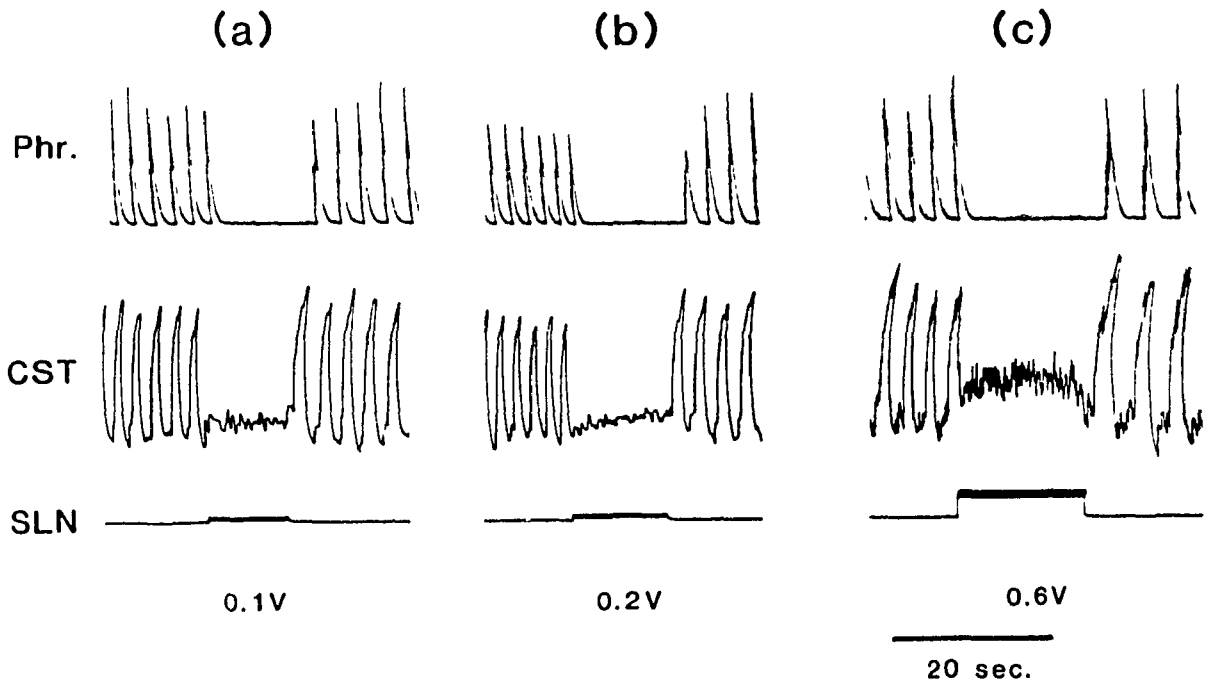
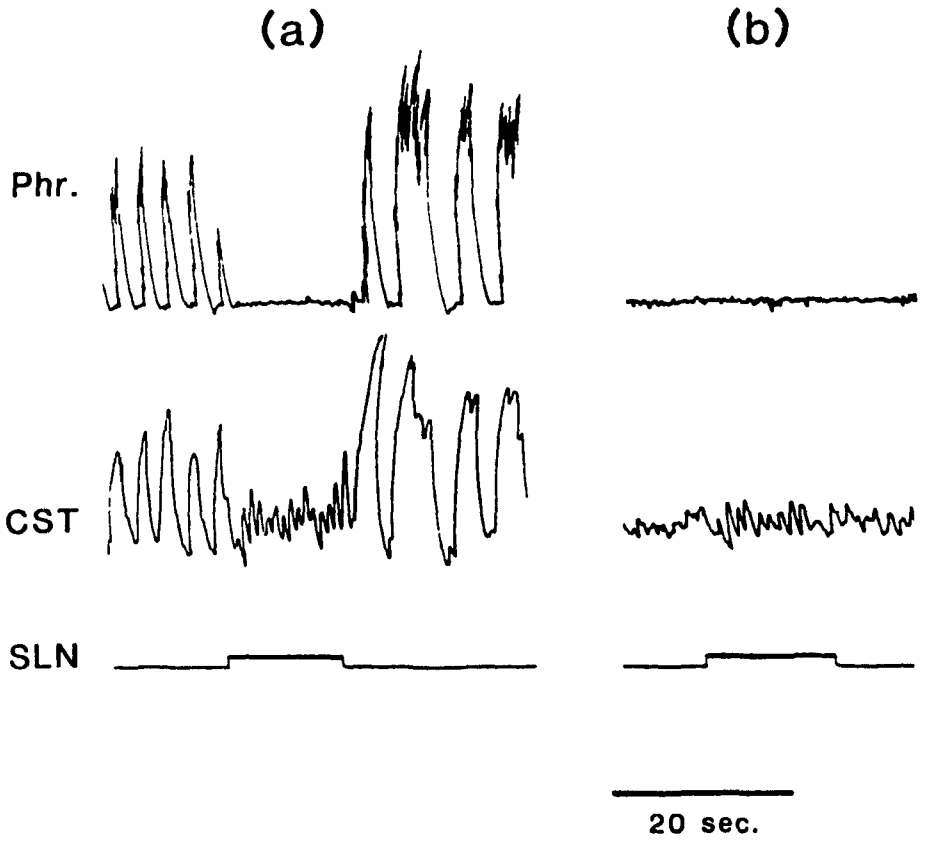


Fig. 2 Lack of effect of superior laryngeal nerve stimulation, at threshold for phrenic nerve activity suppression, on sympathetic activity in hypocapnia. From above: integrated phrenic nerve activity (Phr), integrated sympathetic cervical trunk activity (CST), stimulus. Under control conditions (a) (end-tidal PCO_2 42 mmHg) superior laryngeal nerve stimulation (0.2 mV, 50 Hz, 0.2 ms) for the duration shown causes suppression of phrenic nerve activity and of inspiration-synchronous sympathetic discharge. After hyperventilation in air (b) (end-tidal PCO_2 12 mmHg) phrenic nerve activity and inspiration-synchronous sympathetic discharge are absent: superior laryngeal nerve stimulation with the same parameters as in (a) is without effect.

FIG. 2

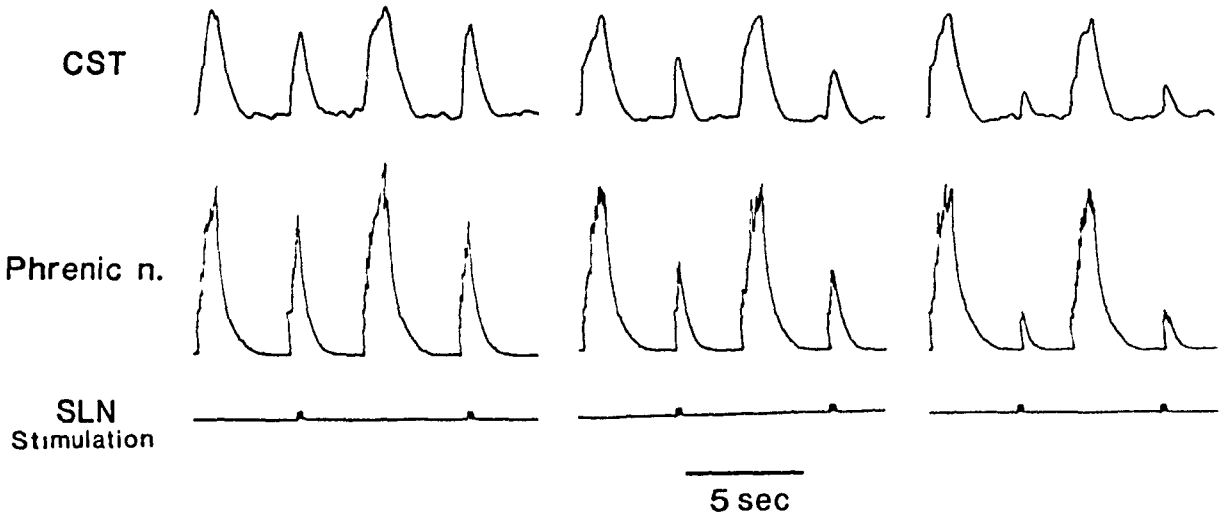


never observed during superior laryngeal nerve stimulation when the inspiration- synchronous sympathetic discharge was absent. Return to control conditions restored the inspiratory discharge of both nerves and of the inhibitory effectiveness, on both, of superior laryngeal nerve stimulation.

The effect of superior laryngeal nerve stimulation on the inspiration-synchronous sympathetic discharge was closely similar to the effect stimulation had on phrenic nerve activity. This was shown by experiments in which trains of stimuli (50 Hz, 0.2 ms pulses), which were of short duration (100-200 ms) with respect to the duration of a respiratory cycle, were delivered at various times during the cycle. By appropriate timing it was possible to grade the inspiration-suppressing action of the stimulus, i.e., it was possible to eliminate a variable fraction of the phrenic nerve burst: the concomitant effect on the inspiration-synchronous sympathetic discharge was always analogous to that on the phrenic burst. A train given at any time during expiration delayed the onset, without modifying the amplitude, of the following phrenic nerve burst and of the concurrent inspiration-synchronous sympathetic discharge. The same stimulus during the last 10-20% of inspiration anticipated the onset of the subsequent inspiration, without modifying the amplitude of the ongoing, or of the following, phrenic burst and concurrent inspiration-synchronous sympathetic discharge appreciably. On the other hand, stimulation during the initial 80% of inspiration eliminated the remainder of

Fig. 3 The effect of superior laryngeal nerve stimulation on inspiration- synchronous sympathetic discharge is closely related to the effect on phrenic nerve activity. Two hundred ms trains of stimuli (50 Hz, 0.2 ms, 0.18 V) delivered every second phrenic burst at 50 % (a), 35 % (b) and 21 % (c) of inspiration.

FIG. 3



the phrenic nerve burst and concurrent inspiration-synchronous sympathetic discharge. The fraction of the discharge which was eliminated depended on the timing of the stimulus. This graded inhibition of phrenic burst duration and amplitude by superior laryngeal nerve stimulation, and the associated effects on the inspiration-synchronous sympathetic discharge, are shown in Fig. 3.

With stimulus trains of 100-200 ms duration, given during the first 50% of inspiration, the inspiration-suppressing effect first appeared at 0.10 · 0.04 V (n = 12) as a small transient depression of the phrenic burst and became maximal at 0.16 ± 0.05 V, at which intensity the phrenic burst was terminated.

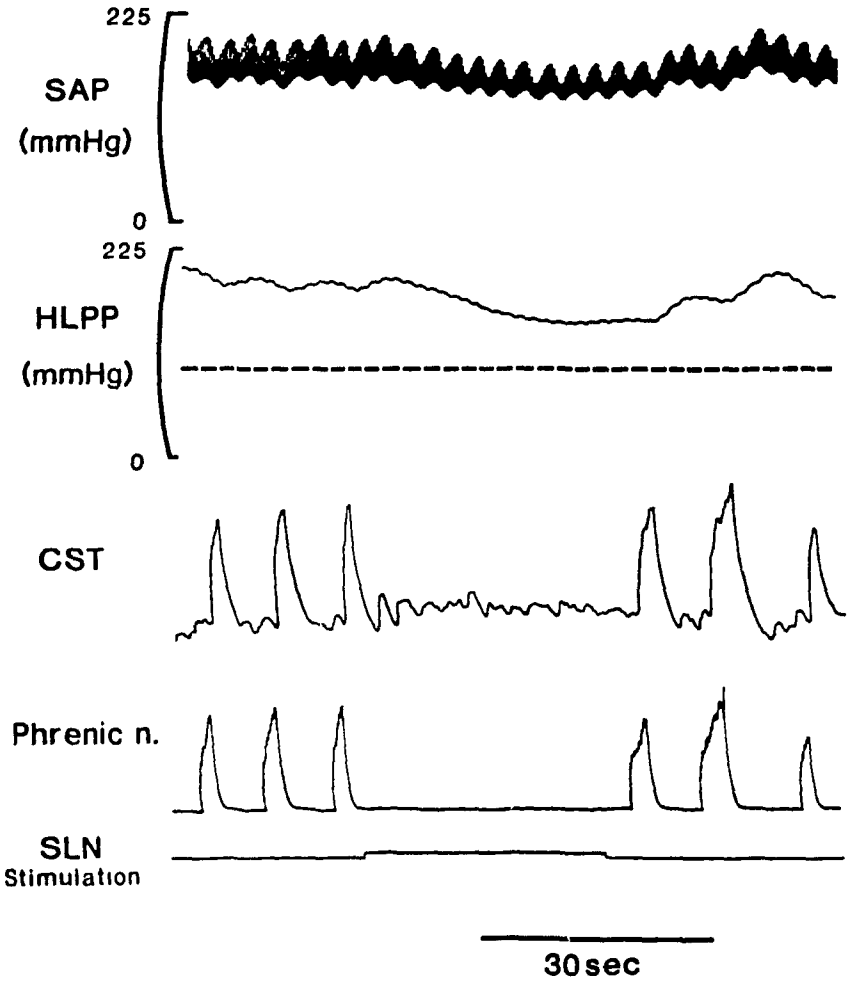
b) Vasodilator Effect of Superior Laryngeal Nerve Stimulation

Under control conditions, repetitive stimulation (50 Hz, 0.2 ms pulses) for 20-30 s at maximal intensity for inspiration-suppression caused a decrease in hindlimb perfusion pressure (Fig. 4), in addition to suppressing the inspiration-synchronous sympathetic discharge. In 18 experiments in sino-aortic denervated cats, the peak decrease in perfusion pressure was 23.0 ± 10.3 mmHg from a control value of 187.0 ± 40.0 mmHg ($P < .001$). A vasodilator response was also shown by 5 cats with intact carotid sinus nerves (e.g. Fig. 6a).

The vasodilatation was abolished by the ganglion-blocker hexamethonium (18 cats, 10 mg/kg i.v.) or by the alpha-adrenergic

Fig. 4 Repetitive superior laryngeal nerve stimulation (50 Hz, 0.2 ms) at intensity just sufficient to suppress phrenic nerve activity (0.3 V) causes suppression of inspiration-synchronous sympathetic discharge, decrease in hindlimb vascular resistance and decrease of systemic arterial pressure. From above: systemic arterial pressure, hindlimb perfusion pressure (constant flow), cervical sympathetic and phrenic neurograms, stimulus signal. Dashed line shows level to which hindlimb perfusion pressure fell when hexamethonium bromide (10 mg/kg) was given i.v. later in the experiment.

FIG. 4


225
SAP
(mmHg)
0
225
HLPP
(mmHg)
0
CST
Phrenic n.
SLN
Stimulation
30sec

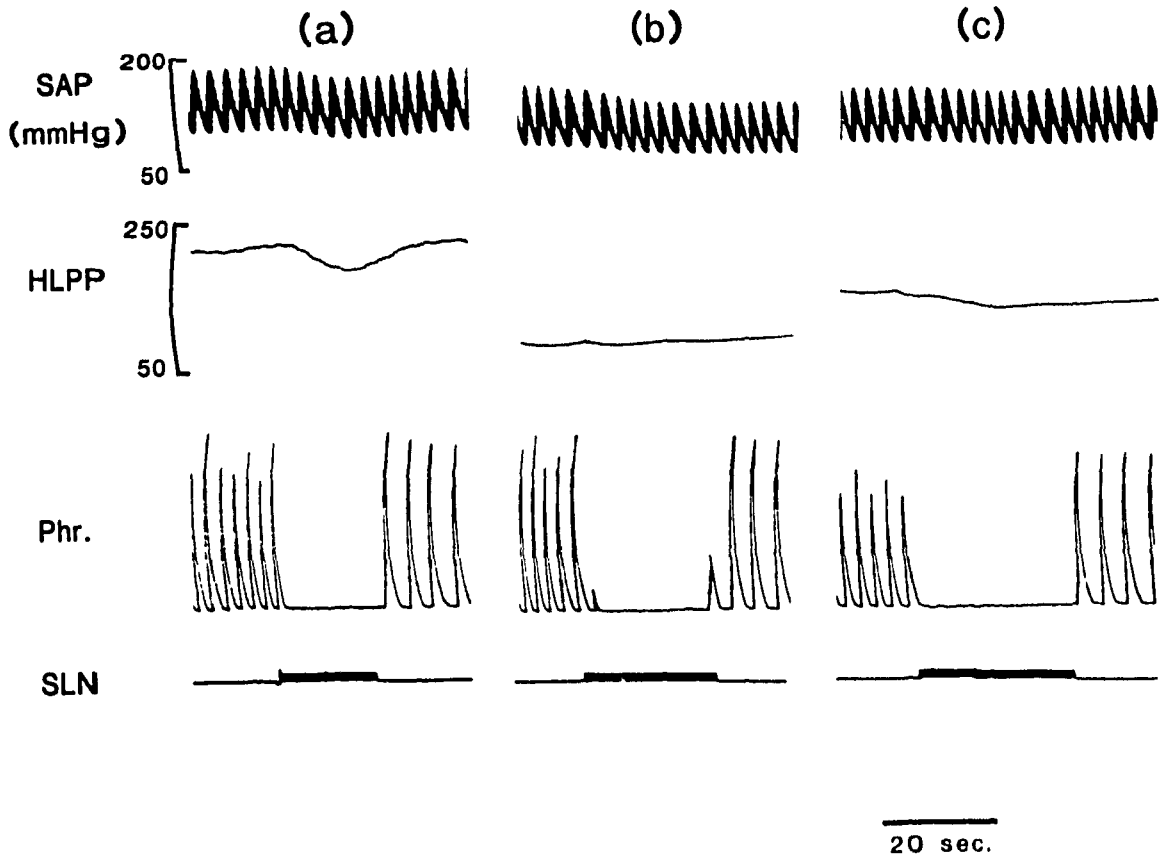
receptor antagonist phentolamine mesylate (2 cats, 450 μg into the perfusion line, Fig. 5). The vasodilatation was not affected by atropine sulphate (2 cats, 0.5 mg/kg i.v.) or by propranolol (2 cats, 100 μg/kg i.v.). The fall in perfusion pressure, during repetitive laryngeal nerve stimulation, was 24.2 ± 9.8% of that caused by hexamethonium (n = 18). On the assumption (see Methods) that the fall in perfusion pressure caused by hexamethonium was due to loss of the neurogenic component of vasoconstrictor tone, this value represents the fraction of neurogenic tone suppressed by superior laryngeal nerve stimulation.

In many cases perfusion pressure remained steady at the lowest level to to which it had fallen during the whole period of repetitive superior laryngeal nerve stimulation (as in Fig. 4). In other cases, however, the initial fall in perfusion pressure was followed by gradual recovery towards control values, in spite of continuous stimulation (Fig. 5a).

An oscillation in perfusion pressure, at the same frequency as that of the phrenic nerve burst and inspiration-synchronous sympathetic discharge of the cervical trunk, but independent of the ventilation pump frequency, was evident in 9 of the sino-aortic denervated cats. This respiratory oscillation in perfusion pressure disappeared during the vasodilatation caused by superior laryngeal nerve stimulation (Fig. 4 and 8, middle and right panels) as well as at low end-tidal PCO_2 levels (10-15 mmHg) which were associated with disappearance of the phrenic nerve burst and of the

Fig. 5 Hindlimb vasodilatation caused by superior laryngeal nerve stimulation is abolished by phentolamine mesylate. From above: systemic arterial pressure, hindlimb perfusion pressure, (constant flow) phrenic neurogram, stimulus signal. (a) control. (b) one min after administration of phentolamine (450 μg) into perfusion line. (c) 5 min after administration of the drug.

FIG. 5



inspiration-synchronous sympathetic discharge.

Several observations suggest that the reflex vasodilatation is related to the reflex inhibition of the inspiration-synchronous sympathetic discharge, described in the preceding section.

First, the superior laryngeal nerve afferents mediating the vasodilator reflex and the suppression of the inspiration-synchronous sympathetic discharge exhibited similar excitability. With repetitive stimulation at 50 Hz, voltages which were subthreshold for suppression of the inspiration-synchronous sympathetic discharge caused no fall in perfusion pressure, while voltages which were maximal or supramaximal for the former were also respectively maximal or supramaximal for the latter.

Secondly, when the inspiration-synchronous sympathetic discharge (together with phrenic nerve activity) was eliminated by hyperventilation in air (end-tidal PCO_2 10-15 mmHg), stimulation of the inspiration-suppressing afferents caused no vasodilatation. Loss of the vasodilator reflex could not be attributed to a vasoconstrictor action of hypocapnia on the hindlimb vasculature because it occurred even when the PCO_2 of the arterial blood perfusing the hindlimb was maintained within the normocapnic range by the use of a normocapnic donor animal (5 experiments, Fig. 6). In these hypocapnic preparations the tonic component of the discharge of the cervical sympathetic trunk was not significantly different from that of the controls. Moreover, significant neurogenic vasoconstrictor tone was still present, as shown by a

Fig. 6 Loss of the vasodilatation evoked by superior laryngeal nerve stimulation during hypocapnia and persistence of the vasoconstriction Cross-perfusion of the hindlimb by donor animal (end-tidal PCO_2 38 mmHg). From above: systemic arterial pressure of recipient animal, hindlimb perfusion pressure (constant flow), systemic arterial pressure of donor animal, cervical sympathetic and phrenic neurograms of recipient animal, stimulus signal. (a) End-tidal PCO_2 of recipient animal 38 mmHg. Notice suppression of phrenic nerve activity and inspiration-synchronous sympathetic discharge, and fall in hindlimb perfusion pressure during repetitive superior laryngeal nerve stimulation (0.14 V, 50 Hz, .2 ms). (b) Recipient animal hypocapnic (end-tidal PCO_2 14 mmHg). Notice absence of phrenic nerve activity and inspiration-synchronous sympathetic discharge. No effect of superior laryngeal nerve stimulation on cervical sympathetic trunk activity or hindlimb perfusion pressure. (c) Recipient animal as in (b). Superior laryngeal nerve stimulus intensity raised to 0.6 V: notice increased firing in cervical sympathetic trunk and increased perfusion pressure.

FIG. 6

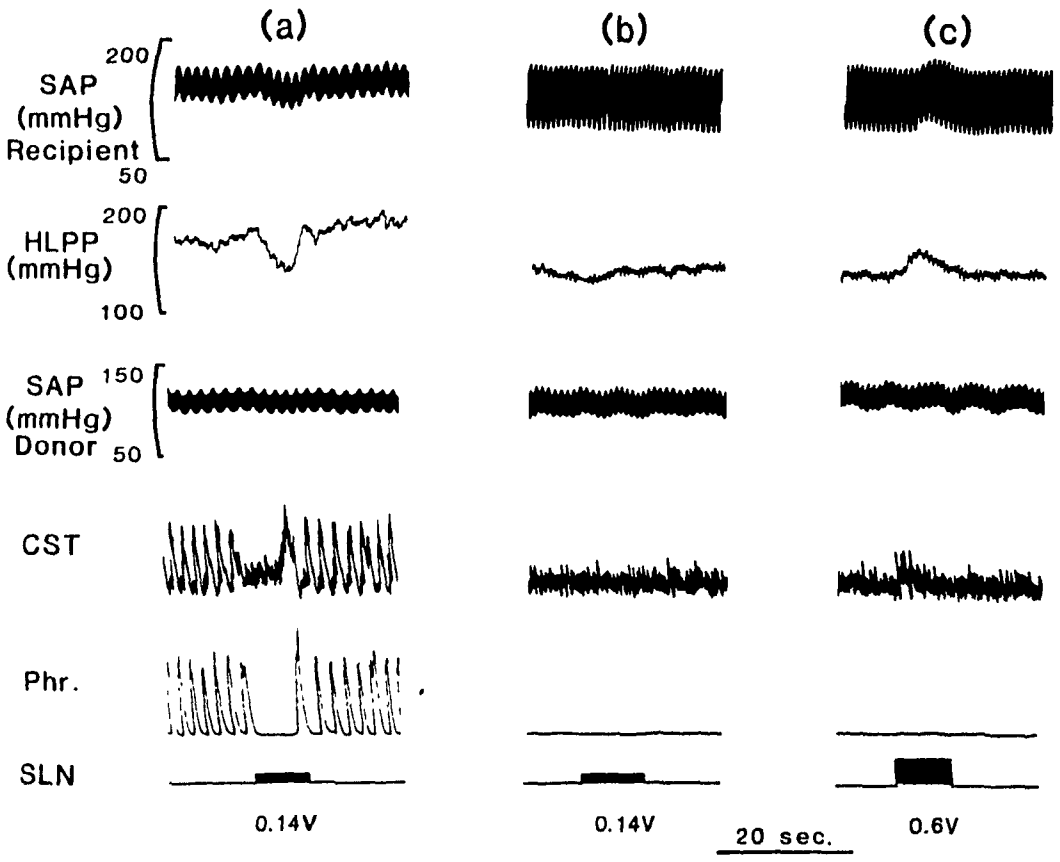
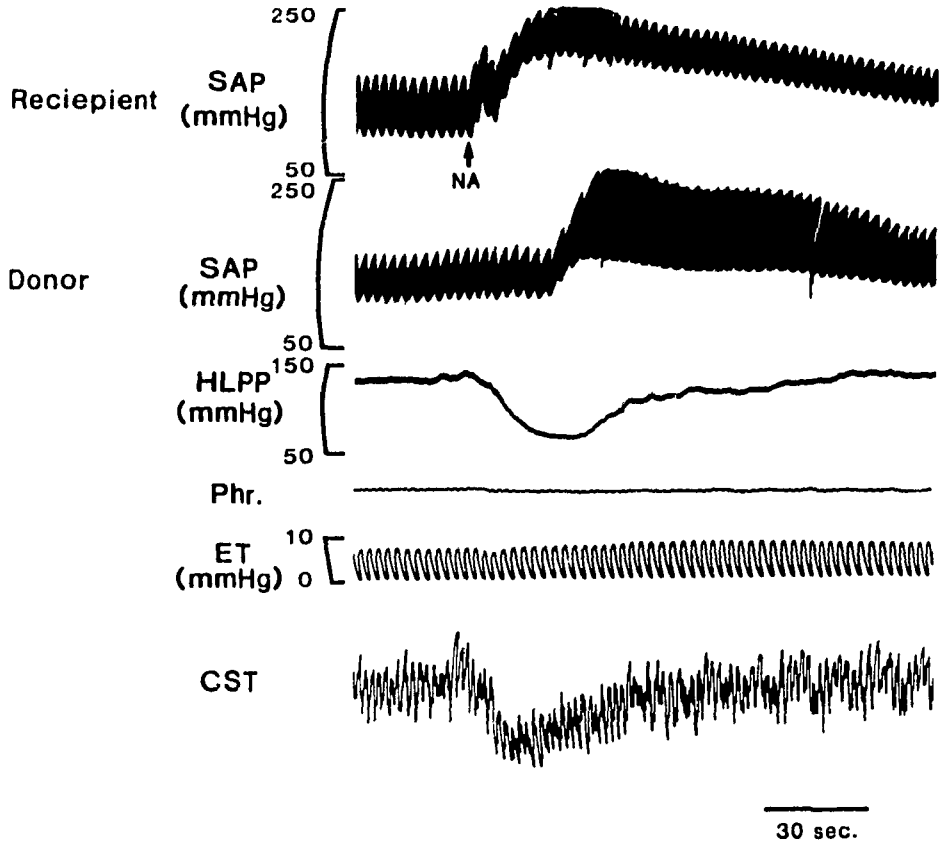

(a)
(b)
(c)
200
SAP
(mmHg)
Recipient
50
200
HLPP
(mmHg)
100
150
SAP
(mmHg)
Donor
50
CST
Phr.
SLN
0.14V
0.14V
20 sec.
0.6V

Fig. 7 Baroreceptor-evoked sympatho-inhibition and vasodilatation persist during systemic hypocapnia. Cross-perfusion of hindlimb of recipient animal with intact baroreceptor nerves by donor animal (end-tidal PCO_2 40 mm Hg). From above: systemic arterial pressure of recipient animal, systemic arterial pressure of donor animal, hindlimb perfusion pressure (constant flow), phrenic neurogram, end-tidal PCO_2, cervical sympathetic neurogram, all of recipient. At arrow, 5 µg noradrenaline injected i.v. into recipient. Notice decrease in cervical sympathetic activity and hindlimb perfusion pressure associated with rise in recipient systemic arterial pressure.

FIG. 7


250
Reciepient
SAP
(mmHg)
50
NA
250
Donor
SAP
(mmHg)
50
150
HLPP
(mmHg)
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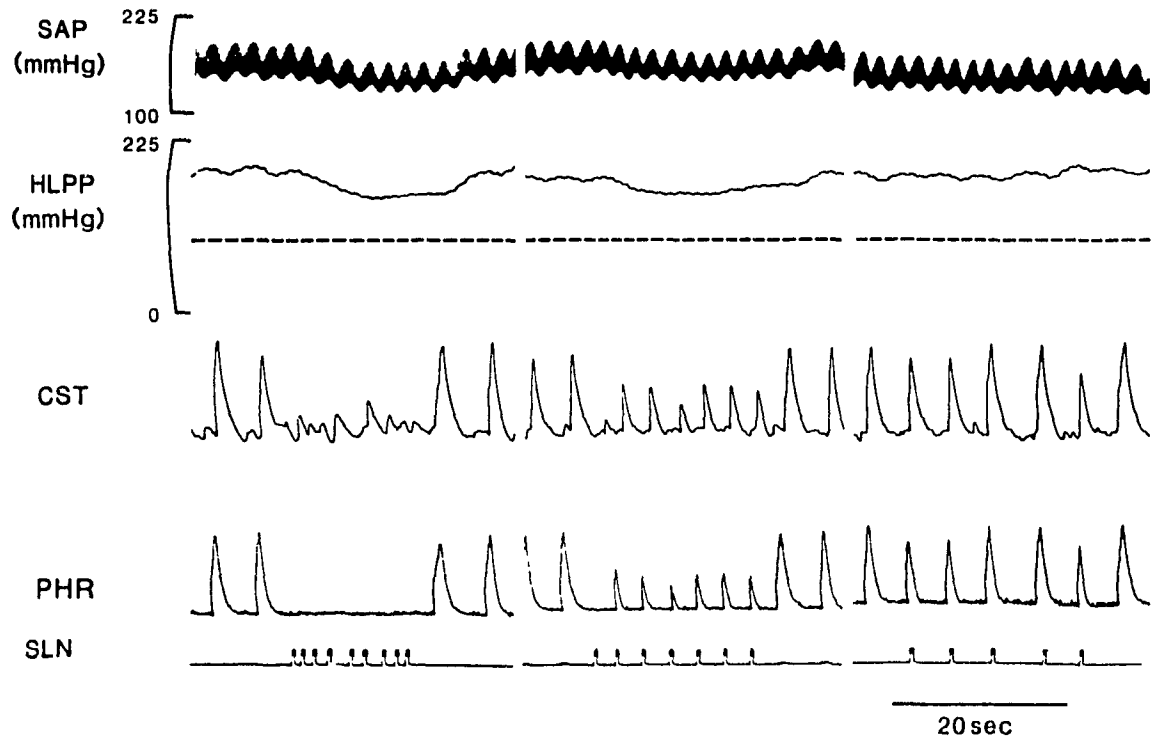
Phr.
10
ET
(mmHg) 0
CST
30 sec.

fall in perfusion pressure following hexamethonium administration. Furthermore, reflex vasodilatation could still be evoked by arterial baroreceptor afferent stimulation (3 experiments, Fig. 7). This degree of systemic hypocapnia also caused vasodilatation which was associated with disappearance of the respiratory oscillation in perfusion pressure in those cats which exhibited this phenomenon. As shown in Fig. 6 (compare panel a and b) the vasodilatation caused by repetitive stimulation of the superior laryngeal nerve under control conditions was of similar magnitude to that caused by hypocapnia. Similar observations were made in the other 4 cats with cross-perfused hindlimbs.

Third, the magnitude of the reflex vasodilatation could be graded by stimulating the inspiration-suppressing afferents of the superior laryngeal nerve with short bursts of stimuli (100-200 ms train duration, 50 Hz, 0.2 ms pulses), delivered during various phases of the respiratory cycle, once during each of 5-10 consecutive respiratory cycles. No vasodilatation occurred if the stimulus was given during the last 10-20% of inspiration or during expiration (Fig. 8, right panel). Vasodilatation occurred with stimulation during the first 80% of inspiration, the magnitude of which depended on the timing of the stimulus train during this phase. Vasodilatation was maximal when the stimulus was given during early inspiration (first 10-20%, Fig. 8, left panel), but was less when the stimulus was delivered during mid-inspiration (Fig. 8, middle panel). These effects of the timing of the

Fig. 8 Effect of stimulus timing during the respiratory cycle on the vasodilator response to superior laryngeal nerve stimulation. From above: systemic arterial pressure, hindlimb perfusion pressure (constant flow), cervical sympathetic and phrenic neurograms, stimulus. Dashed line shows level of hindlimb perfusion pressure after hexamethonium. Superior laryngeal nerve stimulus (200 ms trains, 50 Hz, 0.2 ms, 0.1 V) triggered at selected delays from onset of phrenic nerve burst and delivered for several consecutive respiratory cycles. Left panel: stimulus given in early inspiration. Suppression of phrenic nerve burst and inspiration-synchronous sympathetic discharge. Marked vasodilation. Number of trains greater than predicted from control duration of respiratory cycle because stimulation in early I greatly shortens the post-stimulus cycle. Middle panel: stimulus given in mid-inspiration. Suppression of a fraction of phrenic nerve burst and inspiration-synchronous sympathetic discharge. Vasodilatation present but less than in left panel. Evident shortening of respiratory cycle during stimulation. Right panel: stimulus given in early expiration. No effect on phrenic burst or inspiration-synchronous sympathetic discharge. No effect on hindlimb perfusion pressure.

FIG. 8



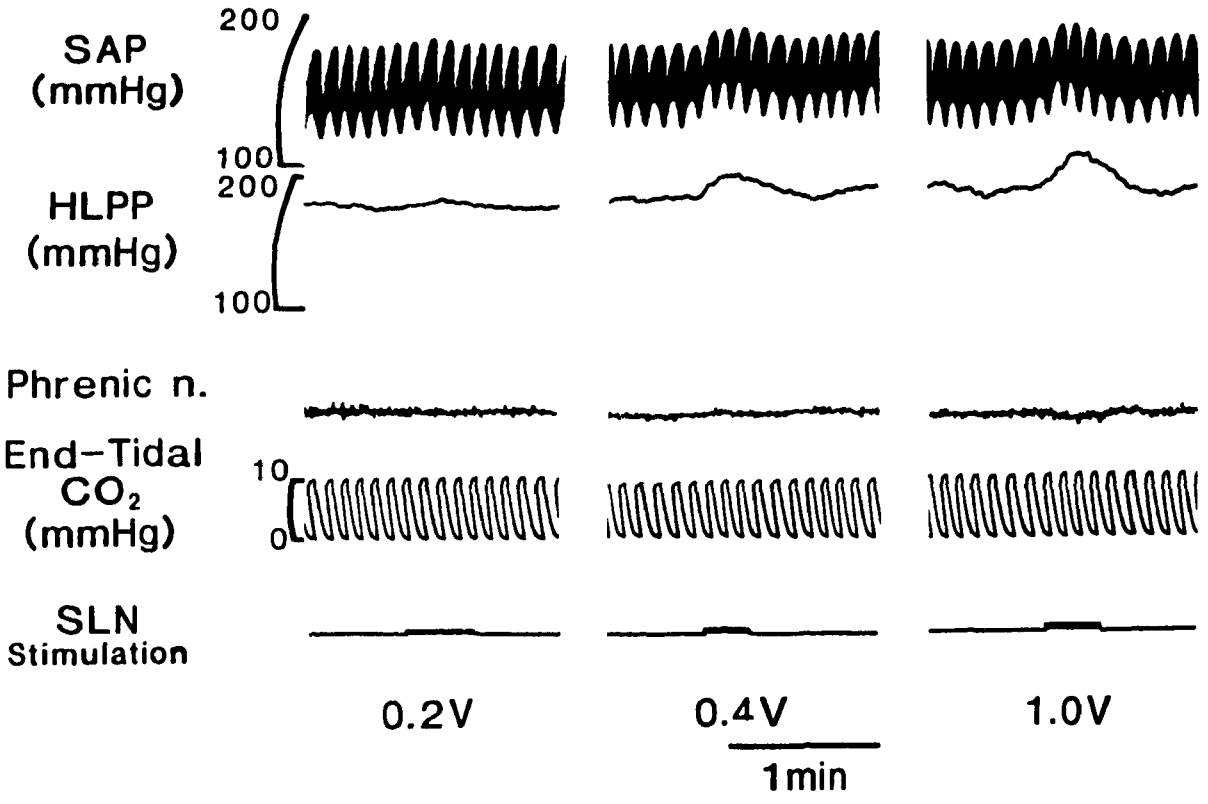
stimulus on the magnitude of reflex vasodilatation are strongly reminiscent of the effects of stimulus timing on the magnitude of the depression of the inspiration-synchronous sympathetic discharge, shown in Fig. 3.

c) Sympatho-excitatory and Vasoconstrictor Effects of SLN Stimulation

As mentioned in the section describing the sympatho-depressant effects, repetitive stimulation of the superior laryngeal nerve (50 Hz, 0.2 ms pulses), with intensity greater than that which was just sufficient for inspiration- suppression, evoked a marked increase in tonic sympathetic activity (Fig. 1c) together with suppression of the inspiration-synchronous sympathetic discharge. Since the sympatho-inhibitory effect is absent at end-tidal PCO_2 levels low enough to abolish phrenic nerve and inspiration- synchronous sympathetic discharge, this sympatho-excitatory effect was the only response seen during repetitive stimulation in hypocapnia (Fig. 6c). The properties of the excitatory component of the reflex evoked in the cervical sympathetic trunk by superior laryngeal nerve afferents were therefore studied by stimulation during hypocapnia. Stimulation with short trains of stimuli (200 ms train duration, 50 Hz, 0.2 ms pulses) caused a discharge with a threshold of 0.18 ± 0.03 V and a maximum amplitude at 0.80 ± 0.10 V (n = 3). The discharge had a latency of 76 ± 4 ms (n = 3), measured from the first stimulus. Repetition of stimulation at a

Fig. 9 Vasoconstrictor effect of superior laryngeal nerve stimulation. From above: systemic arterial pressure, hindlimb perfusion pressure (constant flow) phrenic neurogram, end- tidal PCO_2, stimulus. End-tidal PCO_2:10 mmHg. Stimulus train: 0.2 ms, 50 Hz. Vasoconstrictor response did not increase with stimulation intensity greater than 1.0V (not shown).

FIG. 9


SAP
(mmHg)
200
100
HLPP
(mmHg)
200
100
Phrenic n.
End-Tidal
CO_2
(mmHg)
10
0
SLN
Stimulation
0.2V
0.4V
1.0V
1min

rate of 0.5 train/s or more caused a rapid attenuation of the amplitude of the response. There was an increase in hindlimb perfusion pressure associated with the discharge of the cervical sympathetic trunk. This vasoconstrictor effect was also studied in isolation by repetitive stimulation of the superior laryngeal nerve during hypocapnia in 8 cats in which one hindlimb was autoperfused. Fig. 9 shows the results of such an experiment. The threshold was 0.2 V, the vasoconstriction increased at 0.4 and was maximal at 1.0 V. With maximal stimulation the increase in perfusion pressure was 17.1 ± 6.4 mmHg ($n = 8$, $P < .001$). Vasoconstriction was not sustained through the period of stimulation.

d) Effects of SLN Stimulation on Heart Rate and Systemic Arterial Pressure

Instantaneous heart rate was monitored in 5 cats. Under control conditions heart rate was 192 ± 23 beats/min. During repetitive stimulation (50 Hz, 0.2 ms pulses), of intensity just sufficient to suppress the phrenic nerve burst and the inspiration-synchronous sympathetic discharge, heart rate decreased by 6 ± 3 beats/min ($P < .005$). Increasing the intensity of the stimulus up to 1.0 V did not modify this response. Since all cats were vagotomized, this small bradycardia was presumably mediated by the sympathetic nerves. During hypocapnia, repetitive superior laryngeal nerve stimulation at up to 1.0 V intensity had no effect on heart rate. As mentioned above, the vasodilator response to

superior laryngeal nerve stimulation was also lost under these conditions (Fig. 6b).

Superior laryngeal nerve stimulation evoked changes in systemic arterial pressure which were consistent with the changes observed in the electrical activity of the cervical sympathetic trunk and in hindlimb perfusion pressure. Systemic arterial pressure decreased when repetitive stimulation resulted in inspiration-suppression, suppression of the inspiration-synchronous sympathetic discharge and vasodilatation (Fig. 4, 5a, 6a, 8 left and middle panels), but did not change when the same stimulation was employed either during hypocapnia (Fig. 6b) or with such timing that no depression of the inspiration-synchronous sympathetic discharge occurred (Fig. 8 right panel). Systemic arterial pressure increased when the superior laryngeal nerve was stimulated at intensities greater than those required for inspiration-suppression, and caused sympatho-excitation and vasoconstriction (Fig. 6c, 9b and c).

DISCUSSION

This study has shown that one of the reflex actions of superior laryngeal afferent fibres on sympathetic activity is a selective suppression of the inspiration-synchronous component. The selectivity of this action is indicated by the fact that depression of this component occurred without any depressant action

on the tonic component of the firing of these neurones. In fact, the latter was often enhanced. This reflex appears to be mediated by the same low threshold afferents which suppress the phrenic burst. In addition, electrical stimulation of low threshold afferents in the superior laryngeal nerve evokes a vasodilatation of the hindlimb which is abolished by hexamethonium. The vasodilatation is presumably due to a decrease in neural vasoconstrictor tone, since it is abolished by the alpha-adrenergic blocker phentolamine and unaffected by either atropine or propranolol.

This reflex vasodilatation has some unique properties which suggest that the underlying mechanism is not a process reducing the responsiveness of the sympathetic preganglionic neurones to excitatory inputs but a selective suppression of the inspiration-synchronous sympathetic discharge. First, the vasodilatation was present only when there was an inspiration-synchronous wave in the sympathetic discharge and this wave was abolished by superior laryngeal nerve stimulation. The vasodilatation disappeared when end-tidal PCO_2 was lowered to a level below threshold for the occurrence of phrenic nerve activity and of the inspiration-synchronous sympathetic discharge. Secondly, the magnitude of the vasodilatation was influenced by the timing of the stimulus with respect to the respiratory cycle, being maximal when the stimulus was delivered during early or middle inspiration and minimal when the stimulus was delivered in late

inspiration or in expiration. Both these properties are at variance with those of other sympatho-inhibitory and vasodilator reflexes, e.g. that evoked by arterial baroreceptor afferents. The latter reflex occurs whether or not there is an inspiration-synchronous component of sympathetic discharge (Gerber & Polosa, 1978) and during hypocapnia (present experiments). Moreover, baroreceptor inhibition of sympathetic neural discharge is greater in expiration than in inspiration (Seller, Langhorst, Richter & Koepchen, 1968).

The observation that a respiratory oscillation in hindlimb perfusion pressure, which was present under control conditions in a number of cats, disappeared during the vasodilatation evoked by stimulation of the superior laryngeal nerve is also consistent with the hypothesis that the vasodilatation is due to suppression of the inspiration-synchronous sympathetic discharge. Since this respiratory oscillation was independent of the frequency of the respiratory pump and the preparations were paralyzed, sino-aortic denervated and vagotomized, it could not have been caused by mechanical or reflex effects of the inflation-deflation cycle of the pump. On the other hand, as the frequency of this perfusion pressure oscillation was related to that of the phrenic nerve burst and of the inspiration-synchronous sympathetic discharge, it is more likely that it was due to respiratory modulation, generated within the central nervous system, of the activity of hindlimb vasoconstrictor neurones. The same mechanism was postulated by

Koepchen, Seller, Polster & Langhorst (1968) to account for an oscillation in hindlimb blood flow observed under similar experimental conditions. During hypocapnia this oscillation in perfusion pressure disappeared, as did the inspiration-synchronous sympathetic discharge. During vasodilatation caused by superior laryngeal nerve stimulation this oscillation also disappeared, along with the inspiration-synchronous sympathetic discharge. The simplest interpretation of this parallelism is that the loss of the vasodilator reflex is the consequence of the suppression of the inspiration-synchronous sympathetic discharge. In other words, the loss of the respiratory oscillation in perfusion pressure during superior laryngeal nerve stimulation suggests that respiratory modulation has disappeared from the activity pattern of the sympathetic neurones supplying the hindlimb vasculature. Moreover, since systemic hypocapnia caused vasodilatation (Fig. 6b) which was associated with loss of the respiratory oscillation in perfusion pressure and caused loss of the inspiration-synchronous sympathetic discharge as the dominant effect on sympathetic activity, it can be suggested that hypocapnic vasodilatation is due to removal of the same component of sympathetic discharge, namely the inspiration-synchronous sympathetic discharge, as is the vasodilatation evoked by superior laryngeal nerve stimulation. The observation that the hypocapnic and the superior laryngeal nerve-evoked vasodilatation were of similar magnitude is consistent with this interpretation.

The set of superior laryngeal nerve afferents responsible for the vasodilatation is excited by stimulus intensities within the same range as that which terminates the phrenic nerve burst, causing apnea when repetitively activated, and depressing the inspiration-synchronous sympathetic discharge. The properties of the inspiration-inhibitory afferents in the superior laryngeal nerve have been studied by Miller and Loizzi (1974). These afferents represent 25% of the myelinated axons in this nerve and have conduction velocities of about 40 m/s.

In some cases, during superior laryngeal nerve stimulation at low intensity, perfusion pressure fell initially followed by recovery towards or above control during the stimulation period. This biphasic time course of the response may result from the simultaneous occurrence of two opposing central effects, suppression of the inspiration-synchronous sympathetic discharge and the sympatho-excitation, due to overlap in the excitability properties of the inspiration-suppressing and sympatho-excitatory axons.

Associated with the fall in hindlimb perfusion pressure, caused by stimulation of the inspiration-suppressing afferents in the superior laryngeal nerve, there was invariably a fall in systemic arterial pressure (e.g. Fig. 4, 5a, 6a, 8 left and middle panels). If stimulation did not cause a fall in perfusion pressure, no fall in systemic arterial pressure occurred. The same explanation offered for the vasodilator effect of superior

laryngeal nerve stimulation could account for the depressor effect. Namely, a suppression of the inspiration-synchronous component of sympathetic discharge to the heart and vascular beds other than the hindlimb is likely to result in a fall in systemic arterial pressure due to a reduction in both cardiac output and total peripheral resistance. The depressor effect could be used to estimate the component of systemic arterial pressure which is maintained by the inspiration-synchronous sympathetic discharge. The fall in systemic arterial pressure, observed during superior laryngeal nerve stimulation, therefore suggests that the inspiration-synchronous sympathetic discharge contributes to vasoconstrictor activity in vascular beds other than that under study. Under the conditions of the present study the contribution of the decrease in heart rate to the fall in systemic arterial pressure was negligible.

Excitation of sympathetic preganglionic neurones of the cervical sympathetic trunk and an increase in systemic arterial pressure in response to mechanical stimulation of the larynx has been described previously by Nadel and Widdicombe (1962) and Tomori and Widdicombe (1969). Some properties of an excitatory sympathetic reflex, evoked by stimulation of superior laryngeal nerve afferents and presumably underlying these effects, were described by Gerber and Polosa (1979). The present results extend these findings by providing evidence that this sympatho-excitatory reflex involves hindlimb vasoconstrictor neurones and is evoked at

intensities of superior laryngeal nerve stimulation greater than those required for inspiration-suppression, hence presumably by myelinated afferents of smaller diameter than those causing inspiration-suppression.

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SUMMARY

1. The background discharge of sympathetic preganglionic neurones shows a marked inspiration-synchronous component which is known to originate from within the central nervous system. The contribution of this component to total neurogenic vasoconstrictor tone is unknown.
2. In order to estimate its extent we have exploited the inspiration- suppressing effect of a group of low threshold afferent fibres in the superior laryngeal nerve.
3. The electrical activities of the cervical sympathetic trunk and of the phrenic nerve were recorded in pentobarbital-anaesthetized, paralyzed, artificially ventilated, sino-aortic denervated and vagotomized cats, together with the perfusion pressure of an innervated hindlimb perfused at a constant flow rate.
4. Repetitive stimulation of the superior laryngeal nerve at an intensity just sufficient to suppress phrenic nerve activity inhibited the inspiration- synchronous sympathetic discharge and caused hindlimb vasodilatation. This vasodilatation was abolished by hexamethonium or phentolamine, but was not affected by atropine or propranolol.
5. Following the elimination of phrenic nerve activity and inspiration-synchronous sympathetic discharge by systemic hypocapnia, repetitive stimulation of the superior laryngeal

nerve either failed to affect the residual sympathetic activity and hindlimb perfusion pressure, or caused an increase of both.

6. Stimulation of the superior laryngeal nerve with short (0.2s) trains of stimuli, delivered at selected times of the respiratory cycle for several consecutive cycles, had similar effects on phrenic nerve bursts, inspiration synchronous sympathetic discharge and hindlimb perfusion pressure. Stimulation at progressively earlier times during inspiration produced a graded reduction in all three variables, while stimulation during late inspiration or early expiration had no effect on any of them.

7. The results suggest that the vasodilator reflex, elicited by inspiration- suppressing afferents in the superior laryngeal nerve, results from selective abolition of the excitatory input which causes the inspiration-synchronous discharge of sympathetic neurones. The magnitude of the hindlimb vasodilatation can therefore, be taken as an indication of the extent of control of hindlimb vasoconstrictor tone exerted by this particular input. By comparing the magnitude of the reflexly-evoked vasodilatation with that of the vasodilatation resulting from ganglionic blockade, it was estimated that 24.2% of the neurogenic vasoconstrictor tone of the hindlimb was attributable to the inspiration-synchronous component of sympathetic discharge.

REFERENCES

Adrian, E.D., Bronk, D.W. & Phillips, G. (1932). Discharges in mammalian sympathetic nerves. Journal of Physiology 74, 115-133.

Barman, S.M. & Gebber, G.L. (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. American Journal of Physiology 231, 1601-1607.

Cohen, M.I. & Gootman, P.M. (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. American Journal of Physiology 218, 1092-1101.

Connelly, C.A. & Wurster, R.D. (1982). Spinal organization of respiratory influences on sympathetic nerve discharge patterns in cats. Proceedings of the 12th Meeting of the Neuroscience Society 78.

Gerber, U. & Polosa, C. (1978). Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 56, 191-198.

Gerber, U. & Polosa, C. (1979). Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 57, 1073-1081.

Hillenbrand, C.J. & Boyd, T.E. (1936). Reflex respiratory effects from intermittent stimulation of the vagus and superior

laryngeal nerves. American Journal of Physiology 116, 380-386.

Koepchen, H.P., Seller, H., Polster, J. & Langhorst, P. (1968). Uber die Fein- Vasomotorik der Muskelstrohmbahn und ihre Beziehung zur Ateminnervation. Pflügers Archiv 302, 285-299.

Kubin, L., Trzebski, A. & Lipski, J. Split medulla preparation in the cat: arterial chemoreceptor reflex and respiratory modulation of the renal sympathetic nerve activity. Journal of the Autonomic Nervous System (in press).

Larrabee, M.G., & Hodes, R. (1948). Cyclic changes in the respiratory centers, revealed by the effects of afferent impulses. American Journal of Physiology 155, 147-164.

Miller, A.J. & Loizzi, R.F. (1974). Anatomical and functional differentiation of superior laryngeal nerve fibers affecting swallowing and respiration. Experimental Neurology 42, 369-387.

Miserocchi, G. (1976). Role of peripheral and central chemosensitive afferents in the control of depth and frequency of breathing. Respiration Physiology 26, 101-111.

Nadel, J.A. & Widdicombe, J.G. (1962). Reflex effects of upper airway irritation on total lung resistance and blood pressure. Journal of Applied Physiology 17, 861-865.

Preiss, G., Kirchner, F. & Polosa, C. (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. Brain Research 87, 363-374.

Preiss, G. & Polosa, C. (1977). The relation between end-tidal CO_2 and discharge patterns of sympathetic preganglionic neurons. Brain Research 122, 255-267.

Seller, H., Langhorst, P., Richter, D. & Koepchen, H.P. (1968). Uber die Abhangigkeit der pressoreceptorischen Hemmung des sympathicus von der atemphase und ihre auswirkung in der Vasomotorik. Pflügers Archiv. 302, 300-314.

Tang, P.C., Maire, T.W. & Amassian, V.E. (1957). Respiratory influences on the vasomotor center. American Journal of Physiology 191, 218-224.

Tomori, Z. & Widdicombe, J.G. (1969). Muscular, bronchomotor, and cardiovascular reflexes evoked from stimulation of the respiratory tract. Journal of Physiology 200, 25-51.

Wallin, B.G. (1981). New aspects of sympathetic function in man. In: International Medical Review of Neurology 1; Clinical Neurophysiology, ed. Stalberg, E. and Young, R.R. pp. 145-167. London, Butterworths.

CHAPTER V

THE PATTERN OF SYMPATHETIC NEURONE ACTIVITY DURING EXPIRATION IN THE CAT

1. INTRODUCTION

Previous studies have firmly established that the firing pattern of single sympathetic preganglionic units, or of whole sympathetic nerves, has a rhythmic component, which is synchronous with the inspiratory phase of the respiratory cycle at normal respiratory frequencies and has properties similar to those of the phrenic nerve burst (Cohen & Gootman, 1970; Preiss, Kirchner & Polosa, 1975; Barman & Gebber, 1976; Preiss & Polosa, 1977; Gerber & Polosa, 1978 & 1979; Polosa, Gerber & Schondorf, 1980). In contrast, there are few observations on the properties of the sympathetic discharge which occurs during the expiratory phase of the respiratory cycle. Published records of the electrical activity of whole sympathetic nerves in vagotomized animals show that the level of activity increases from early to late expiration (Cohen & Gootman, 1970; Barman & Gebber, 1976). Some of these records show a monotonic increase in activity throughout expiration, while others show an early increase followed by a plateau. Various interpretations of these incrementing patterns of sympathetic activity during expiration are possible, but none has been subjected to experimental test as yet. The incrementing pattern may represent a recovery of activity from an early expiratory depression or an incrementing excitation synchronous with the phase of activity of brainstem expiratory neurones (Bainton, Richter, Seller, Ballantyne & Klein, 1985) or of

phase-spanning respiratory neurones (Gootman, Cohen, Piercey & Wolotsky, 1975).

The present experiments were directed to the question of whether the background discharge of sympathetic preganglionic neurone contains rhythmic components related to the activity of brainstem expiratory neurones. The existence of such components would suggest the existence of synaptic connections between these two sets of neurons. The activity level of brainstem expiratory neurones is known to be enhanced during moderate lung inflation (Sears, 1964; Bishop, 1967) and during systemic hypercapnia (Bainton, Kirkwood & Sears, 1978; Bainton & Kirkwood, 1979). Therefore in the present experiments we have investigated the effect of these expiratory- facilitating maneuvers on the pattern of firing of the cervical sympathetic trunk in expiration.

2. METHODS

Thirty one cats of both sexes were used (2.3 to 4.0 kg). Five of the cats were decerebrated at midcollicular level under ether anesthesia. In the remainder, anaesthesia was obtained with i.p. sodium pentobarbitone (35 mg/kg initial dose, followed by a maintenance dose of 9 mg/kg i.v. every 3 hrs). With this dose the withdrawal reflex on pinching forepaw or hindpaw was suppressed for the duration of the experiment. The trachea was cannulated and the animals were artificially ventilated, while continuously monitoring

tidal CO_2 concentration with an infrared gas analyser and tracheal pressure with a strain gauge. All cats were paralyzed with pancuronium bromide (initial dose 200 µg/kg followed by maintenance doses of 100 µg/kg which were given every 2-3 hours, when the effect of the previous dose had worn off, as evidenced by the appearance of spontaneous breathing movements, and after testing for adequacy of the level of anaesthesia). Frequency and tidal volume of the respiratory pump were adjusted to obtain, in control conditions, a end-tidal PCO_2 of between 35 and 40 mmHg. In ten experiments a phrenic-triggered respiratory pump was used (Remmers & Gauthier, 1976). Central respiratory cycle is defined as the interval between two successive phrenic nerve bursts. Inspiration is defined as the time between onset of phrenic nerve activity and beginning of rapid decline, expiration as the remainder of the cycle. Inflation is defined as the increase in tracheal pressure caused by the respiratory pump, deflation as the return of tracheal pressure to pre-inflation level from peak inflation. For consistency with the terminology used in respiratory physiology (Grippi, Pack, Davies & Fishman, 1985) the value of tracheal pressure or of lung volume at the end of the deflation phase of the ventilation cycle is referred to as end-expiratory level. A variable load to passive deflation (a water column of various heights), which produced graded increases in end-expiratory lung volume (FRC), was introduced in the circuit when required (Bishop, 1967, Russell & Bishop, 1976). Systemic hypercapnia was produced

by ventilation with gas mixtures containing various CO_2 concentrations in O_2, systemic hypocapnia by hyperventilation in room air. Catheters were placed in a femoral artery and vein for continuous recording of systemic arterial pressure and for administration of drugs, respectively. Rectal temperature was maintained at 37-38°C using an infrared lamp controlled by a feedback circuit.

The electrical activity of the phrenic nerve, of the cervical sympathetic trunk and, in three cats, of the recurrent laryngeal nerve was recorded monophasically with silver hook electrodes, amplified (bandpass 10 Hz to 10 KHz) and stored on magnetic tape. After half-wave rectification and low-pass filtering (time constant 100 ms) these signals were also displayed on a storage oscilloscope and pen recorder. These rectified, low-pass filtered, records of neural activity are usually referred to in the literature as "integrated" activity. The level of "zero" activity for the recording of cervical sympathetic trunk activity was obtained by applying procaine to the nerve or by crushing the nerve proximal to the recording electrodes.

The carotid bifurcation was exposed bilaterally in all animals, after resection of a segment of the esophagus and trachea, and both carotid sinus nerves were identified at their junction with the IX cranial nerve and cut. The aortic nerves were also bilaterally cut when they were identified as separate from the cervical vagus nerves. The vagus nerves were cut in some cases

before the beginning of the experiments. In the majority of cases they were prepared for section during the experiment. In two cats, after a left thoracotomy, a catheter was inserted into the pericardial sac for injection of procaine (2 ml of a 2% solution) (Arndt, Pasch, Samodelov & Wiebe, 1981) at the appropriate time during an experiment.

3. RESULTS

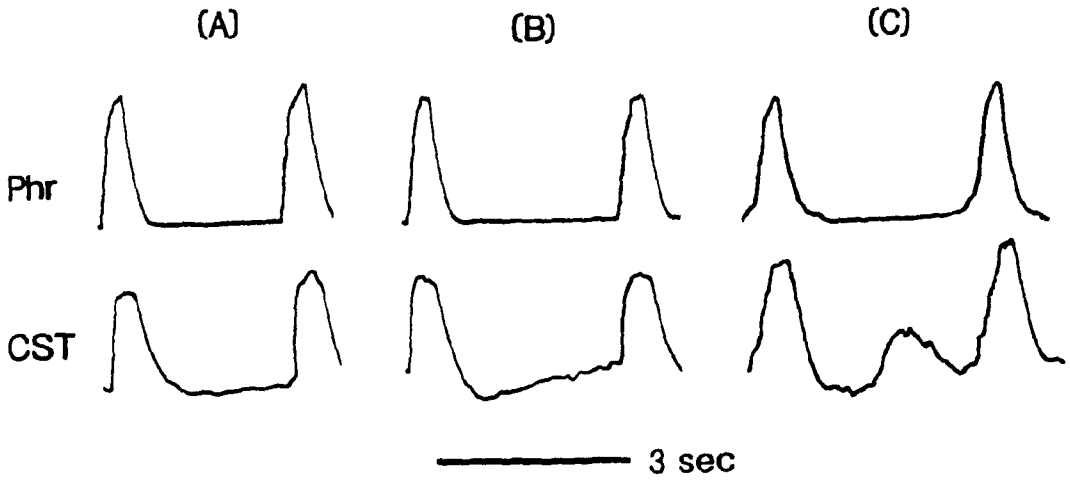
a) Pattern of Activity During Expiration of the Cervical Sympathetic Trunk (CST) at Normal FRC

The pattern of sympathetic activity during expiration was examined in 9 vagotomized cats and in 22 cats with intact vagus nerves. In the latter group the observations were made in the absence of artificial ventilation during a period equal to two respiratory pump cycles. All animals were normocapnic and showed a pronounced inspiration-synchronous sympathetic discharge. In 11 of the cats the mean level of sympathetic activity was approximately constant throughout expiration. This will be referred to as a "tonic" pattern (Fig. 1A). In the majority of cats (17) the mean level of activity increased from early expiration to middle or late expiration. This will be referred to as an "incrementing" pattern. In some cats showing an incrementing pattern the increase was continuous throughout expiration, in others a plateau was formed in late expiration (Fig. 1B). These differences in the pattern of

FIGURE LEGENDS

Fig. 1 Patterns of sympathetic activity during expiration in anaesthetized, vagotomized, paralyzed normocapnic cats. From above, averaged (20 sweeps) integrated phrenic nerve and cervical sympathetic trunk activity. In this and all subsequent figures all averages shown were triggered from the onset of phrenic nerve discharge. An upward deflection indicates increased discharge. A, B & C: recordings from three different cats, all showing a pronounced inspiratory sympathetic discharge. Panel A: mean level of sympathetic activity remained constant throughout expiration (tonic type). Panel B: mean level of sympathetic activity increased continuously during expiration (incrementing type). Panel C: a distinct wave-like discharge of sympathetic activity appeared during the second half of expiration.

FIG. 1



expiratory firing between animals were not related to differences in the duration of the respiratory cycle or of its phases, nor to presence or absence of the vagus nerve. Each pattern was invariant for a given set of experimental conditions. Three cats showed a distinct burst of activity in late expiration (Fig. 1C). Thus, in these cases sympathetic activity showed two peaks for each respiratory cycle. Similar observations have been recently described by Bainton et al. (1985). Peak amplitude and rate of rise of this expiratory burst, in the low-pass filtered record, were lower than for the inspiration-synchronous sympathetic burst. Since these animals were sino-aortic denervated and vagotomized or at constant lung volume, it may be assumed that this pattern of sympathetic activity in expiration was not generated reflexly but by mechanisms within the CNS. A possible explanation for this pattern is that it results from excitatory input from brainstem expiratory neurones to sympathetic preganglionic neurones. If this was the case, it should be possible to evoke an increase of sympathetic activity during expiration by performing manoeuvers which are known to increase the level of activity of brainstem expiratory neurones.

b) Pattern of Activity in Expiration of the CST During Ventilation with Positive End-expiratory (PEEP) (increased FRC)

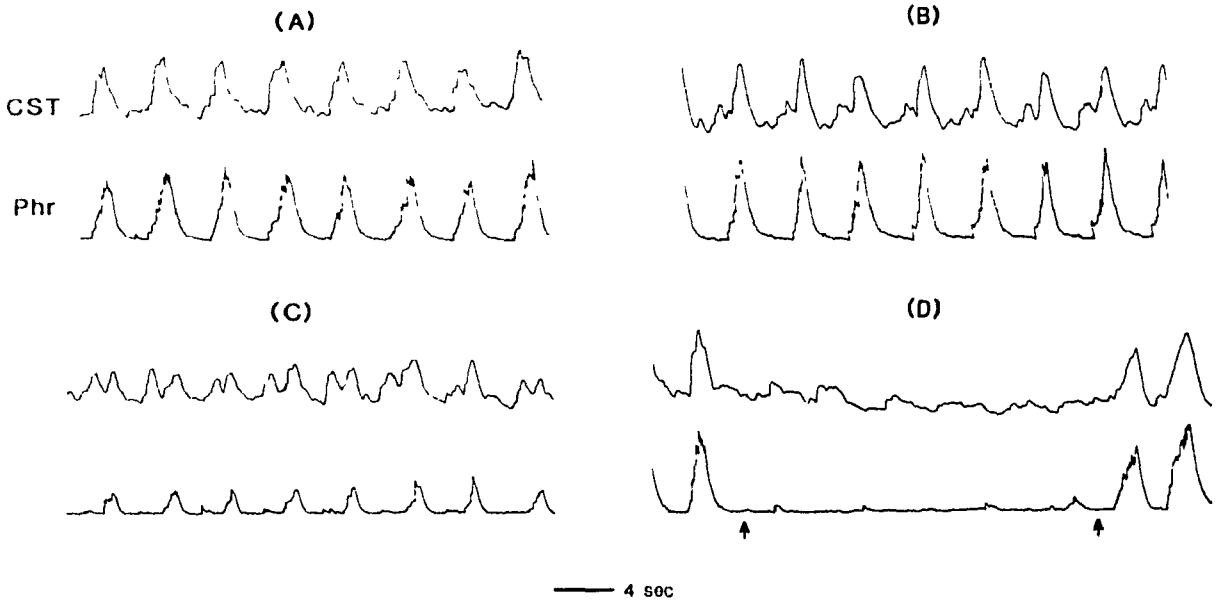
Positive end-expiratory pressure within the range from 2.1 ±

0.4 to 6.7 ± 0.6 cm H_2O caused an increase in the level of activity during expiration of the cervical sympathetic trunk in 19 of 22 cats with intact vagus nerves tested. In addition, expiratory loading caused attenuation of the inspiration-synchronous sympathetic discharge. Fig. 2 shows the results of such an experiment. At zero end-expiratory pressure the pattern of expiratory activity of the cervical sympathetic trunk was tonic (panel A). Panels B & C show the effect of ventilation with end-expiratory pressures of 2.5 and 5.0 cm H_2O, respectively. An expiratory wave appeared, reminiscent of that shown in Fig. 1C. The expiratory wave appeared with some delay with respect to the onset of expiration, incremented first and then decremented somewhat before the onset of the following inspiration. The amplitude of this expiratory wave of sympathetic activity was greater at 5.0 (Fig. 2C) than at 2.5 (Fig. 2B) cm H_2O. At 5.0 cm H_2O end-expiratory pressure a decrease in peak amplitude of the phrenic nerve burst and of the inspiration-synchronous wave of sympathetic discharge was also observed. The inspiratory and expiratory waves were of similar amplitude at this value of end-expiratory pressure. With a pressure of 10 cm H_2O (Fig. 2D), phrenic nerve activity disappeared, together with the inspiratory and expiratory components of sympathetic discharge. At this value of end-expiratory pressure the sympathetic record showed waves of depression which were related in time to each inflation phase of the respiratory pump cycle and which appeared to summate, resulting

in a progressively decreasing level of sympathetic activity. Positive end-expiratory pressure produced the expected (Cohen, 1975) excitation of expiratory neurones: a burst of activity in late expiration appeared in the record of recurrent laryngeal nerve activity during ventilation with end-expiratory pressures of 2.5 and 5.0 cm H_2O. All these effects on sympathetic, recurrent laryngeal and phrenic nerve activity disappeared after bilateral cervical vagotomy. Effects similar to those just described were obtained in cats in which procaine was injected in the pericardial sac. This result rules out the possibility that sensory receptors in the heart, responsive to changes in transmural pressure and/or shape of the cardiac chambers resulting from positive end-expiratory pressure ventilation (Cassidy & Mitchell, 1981), could be the source of the observed excitation in expiration of sympathetic neurones. In these experiments, in which ventilation with positive end-expiratory pressure was used, lung inflation started in inspiration and terminated in early expiration, and inspiration was terminated by the inflation, i.e. phrenic nerve activity was entrained to the respiratory pump cycle. This is the most commonly observed phase-relation during entrainment of phrenic nerve activity to lung inflation in cats with intact vagus nerves artificially ventilated at inflation frequencies close to, or higher than, the spontaneous frequency of the respiratory pattern generator. (Cohen, 1969; Vibert, Caille & Segundo, 1981; Petrillo, Glass & Trippenbach, 1983).

Fig. 2 Effect of positive end-expiratory pressure on the pattern of cervical sympathetic trunk activity in expiration. Each panel shows integrated cervical sympathetic trunk (above) and phrenic nerve activity. Panel A: control records at zero end-expiratory pressure. Sympathetic activity in expiration is of the tonic type. Panels (B), (C) & (D) show the effects of end-expiratory pressures of 2.5, 5.0 and 10.0 cm H_2O respectively. In B, a small wave-like increase in sympathetic activity appears late in expiration. In C, the wave of sympathetic activity in expiration increases in size while the phrenic burst and inspiration-synchronous sympathetic discharge are markedly reduced. In D, after one cycle of ventilation at zero end-expiratory pressure, end-expiratory pressure of 10 cm H_2O is applied between the arrows. The phrenic nerve burst is completely suppressed. Cervical sympathetic trunk activity shows a progressive slow decline, on which are superimposed waves of depression, presumably synchronous with each lung inflation. Returning to zero end-expiratory pressure (second arrow) causes the reappearance of the phrenic burst and of the inspiration synchronous sympathetic discharge.

FIG. 2



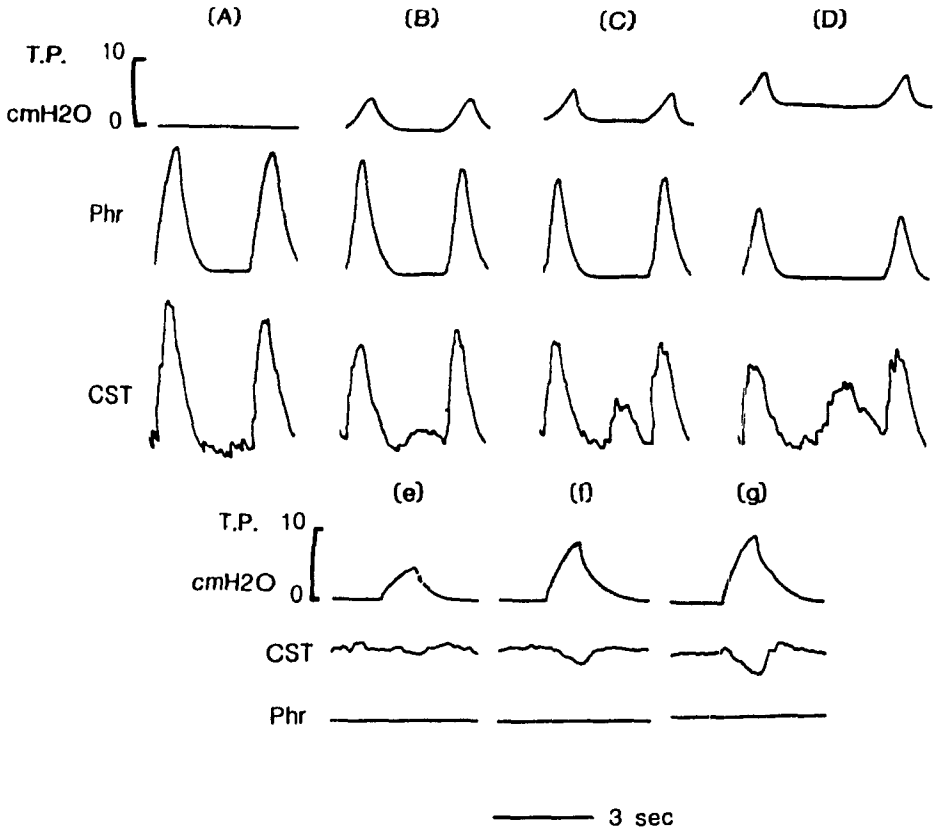
Results similar to those shown in Fig. 2 were obtained during ventilation with positive end-expiratory pressure when lung inflation was limited to inspiration by using a phrenic-triggered respiratory pump, thus simulating the phase relation of inflation to central respiratory activity existing during spontaneous breathing. Onset and end of lung inflation coincided approximately with onset and peak of the phrenic nerve burst. The results of such an experiment are shown in Fig. 3 in which panel A shows the waveform of the cervical sympathetic trunk recorded with the respiratory pump turned off and panels B-D show the effect of lung inflation during inspiration at increasing FRCs caused by increasing levels of positive end-expiratory pressure. Panels e-g show the effect of lung inflations, causing peak tracheal pressures in the same range (4.4 to 9.4 cm H_2O) as in panels B-D on cervical sympathetic trunk activity during systemic hypocapnia. In panel A, with the respiratory pump turned off and zero tracheal pressure, the activity of the cervical sympathetic trunk in expiration was of the tonic type and at a low level. When the respiratory pump was turned on (panel B) a small amplitude expiration- synchronous sympathetic discharge appeared. At increasing FRC values (panels C & D) the peak amplitude of the expiration-synchronous sympathetic discharge increased; in addition, there was a progressive decrease in the amplitude of the inspiration-synchronous sympathetic discharge and phrenic burst. Duration of expiration increased. All these effects disappeared after bilateral cervical vagotomy.

In the experiments shown in Fig. 2 and 3 (panels B-D), the deflation phase of the ventilation cycle occurred in expiration. This fact suggests the possibility that the appearance of the expiration-synchronous sympathetic discharge during ventilation with positive end-expiratory pressures in the range from 2.1 ± 0.4 to 6.7 ± 0.6 cm H_2O was the result of reflex excitation of the sympathetic preganglionic neurone by vagal afferents activated by lung deflation (Daly, Hazzledine & Ungar, 1967). This possibility is made unlikely by two sets of observations. One is that for the range of lung volumes used in these experiments, when the rhythmic activity of the central respiratory pattern generator was eliminated by systemic hypocapnia (Fig. 3, panels e-g), no excitation of sympathetic activity on deflation, but only depression on inflation, was observed. The other observation is that at constant, elevated lung volume, in normocapnia, a similar sympatho-excitation in expiration was recorded (see next section).

The lack of sympatho-excitation by positive end-expiratory pressure in the absence of central respiratory activity (Fig. 3e-g) suggests that the expiration-synchronous sympathetic discharge evoked by positive end-expiratory pressure in normocapnia results from an effect mediated by brainstem respiratory neurones. Thus, the periodicity of the expiration-synchronous sympathetic discharge appears to be determined not by the inflation-deflation cycle of the respiratory pump, but by the rhythm of the activity of these neurones. If this inference was correct, then during constant

Fig. 3 Effect of increasing FRC on cervical sympathetic trunk activity in expiration. Ventilation with phrenic-triggered pump. In each panel, from top tracheal pressure, integrated phrenic nerve and cervical sympathetic trunk activity (averages of 20 sweeps). A-D: normocapnia (end-tidal P_{CO} 40 mm Hg). e-g: hypocapnia (end-tidal P_{CO} 15 mm Hg). A: respiratory pump off. B: ventilation with zero end-expiratory pressure. Notice small expiratory wave of sympathetic activity. C & D: ventilation with positive end-expiratory pressure of 2 and 6 cm H_2O. Notice graded increase in the expiratory wave of sympathetic activity, together with depression of phrenic burst and inspiration-synchronous sympathetic discharge and prolongation of expiration. e-g show the effect of lung inflation on sympathetic activity in the absence of rhythmic phrenic nerve activity.

FIG. 3



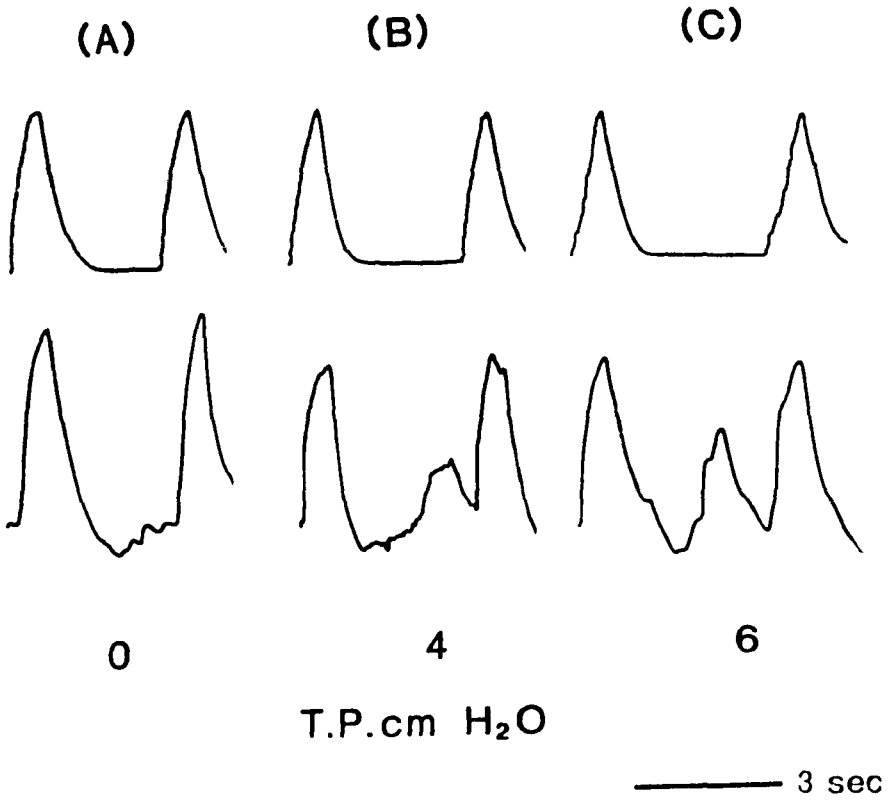
elevated lung volume in normocapnia an expiration-synchronous sympathetic discharge should be observed.

c) Pattern of Activity in Expiration of the CST at Constant, Elevated Lung Volume

In 22 cats with intact vagus nerves, at the end of the first pump cycle following the application of positive end-expiratory pressure, the respiratory pump was turned off for the duration of 2-3 respiratory cycles: the lungs remained inflated at the higher FRC. The results of a typical experiment are shown in Fig. 4. Fig. 4A shows the control sympathetic waveform at zero end-expiratory pressure and at constant lung volume: there was a prominent inspiration-synchronous sympathetic discharge while activity in expiration was of the 'incrementing' type. Positive end-expiratory pressure (4 cm H_2O, Fig. 4B and 6 cm H_2O, Fig. 4C) caused the appearance of an expiration-synchronous sympathetic discharge. In addition, there was an increase in duration of expiration and some attenuation of the peak amplitude of the phrenic nerve burst, as described by Bartoli, Bystrzycka, Guz, Jain, Noble and Trenchard (1973). The peak amplitude of the inspiration-synchronous sympathetic discharge was also somewhat attenuated. These effects were observed in 19 out of 22 cats over the range of positive end-expiratory pressure values from 2.1 $\pm$ 0.4 to 6.7 $\pm$ 0.6 cm H_2O. Bilateral cervical vagotomy abolished all these effects, which were also lost at levels of systemic

Fig. 4 Effect of static lung inflation on activity pattern of cervical sympathetic trunk in expiration. From above, integrated phrenic nerve and cervical sympathetic trunk activity (averages of 8 sweeps). Panel A: Control at constant lung volume and zero end-expiratory pressure. Incrementing pattern of cervical sympathetic trunk activity in expiration. Panels B and C: An increase in static lung volume (tracheal pressure of 4 and 6 cm H_2O) caused the appearance of a wave-like discharge during the second half of expiration, which was greater at 6 than at 4 cm H_2O tracheal pressure.

FIG. 4



hypocapnia associated with abolition of phrenic nerve activity. With tracheal pressures in excess of 10.2 cm H_2O phrenic nerve activity disappeared together with the inspiration and expiration synchronous sympathetic discharge, and the level of sympathetic activity was markedly depressed.

d) Pattern of Activity During Expiration of the CST at Normal FRC when Phasic Lung Inflations Occurs in Expiration

During the experiments of static lung inflation, described in the preceding section, the expiration-synchronous sympathetic discharge appeared at tracheal pressure values well within the range of inflation pressures occurring during "normal" artificial ventilation. It was of interest to test whether lung inflation with normal tidal volumes during expiration, in cats with intact vagus nerves, would cause an expiration-synchronous sympathetic discharge. This test was performed in 15 cats with intact vagus nerves. Inflation in expiration was obtained in some of the cases by setting the frequency of the respiratory pump at a value lower than the intrinsic central respiratory frequency determined with respiratory pump off. With intact vagus nerves, one to one phase-locking of the central respiratory cycle to the slower respiratory pump cycle occurs, within a limited range of frequencies, with inflation occurring in expiration (Petrillo et al., 1983). In other cases the experiment was done with the

Fig. 5 Effect of phasic lung inflation in expiration on cervical sympathetic trunk activity in expiration. From above, tracheal pressure, integrated phrenic nerve and cervical sympathetic trunk activities (averages of 15 sweeps). Panel A shows the cervical sympathetic trunk activity in expiration, in control conditions during apnoea. Panel B shows that during ventilation with lung inflation occurring in expiration there is a wave-like sympathetic discharge, together with prolongation of the phase.

FIG. 5

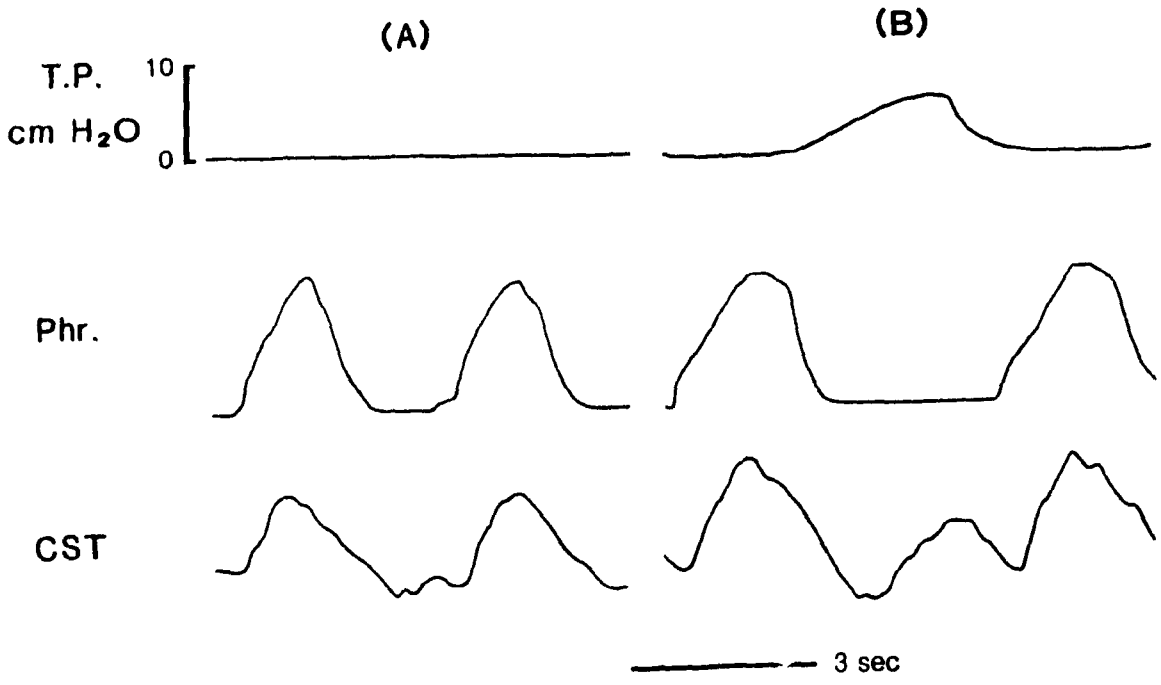
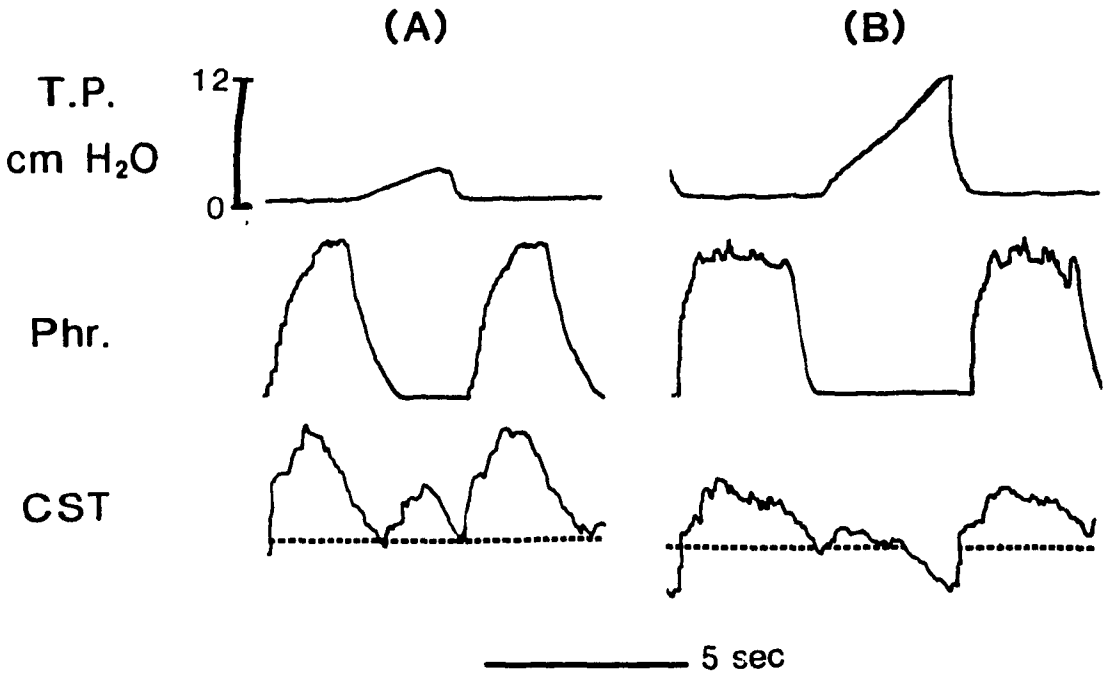


Fig. 6 The effect of lung inflation in expiration on cervical sympathetic trunk activity in this phase depends on lung volume. From above, tracheal pressure, integrated phrenic and cervical sympathetic trunk activities (average of 10 sweeps). Lung inflation triggered by phrenic burst. Dotted line marks the level of cervical sympathetic trunk activity at a value of systemic hypocapnia (end-tidal P_{CO_2} 15 mmHg) at which phrenic nerve activity was absent. A: lung inflation with low tidal volume (peak tracheal pressure 3.5 cm H_2O) caused an expiratory sympathetic discharge. B: lung inflation with large tidal volume (peak tracheal pressure 12 cm H_2O) produced an inhibition of cervical sympathetic trunk activity to a level lower than the hypocapnic level (i.e. below the level obtained by suppression of rhythmic respiratory activity). The increased duration and steeper rise of the integrated phrenic record in (B) are probably the result, respectively, of the dependence of inspiratory duration on the duration of the preceding expiration (Zuperku & Hopp, 1985) and of the reflex excitatory effect of large, rapid deflations on brainstem inspiratory neurone activity (Sellick & Widdicombe, 1970).

FIG. 6



phrenic-triggered pump by triggering inflation from the offset of the phrenic burst. Inflation pressures of 5-6 cm H O were used. Fig. 5 shows the results of such an experiment. An expiration-synchronous sympathetic discharge, of shape and time course comparable to those of the expiration- synchronous sympathetic discharge shown in Fig. 2-4, was observed in 13 out of 15 cases tested. In addition, a prolongation of expiration over the control value in apnea was observed. It must be mentioned that when inflation pressures in excess of 10.2 cm H O were used, inhibition, rather than excitation, of sympathetic activity in expiration occurred (Fig. 6) as previously shown by Gootman, Feldman & Cohen (1980). When the animal was ventilated with peak tracheal pressure of 7 cm H O at various repetition rates, such that the inflation occurred entirely in inspiration (Fig. 7A), in late inspiration and early expiration (Fig. 7B) or entirely during expiration (Fig. 7C), marked expiration-synchronous discharge was only observed when inflation was entirely in expiration. This discharge, as well as the phase-locking between respiratory and pump cycle, disappeared after bilateral cervical vagotomy. When central rhythmic respiratory activity was abolished by hyperventilation in air (end-tidal P_{CO} 15 mm Hg) lung inflation at the same repetition rate and with the same peak tracheal pressure caused a small depression of sympathetic discharge.

Fig. 7 Effect of lung inflation during various phases of the central respiratory cycle on cervical sympathetic trunk activity in expiration. Stable 1:1 locking of the lung inflation cycle to various phases of the respiratory cycle was produced by changing the frequency of the respiratory pump. From above, tracheal pressure, integrated phrenic nerve and cervical sympathetic trunk activities (averages of 12 sweeps). A: lung inflation is synchronous with the phrenic nerve discharge. Sympathetic activity in expiration is of the tonic type and at a low level. B: lung inflation occurs during late inspiration and early expiration. Cervical sympathetic trunk discharge in expiration similar to A. C: lung inflation is entirely in expiration. A marked expiration synchronous sympathetic discharge appears (absent when respiratory pump was turned off). Same explanation as in legend fig. 6 for the difference in phrenic burst shape between panel B and C. The difference in phrenic burst shape between panel A and B is likely due to the different phase relation of lung inflation to phrenic burst.

FIG. 7

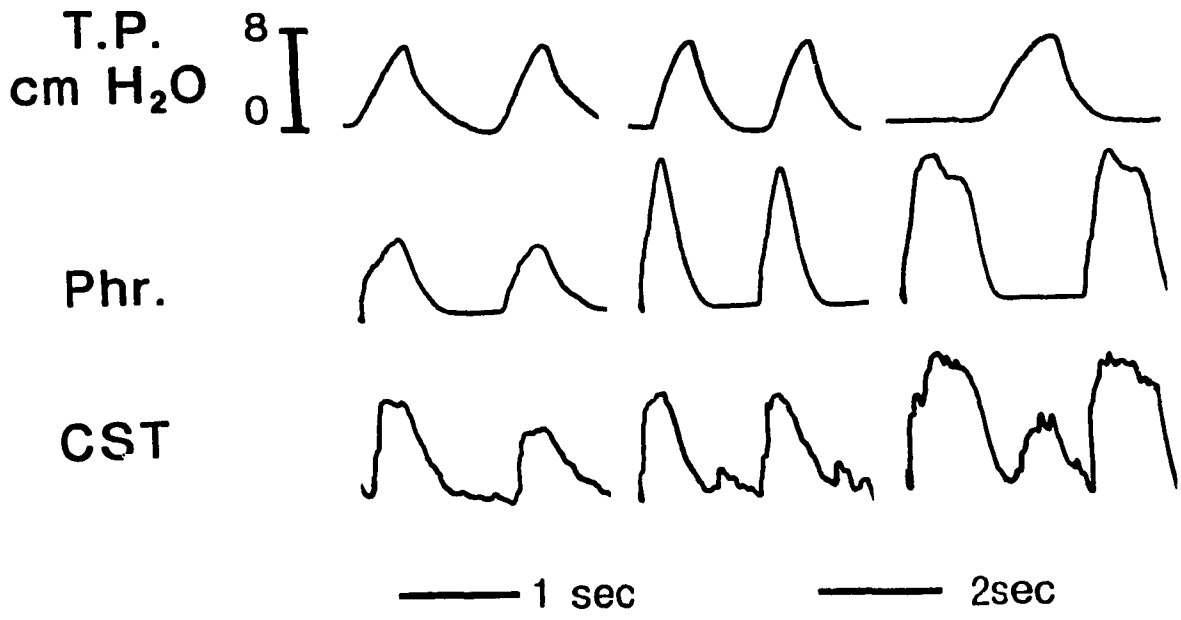
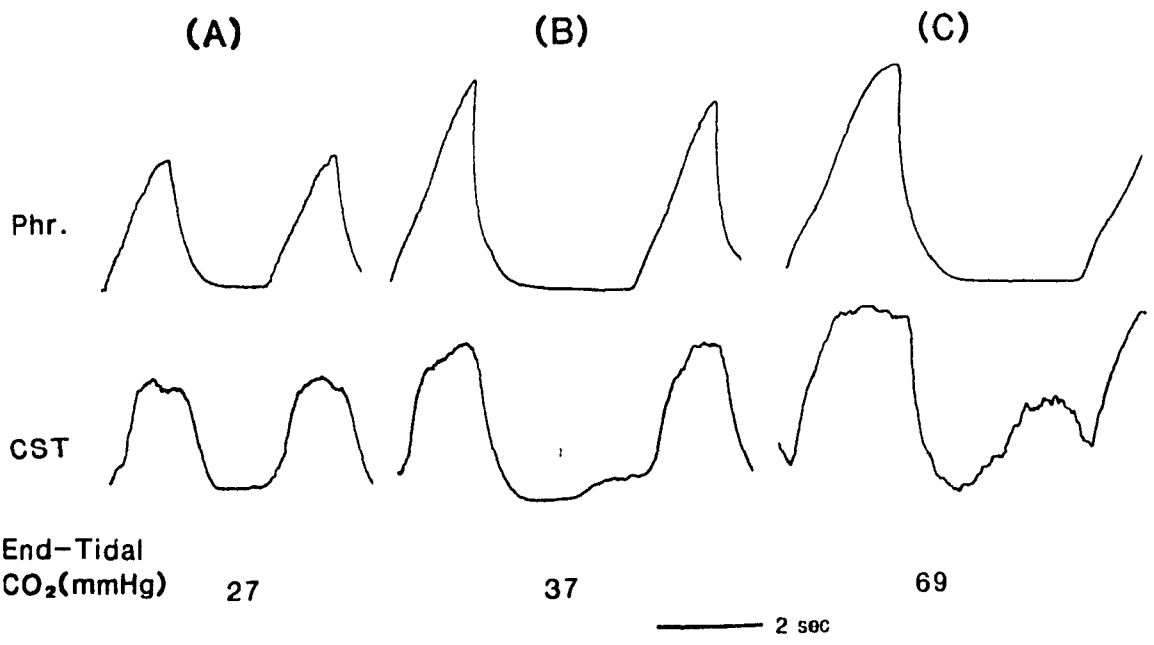


Fig. 8 Effect of systemic hypercapnia on the discharge of the cervical sympathetic trunk in expiration. Decerebrate sino-aortic denervated, vagotomized cat. From above, integrated phrenic nerve and cervical sympathetic trunk activities (average of 20 sweeps). A: in normocapnia, cervical sympathetic trunk activity in expiration is of the tonic type. During progressive hypercapnia, the level of cervical sympathetic trunk activity in expiration increases in B and assumes a wave-like shape at the highest end-tidal P_{CO_2} values (C). Slowing of the central respiratory rhythm in hypercapnia (as in panels B & C) in a similar preparation has been described before (St. John, 1979).

FIG. 8



e) Pattern of CST Activity in Expiration During Systemic Hypercapnia

In eleven vagotomized, sino-aortic denervated cats, the pattern of cervical sympathetic trunk activity was studied at various levels of arterial P_{CO_2}. Records from such an experiment in a decerebrate cat are shown in Fig. 8. With increasing CO_2 levels, in addition to the progressive increase of the peak amplitude of the inspiration-synchronous component of sympathetic discharge, previously described (Preiss & Polosa, 1977), there was also a progressive increase of the discharge in expiration, which had a wave-like appearance as in the Fig. 2-7.

DISCUSSION

This paper reports the observation that the discharge, recorded in the cervical sympathetic trunk, has a component, time-locked to the respiratory cycle, which occurs in expiration. This component is detected infrequently in control conditions but is consistently observed during moderate increases in FRC and during hypercapnia.

Concerning the mechanism by which the increase in FRC evokes the expiratory sympathetic discharge, the range of effective tracheal pressures and the abolition of this discharge by bilateral cervical vagotomy suggest that these sympatho-excitatory effects of increased FRC are mediated by pulmonary stretch receptors (Sant'Ambrogio, 1982). The fact that systemic hypocapnia, which

has negligible influence on pulmonary stretch receptor discharge (Kunz, Kawashiro & Scheid, 1976; Bradley, Noble & Trenchard, 1976), eliminates the expiratory sympathetic discharge caused by an increase in FRC, suggests that not only the discharge of pulmonary stretch receptors, but also a certain level of activity of brainstem respiratory neurones is necessary for the increase in FRC to cause the expiration-synchronous sympathetic discharge. The observation of rhythmic expiratory sympathetic discharge during static lung inflation in normocapnic animals with intact vagus nerves shows that the facilitation of sympathetic discharge in expiration caused by an increase in lung volume is locked to the central respiratory cycle rather than to the pump cycle. This set of observations suggests the inference that the expiratory sympathetic discharge results from the activity of neurones, with the rhythmicity of respiration, which are activated by sensory input from the lungs (presumably originating from pulmonary stretch receptors) and, in addition, by CO_2.

A number of analogies between the properties of the expiratory sympathetic discharge on one hand and the properties of the activity of expiratory motoneurones and brainstem expiratory neurones on the other suggest the brainstem expiratory neurones as the source of the expiratory sympathetic discharge. Expiratory alpha-motoneurones show little or no activity during normal ventilation (Sears, 1964; Bishop, 1967). This is consistent with the low level of sympathetic activity during expiration in

normocapnia (see results). Sympathetic activity increases with moderate increases in FRC which have been shown to recruit expiratory motoneurones (Bishop, 1967; Russell & Bishop, 1976; Dimarco, Dimarco, Stroml & Altose, 1984; the present study). At large values of FRC, which cause inhibition of expiratory motoneurons (Sommer, Feldman & Cohen, 1979), expiration-synchronous sympathetic discharge was never observed. In hypocapnia, expiratory motoneurones lose their rhythmicity and at very low values of arterial PCO they become silent (Bainton et al., 1978; Bainton & Kirkwood, 1979). In this condition, the excitatory effect of moderate lung inflation on these neurones is lost (Barillot & Dussardier 1976) as it is on the sympathetic preganglionic neurones (see results). Brainstem expiratory neurones show responses similar to those of expiratory alpha motoneurones. During lung inflation above FRC brainstem expiratory neurones show facilitation at transpulmonary pressures in the normal tidal range or slightly above it (Cohen 1969; Bianchi & Barillot, 1975; Feldman & Cohen 1978; Baker, Frazier, Hanley & Zechman, 1979) and inhibition at transpulmonary pressures markedly higher than normal (Koepchen, Klussendorf & Phillip, 1970; Bianchi & Barillot, 1975; Cohen, Sommer & Feldman, 1982). The firing rate and burst duration of these neurones increase during systemic hypercapnia (Cohen 1968).

As stated above, the vagal afferents evoking the expiration-synchronous sympathetic discharge during the experiments

of increased FRC and of inflation in expiration are probably those associated with the low threshold, slowly adapting pulmonary stretch receptors which cause the Hering-Breuer expiratory-facilitatory reflex (Fishman, Phillipson & Nadel, 1973; Farber, 1982). These maneuvers caused shortening of the phrenic nerve discharge and prolongation of expiration (see results; also Fig. 3-7) consistent with the hypothesis that they were evoking the Hering-Breuer reflex (Bartoli et al., 1973).

The present results that large inflations in expiration (tracheal pressure in excess of 10 cm H_2O) cause sympathetic inhibition are consistent with those of Gootman et al. (1980). Thus, lung inflation, in the present experiments, had a dual effect on sympathetic preganglionic neurones in normocapnic cats with intact vagus nerves: it caused facilitation at low transpulmonary pressures, and inhibition at higher transpulmonary pressures. This dual effect can be explained with the hypothesis that the excitation, caused by inflation in expiration, is probably secondary to activation of expiratory neurones, as discussed above, whereas the inhibition is due, at least in part, to a primary reflex effect, independent of the respiratory neurones, since the inhibition is present in hypocapnia.

The observation, described in the present paper, that lung inflation may cause excitation of sympathetic neurones, leads to the prediction that under appropriate conditions, namely at some inflation volumes, the expiration-synchronous excitation may

outweigh the depressant effect, with the net result that the activity level of these neurones may increase during lung inflation. Some observations in the literature (Hainsworth 1974) are consistent with this prediction. In the dog static inflation of an innervated lung, while the other lung was denervated and ventilated, caused a small but significant increase in hindlimb vascular resistance at tracheal pressure values of 5-20 cm H_2O, while at higher tracheal pressures (20-40 cm H_2O) hindlimb vascular resistance decreased. These data show, therefore, a reversal of the effects of lung inflation, reminiscent of the reversal observed in some of the present experiments (e.g. Fig. 2D & 6B). It must be pointed out, however, that no such reversal was obtained in the experiments of Daly et al. (1967) who found in dogs that lung inflation with transpulmonary pressures up to 7 cm H_2O (estimated tracheal pressure 11 cm H O, Spells, 1970) had no effect on hindlimb vascular resistance, while lung inflation with transpulmonary pressures in excess of 7 cm H O caused vasodilation. While the mechanism of this reversal has not been investigated yet, the observation that in order to block the reflex vasodilator effect of large lung inflations requires vagal cooling to 1°C (Daly et al., 1967), while it is known that the vagal afferents causing the Hering-Breuer reflex are blocked at between 8 and 5°C (Fishman et al., 1973) suggests that receptors associated with unmyelinated afferents could be responsible for the sympatho-inhibitory effect of lung hyper inflation. Pulmonary receptors associated with

unmyelinated afferents have been shown in open chest dogs to be excited by hyperinflation (threshold 7 cm H_2O transpulmonary pressure), to be non-adapting and to cause reflexly apnea, hypotension and bradycardia (Coleridge, Coleridge and Luck, 1965; Green, Schmidt, Schultz, Roberts, Coleridge & Coleridge, 1984).

Finally, in the case of the CO_2-evoked increase of sympathetic discharge in expiration, its mechanism may include, in addition to an increased facilitatory input from brainstem expiratory neurones, an increase in sympathetic preganglionic neurone excitability caused by CO_2 (Zhang, Rohlicek & Polosa, 1982) resulting in increased responsiveness to excitatory input.

ACKNOWLEDGEMENTS

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SUMMARY

1. The properties of sympathetic preganglionic neurone activity during expiration were studied in pentobarbitone-anaesthetized (n = 26) and in non-anaesthetized, midcollicular decerebrate (n = 5), paralyzed, artificially ventilated cats in which the electrical activity of the phrenic nerve and of the cervical sympathetic trunk was recorded.

2. In control conditions (end-tidal P_{CO_2} between 35 and 40 mmHg, zero end-expiratory pressure) sympathetic activity during expiration was either steady at a low level (n = 11) or showed a modest progressive increase from a low level in early expiration (n = 17). Very infrequently (n = 3), it showed a transient increase during the second half of expiration.

3. Artificial ventilation with positive end-expiratory pressures in the range from 2.1 · 0.4 (mean · S.D.) to 6.7 · 0.6 cm H_2O caused, in cats with intact vagus nerves, an increase in sympathetic neurone activity during the second half of expiration. Within this range of pressures, the magnitude of the increase was related to the magnitude of the positive end-expiratory pressure. This effect reversed at higher positive end-expiratory pressures. Pressures in excess of 10.2 · 1.8 cm H_2O caused inhibition of sympathetic activity.

4. The sympatho-excitatory effect of positive end-expiratory pressure disappeared after bilateral cervical vagotomy. With

intact vagus nerves, it also disappeared at levels of systemic hypocapnia (end-tidal P_{CO_2} 15 mm Hg) which abolished phrenic nerve activity. In hypocapnia, artificial ventilation with peak tracheal pressures greater than 7.2 ± 1.1 cm H_2O caused inhibition of sympathetic activity, while ventilation with lower end-expiratory pressures had no effect on sympathetic activity. It may be concluded that the sympatho-excitatory effect of positive end-expiratory pressure is mediated by vagal afferents and requires a certain level of brainstem respiratory neurone activity.

5. Sympatho-excitation during expiration was also observed, in normocapnic conditions, during short-duration static lung inflation with tracheal pressures in the range from 2.5 ± 0.3 to 7.0 ± 0.8 cm H_2O as well as during artificial ventilation with zero end expiratory pressure when lung inflation occurred in expiration. These responses were abolished by bilateral cervical vagotomy and during systemic hypocapnia.

6. Sympatho-excitation during expiration was also observed when systemic hypercapnia was produced in vagotomized cats by artificial ventilation with gas mixtures containing 5 or 10% CO_2.

8. These results can be explained by the hypothesis that some brainstem expiratory neurones are a source of facilitatory synaptic input to sympathetic neurones. The activity of

REFERENCES

Arndt, J.O., Pasch, U., Samodelov, L.F. and Wiebe, H. (1981). Reversible blockade of myelinated and non-myelinated cardiac afferents in cats by instillation of procaine into the pericardium. Cardiovascular Research 15, 61-67.

Bainton, C.R., Kirkwood, P.A. and Sears, T.A. (1978). On the transmission of the stimulation effects of carbon dioxide to the muscles of respiration. Journal of Physiology 280, 249-272.

Bainton, C.R. and Kirkwood, P.A. (1979). The effects of carbon dioxide on the tonic and the rhythmic discharges of respiratory bulbospinal neurones. Journal of Physiology 296, 291-314.

Bainton, C.R., Richter, D.W., Seller, H., Ballantyne, D. and Klein, J.P. (1985). Respiratory modulation of sympathetic activity. Journal of the Autonomic Nervous System 12, 77-90.

Baker, J.. Jr., Frazier, D.T., Hanley, M. and Zechman, F.W. (1979). Behavior of expiratory neurons in response to mechanical and chemical loading. Respiration Physiology 36, 337-351.

Barillot, J.C. and Dussardier, M. (1976). Activité des motoneurones laryngés expiratoires. Journal de Physiologie (Paris) 72, 311-343.

Barman, S.M. and Gebber, G.L. (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. American Journal of

Physiology 231, 1601-1607.

Bartoli, A., Bystrzycka, E., Guz, A., Jain, S.K., Noble, M.I.M. and Trenchard, D. (1973). Studies of the pulmonary vagal control of central respiratory rhythm in the absence of breathing movements. Journal of Physiology 230, 449-465.

Bianchi, A.L. and Barillot, J.C. (1975). Activity of medullary respiratory neurones during reflexes from the lungs in cats. Respiration Phyisology 25, 335-352.

Bishop, B. (1967). Diaphragm and abdominal muscle responses to elevated airway pressures in the cat. Journal of Applied Physiology 22, 959-965.

Bradley, A.W., Noble, M.I.M. and Trenchard, D. (1976). The direct effect on pulmonary stretch receptor discharge produced by changing lung carbon dioxide concentration in dogs on cardiopulmonary bypass and its action on breathing. Journal of Physiology 261, 359-373.

Cassidy, S.S. and Mitchell, J.H. (1981). Effects of positive pressure breathing on right and left ventricular preload and afterload. Federation Proceedings 40, 2178-2181.

Cohen, M.I. (1968). Discharge patterns of brain-stem respiratory neurons in relation to carbon dioxide tension. Journal of Neurophysiology 31, 142-155.

Cohen, M.I. (1969). Discharge patterns of brain-stem respiratory neurons during Hering-Bruer reflex evoked by lung inflation. Journal of Neurophysiology 32, 356-374.

Cohen, M.I. (1975). Phrenic and recurrent laryngeal discharge patterns and the Hering-Breuer reflex. American Journal of Physiology 228, 1489-1496.

Cohen, M.I. and Gootman, P.M. (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. American Journal of Physiology 218, 1092-1101.

Cohen, M.I., Sommer, D. and Feldman, J.L. (1982). Relation of expiratory neuron discharge to lung inflation and expiratory duration. Proceedings International Symposium: Central Neural Production of Respiratory Movements. Lake Bluff, Illinois, p. 141.

Coleridge, H.M., Coleridge, J.C.G. and Luck, J.C. (1965). Pulmonary afferent fibres of small diameter stimulated by capsaicin and by hyperinflation of the lungs. Journal of Physiology 179, 248-262.

Daly, M. de Burgh, Hazzledine, J.L. and Ungar, A. (1967). The reflex effects of alterations in lung volume on systemic vascular resistance. Journal of Physiology 188, 331-351.

Dimarco, A.F., Dimarco, M.S., Stroml, K.P. and Altose, M.D. (1984). Effects of expiratory threshold loading on thoraco abdominal motion in cats. Respiration Physiology 57, 247-257.

Farber, J.P. (1982). Pulmonary receptor discharge and expiratory muscle activity. Respiration Physiology 47, 219-229.

Feldman, J.L. and Cohen, M.I. (1978). Relation between expiratory

duration and rostral medullary expiratory neuronal discharge. Brain Research 141, 172-178.

Fishman, N.H., Phillipson, E.A. and Nadel, J.A. (1973). Effect of differential vagal cold blockade on breathing pattern in conscious dogs. Journal of Applied Physiology 34, 754-758.

Gerber, U. and Polosa, C. (1978). Effects of pulmonary stretch receptor afferent stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 56, 191-198.

Gerber, U. and Polosa, C. (1979). Some effects of superior laryngeal nerve stimulation on sympathetic preganglionic neuron firing. Canadian Journal of Physiology and Pharmacology 57, 1073-1081.

Gootman, P.M., Cohen, M.I., Piercey, M.F. and Wolotsky, P. (1975). A search for medullary neurons with activity patterns similar to those in sympathetic nerves. Brain Research 87, 395-406.

Gootman, P.M., Feldman, J.L. and Cohen, M.I. (1980). Pulmonary afferent influences on respiratory modulation of sympathetic discharge. In: Central interaction between respiratory and cardiovascular control systems. ed. Koepchen, H.P., Hilton, S.M. and Trzebski, A. pp. 172-178, Berlin, Springer.

Green, J.F., Schmidt, N.D., Schultz, H.D., Roberts, A.M., Coleridge, H.M. and Coleridge, J.C.G. (1984). Pulmonary C-fibers evoke both apnea and tachypnea of pulmonary chemoreflex. Journal of Applied Physiology 57, 562-567.

Grippi, M.A., Pack, A.I., Davies, R.O. and Fishman, A.P. (1985). Adaptation to reflex effects of prolonged lung inflation. Journal of Applied Physiology 58, 1360-1371.

Hainsworth, R. (1974). Circulatory responses from lung inflation in anesthetised dogs. American Journal of Physiology 226, 247-255.

Knox, C.K. (1973). Characteristics of inflation and deflation reflexes during expiration in the cat. Journal of Neurophysiology 36, 284-295.

Koepchen, H.P., Klussendorf, D. and Phillip, U. (1970). The discharge pattern of expiratory neurons during various states of apnea. Pflügers Archiv 319, R51.

Kunz, A.L., Kawashiro, and Scheid, P. (1976). Study of CO_2-sensitive vagal afferents in the cat lung. Respiration Physiology 27, 347-355.

Ledlie, J.F., Pack, A.I. and Fishman, A.P. (1983). Effect of hypercapnia and hypoxia on abdominal expiratory nerve activity. Journal of Applied Physiology 55, 1614-1622.

Petrillo, G.A., Glass, L. and Trippenbach, T. (1983). Phase locking of the respiratory rhythm in cats to a mechanical ventilator. Canadian Journal of Physiology and Pharmacology 61, 599-607.

Polosa, C., Gerber, U. and Schondorf, S. (1980). Central mechanisms of interaction between sympathetic preganglionic neurons and the respiratory oscillator. In: Central

interaction between respiratory and cardiovascular control systems. ed. Koepchen, H.P., Hilton, S.M. & Trzebski, A. pp. 137-142, Berlin, Springer.

Preiss, G., Kirchner, F. and Polosa, C. (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. Brain Research 87, 363-374.

Preiss, G. & Polosa, C. (1977). The relation between end-tidal CO_2 and discharge patterns of sympathetic preganglionic neurons. Brain Research 122, 255-267.

Remmers, J.E. and Gauthier (1975). Servo respirator constructed from a positive-pressure ventiltor. Journal of Applied Physiology 41, 252-255.

Russel, J.A. and Bishop, B. (1976). Vagal afferents essential for abdominal muscle activity during lung inflation in cats. Journal of Applied Physiology 41, 310-315.

Sant'Ambrogio, G. (1982). Information arising from the tracheobronchial tree of mammals. Physiological Reviews 62, 531-569.

Sears, T.A. (1964). Efferent discharge in alpha and fusimotor fibers of intercostal nerves of the cat. Journal of Physiology 174, 295-315.

Sellick, H. and Widdicombe, J.G. (1970). Vagal deflation and inflation reflexes mediated by lung irritant receptors. Quarterly Journal of Experimental Physiology 55, 153-163.

Sommer, D., Feldman, J.L. and Cohen, M.I. (1979). Responses of

caudal medullary expiratory neurons to lung inflation. Federation Proceedings 38, 1144.

Spells, K.G. (1970). Comparative studies in lung mechanics based on a survey of literature data. Respiration Physiology 8, 37-57.

St. John, W.M. (1979). An analysis of respiratory frequency alterations in vagotomized, decerebrate cats. Respiration Physiology 36, 167-186.

Vibert, J.F., Caille, D. and Segundo, J.P. (1981). Respiratory oscillator entrained by periodic vagal afferents: An experimental test of a model. Biological Cybernetics 41, 119-130.

Zhang, T.X., Rohlicek, C.V. and Polosa, C. (1982). Responses of sympathetic preganglionic neurons to systemic hypercapnia in the acute spinal cat. Journal of the Autonomic Nervous System 6, 381-389.

Zuperku, E.J. and Hopp, F.A. (1985). On the relation between expiratory duration and subsequent inspiratory duration. Journal of Applied Physiology 58, 419-430.

CHAPTER VI. GENERAL DISCUSSION

1. SUMMARY OF EXPERIMENTAL FINDINGS

The work presented in this thesis explores the properties of two components of sympathetic neuron discharge which are time-locked to the central respiratory cycle but independent of the ventilation cycle and are therefore presumably generated by mechanisms operating within the CNS. Three of the papers concern the inspiration-synchronous burst of sympathetic discharge, the fourth is about a previously unknown, late expiration (E), component of sympathetic discharge.

Paper #1, published in Journal of Physiology (London) 385: 545-564, 1987, describes some properties of the inspiration-related sympathetic discharge (IRSD) and explores the basis of the relationship between sympathetic discharge and phrenic nerve discharge (PND). The IRSD was recorded in the whole cervical sympathetic trunk (CST) or in small CST strands containing single units. In whole nerve recording the IRSD appears as a burst of firing which is observed in all animals studied. Onset and termination of the sympathetic burst had a fixed delay, for a given set of experimental conditions, from onset and termination of the phrenic nerve burst. In contrast with the inspiratory burst of the phrenic nerve, which has a typical incrementing envelope, suggestive of a staggered recruitment of the neurons over the whole of inspiration (I) and of a progressive increase in firing frequency of the recruited neurons during I, the envelope of the

inspiratory burst of the CST is usually square-wave-like. This shape could arise from the combination of various recruitment patterns and firing frequency behaviours. Single-unit recording provides the explanation for the square-wave shape of the envelope of the inspiratory sympathetic burst: the majority of units are recruited during the initial 30% of I and, once recruited, their firing frequency stays approximately constant until the end of I. Experimental procedures, like changes in CO_2 or in anesthetic levels, which modify the level of central I activity, resulted in a roughly proportional change in the amplitude of the phrenic nerve burst and of the I burst of sympathetic firing. These data are consistent with the hypothesis that phrenic motoneurons and sympathetic preganglionic neurons (SPNs) that generate the I burst are driven by a common rhythmic input. Differences in burst shape may result from differences in the properties of the pathways delivering the rhythmic input to the two sets of neurons and/or of the neurons themselves. Interestingly, the I-burst of hypoglossal motoneurons (described by others) is similar to that of SPNs and is explained in the same way (early recruitment in I, constant firing frequency). In other experimental conditions, however, the amplitude of the burst of the phrenic nerve and CST could be quite different. One such condition is baroreceptor stimulation: the I bursting of SPNs was suppressed while the phrenic nerve bursting was only slightly depressed. This is probably the result of the strong, selective, inhibitory effect of the baroreceptor input on

SPNs. Another condition is when a phrenic burst occurs at a short interval (e.g. 0.5 sec) after a preceding one. In these conditions the I-burst of the CST is either missing or greatly attenuated. This is possibly the result of a postulated inhibitory action of early-E brainstem neurons on SPNs. The hypothesis of a common input does not require that both sets of neurons be always simultaneously active. Thus, differences in excitability of the neurons themselves or of the pathways connecting them to the rhythmic driver, as a result of the action of other synaptic inputs, may cause presence of rhythmic bursting in one set and absence in the other, as observed during baroreceptor stimulation or during spontaneous instantaneous high frequency bursting of the phrenic nerve. The hypothesis of a common rhythmic driver for both phrenic motoneurons and SPNs was further supported by experiments in which the respiratory rhythm was altered by electrical stimulation of the afferent axons in the superior laryngeal nerve (SLN), by varying the frequency of the respiratory pump in cats with intact vagus nerves in which there was entrainment of the respiratory pattern generator to the frequency of the respiratory pump and by hyperthermia-hypocapnia. These experiments showed that the IRSD had always the same behaviour as the phrenic burst, over a wide range of experimental conditions. Electrical stimulation of afferents in the SLN at various times (phases) of the respiratory cycle produced equivalent, phase-dependent, resetting patterns for both phrenic and sympathetic burst. When the phrenic nerve burst

frequency was changed between 13 and 39 cycles per minute (cpm) by changes in respiratory pump frequency, the sympathetic burst remained locked in a 1:1 ratio to the phrenic nerve burst with a constant delay. Similar results (1:1 ratio and constant delay between phrenic and sympathetic burst) were obtained when the phrenic burst frequency increased from 15 to 300 cpm by increasing body temperature to 42 C° in hypocapnia.

Paper #2, published in the monograph: Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms, pp. 189-202, 1987, analyzed some of the experimental data presented in paper #1 for evidence of coupled oscillator mechanisms in the generation of the respiratory rhythm of sympathetic discharge. The justification for undertaking this analysis is the proposal, made by some authors in the absence of an appropriate experimental basis, that the respiratory rhythm of sympathetic discharge results from intrinsic rhythmicity of neuron networks specifically antecedent to the SPN which, under most experimental conditions, becomes entrained to the rhythm of activity of brainstem respiratory neurons. The proponents of this hypothesis seem not to be aware of the fact that the hypothesis is testable. The system made of the hypothetical specific antecedent circuitry to the SPN and the respiratory pattern generator (RPG) may be considered, according to this hypothesis, analogous to an oscillator (the rhythmic circuitry antecedent to the SPN) which receives rhythmic input from an external source (the RPG). Such a system is referred

to as a coupled oscillator system. From a survey of theoretical and experimental literature it is clear that the dynamics of coupled oscillator behaviour is predictable and is remarkably similar over a wide variety of biological oscillator types. Three features of the steady-state behaviour of an oscillator acted upon by a rhythmic input are of diagnostic value and can be used as criteria to define a biological system as a coupled oscillator system: i) the oscillator can be stably entrained in a one-to-one ratio to the frequency of the driving input over a narrow range of frequencies only, ii) at each frequency within the one-to-one entrainment range the input occurs at a unique phase of the cycle, i.e. the time of occurrence (phase) of the input during the oscillator cycle is a function of frequency, and iii) when the frequency of the driving input exceeds the limits of the one-to-one range, entrainment patterns involving small integer ratios are established between the frequency of the input and the frequency of the driven oscillator. The experimental data obtained when the phrenic burst frequency was changed by changing the respiratory pump frequency or by hyperthermia-hypocapnia and presented in the preceding paper were examined for these criteria. The data did not meet any of these criteria. In fact, phrenic and inspiration-related sympathetic burst maintained a one-to-one relation over all frequencies tested. In addition, the delay between the two bursts was independent of frequency. Thus, the data are inconsistent with the behaviour expected from a system of

coupled oscillators. Instead, the results are compatible with the hypothesis of a common oscillator which drives both the phrenic and the sympathetic neurons.

Paper #3, published in Journal of Physiology (London) 364: 183-198, 1985, describes an estimate of the contribution of the inspiration-synchronous component of SPN discharge to neurogenic vasoconstrictor tone. The experimental approach consisted of comparing tone before and after "ablation" of this component of sympathetic discharge. Ablation was obtained by exploiting the I-suppressing effect of a group of low threshold afferent axons in the SLN. Estimates of changes in neurogenic vasoconstrictor tone were obtained by recording, in the cat, perfusion pressure of an innervated hindlimb perfused at a constant flow rate. At constant flow, perfusion pressure is a measure of vascular resistance of the hindlimb. The electrical activity of the CST and phrenic nerve (PN) was also recorded. The main finding of this study is that when the SLN is stimulated with a train of stimuli of intensity, frequency and duration just sufficient to suppress phrenic nerve discharge for 20-30 s, the IRSD is also suppressed and vasodilation of the hindlimb is observed. The vasodilation is neurogenic and is abolished by nicotinic block with hexamethonium or by the alpha-adrenergic blocker phentolamine. The vasodilation evoked by SLN stimulation was graded by patterning the SLN stimulus in such a way that it did not suppress the phrenic nerve burst but produced only premature termination of it for several consecutive cycles.

By adjusting the timing of the stimulus train in the respiratory cycle it is possible to grade the extent of shortening of the phrenic burst. The effects on the IRSD were always parallel to those on the phrenic burst. Under these conditions, the magnitude of the vasodilation increased in relation to the extent of stimulus-induced shortening of the phrenic nerve burst. Stimulation in late I or early E, which had no effect on the phrenic burst, had no effect on the IRSD or on hindlimb vascular resistance (HLVR). At low CO_2 levels, at which phrenic burst and IRSD are absent, SLN stimulation produced either no change in sympathetic discharge and HLVR or an increase in both. These results can be explained with the hypothesis that the vasodilator reflex, evoked by the inspiration-suppressing afferents in the SLN, results from selective suppression of the excitatory input which causes the IRSD. This explanation is based on the just reviewed observations that in the absence of IRSD (e.g in hypocapnia) SLN stimulation caused no vasodilation and that vasodilation is some how in proportion to the SLN effects on the IRSD. The magnitude of the SLN-evoked hindlimb vasodilation can therefore be taken as an indication of the extent of control of HLVR exerted by the IRSD. The ratio of the maximum SLN-evoked vasodilation to the maximum vasodilation resulting from ganglionic block (the latter an indication of total neurogenic tone) provides an estimate of that fraction of neurogenic vasoconstrictor tone of the hindlimb that is due to the IRSD. In normocapnic, normoxic conditions this

component accounted for 24% of hindlimb vascular resistance.

Paper #4 published in Journal of Physiology (London) 378: 375-390, 1986, describes the discovery of another component of sympathetic discharge which is locked to the respiratory cycle. The initial observation was that in some cats, under control conditions, sympathetic activity recorded in the CST shows a transient increase during the second half of E. In these animals, then, there were two sympathetic bursts for each respiratory cycle, one in I, the other in late E. The late expiratory burst was always smaller than that associated with inspiration. Manoeuvres which are known to increase the level of activity of late E brainstem neurons resulted in the appearance of a late expiratory burst in all the cats tested. Artificial ventilation with positive end-expiratory pressure (PEEP) in the range from 2.1 to 6.7 cm H_2O, with intact vagus nerve, caused the appearance of a late E burst. Within this range, late-E burst amplitude was related to magnitude of PEEP. With higher values of PEEP sympathetic activity was inhibited. The late-E sympathoexcitatory effect of PEEP disappeared after bilateral vagotomy and, with intact vagus nerve, when the arterial PCO_2 was lowered by hyperventilation in air to a level at which phrenic nerve activity was abolished. It seems, therefore, that the late E excitatory effect of PEEP on SPNs is mediated by vagal afferents and requires a normal level of brainstem respiratory neuron activity. A late E burst was also observed in normocapnia, during static lung inflation for the

duration of 2-3 respiratory cycles with pressures in the 2-7 cm H_2O range, while the respiration pump was off, as well as during artificial ventilation with zero end-expiratory pressure when inflation was made to occur in E. These responses, too, were abolished by bilateral cervical vagotomy and during systemic hypocapnia. A late E burst of SPN firing was also observed in all vagotomized cats made hypercapnic by ventilation with gas mixtures containing 5-10% CO_2. Since it is known that the activity level of brainstem late E neurons is enhanced by moderate degrees of lung inflation and by increased chemical drive, and since in these conditions a late E burst appears in the discharge of SPNs, it may be concluded that late E neurons are a source of excitatory input to the SPN.

2. PHYSIOLOGICAL SIGNIFICANCE OF THE RESPIRATORY MODULATION OF SYMPATHETIC FIRING

a) Optimization of O_2 Delivery?

It may be argued that since the function of the respiratory and cardiovascular systems is to meet the metabolic needs of tissue, central coupling between brainstem neurons controlling respiration and the cardiovascular system may help coordinate the actions of the two systems. This proposition, at least as far as

mammals are concerned, is not entirely logical. Many of the circulatory variables which are involved in controlling the delivery of O_2 to tissue are not under CNS control: for example, the decrease of vascular resistance associated with increased metabolism, the O_2-extraction, the ventricular stroke volume (in part). On the other hand, ventilation is entirely under CNS control. Thus, the central coupling would only have a minor influence on the cardiovascular steps involved in O_2 transport. A striking demonstration of the fact that O_2 transport is relatively independent not only of any synchronization between sympathetic and respiratory neuron activity but even of sympathetic activity in general is provided by the experimental finding that cardiac denervation in greyhounds results in only modest reductions in maximal aerobic work rates and maximal cardiac output values (Donald et al., 1963, 1968). Another point to be mentioned in this regard is the large difference between the delay associated with activation of respiratory muscles, which is relatively short, and that associated with activation of cardiovascular effectors, which is relatively long. Thus, synchronous activity of the neurones controlling the two systems results in asynchronous activation of the two sets of peripheral effectors. It is possible, on the other hand, to argue for the usefulness of the coordination between heart rate and ventilation in fish (Satchell, 1960; Shelton and Randall,

1962). In both elasmobranchs (e.g. dogfish) and teleosts (e.g. trout) the only neural mechanism of sinus rate control is a vagal inhibitory action which can increase or decrease heart rate (Taylor and Butler 1982). There is no evidence for any role in the control of heart rate of adrenergic nerve fibers (Short et al. 1977). Ventilation (i.e. flow of water over the gills) is produced by closing of the mouth and generating a positive orobranchial pressure (this is equivalent to inspiration in mammals). In dogfish (and fish in general), under resting conditions, the heart beats at the same frequency as ventilation. Satchell (1960) reported that a heart beat was three times more likely to occur during the closed mouth phase than during the open mouth phase of the ventilatory cycle. This coordination is the result of a periodic inhibition of the parasympathetic cardiac inhibitory neurons, which allows the sinus rate to accelerate to its intrinsic rate. The inhibition of parasympathetic cardiac neurons is presumably mediated by the same neural network which generates the mechanical act of ventilation. Due to the intermittency of the water flow over the gills, this coordination ensures that blood flow is maximum when water flow is maximum. This matching of blood and water flow is likely to increase the effectiveness of respiratory gas exchange across the gill epithelium. This may be particularly important in aquatic animals due to the high energy expenditure involved in irrigating the gills (Shelton and Randall, 1962). The coupling between respiratory neurons and some autonomic

neurons may therefore be interpreted as a mechanism with a definite metabolic function in some species of fish, which has been retained through evolution in higher species like the mammals even though a case for a similar function as in fish cannot be made.

b) Brainstem Respiratory Neurons Contribute to the Synaptic Activity That Underlies the Background Firing of SPNs

It is possible to take the view that the synchronization of PNA and IRSD is a trivial consequence of the fact that inspiratory brainstem neurons are connected to the SPN or to antecedent facilitatory neurons. The fact that the signals carried by this pathway are rhythmic and hence impose a rhythm on SPN firing may not be important. What may be important is the mean level of excitatory drive this input provides to the SPN population. An early single unit study of SPNs projecting to the CST (Preiss et al., 1975) showed that when the respiratory drive was decreased by lowering $PaCO_2$ all SPNs, the activity of which consisted exclusively of an I-synchronous discharge, became silent. This effect was attributed to the decrease in drive from brainstem I neurons, and not to a decrease in excitatory chemoreceptor input directly to the SPN, because SPNs with firing unrelated to respiration were not significantly affected by this manoeuvre (Preiss and Polosa 1977). The striking difference in the response to hypocapnia of respiratory-modulated and non-modulated SPNs

suggested that the CO_2-sensitivity of SPNs, which is mainly mediated by peripheral and central chemoreceptors (Lioy et al., 1978; Hanna et al., 1979), is the result not of direct connections between chemoreceptors and SPNs, which would be unlikely to select respiratory modulated neurons only, but of the connections between chemoreceptors and brainstem respiratory neurons and between the latter and the SPN (Polosa et al., 1980). On the basis of these data and interpretations, the loss of I-synchronous sympathetic discharge in hypocapnia may be attributed, therefore, to the loss by the SPNs of the excitatory drive deriving from brainstem I neurons. Based on this assumption, in a study of the effect of CO_2 on single SPN firing (Preiss and Polosa, 1977) it was estimated that in normocapnia 50-60% of the firing was due to input from brainstem I neurons. Experiments in which the effect of hypocapnia on the neurogenic component of hindlimb vascular resistance (HLVR) was measured, gave a value of 30% for the neurogenic component of HLVR which can be eliminated by hypocapnia and which therefore, on the assumptions stated above, can be attributed to input from brainstem I neurons (Lioy et al., 1978). This value is close enough to the estimate of the neurogenic component of HLVR due to I-activity, obtained in the experiments of I-suppression by SLN stimulation reported in chapter 4, to suggest that both hypocapnia and SLN stimulation remove the same component of SPN discharge.

c. IRSD and Synaptic Transmission in Sympathetic Ganglia and at Neuro-effector Junctions

In addition to contributing to the mean activity level of SPNs, as discussed in the previous section, the respiratory input by virtue of the burst firing it produces may activate (or enhance) synaptic transmission mechanisms at the ganglionic synapse and at the neuro-effector junction.

i) Input-Output Relation of Sympathetic Ganglia: The Amplifying Function of the Ganglia

The level of sympathetic input to cardiovascular effectors is determined by the number of sympathetic postganglionic neurons which are firing and by their firing frequency. In turn, the level of activity in the postganglionic neuron population is determined by the number, and discharge rate, of active SPNs as well as by the input - output relation of the ganglion. The latter can be modified by a number of pre - and post - synaptic mechanisms, some of which are sensitive to the pattern of SPN firing and hence can be markedly affected by the respiratory modulation of SPN firing.

It is estimated that in CNS intact, anesthetized or decerebrate, unanesthetized cats, only 20-30% of the total population of SPN which projects to the CST are tonically active at a mean firing rate of 1.4 Hz (Polosa 1968; see also Jänig 1985).

Approximately half of these tonically active SPNs show respiratory modulation, i.e. receive input from the respiratory pattern generator (Preiss and Polosa, 1977). The most prominent feature of the respiratory- modulated activity (as discussed in chapters 1 and 2) is a burst-like discharge in I time-locked to the burst discharge of the phrenic nerve. The instantaneous discharge rate of a single SPN during the burst can be as high as 40 Hz (Bachoo and Polosa, 1987). The inspiratory burst of 2-40 spikes is followed by quiescence or low level activity during expiration such that the mean firing frequency remains relatively low.

The fraction of active neurons, and their mean firing rate, are both higher for the postganglionic than for the preganglionic sympathetic neurons. In chloralose-anesthetized cats 75-100% of postganglionic vasoconstrictor axons of the hindlimb are tonically firing at a mean frequency of 3.4 Hz, (0.5 - 5.0 Hz, Jänig 1985). Most of these postganglionc neurons ($\geq$ 90%) display a respiratory-related discharge (Jänig 1985). Similarly, in cats and rabbits anesthetized with chloralose or urethane, the majority (80%) of the superior cervical ganglion (SCG) neurons are tonically active and discharge at a mean rate of 3-4 Hz. Approximately half of these neurons display a respiratory-modulated discharge pattern (Mirgorodski and Skok, 1969).

The rate of firing of sympathetic postganglionic neurons controlling hindlimb vascular resistance under normal conditions was indirectly estimated as 3-5 Hz by the frequency of repetitive

supramaximal stimulation of the distal, cut end, of postganglionic axons required to produce the same level of vascular resistance as before section of the axons (Folkow, 1952). In anesthetized vagotomized cats, in which surgical decentralisation of the right stellate ganglion or ganglionic block of cholinergic transmission reduced heart rate to 120-140 bpm from a control value of 200 bmp, supramaximal stimulation of the right inferior cardiac nerve at 2-4 Hz is required to obtain the control heart rate of 200 bpm (Bachoo et al., 1988). A comparison of the proportion of tonically active pre- and post-ganglionic neurons and of their respective firing frequency suggests that ganglionic transmission results in amplification of the input. This is the consequence of the recruitment of a greater number of output that input neurons and of the firing of the former at higher frequencies than the latter.

ii) Organization of Sympathetic Ganglia

The amplifying properties of sympathetic ganglia may be attributed in large part to the organization of the synaptic connections between preganglionic and postganglionic neurons. There is no evidence of connections between ganglion cells which could act as a positive feedback mechanism and account for the amplification (based on 562 cell pair recordings in the guinea-pig

SCG (Purves and Lichtman, 1978). On the other hand, a number of postganglionic neurons greater than that of preganglionic neurons could account for the amplification. Anatomical data in mammals suggest that the ratio of pre- to post-ganglionic neurons is in the range of 1:10 to 1:20. For the SCG of the guinea-pig, the number of preganglionic axons that project to the ganglion, based on counts of retrogradely labelled cells in the spinal cord following horseradish peroxidase application to the cut CST, was 1600. The number of neurons in the SCG is estimated at 16,000. If each ganglion cell were innervated by a single preganglionic axon, then each preganglionic axon would contact on average 10 cells. However, SCG cells are, on average, innervated by 12 different preganglionic axons (Nja and Purves, 1977). Therefore, a preganglionic axon, on average, innervates 10 x 12 ganglion cells in the SCG. The set of ganglion cells innervated by one preganglionic axon is called a structural unit (Purves and Wigston, 1983). Thus, this pattern of innervation demonstrates considerable divergence and convergence of SPN input.

iii) Effects of the Convergence of SPN on Ganglion cells

In vivo intracellular recordings from postganglionic neurons reveal that the spikes generated by these neurons during their tonic firing are produced by fast compound EPSPs due to activation of nicotinic receptors. The ionic mechanisms underlying the fast, nicotinic EPSP have been studied in amphibian and mammalian

ganglion cells (Selyanko et al., 1979; Skok, 1986). The spontaneous fast suprathreshold EPSPs are likely due to the synchronous firing of several converging presynaptic axons while the ineffective EPSPs may be due to the firing of fewer such axons. That input from several preganglionic axons is involved in producing spontaneous firing in a sympathetic ganglion cell is indicated by the finding that the frequency of occurrence of spontaneous fast EPSPs recorded from ganglion cells decreased in a stepwise manner during sequential acute section of the various nerves that provide input to the mesenteric ganglion (Crowcroft and Szurszewski, 1971). Furthermore, with graded anodal block of the cervical sympathetic trunk, current that selectively blocked conduction in low-treshold preganglionic axons reduced the frequency of, but did not abolish, the spontaneous firing of postganglionic neurons (Mirgorodski and Skok, 1970).

Consider the case of a postganglionic neuron which, as described above, may receive synaptic contacts from 10-20 preganglionic axons of which approximately 20% i.e. 2-4 are active at any given time. Each active preganglionic axon evokes nicotinic EPSPs (of 10-30 ms duration) at a mean rate of 1-2 Hz. It is assumed that the nicotinic EPSP generated by one preganglionic axon is subthreshold (Erulkar and Woodward, 1968; Blackman and Purves, 1969; Perri et al., 1970; Sacchi and Perri, 1971; Mirgorodski and Skok, 1970; Lebedev et al., 1977), therefore threshold can only be reached by "superposition" (Blackman 1974) i.e. by spatio-temporal

summation of nicotinic EPSPs (but cf. Skok, 1983). If the time of firing of one preganglionic axon were independent of the time of firing of the others, intuitively the probability that the required number of input axons fired within the required 10-30 ms would be very low.

A possible mechanism for increasing the chance of summation is by synchronization of a large fraction of the tonically firing preganglionic neurons, as occurs during the I-related discharge of SPNs. Synchronization means that the population of SPNs firing with an inspiratory pattern will simultaneously and periodically increase their mean firing rate. Thus, the inspiration-related burst of SPN firing is likely to enhance ganglionic transmission by increasing the probability of spatial summation of nicotinic EPSPs. In additon, it has been shown that burst firing can enhance transmission by activating a number of pre- and post-synaptic ganglionic mechanisms.

iv) Presynaptic Ganglionic Mechanisms Which May be Activated by The IRSD

In anesthetized cats ventilated for 45 minutes with hypercapnic-hypoxic gas mixtures the acetylcholine content of the SCG, determined mainly by content in preganglionic axon terminals, increases by up to 33% (Birks, 1978). This manoeuvre increases inspiratory neuron activity and hence the IRD of SPNs (Preiss and

Polosa 1977). Electrical stimulation of the CST with burst stimulus patterns that mimic the respiratory burst produced a similar result, i.e. an increase in ACh content of the SCG by up to 70% over a period of 1-4 hrs., compared to stimulation for the same duration and with the same mean frequency (0.2 - 4.0 Hz) but with constant interstimulus intervals. These mean frequencies are within the spectrum of physiological SPN discharge frequencies (Bachoo and Polosa, 1987). Burst-patterned stimulation also increases ACh output by up to 3-fold over that produced by constant interval stimuli of the same mean rate (Birks 1978, 1982). Thus, a pattern of SPN stimulation which mimics the respiratory-modulated pattern of discharge of these neurons can markedly increase ACh output as well as ACh synthesis and storage.

The increase in ACh output produced by burst stimulation exerts a significant effect on transmission. Stimulation of the preganglionic input to the stellate ganglion of the cat with a burst pattern similar to that which increased ACh release from the SCG, evoked compound action potentials in the inferior cardiac nerve larger than those evoked by unpatterned stimulation of the same mean frequency (Birks et al., 1981). The potentiation of the evoked response by the burst pattern is likely due, in part, to an increase in the amplitude of the nicotinic EPSP resulting from the demonstrated increase in ACh output. Other possible mechanisms contributing to the potentiation include activation of a slow muscarinic EPSP by the increased ACh output (Shulman and Weight,

1976) and of a late-slow non-cholinergic, possibly peptidergic, EPSP (Nishi and Koketsu, 1968). Both mechanisms can produce a prolonged increase in excitability of the postganglionic neuron (Libet, 1964, Jan et al., 1980; Hartzell 1981; see below). With regard to the just mentioned non-cholinergic EPSP, over the last few years neuropeptides have become the object of investigation as putative neurotransmitters in the sympathetic nervous system. It has recently been shown that SPNs of the cat may contain up to 6 different neuropeptides (Krukoff et al., 1985). These peptides are presumably co-localised with ACh within the same nerve terminal. A role for peptides in ganglionic transmission is suggested by the analogies between the mechanism of the late-slow EPSP and that of peptide-evoked depolarization (Jan and Jan, 1982) as well as by the demonstration of peptide depletion by prolonged preganglionic stimulation (Bachoo et al., 1987). Because of the frequency-dependence of their release (Anderson et al., 1983; Dutton and Dyball, 1979; Lundberg, 1981; Lundberg et al., 1986), peptides in preganglionic axon terminals may be particularly affected by the respiratory modulation of SPN activity.

v) Post-synaptic Ganglionic Mechanisms Which May Be Activated by The IRSD

The muscarinic and the non-cholinergic mechanism of ganglionic

transmission were mentioned in the preceding section because of their possible importance in explaining the facilitation of ganglionic transmission by burst patterning, mimicking the IRSD, of the preganglionic input. Here more details about both mechanisms, in particular details concerning their frequency-dependence, are given.

a) Muscarinic mechanisms

Activation of muscarinic receptors by the released ACh may produce long-lasting depolarization of ganglion cells (North and Tokimasa, 1984; Brown and Selyanko, 1985; Percy and Krier, 1987). The depolarization is due to block of a voltage dependent K+ current (Brown and Adams, 1980; Adams and Brown, 1982). It has been shown that electrical activation of the preganglionic input to the cat stellate ganglion can evoke firing of cardioaccelerator neurons by a muscarinic mechanism (Brown, 1967; Flacke and Gillis, 1968). However, in order to demonstrate muscarinic actions activation of preganglionic axons at high frequencies is required. For instance, in the rabbit SCG and guinea-pig inferior mesenteric ganglion the lowest stimulation frequencies capable of producing a slow muscarinic EPSP were in the 8-10 Hz range (train duration 2s) (Nield, 1978; Percy and Krier, 1987). These frequencies are greater than the mean firing frequencies of tonically active SPNs but within the range of peak frequencies reached during the inspiration-related discharge (Polosa, 1968; Bachoo and Polosa,

1987). As mentioned above, cardiovascular effectors can be activated exclusively by ganglionic muscarinic mechanisms. There is evidence in the literature of reflex activation of these mechanisms. Thus, sympathetic vasoconstrictor (Freyburger et al., 1950; Brown, 1967) and cardioaccelerator neurons (Freyburger et al., 1950) can be activated in the presence of a nicotinic antagonist by cerebral ischemia, a manoeuvre which is known to produce an intense sympathetic discharge. Henderson and Ungar (1978) have shown that the reflex increase in vascular resistance in skeletal muscle observed during stimulation of the arterial chemoreceptors persists when hexamethonium at doses of 1-2 mg/kg is administered, whereas the reflex increase in resistance evoked by unloading the baroreceptors in abolished. The response to chemoreceptor stimulation is abolished by atropine. These observations can be explained by the hypothesis that peripheral chemoreceptor excitation produces an increase in sympathetic activity mostly in the form of an enhanced inspiration-related discharge which is capable of activating muscarinic mechanisms of ganglionic transmission by virtue of its high frequency components. Unloading the baroreceptors, on the other hand, would likely lead to a predominantly asynchronous increase in sympathetic activity which may lack the high frequency components required for activation of ganglionic muscarinic mechanisms. This interpretation is supported by the observations of Jänig et al., (1983) who showed that during blockade of nicotinic transmission

postganglionic muscle vasoconstrictor neurons could be reflexly activated by peripheral arterial chemoreceptor stimulation. This activation was in most cases atropine-sensitive and hence, presumably, muscarinic. In a few notable exceptions, the reflex activation of the neurons was resistant to muscarinic antagonists, suggesting that it was mediated by a non-cholinergic mechanism.

b) Non-cholinergic mechanisms

In the presence of blocking doses of nicotinic and muscarinic antagonists, activation of postganglionic neurons by preganglionic axons may still occur, but requires repetitive stimulation at even higher frequency than required for activating muscarinic transmission. We have studied non-cholinergic transmission in the cat stellate ganglion (Bachoo et al., 1988). Following brief activation (40 Hz 5-30s) of the preganglionic input the non-cholinergic mechanism can produce a persisting (10 min) afterdischarge of ganglion cells. The lowest frequency of preganglionic stimulation which could evoke a detectable cardioacceleration mediated by non-cholinergic ganglionic transmission depended on the number of activated preganglionic axons. Stimulation with supramaximal intensity of the sympathetic trunk just below T3WR, which contains a large number of preganglionic axons en route to the stellate ganglion, required 10 Hz. On the other hand, stimulation of T1WR or T2WR, which contain

a smaller number of preganglionic axons projecting to the SG, required a higher frequency. The frequency and temporal pattern of the stimulus were also critical for the activation of the non-cholinergic mechanism. A long (5 min) train of pulses at 5 Hz (1500 pulses) failed to produce cardioacceleration, whereas a brief (3.8 sec) higher frequency train (40 Hz, 152 pulses) produced cardioacceleration. A stimulus pattern mimicking the IR-discharge (40 Hz 1 sec train every 10 sec) produced a cardioacceleration while the same number of stimuli delivered at the same mean rate (4 Hz) with constant interstimulus interval failed to produce cardioacceleration. These results suggest that the inspiration-related bursting pattern of SPN firing may be important for activating this non-cholinergic mechanism of ganglionic transmission.

An important question is what is the role of this non-cholinergic mechanism of ganglionic transmission under normal conditions, when cholinergic mechanisms are intact. In the case of the heart, under normal experimental conditions block of nicotinic transmission with the appropriate dose of antagonists eliminates all neurogenic cardioaccelerator tone, as shown by the observation that subsequent bilateral stellectomy produces no further drop in heart rate (Bachoo and Polosa, unpublished observations). Thus, ganglionic transmission of the background SPN activity requires the nicotinic mechanism and, under the same conditions, the non-cholinergic mechanism, on its own, is inadequate to fire the

ganglion cells. A possible role for the non-cholinergic mechanism, under these conditions, is to enhance the efficacy of nicotinic transmission by increasing the excitability of the post-ganglionic neuron, i.e. to modulate nicotinic transmission. We tested this possibility in experiments in the cat in which we studied the effect of stimulation with a short-train (10-40 Hz for 1 s) of one set of preganglionic inputs to the decentralized stellate ganglion on the firing of a pool of ganglion neurons which were activated by single shock stimulation of a converging input via a nicotinic mechanism. The conditioning train produced a long-lasting facilitation (Bachoo & Polosa, unpublished observations). This heterosynaptic facilitation persists during muscarinic and partial nicotinic block, hence is mediated by a non-cholinergic mechanism. The facilitation is not modified by blockers of adrenoceptors or dopamine receptors. Evidence of heterosynaptic facilitation of nicotinic transmission is also provided by the observations of Blumberg and Janig (1983). They showed that the background activity of postganglionic neurons supplying the cat hindlimb was enhanced for periods of up to 40 min following a short train of repetitive stimulation of the peripheral cut end of a white ramus which provides part of the input to the ganglion where the neuron cell bodies are situated. In the cat stellate ganglion in vitro, Mochida and Libet (1985) described a longlasting facilitation of muscarinic responses following conditioning by heterosynaptic input. This facilitation was not mediated by a cholinergic

mechanism. Thus both nicotinic and muscarinic mechanism can be heterosynaptically facilitated by a non-cholinergic mechanism following a brief high frequency stimulation of SPN input.

In addition to influencing the excitability of postganglionic neurons, the inspiration-related burst of SPNs may also be important in the regulation of catecholamine synthesis in postganglionic neurons. Ip and Zigmond (1984) showed that in the rat SCG, preganglionic nerve stimulation at 10 Hz for 30 min resulted in a 5-fold increase in DOPA synthesis, suggesting that tyrosine hydroxylase (TH) activity, the rate-limiting step in NA synthesis, had increased. Hexamethonium and atropine reduced this increase by only 50%, suggesting that the action on TH was mediated in part by a non-cholinergic mechanism. If however, preganglionic nerve stimulation was performed with a burst pattern (10 Hz, 1 sec every 6s for 30 mins, i.e. 6 times fewer pulses than in the case of 10 Hz for 30 min) DOPA synthesis was 3 times greater than with continuous stimulation. Furthermore, under these conditions, the increase in TH activity was not reduced by nicotinic and muscarinic antagonists, which indicates that burst-pattern stimulation activated DOPA-synthesis entirely by a non-cholinergic mechanism. Thus the relative importance of cholinergic and non-cholinergic transmission in the transynaptic regulation of TH activity may vary with the pattern of SPN firing.

(vi) Cellular Mechanisms of Presynaptic Effects Produced in Sympathetic Ganglia by High Frequency Bursts

At many synapses the release of transmitter by the axon terminal is facilitated by previous impulse activity in the same axon, especially if activity is of the burst type (Larrabee and Bronk, 1947; Del-Castillo and Katz, 1954; Brimble et al., 1972; Bliss and Lomo, 1973). The transient increase in intracellular Ca^{2+} concentration associated with the action potential, and which is required for exocytosis of transmitter, may persist for several tens of milliseconds (Magleby and Zengel, 1975a,b; Erulkar and Rahamimoff, 1978). Summation of consecutive Ca^{2+} transients is thought to be responsible for the facilitation of transmitter release which is observed when the nerve terminal is invaded by an action potential at a short interval after a preceding one. Such a mechanism has been proposed as the explanation for the phenomenon of post-tetanic potentiation (PTP), which has been particularly well studied in sympathetic ganglia, at the vertebrate neuromuscular junction and in Ia afferent nerve terminals. This phenomenon of facilitation restricted to the tetanized input (homosynaptic) may have more than one component. Following a brief (10 sec) conditioning tetanic (5-10 Hz) stimulation of the preganglionic input to a sympathetic ganglion, the homosynaptic facilitation of synaptic transmission demonstrates two phases of

decay, a fast phase, prominent with shorter trains and a slow phase, prominent with repeated bursts or longer trains (Zengel et al., 1980). Lev-Tov and Rahaminoff (1980) suggest that the quickly decaying phase of facilitation may be due to the above described transient accumulation of Ca^{2+} ions and should be labeled PTP. The slowly-decaying phase, they suggest, may be due to accumulation of Na^{+} ions in the nerve terminals and should be labeled long term facilitation (LTF). Birks and Cohen (1968a,b) demonstrated that miniature EPP frequency, and by inference ACh output, increased at the frog neuromuscular junction under conditions that produced Na^{+} accumulation (e.g. pharmacologically blocking the Na^{+}-pump) in the nerve terminals.

An accumulation of Na^{+} ions inside the nerve terminals during and after a high frequency burst of firing is thought to enhance the release of neurotransmitter through an increase in intracellular Ca^{2+}. How does the increase in intracellular Na^{+} lead to a rise in free intracellular Ca^{2+}, which must be the ultimate cause of the increased frequency of m.epp's and potentiation of the epp? Blaustein (1976) demonstrated that in the squid giant axon an exchange reaction occurs across the cell membrane that exchanges internal Ca^{2+} for external Na^{+}. The stoichiometry for this reaction is not clear, ratios of 1:3 or 1:4 have been suggested (Blaustein, 1976). It is assumed that the Na^{+}-Ca^{2+} exchange is mediated by a carrier molecule which shuttles back and forth across the membrane whenever the internal and

external binding sites are occupied by Ca^{2+} and Na^{+}, respectively. The energy for this Na^{+}-Ca^{2+} pump comes from the Na^{+} electrochemical gradient maintained by the ATP-dependent Na^{+}-K^{+} pump. In principle, the Na^{+}-Ca^{2+} pump can not only exchange internal Ca^{2+} for external Na^{+} but also external Ca^{2+} for internal Na^{+}. This latter exchange could account for the well known ouabain-insensitive Na^{+} efflux which is markedly reduced when the external Ca^{2+} concentration is zero (Blaustein, 1976). Which ion (Na^{+} or Ca^{2+}) is exchanged depends on the concentration of Ca^{2+} and Na^{+} on each side of the membrane and the relative affinities. Thus under conditions when intracellular Na^{+} is high, following repetitive stimulation, internal Na^{+} is exchanged for external Ca^{+2}. Similar exchange reactions can also take place across mitochondria (Blaustein et al., 1980). A 35 Hz 5 sec train increases internal Na^{+} concentration by 10 mM and Ca concentration by a factor of 4 over the resting levels (Birks, 1985).

Birks (1985) has shown that choline uptake by a high affinity transporter in the axon terminal membrane is the rate-limiting step for ACh synthesis in sympathetic ganglia and that this transport process is intimately linked to the activity of the Na^{+}-K^{+} pump i.e. depression and activation of the Na^{+}-K^{+} pump is associated with depression and enhancement, respectively, of choline uptake. This has led Birks (1985) to suggest that the influx of Na^{+} ions with the action potential not only gives rise to an increase in

intracellular Ca^{2+} through the Na^{+}-Ca^{2+} exchange mechanism, but may also be important in controlling ACh synthesis by its effect on choline uptake.

Redman and Walmsley (1983) have shown that some boutons of Ia afferents which synapse on motoneurons have a very low probability of release when the axons are stimulated at low frequencies but can be recruited to release transmitter by a high frequency burst. Wojtowicz and Atwood (1985, 1986) have described an analogous phenomenon at the crayfish neuromuscular junction. This neuromuscular junction is made of as many as 25-50 morphologically defined synapses distributed along a 5-10 μm length of axon terminal. However, a surface focal electrode which can record from the whole length of the neuromuscular junction detects transmission from far fewer synapses at low frequencies of stimulation. This led Wojtowicz and Atwood (1985, 1986) to conclude that relatively few release sites contribute to the epp at low stimulation frequencies. Following a high frequency train of stimuli, the quantal content of the epp increased presumably as a consequence of an increase in the number of boutons which were invaded by the action potential and released transmitter. A similar situation exists in adrenergic nerve varicosities where the probability of release of noradrenaline from a particular release site is very low but can be increased by a previous tetanus (Cunnane and Stjarne, 1984). Birks and Isacoff (1988) suggest that a similar situation may exist in the rat superior cervical ganglion where burst-pattern

stimulation, resembling the IRSD, produced a potentiation of nicotinic transmission by a presynaptic mechanism which involved an increase in the statistical parameter for probability of release (p). If this general picture is correct, a possible mechanism of long term potentiation is the recruitment of previously inactive synapses into the pool of synapses which release transmitter following invasion of the axon terminal by an action potential. The mechanism by which high frequency stimulation recruits the previously inactive synapses at the crayfish neuromuscular junction is unknown. One possibility is the co-release of a peptide that acts on the axon terminal to increase the probability of invasion of the boutons by the action potential.

The phenomenon of "spike broadening" i.e. increased duration of the action potential during a burst has been proposed (Holz et al., 1988) as a mechanism for allowing a greater Ca^{2+} influx in neurons showing this feature (hypothalamic neurons, others) and thereby for facilitating transmitter release. The basis of spike broadening is inactivation of K^{+}-channels responsible for spike repolarization. However, since no electrical recording from preganglionic axon terminals is available, there is no evidence of whether or not such phenomenon occurs in ganglia.

vii) Effect of Burst Patterning of Postganglionic Neuron Activity on Neuro-effector Transmission

Burst patterning of postganglionic neuron activity also has an enhancing effect on transmission to the effectors. A number of investigators have compared transmitter release or the responses of autonomic effector cells produced by burst pattern stimulation with those produced by continuous stimulation at the same mean frequency. Nilsson et al (1985) have reported that larger vasoconstrictor responses were obtained in isolated mesenteric arteries in vitro upon stimulation with trains of irregularly spaced stimuli containing high frequency components, compared to trains of regularly spaced stimuli of the same mean frequency (2 Hz). Whether this increase in sympathetic vasoconstriction is related to enhanced release of noradrenaline or neuropeptide Y is not known. Both these agents are capable, at least in the cat spleen, of mimicking the functional responses to nerve stimulation (Lundberg et al., 1985). Release of neuropeptide Y (NPY) from the splenic nerve is potentiated compared to catecholamine release, in pigs, by burst patterned stimulation at the same mean frequency with equally spaced stimuli (Lundberg et al., 1986). Andersson et al (1983) described the response of resistance and capacitance vessels in the cat hindlimb to electrical stimulation of the lumbar sympathetic trunk. Stimulation in bursts (1.0 s duration) or at a constant frequency, at mean rates from 0.5 Hz to 16 Hz, produced

constrictor responses of similar magnitude in resistance vessels, whereas the response of capacitance vessels (measured as a change in blood volume of the vascularly isolated hindlimb muscle) was markedly greater with the burst pattern of stimulation. Similar effects were obtained with postganglionic axons innervating the SA node (unpublished data from our laboratory). In cats with decentralized right stellate ganglion, stimulation of the right inferior cardiac nerve with burst patterned stimulation over a mean frequency range from 0.5 to 4 Hz, consistently produced a 30 to 60% greater heart rate increase than that produced by continuous stimulation at the same mean frequency. It therefore appears that high frequency burst patterned discharge of postganglionic neurons increases the magnitude of cardiovascular effector response.

Lundberg et al (1986) have emphasized the importance of burst stimulation for the release of NPY from sympathetic postganglionic axon terminals. In anesthetized pigs, splenic nerve stimulation with bursts induced a greater increase in splenic vascular resistance and a 5 fold higher NPY output than continuous stimulation of the same mean frequency. Blockade of α and β adrenoceptors or depletion of releasable catecholamines failed to prevent the increase in vascular resistance evoked by burst stimulation. This suggests that the burst released a non-adrenergic constrictor agent, possibly NPY. NPY has a potent vasoconstrictor action in a number of isolated vascular preparations and potentiates the vasoconstrictor action of NA

(Lundberg et al., 1986). Fried et al (1985) showed that in sympathetic nerve terminals of the spleen NPY is stored mainly in large dense core vesicles, whereas NA is present in both large and small dense core vesicles. Continuous stimulation at low frequency releases mainly NA, while intermittent high frequency bursts preferentially enhance the release of NPY (Lundberg, 1985). Therefore, under physiological conditions, the burst-like discharge pattern of SPNs, relayed to the postganglionic neurons, may preferentially release the peptide content of the larger dense core vesicles of postganglionic axon terminals.

The amplitude of excitatory junction potentials (EJPs) generated by the release of NA at the smooth muscle-nerve junction is influenced by the pattern of action potentials arriving at the nerve terminals. A burst pattern has been shown to produce on average larger EJPs than constant interval stimuli at the same mean frequency (Cunnane and Stjarne, 1984). In fact, the efficacy of the impulses in a patterned train can be more than 50% greater than that of impulses at a constant interstimulus interval in terms of the EJP amplitude contributed by each impulse. However, the junctional potential are not solely responsible for the greater effectiveness of the patterned discharge. The slow time course of relaxation of the smooth muscle is also likely to play a role in this regard. The development of tension would be considerably less were it not for the slow time course of relaxation relative to the decay constant of the EJPs. This slow time course permits

summation of the contractions evoked by each action potential in the burst and results in a greater net contraction.

In summary, one consequence of the respiratory-modulated pattern of SPN discharge is an increase in the efficacy of synaptic transmission at ganglionic synapses and at the neuroeffector junction. This results in a more sensitive control of the cardiovascular system by the CNS.

3. ORGANIZATION OF THE RESPIRATORY RHYTHM GENERATING NETWORK

a) Generalities

As already mentioned the average contour of the SPN discharge can show several rhythmic components locked to various phases of the respiratory cycle in addition to the discharge synchronous with phrenic activity (i.e. the IRSD). These additional components are a marked depression in early expiration (Bainton et al., 1985), a facilitation in late expiration (Bainton et al., 1985; Bachoo and Polosa, 1986; Koizumi and Kollai, 1987) and a weak, short-lived depression in early inspiration (Cohen and Gootman, 1970; Gootman and Cohen, 1974; Bainton et al., 1985). The implication of this complex respiratory modulation of sympathetic activity is that several respiratory neuron types which are thought to be part of the respiratory rhythm generating network may be capable of influencing sympathetic activity, in addition to the inspiratory neurons, for which the evidence is reasonably good. It seems appropriate, therefore, to outline current thoughts on how

the respiratory rhythm is generated. This is relevant in view of the fact that the same hypothetical network is likely to generate the respiratory modulation of sympathetic activity. A number of recent reviews provide a thorough description of the work aimed at identifying the cellular elements of the rhythm-generating network and their synaptic organization (Cohen, 1979, 1981; Euler, 1983; Feldman, 1981, 1986; Long and Duffin, 1986; Merrill, 1981; Mitchell, 1977; Richter, 1982; Richter and Ballantyne, 1983). The evidence on the basis of which a neuron is classified as a member of the network is, essentially, the existence of a temporal relationship between the firing pattern of the neuron and phrenic discharge (cf. Netick and Orem 1981). A stricter criterion for a neuron to be considered a constituent of the respiratory network is that its activation or inhibition should reset the respiratory rhythm (see Pinsker and Ayers 1983). However, in the mammalian CNS the action of a single neuron has rarely been shown to be consequential and this makes it uncertain whether the stricter criterion could be met by a real neuron. This section reviews, in brief, current notions concerning the neural network which generates the respiratory rhythm. Features of rhythm-generating neural circuits of invertebrates are also briefly reviewed, because for the invertebrates the make-up and function of some of these circuits is known, and hence these circuits provide possible models of how the respiratory pattern generator may be made and may work.

However inadequate our current concepts may be, the existence of a temporal relationship between the firing pattern of particular respiratory neurons and a particular component of sympathetic activity has led to the hypothesis that the latter may be a consequence of the former. This hypothesis does not presuppose that respiratory neurons which are thought to be modulating sympathetic activity be part of the hypothetical respiratory rhythm generating network. Indeed they may be interneurons which simply relay input from the rhythm generating network to brainstem neurons generating sympathetic activity.

It is generally agreed that the respiratory rhythm in mammals is generated in the lower brainstem (see, Feldman 1986 for further discussion, especially on the possible role of the spinal cord in rhythmogenesis). This is most dramatically illustrated by the observation that although transection of the spinal cord at high cervical level abolishes the respiratory motor outflow to the diaphragm and intercostal muscles (but cf. Aoki et al., 1980) periodic respiratory activity persists in cranial nerves VII, IX, X and XII (St. John et al., 1981). The respiratory rhythm, e.g. the burst firing of the phrenic nerve, persists after midcollicular decerebration, paralysis and vagotomy in preparations with intact brainstem-spinal cord connections, suggesting that it is generated in the medulla in the absence of rhythmic sensory feedback from the lungs. Summaries of studies which have attempted to outline the

neural circuitry of the respiratory rhythm generator based on studies of neurons displaying a respiration-related firing pattern or on neuroanatomical mapping studies can be found in the review by Long and Duffin (1986).

b) Features of the Output of the Respiratory Rhythm Generating Network

The major features of the output of the respiratory rhythm generator directed to inspiratory motoneurons are illustrated by the discharge of the phrenic nerve, which innervates the diaphragm and subserves a purely inspiratory function. The timing of the activity of brainstem inspiratory motoneurons which send their axons to the hypoglossal, glossopharyngeal, recurrent laryngeal and superior laryngeal nerves is the same as that of phrenic motoneurons, suggesting that these neurons are all driven by a common input (Fukuda and Honda, 1983).

Whole phrenic nerve activity characteristically shows an abrupt onset, followed by an augmenting discharge involving both a progressive recruitment of the motor neuron population and an increasing firing frequency (Iscoe et al., 1976). This discharge pattern is produced by a ramp-like depolarization of the phrenic motoneuron membrane (Berger, 1979) which terminates abruptly. The ramp-like depolarization is thought to be the product of the activity of a particular population of brainstem inspiratory

neurons which are therefore called ramp-inspiratory neurons (Feldman, 1986). The output of these brainstem neurons is relayed to the inspiratory motoneurons through premotor bulbospinal neurons and, in parallel, to a population of late inspiratory 'off-switch' brainstem neurons, which are thought to be responsible for terminating the firing of the ramp-generating inspiratory neurons by producing, with a delay, a powerful inhibitory shunting of their membrane (Cohen, 1979). This inhibitory action, which is short-lasting, shuts off all phrenic activity for approximately 50-100 ms and marks the end of the inspiratory phase. This phase of inhibition is followed by a period of post-inspiratory phrenic activity, characterized by abrupt onset (marking the onset of expiration) and gradual decline (i.e. end of stage I expiration, Richter and Ballantyne, 1983). Expiratory motoneurons innervating the internal intercostal and abdominal muscles are activated only after all post-inspiratory activity of phrenic motoneurons has ceased.

c) Firing Patterns of Brainstem Neurons Presumed To Be Components of the Respiratory Rhythm Generating Network

At present there is no generally accepted scheme for classifying respiratory neurons and moreover there is little indication about what might constitute a unifying classification scheme; schemes based on firing pattern, anatomy, morphology and

responses to inputs have been used. For the purpose of this discussion it seems most relevant to describe the firing pattern of respiratory neurons, their sequence of activation and their interaction. Since bulbospinal neurons are pre-motor and are not likely to be part of the pattern generator mechanism we will concentrate on the firing pattern of those respiratory neurons whose axons do not extend beyond the brainstem, i.e. propriobulbar respiratory neurons.

Medullary neurons with discharge pattern phase-locked to the phrenic discharge are mainly concentrated in two areas, the dorsal and ventral respiratory group. The 'dorsal respiratory group' (DRG) anatomically corresponds to the ventrolateral nucleus of the tractus solitarius. The 'ventral respiratory group' (VRG) encompasses the nucleus ambiguus and retroambiguus (Long and Duffin 1986). Many of these respiratory neurons send their axons to the spinal cord (bulbospinal neurons), as shown by their antidromic excitation in response to spinal cord stimulation, and function as premotor neurons, projecting to the spinal motoneurons which innervate the diaphragm, intercostal and abdominal muscles (Hilaire and Monteau, 1976; Feldman and Speck, 1983; Davies et al., 1985a,b). Whether these bulbospinal neurons are involved in generating the respiratory rhythm or act as relay neurons for a respiratory command signal they receive from antecedent neurons has not been resolved. As already mentioned, in theory the crucial

test is whether or not selective activation of these neurons alters (resets) the respiratory rhythm. The available experimental data are contradictory, since antidromic activation of these bulbospinal respiratory neurons reset the respiratory rhythm in one study (Gauthier and Monteau, 1986) but failed to reset it in another (Feldman et al., 1984). However, it must be realized that with stimulation within the CNS it is practically impossible to selectively antidromically activate a set of axons without simultaneously activating, antidromically or orthodromically, other axon sets which can synaptically influence neurons antecedent to the system under study. When dealing with single neurons it is possible to differentiate an antidromic from an orthodromic response because the former has a sharp threshold and an all-or-nothing character (Lipski, 1981). These criteria no longer apply in the case of neuron populations, which give graded responses to both orthodromic and antidromic stimulation.

Among propriobulbar inspiratory neurons, many fire throughout inspiration with an augmenting firing frequency, similar to the augmenting phrenic discharge (ramp-inspiratory neurons). Few fire with a decrementing frequency during the same phase (Cohen, 1968; Cohen and Feldman, 1984) and are recruited early in inspiration (early inspiratory neurons). Others are recruited late (late inspiratory neurons) during inspiration (Baker and Remmers, 1982; Feldman et al., 1976; Marino et al., 1981). Another population of propriobulbar respiratory neurons discharges only during expiration

(Lipski and Merrill, 1979). Among these, some fire only during the post inspiratory phase (early expiratory or post-inspiratory neurons) while others fire only during the second half of expiration (late expiratory neurons) (Ballantyne and Richter, 1982; Feldman and Cohen, 1978; Richter and Ballantyne, 1983). Expiratory neurons may fire with an augmenting or decrementing pattern or at constant frequency. Thus propriobulbar respiratory neurons can be classified (on the basis of the phase of the respiratory cycle in which they discharge) as ramp inspiratory, early inspiratory, late inspiratory, post inspiratory and late expiratory neurons. The early and late inspiratory neurons are thought to be reciprocally inhibitory and to make up the mechanism for switching inspiration off (Bradley et al., 1975; Cohen, 1979; Feldman et al., 1976; Richter and Ballantyne, 1983; Sears et al., 1982).

It is this wide variety of firing patterns which is thought to be indicative of an organized neural network in which interactions between the various types of respiratory neurons results in the generation of a rhythmic output, the respiratory rhythm. A comparison of firing patterns and membrane potential trajectories of the various propriobulbar respiratory neurons, bulbospinal respiratory neurons and respiratory motoneurons suggests a limited number of possible synaptic interactions between the various types of neurons. However, the unresolved issue remains whether or not any of the currently identified propriobulbar respiratory neurons plays a direct role in generating the respiratory rhythm. It

cannot be ruled out that these neurons are simply follower neurons downstream of as yet unidentified pattern generator neurons.

The following is a hypothetical temporal sequence, consistent with observed data, of respiratory neuron interactions that would result in a rhythmic output appropriate for generating a respiratory cycle (Euler, 1983; Richter et al., 1986). Tonic excitation of central and peripheral chemoreceptors by arterial pCO_2 leads to tonic activation of the brainstem reticular activating system which in turn excites propriobulbar inspiratory neurons. In contrast, E-neurons are weakly excited by increasing CO_2 (Sears et al., 1982). The ramp-inspiratory neurons fire with an augmenting discharge and feed this activity into bulbospinal inspiratory neurons and late inspiratory neurons. The latter neurons are silent initially, however, due to inhibitory input from early inspiratory neurons. Early inspiratory neurons, which fire with a decrementing pattern, inhibit during their discharge late inspiratory neurons, post-inspiratory neurons and late expiratory neurons. Their discharge, however, fades with time, possibly due to intrinsic membrane properties resulting in firing adaptation. When these neurons stop discharging, late inspiratory neurons are released from inhibition and discharge a short burst of action potentials, which results in a transient but powerful inhibition of bulbospinal, propriobulbar, and inspiratory motoneurons. This marks the end of the inspiratory phase of the cycle and corresponds

in time to the period of brief interruption of phrenic nerve activity (Richter's 'reversible off-switch'). At this time post-inspiratory neurons are also transiently hyperpolarized followed by a pronounced rebound excitation. Once post-inspiratory neurons start to discharge they inhibit all inspiratory and late expiratory neurons of the network (irreversible off-switch). The discharge of post-inspiratory neurons fades with time presumably due to intrinsic membrane properties (Richter and Heyde, 1975), expiratory neurons are released from inhibition, start to discharge and inhibit the inspiratory and post-inspiratory neurons of the network. What terminates late, E-neuron activity is not clear. Switching between E-I phases may occur when the E-neuron population fatigues, so that its level of activity spontaneously declines and by a process of disinhibition allows a buildup of activity in the I population. Alternatively, Feldman (1986) has described a population of very late E-neurons, which fire a brief burst of spikes before the onset of the phrenic burst and may hypothetically subserve a role equivalent to the late-inspiratory neurons.

d) Central Motor Pattern Generator Mechanisms in Simple Nervous Systems: Possible Models for Respiratory Rhythmogenesis in Mammals. The Role of Re-excitation and Reciprocal Inhibition in Rhythogenesis

Vertebrate and invertebrate animals can produce a number of

rhythmic motor patterns like those underlying walking, swimming and feeding. The neuronal circuitry generating these rhythmic motor patterns has been reasonably well identified and analyzed in a small number of invertebrate motor systems. From the analysis of these rhythmic motor pattern generators it is clear that patterned activity can be generated by neural circuits in two ways, (i) by the pacemaker properties of constituent neurons or (ii) by a set of non-pacemaker neurons connected in such a way that a regular alternation of activity and quiescence results. Until recently it was believed that pacemaker and non-pacemaker neurons are fundamentally different. Recent in vitro experimental data show that non-pacemaker neurons, for example neurons from the guinea-pig nucleus tractus solitarius (NTS) (Dekin et al., 1985) and SPNs from the cat thoracic spinal cord (Yoshimura et al., 1987) can show pacemaker properties in the presence of particular neurotransmitters. The term conditional pacemaker has been introduced to refer to these cases. Although the physiological significance of conditional pacemakers in rhythmic pattern generation is not yet clear, the notion of conditional pacemaker suggests that the distinction between the two ways of generating rhythmic motor patterns should not be considered too rigidly.

Examples of pattern generators in which the rhythm is produced by identifiable pacemaker neurons, which drive a set of other neurons eventually relaying the output to the effector cells, are neural networks which regulate the pyloric rhythm of the lobster

(Selverston and Miller, 1980) and the heart beat of the leech (Calabrese and Peterson, 1983). Both networks demonstrate a property of redundancy which seems to apply as well to the respiratory pattern generator. Redundancy is shown by the fact that after selective destruction of the pacemaker neurons, a pattern of rhythmic activity similar to that existing before continues to be produced by the remainder of neurons in the network. Presumably the post-lesion rhythm is due to the capacity of certain neurons in the network to become pacemakers when the dominant pacemakers elements of the network are removed. This phenomenon is presumably prevented, under normal conditions, by a mechanism analogous to the overdrive suppression experienced by sino-atrial cells whose intrinsic pacemaker rate is slower than the frequency of the fastest sino-atrial pacemaker cells (Vassalle, 1977). These cells assume a pacemaker role only when the faster pacemaker cells are eliminated. Several observations suggest that redundancy may also occur in the neural network generating the respiratory rhythm. In the cat brainstem, lesions of either the DRG or VRG or cold block of the Botzinger-C complex, sites which are thought to contain respiratory neurons directly involved in generating the respiratory rhythm, do not abolish the rhythm (Speck and Feldman, 1982; Budzinska et al., 1985). Complete midsaggital transection of the brainstem can give rise to what appears to be normal respiratory activity from the two half brainstems in a number of experimental animals, including rabbit, (Gromysz and

Karczewski, 1980), monkey and cat (Gromysz and Karczewski, 1982). Furthermore, rhythmic phrenic discharge, resembling the normal discharge, can be seen following transection of the spinal cord at the first cervical level (Aoki et al., 1980). The inference from these observations is that a respiratory rhythm can still be obtained after significant reduction of the amount of nervous tissue which is presumed to be generating it, that is, the respiratory pattern generator may be characterized by a similar redundancy as displayed by the generators of the pyloric rhythm in the lobster and the heart rhythm in the leech.

Examples of pattern generators in which the rhythm is produced by the interactions of non-pacemaker neurons are the generators of the swimming rhythm in the mollusks clione and tritonia. The central pattern generator (CPG) for swimming in the mollusk clione (Satterlie, 1985) is relatively simple and consists of four non-pacemaker interneurons, two of which control the parapodial upstroke and two the downstroke. These two sets of interneurons are linked by reciprocal monosynaptic inhibitory synapses. All four interneurons show post-inhibitory rebound. This simple neuronal network produces a rhythmic output in the presence of tonic input. The CPG for the swimming rhythm in the mollusk tritonia (Getting, 1983a,b) is more complex than that for clione. In Tritonia, the swim oscillator consists of two types of cells, interneurons which are premotor to the dorsal and ventral swim muscles (DSI and VSI, respectively) and other interneurons (C2)

(Getting, 1981, 1983a,b). Neurons within each group (DSI, VSI and C2) are connected by reciprocal excitatory synapses, C2-neurons are excited by VSI and inhibited by DSI. The interneurons of these 3 groups are part of the swim central pattern generator (CPG) shown by the fact that advancing or delaying their burst with intracellular current injection can reset the entire motor pattern (Getting et al., 1980; Getting, 1983b). The sequence of activation of these interneurons during the swim cycle begins with firing of the DSIs. This can be produced by tactile stimulation in-vivo or by electrical stimulation of an appropriate sensory nerve. The firing of DSIs inhibits the VSIs and attempts to excite the C2 interneurons. The C2 interneurons' membrane properties are such that there is an initial (~ 100 ms) period of rectification (A-current) which effectively delays onset of firing; C2 interneurons in turn excite the VSI, so although the VSI are being inhibited by the DSI, the C2-mediated excitatory action eventually prevails over the DSI-mediated inhibition. Once the VSI fire, they inhibit both the DSI and C2 thus terminating the DSI burst. However, with the DSI silenced the VSI no longer receive an excitation from C2 and their spike frequency declines, disinhibiting the DSI and thus allowing the cycle to repeat. This pattern of activity persists so long as the depolarisation is sufficient to make the DSI fire at a frequency sufficient to bring the C2 interneurons to threshold. The most important element in

this network which allows a cyclic pattern of activity to be sustained is the role of C2 interneuron which produce, with a delay, an amplified feedforward excitation of VSI. A number of other invertebrate rhythm-generating circuits also show a similar degree of complexity (see Elliot and Benjamin, 1985). Two principles of possible importance for the respiratory pattern generator which are illustrated by CPGs of simple nervous systems are feedback excitation and reciprocal inhibition.

As far as feedback excitation is concerned, Salmoiraghi and Burns (1960) suggested that within the set of inspiratory or expiratory brainstem neurons self-re-excitation occurs such that once excitation takes place in some members of the set it quickly spreads to the other members as well as returning, with amplification, to the originally excited neurons. This would result in a progressive increase in the level of excitation within each set. In CPGs of simpler nervous systems, the basis for self-re-excitation is provided by mutual excitatory connections. This has been demonstrated for CPGs which control swimming in Tritonia (Getting, 1981), feeding in snails (Kaneko et al., 1978), ventilation in Aplysia (Byrne, 1983), the pyloric rhythm in lobsters (Miller and Selverston, 1982) and the heart beat in leech (Tazaki and Cooke, 1979). In the majority of these systems, the mutual excitatory coupling is by means of electrical synaptic junctions. The one exception is the CPG which controls swimming in Tritonia, where chemical rather than electrical synapses mediate

mutual excitatory coupling between interneurons active during dorsal flexion. The function of this kind of positive feedback mechanism in these CPGs appears to be that of synchronizing the activity of a population of neurons as well as of reinforcing excitation, whether endogenous (pacemaker CPGs) or exogenous (non-pacemaker CPGs). Miles and Wong (1983) have provided an example of the operation of mutual excitation in the rat hippocampal slice, in which activation of a single pyramidal cell by intracellular stimulation produces synchronized firing of a large population of hippocampal neurons, if synaptic inhibition is suppressed by picrotoxin. The effect was attributed to excitation spreading through synapses between cells. As far as evidence that feedback excitation exists in the RPG, the autocorrelogram of the spike activity of bulbospinal inspiratory neurons and of phrenic discharge is characterized by a sustained oscillation with a frequency in the range of 50-100 Hz (Cohen, 1973, 1976; Feldman and Speck, 1983; Richardson and Mitchell, 1982). These high frequency oscillations have been reported in diaphragmatic EMGs from awake cats and humans (Bruce and Goldman, 1983). This suggests that the level of input to the neurons or the neuron excitability fluctuates in a regular fashion at a frequency within this range. Cross-correlation analysis of brainstem inspiratory neurons against phrenic discharge shows that in most inspiratory neurons the oscillation is phase-locked to the phrenic oscillation. Cohen (1973) suggested that this synchrony of spike activity arises from

reexcitant connections. The membrane potential of bulbo-spinal inspiratory neurons shows a slow, ramp-like, depolarizing wave in inspiration. Superimposed on the first two-thirds of the slow depolarization is an oscillation of up to 5 mV amplitude and frequency of 50 - 100 Hz (Mitchell and Herbert, 1974a). Action potentials occur at the peak of these oscillations of membrane potential, resulting in an oscillating pattern of the neurons discharge which is then synaptically transmitted to phrenic motoneurons. During the last third of inspiration the oscillation disappears, possibly due to desynchronization of the synaptic input which generates the oscillation. Mitchell and Herbert (1974a,b) however showed that the oscillation in membrane potential begins before the appearance of action potentials in all bulbo-spinal inspiratory neurons studied. This observation is inconsistent with the view that bulbo-spinal inspiratory neurons are synchronized by self-reexciting loops. On the assumption that the I ramp-generating neurons are connected to each other by excitatory connections, intracellular stimulation of a single cell, or of a few cells, should lead, under appropriate conditions, to excitations of the whole homonymous population, as found in the hyppocampal slice by Miles and Wong (1983).

As far as reciprocal inhibition is concerned, Burns and Salmoiraghi (1960) proposed that the inspiratory and expiratory neuron sets of the respiratory pattern generator are reciprocally

inhibitory, so that as one group becomes active it inhibits the other and simultaneous excitation of both sets does not occur. Reciprocal inhibition between neurons discharging in opposite phases is a common feature of CPGs in simple nervous systems (Pinsker and Ayers, 1983). Concerning the question of whether reciprocal inhibition is essential in the generation of the rhythmicity in all these CPGs, presently this question has been answered in the affirmative only for the CPG in the mollusk clione (Satterlie, 1985). As mentioned previously, the CPG for swimming in this mollusk consists of two groups of antagonistic interneurons that are coupled by reciprocal, monosynaptic inhibitory connections. This network is capable of stable oscillatory activity driven by rebound firing evoked on the decay phase of ipsps. Cycling can be initiated by injection of a short depolarizing or hyperpolarizing current pulse into one cell of the network. Hyperpolarizing the membrane of an interneuron with a long current pulse abolishes swimming activity for the duration of the pulse. In at least two other CPGs reciprocal inhibition has been shown to have only a partial role in rhythm generation. In the CPG regulating the heart beat of the leech, reciprocal inhibition between a pair of command oscillator neurons has a role in terminating the endogenously initiated burst activity. If one member of the pair is removed, the cycle duration of the remaining one increases dramatically (Peterson, 1983). The burst activity of

the dorsal swim interneurons in the Tritonia CPG is also terminated by reciprocal inhibition from ventral swim interneurons (Getting, 1983a).

Ever since Burns and Salmoiraghi's (1960) original proposal, reciprocal inhibition between medullary inspiratory and expiratory neurons has been frequently invoked to explain respiratory rhythmogenesis. Richter et al. (1979) provided the first convincing evidence of inhibition of NTS inspiratory neurons during expiration by intracellular recording from these neurons. IPSPs were observed during expiration which were reversed by intracellular chloride injection. The contour of the reversed IPSPs closely resembles the envelope of the augmenting firing pattern of the caudal NRA expiratory neurons (Merril, 1974). The latter neurons were therefore considered the most likely source of the expiratory inhibition of NTS inspiratory neurons. Further study by Fedorko et al. (1983), and Merrill and Fedorko (1984) provided evidence that the expiratory inhibition of NTS inspiratory neurons is due, in part at least, to input from Botzinger expiratory neurons. However, the role of this inhibition in producing the alternating, two phase, pattern of respiratory activity is unclear since ablation of much of Botzinger complex does not abolish the respiratory rhythm.

In summary, this survey of topics related to the mode of operation of the respiratory pattern generator, in particular the

survey of neuron types which are part of the network, leads to the working hypotheses that the synaptic input responsible for the complex respiratory modulation of sympathetic neuron activity derives from ramp inspiratory neurons, which may account for the IRSD, from early inspiratory neurons, which may account for the early inspiratory inhibition (Cohen and Gootman, 1970; Bainton et al., 1985), from post-inspiratory neurons, which may account for post-inspiratory depression, and from late-expiratory neurons, which may account for late expiratory excitation. Only for the first hypothesis, that ramp inspiratory neurons are responsible for the IRSD, there is enough data of similarities in SPN and inspiratory neuron responses to various stimuli to make it look fairly likely. The hypotheses concerning the other three components of respiratory modulation require more data comparing firing pattern and responses of sympathetic neurons with those of the postulated input neurons.

4. SYNTHESIS OF MECHANISMS BY WHICH RESPIRATION INFLUENCES CIRCULATION

In section 1 of the introduction (Chapter I) the various mechanisms by which respiration can influence the operation of the cardiovascular system have been reviewed. In these sections each mechanism has been described and analysed in isolation. Here an attempt is made to fit these various mechanisms together in order

to see how they interact with each other and what is their overall effect in the control of SAP.

During quiet breathing at normal respiratory rates (12-20 breaths/min) and tidal volumes SAP falls in inspiration and increases in expiration (Dornhorst, 1952). This oscillation in SAP is the resultant of the interplay of several mechanical and neural influences. The mechanical influences are a consequence of the changes in pleural pressure, associated with breathing, and affect mainly cardiac function (see the Introduction for details). The neural influences include a centrogenic mechanism, based on a postulated synaptic coupling between brainstem respiratory neurons and sympathetic as well as cardiac parasympathetic neurons, and reflexogenic mechanisms, mediated by sensory neurons associated with cardiovascular and respiratory structures, which connect with both sympathetic and parasympathetic cardiac neurons.

During inspiration, the reduction in pleural pressure increases the gradient for venous blood flow from the extrathoracic systemic veins to the right atrium (Brecher, 1956). This leads to an increased preload of the right ventricle, resulting in an increase in right ventricular stroke volume. Left ventricular stroke volume falls in inspiration, as a result of a number of mechanism(s). (1) The inspiratory increase in right ventricular filling may displace the interventricular septum to the left thereby reducing left ventricular compliance (Olsen et al., 1985; Beyar et al., 1987). As a result, the compressed left ventricle

fills less and its stroke volume will be reduced. (2) An increase in pulmonary vascular capacitance (Trimby and Nicholson, 1924) which will decrease filling of the left ventricle and (3) an increase in left ventricular afterload (Summer et al., 1979).

With the beginning of inspiration there is an increase in sympathetic activity, namely the IRSD, which produces, within a few seconds, an increase in peripheral vascular resistance, heart rate and contractility and hence blood pressure. The inspiratory inhibition of parasympathetic cardiac neurons will make the major contribution to the increase in heart rate. Therefore, by virtue of the coupling between brainstem respiratory neurons and the autonomic neurons which influence the cardiovascular system, the inspiratory phase of the central respiratory cycle will produce an increase in peripheral resistance and cardiac output and hence blood pressure. However, unlike the mechanical effects, which have no appreciable delay, the neurogenic effects have built-in delays, mainly contributed by the slow target cell response to autonomic transmitters. Therefore the onset of the increase in SAP due to this centrogenic mechanism will be delayed with respect to the onset of inspiration. For details of the step response characteristics of cardiovascular effectors see Introduction, section 7 of part II. The delay will determine whether the effect on the targets occurs in the same phase in which the neural modulation occurs or in the next phase. For example, at normal

respiratory rates, the respiratory sinus arrhythmia, which is largely due to I-inhibition of parasympathetic cardiac neurons and to a smaller extent to I-facilitation of sympathetic cardiac neurons, is characterized by increase of heart rate in I and decrease in E. Whether the inspiratory increase in heart rate actually contributes to an increase in cardiac output during inspiration and therefore SAP has not been established (see Barnes et al., 1980). Typical onset delay and time constant of the increase in heart rate and peripheral resistence in response to pre- or postganglionic sympathetic stimulation are 0.5 - 2.0 sec and 3-12 sec (Rosenbaum and Race, 1968; Chess et al., 1975) respectively, depending on intensity and frequency of stimulation, i.e. depending on number of axons recruited and their discharge rate. Hence, depending on the duration of the respiratory cycle and its phases, the peak neurogenic effect on SAP may occur late in inspiration, or during the successive expiration, or even during the successive inspiration. Some properties of the IRSD component of the centrogenic mechanism in open loop conditions, i.e. the absence of sensory feedback from lungs or from arterial baroreceptors, are known (see Introduction part II). We know, for instance, that the magnitude of this component will change in close relationship with changes in the level of phrenic nerve activity (Preiss and Polosa, 1977), that this component involves the activity of approximately half of the discharging SPN pool (Polosa

et al., 1980) and that this component accounts for approximately one fourth of the neurogenic vasoconstrictor tone in the hindlimb in normocapnia (Bachoo and Polosa, 1985).

With the onset of inspiration, air flow starts and lung volume increases. This increase in volume is sensed by pulmonary receptors with afferents in the vagus nerve which feed the lung volume information onto brainstem respiratory neurons as well as sympathetic and cardiac parasympathetic neurons (see introduction part II, reflex mechanisms). The question to consider is how the vagal sensory feedback modifies the centrogenic mechanism when, in normal conditions, the latter operates in closed-loop conditions. Inspiratory lung inflation, within a certain range of tidal volumes, results in shortening of inspiration and of the succeeding expiration, when the vagus nerve is intact (Clarke and Euler, 1972). There is no change in the rate of rise of phrenic activity, hence peak amplitude of the phrenic bursts decreases. Lung inflation with large tidal volumes in inspiration abolishes the IRSD (Gootman et al., 1980). The effect on parasympathetic cardiac neurons is an increase in discharge (Anrep, 1936). However, these effects are likely to be small at normal tidal volume (Bachoo and Polosa, 1986). In addition, there is evidence for an inhibitory action of lung inflation on sympathetic neurons that is not secondary to changes in the activity of brainstem respiratory neurons (Gootman et al., 1980; Bachoo and Polosa, 1986). However, the effect only appears with very large inflations and therefore

may be disregarded when only tidal volumes within the eupneic range are considered. In conclusion, probably the feedback from pulmonary afferents activated by the increase in lung volume occurring in inspiration is of little consequence in modifying the centrogenic mechanism when tidal volume is in the eupneic range. When, however, inspiratory activity increases, e.g. because of increased chemoreceptor drive or during exercise, the vagal feedback may be expected to significantly dampen the amplitude of the IRSD as well of the non-modulated sympathetic discharge, and significantly increase parasympathetic cardiac neuron activity.

During a respiratory cycle the negative swing in intrathoracic pressure occurring in inspiration produces a decrease in cardiac output (result of decrease left ventricular stroke volume and increased heart rate) which is followed by an increase in cardiac output in expiration. This "mechanical" fluctuation in cardiac output results in a synchronous fluctuation in SAP. The relationship between the fluctuations in SAP and the phase of the respiratory cycle is a function of respiratory rate (see introduction). At moderate rates of breathing (12-20 breaths/min) SAP falls during most of inspiration. At lower rates (10 breaths/min) inspiration is associated with an increase in SAP. The fluctuation in SAP is translated in a fluctuation in baroreceptor activity. During inspiration the baroreceptors are unloaded and as a consequence sympathetic activity increases. The increase in sympathetic activity during inspiration may also be

due, in part, to relative refractoriness of the baroreceptor reflex circuit during inspiration (Gilbey et al., 1984). This reflex increase in sympathetic activity in inspiration, coupled with the centrogenic IRSD, will produce an increase in peripheral resistance, heart rate and contractility. Due to the delays associated with sympathetic activation of target cells the phase and cycle in which the effect on SAP will occur depends on the frequency of respiration and the magnitude of the sympathetic oscillations. For postganglionic firing frequencies between 1 and 10 Hz the time constant of the response to a step input is between 3 and 12 s (Rosenbaum and Race, 1968); at normal respiratory rates (10-15 breaths/min) the increase in resistance will coincide with expiration. Simultaneously with the reflex increase in sympathetic activity in inspiration, the inspiratory unloading of the arterial baroreceptors will produce a reflex decrease in the activity of parasympathetic cardiac neurons. For this system, since the delays are shorter than for the sympathetic system (see Introduction, section 7 of part II), the cardiac parasympathetic disfacilitation will result in an increase in heart rate during the same inspiratory phase during which the baroreceptors are unloaded. Both the neurogenic increase in vascular resistance and in cardiac output will act, if they occur in inspiration, to minimize the decrease in SAP produced by the mechanical influence of inspiration on cardiac output. If they occur in expiration they will enhance the increase in SAP produced by the mechanical effect of expiration on cardiac output.

Various authors have reported that oscillations in sympathetic activity at the frequency of respiration may be mediated by the baroreceptor reflex evoked by the respiratory oscillation in SAP (see Wallin and Fagius, 1986; Eckberg et al., 1985). In a number of human studies respiratory modulation of sympathetic activity was only observed when a blood pressure oscillation at the frequency of respiration was present (Eckberg et al., 1985). This suggests that the respiratory modulation of sympathetic discharge in these cases is secondary to the "mechanical" respiratory oscillation in SAP and is mediated by the baroreflex. Wurster and Connelly (1987) have recently reported that in cats with pontine lesions, the baroreflex-induced respiratory oscillations in sympathetic activity, consequence of mechanically induced respiratory swings in arterial pressure of 5-20 mmHg, can be the dominant form of respiratory oscillation in sympathetic activity. It is conceivable that this could occur if the gain of the baroreceptor reflex in the experimental conditions just described was greater than under the experimental conditions in which the centrogenic type of respiratory modulation of SPN discharge is observed.

REFERENCES

Adams, P.R., D.A. Brown (1982). Synaptic inhibition of the M-current: slow excitatory post-synaptic potential mechanism in bullfrog sympathetic neurons. J. Physiol. 332: 263-272.

Adrian, E.D., D.W. Bronk, G. Phillips (1932). Discharges in mammalian sympathetic nerves. J. Physiol. (Lond.) 74: 115-153.

Akasu, T., K. Koketsu. (1986). Muscarinic transmission. In Autonomic and Enteric Ganglia Ed. by A.G. Karczmar, K. Koketsu, S. Nishi. p. 161-180.

Andersson, P.O., S.R. Bloom, J. Jarhault (1983). Colonic motor and vascular responses to pelvic nerve stimulation and their relation to local peptide release in the cat. J. Physiol. 334: 293-307.

Anrep, G.V., W. Pascual, R. Rossler (1936). Respiratory variations of the heart rate. I. The reflex mechanisms of the sinus arrhythmia. Proceedings of the Royal Society (B) 119: 191-217.

Aoki, M, S. Mori, K. Kawahara, H. Watanabe, N. Ebata (1980). Generation of spontaneous respiratory rhythm in high spinal cats. Brain Res. 202: 51-63.

Atwood, H.L., L.E. Swenarchuk, C.R. Aruenwald (1975). Long term synaptic facilitation during sodium accumulation in nerve terminals. Brian Res. 100: 198-204.

Bachoo, M., C. Polosa (1985). Properties of a sympatho-inhibitor and vasodilator reflex evoked by superior laryngeal nerve afferents in the cat. J. Physiol. 364: 183-198.

Bachoo, M., C. Polosa (1986). The pattern of sympathetic neurone activity during expiration in the cat. J. Physiol. 378: 375-390.

Bachoo, M., C. Polosa (1987). Properties of the inspiration related activity of sympathetic preganglionic neurones of the cervical trunk in the cat. J. Physiol. 385: 545-564.

Bachoo, M., J. Ciriello, C. Polosa (1987). Effect of preganglionic stimulation of neuropeptide-like immunoreactivity in the stellate ganglion of the cat. Brain Res. 400: 377-382.

Bachoo, M., E. Isacoff, C. Polosa (1988). Slow cardioacceleration mediated by noncholinergic transmission in the stellate ganglion of the cat. Can. J. Physiol. Pharm. 66: 1066-1074.

Bainton, C.R., D.W. Richter, H. Seller, D. Ballantyne, J.P. Klein (1985). Respiratory modulation of sympathetic activity. J. Auton. Nerv. Syst. 12: 77-90.

Baker, J.P. J.E. Remmers (1982). Responses of medullary respiratory neurons to central pontine stimulation. Resp. Physiol. 50: 197-208.

Ballantyne, D., D.W. Richter (1982). The functional difference between post-inspiratory neurons in the cat. Pflügers Arch. 394:R56.

Ballantyne, D, Richter, D.W. (1984). Post-synpatic inhibition of

bulbar inspiratory neurons in the cat. J. Physiol. (Lond.) 348: 67-87.

Ballantyne, D., D.W. Richter (1986). The non-uniform character of expiratory synaptic activity in expiratory bulbospinal neurones of the cat. J. Physiol. (Lond.) 370: 433-456.

Barman, S.M., G.L. Gebber (1976). Basis for synchronization of sympathetic and phrenic nerve discharges. Amer. J. Physiol. 231: 1601-1607.

Barnes, R.J., E. Bower, T.J. Rink (1980). Hemodynamic responses to stimulation of the cardiac autonomic nerves in the anaesthetized cat with closed chest. J. Physiol. 299: 55-73.

Berger, A.J. (1979). Phrenic motoneurons in the cat: subpopulations and nature of respiratory drive potentials. J. Neurophysiol. 42: 76-90.

Beyar, R., M.J. Hansknencht, H.R. Halperin, F.C.P. Yin, M.L. Weisfeldt (1987). Interactions between cardiac chambers and thoracic pressure in intact circulation. Am. J. Physiol. 253: H1240-H1252.

Birks, R.I. (1978). Regulation by patterned preganglionic neural activity of transmitter stores in a sympathetic ganglion. J. Physiol. 280: 539-572.

Birks, R.I. (1982). Acetylcholine release during burst-patterned stimulation of a sympathetic ganglion: its relation to transmission efficacy. J. Physiol. (Paris) 78: 417-419.

Birks, R.I. (1983). Activation of feline acetylcholine synthesis

in the absence of release: dependence on sodium, calcium and the sodium pump. J. Physiol. 344: 347-357.

Birks, R.I. (1985). Activation of acetylcholine synthesis in cat sympathetic ganglia; dependence on external choline and sodium-pump rate. J. Physiol. 367: 401-417.

Birks, R.I., M.W. Cohen (1968a). The action of sodium pump inhibitors on neuromuscular transmission. Proc. Roy. Soc. B. 170: 381-399.

Birks, R.I., M.W. Cohen (1968b). The influence of internal sodium on the behaviour of motor nerve endings. Proc. Roy. Soc. B. 170: 401-421.

Birks, R.I., E.Y. Isacoff (1988). Burst-patterned stimulation promotes nicotinic transmission in isolated perfused at sympathetic ganglia. J. Physiol. 402: 515-532.

Birks, R.I., W. Laskey, C. Polosa (1981). The effect of burst patterning of preganglionic input to the efficacy of tansmission at the cat stellate ganglion. J. Physiol. 318: 531-539.

Blackman, J.A. (1974). Function of autonomic ganglia. In: The Peripheral Nervous System, ed. by J.I. Hubbard. New York. pp. 257-276.

Blackman, J.A., R.D. Purves (1969). Intracellular recordings from ganglia of the thoracic sympathetic chain of the Guinea Pig. J. Physiol. 203: 173-198.

Blaustein, M.P., (1976). The in's and out's of calcium transport

in squid axons: internal and external ion activation of Ca efflux. Fed. Proc. 35: 2574-2578.

Blaustein, M.P., C.F. McGraw, A.V. Samlyo, E.S. Schweitzer, (1980). How is the cytoplasmic calcium concentration controlled in nerve terminals? J. Physiol. (Paris). 76: 459-470.

Bliss, T.V.P., T. Lomo (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the preforant path. J. Physiol. 232: 331-356.

Blumberg, H., W. Jänig (1983). Enhancement of resting activity in postganglionic vasoconstrictor neurones following short-lasting repetitive activation of preganglionic axons. Pflügers Arch. 396: 89-94.

Bongianni, M.C., G. Fontana, T. Pantaleo (1988). Influence of superior laryngeal afferent stimulation on expiration activity in cats. J. Appl. Physiol. 65: 385-392.

Bradley, A.W., C. von Euler, I. Marttila, B. Roos (1975). A model of the central and reflex inhibition in the cat. Biol. Cybern. 19: 105-116.

Brecher, C.L. (1956). Venous Return. New York: Grune & Stratton.

Brimble, M.J., D.I. Wallis, B. Woodward (1972). Facilitation and inhibition of cell groups within the superior cervical ganglion of the rabbit. J. Physiol. 226: 629-652.

Brown, A.M., (1967). Cardiac sympathetic adrenergic pathways in which synaptic transmission is blocked by atropine sulphate.

J. Physiol. 191: 271-288.

Brown, D.A., P.R. Adams (1980). Muscarinic supression of a novel voltage-sensitive K^+ current in a vertebrate neurone. Nature 283: 673-676.

Brown, D.A., A.A. Selyanko (1985). Membrane currents underlying the cholinergic slow excitatory post-synaptic potential in the rat sympathetic ganglion. J. Physiol. 365: 365-387.

Bruce, E.N. (1988). Correlated and uncorrelated high-frequency oscillations in phrenic and recurrent laryngeal neurograms. J. Neurophysiol. 59: 1188.

Bruce, E.N., M.D. Goldman (1983). High frequency oscillations in human respiratory electromyograms during voluntary breathing. Brain Res. 269: 259-265.

Budzinska, K.C., C. von Euler, F.F. Kao, T. Pantaleo, Y. Yamamaoto (1985). Effect of graded cold block in rostral areas of the medulla. Acta Physiol. Scand. 124: 329-349.

Burns, B.D., A.C. Salmoiraghi (1960). Repetitive firing of respiratory neurones during their burst activity. J. Neurophysiol. 23: 27-46.

Byrne, J.M. (1983). Identification and initial characterization of a cluster of command and pattern generating neurons underlying respiratory pumping in Aplysia californic. J. Neurophysiol. 49: 491-508.

Calabrese, R.L. (1979). Neural generation of the peristaltic and non-peristaltic heartbeat coordination modes in the leech

Mirudo medicinalis. Am. Zool. 19: 87-102.

Calabrese, R.L., E.L. Peterson (1983). Neural control of heartbeat in the leech, Hirudo medicinalis. In: Neural Origin of Rhythmic Movements, eds. A. Roberts, B. Roberts. Symp. Soc. Exp. Biol. 37: 195-221.

Chess, G.F., D.W. Varsey, J.L. Henry, F.R. Calaresu (1975). A mathematical model of the sympathetic heart period system in the cat. Ann. Biomed. Eng. 3: 189-198.

Clarke, F.J., C. von Euler (1972). On the regulation of depth and rate of breathing. J. Physiol. 222: 267-295.

Cohen, M.I., P.M. Gootman (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. Am. J. Physiol. 218: 1092-1101.

Cohen, M.I. (1968). Discharge properties of brain-stem respiratory neurons in relation to carbon dioxide tension. J. Neurophysiol. 31: 142-165.

Cohen, M.I. (1973). Synchronization of discharge, spontaneous and evoked, between inspiratory neurons. Acta Neurobiol. Exp. 33: 189-218.

Cohen, M.I. (1975). Phrenic and recurrent laryngeal discharge patterns and the Hering-Breuer reflex. Am. J. Physiol. 228: 1486-1496.

Cohen, M.I. (1976). Synaptic relations between inspiratory neurons and the Hering-Breuer reflex. In. Respiratory centers and Afferent Systems. Ed. by B. Duran. Paris: INSERM. pp.

19-29.

Cohen, M.I. (1979). Neurogenesis of respiratory rhythm in the mammal. Physiol. Rev. 59: 1105-1173.

Cohen, M.I. (1981). Central determinants of respiratory rhythm. Ann. Rev. Physiol. 43: 91-104.

Cohen, M.I., J.L. Feldman (1977). Models of respiratory phase-switching. Fed. Proc. 36: 2367-2374.

Cohen, MI.I., J.L. Feldman (1984). Discharge properties of dorsal medullary inspiratory neurons: relation to pulmonary affarent and phrenic efferent discharge. J. Neuroph. 51: 753-776.

Cohen, M.I., J.L. Feldman, D. Sommer (1985). Caudal medullary expiratory neurone and internal intercostal nerve discharges in the cat: effects of lung inflation. J. Physiol. 368: 147-178.

Cohen, M.I., M.F. Piercey, P.M. Gootman, P. Wolotsky (1974). Synaptic connections between medullary inspiratory neurons and phrenic motoneurons as revealed by cross-correlation. Brain Res. 81: 319-324.

Cohen, M.I., W.R. See, C.N. Christatos, A.L. Sica (1987). High-frequency and medium frequency components of different inspiratory nerve discharges and their modification by various inputs. Brain Res. 417: 148-152.

Crowcroft, P.J., J.H.A. Szurskewski (1971). A study of the inferior mesenteric ganglion of guinea pigs with intracellular electrodes. J. Physiol. 219: 421-441.

Cunnane, T.C., L. Stjarne (1984). Transmitter secretion from individual varicosities of guinea-pig and mouse vas deferns: highly intermittent and monoquantal. Neuroscience. 13: 1-20.

Czyzyk, M.F., L. Fedorko, A. Trezebski (1987). Pattern of the respiratory modulation of the sympathetic activity is species dependent: synchronisation of the sympathetic outflow over the respiratory cycle in the rat. In. Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms. Ed. J. Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa. pp. 143-152. Alan Liss Inc.

Daly, M. de B. (1972). Interaction of cardiovascular reflexes In: Scientific Basis of Medicine Annual Review, University of Lond, Atholone Press, pp. 307-332.

Davidson, N.S., S. Goldner, D.I. McCloskey (1976). Respiratory modulation of baroreceptor and chemoreceptor reflexes affecting heart rate and cardiac vagal efferent nerve activity. J. Physiol. (Lond.) 259: 523-530.

Davies, J.A., P.A. Kirkwood, T.A. Sears (1985a). The detection of monosynaptic connexions from inspiratory bulbospiral neurons to inspiratory motoneurons in the cat. J. Physiol. 368: 33-62.

Davies, J.A., P.A. Kirkwood, T.A. Sears (1985b). The distribution of monosynaptic connexions from inspiratory bulbospiral neurons to inspiratory motoneurons in the cat. J. Physiol. 368: 63-87.

Dekin, M.S., G.B. Richerson, P.A. Getting (1985). Thyrotropin - releasing hormone induces rhythmic bursting in neurons of nucleus tractus solitarius. Science. 229: 67-69.

Del Castillo, J., B. Katz (1954). Statistical factors involved in neuromuscular facilitation and depression. J. Physiol. 12: 574-585.

Dembowsky, K., J. Czachurski, H. Seller (1985). An intracellular study of the synaptic input to sympathetic preganglionic neurones of the third thoracic segment of the cat. J. Auto. Nerv. Syst. 13: 201-244.

Donald, D.E., S.E. Milburn, J.T. Shepherd (1964). Effect of cardiac denervation on the maximal capacity for exercise in the racing greyhound. J. Appl. Physiol. 19: 849-852.

Dunant, V., M. Dolivo (1968). Plasticity of synaptic functions in the excised sympathetic ganglion of the rat. Brain Res. 10: 272-273.

Dutton, A., R.E.J. Dyball (1979). Phasic-firing enhances vasopressin release from rat neurophypophysis. J. Physiol. 290: 433-440.

Eckberg, D.L., C. Nerhed, B.A. Wallin (1985). Respiratory modulation of muscle sympathetic and vagal cardiac outflow in man. J. Physiol. 365: 181-196.

Ellenberger, H.M., J.L. Feldman (1988). Monosynaptic transmission of respiratory drive to phrenic motoneurons from brainstem bulbospinal neurons in cats. J. Comp. Neurol. 269: 47-57.

Elliot, P.A., B.J. Benjamin (1985). Interaction of pattern generating interneurons controlling feeding in lymnaea stagnalis. J. Neurophysiol. 54: 1396-1422.

Erulkar, S.D., R. Rahamimoff (1978). The role of calcium ions in tetanic and post-tetanic increase in miniature end-plate potential frequency. J. Physiol. 287: 501-511.

Erulkar, S.D., J.K. Woodward (1968). Intracellular recording from mammalian superior cervical ganglion in Situ. J. Physiol. 199: 189-203.

Euler, C. von (1983). On the central pattern generator for the basic breathing rhythmicity. J. Appl. Physiol. 55: 1647-1659.

Euler C. von (1986). Brain stem mechanisms for generation and control of breathing pattern In: A.P. Fishman, N.S. Cherniack, J.G. Widdicombe, S.R. Geiger (eds.). Handbook of Physiology, Section III: The Respiratory System. American Physiological Society, Bethesda, pp. 1-67.

Fedorko, L., E.G. Merrill (1984). Axonal projections from the rostral expiratory neurones of the Bötzinger complex to medulla and spinal cord in the cat. J. Physiol. (Lond.) 350: 487-496.

Fedorko, L., E.G. Merrill, J. Lipski (1983). Two descending medullary inspiratory pathways to phrenic motoneurons. Neurosci. Lett. 43: 285-291.

Feldman, J.L. (1981). Interaction between brainstem inspiratory

neurons. Fed. Proc. 40: 2384-2388.

Feldman, J.L. (1986). Neurophysiology of respiration in mammals. In. Handbook of Physiology, Section: The Nervous System, Vol.V. Ed. by F.E. Bloom pp. 463-524. Am. Physiol. Soc., Bethesda, Maryland.

Feldman, J.L., M.I. Cohen (1978). Relation between expiratory nuration and costral medullary expiratory neuronal discharge. Brain Res. 141: 172-178.

Feldman, J.L., M.I. Cohen, P. Wolotsky (1976). Powerful inhibition of pontine respiratory neurons by pulmonary afferent activity. Brain Res. 104: 341-346.

Feldman, J.L., H. Gauthier (1970). Interaction of pulmonary afferents and pneumotaxic center in control of respiratory patterns in cats. J. Neurophysiol. 39: 31-44.

Feldman, J.L., A.D. Loewy, D.F. Speck (1985). Projections from the ventral respiratory group to phrenic and intercostal motoneurons in the cat: an autoradiographic study. J. Neurosci. 8: 1993-2000.

Feldman, J.L., D.R. McCrimmon, D.F. Speck (1984). Effect of synchronous activation of medullary inspiratory bulbospinal neurons to phrenic nerve discharge. 347: 241-254.

Feldman, J.L., Speck, D. (1983). Interactions among inspiratory neurons in dorsal and ventral groups in cat medulla. J. neurophysiol. 49: 472-490.

Flacke, W., Gillis, R.A. (1968). Impulse transmission via

nicotinic and muscarinic pathways in the stellate ganglion of the dog. J. Pharmacol. Exp. Therap. 163: 266-276.

Folkow, B. (1952). Impusles frequency in sympathetic vasomotor fibers correlated to the release and elimination of the transmitter. Acta. Physiol. Scand. 91: 211-215.

Folkow, B. (1955). Nervous control of blood vessels. Physiol. Rev. 35: 629-663.

Freyburger, W.A., C.C. Gruhzit, B.R. Rennick, G.K. Moe (1950). Pressor pathways not blocked by tetraethylammonium. Am. J. Physiol. 163: 290-293.

Freyburger, W.A., C.C. Gruhzit, B.R. Rennick, G.K. Moe (1950). Action of tetraethylammonium on pressor response to asphyxia. Am. J. Physiol. 163: 554-560.

Fried, G., J.M. Lundberg, E. Theordorsson-Norheim, (1985). Subcellular storage and axonal transport of neuropeptide Y (NPY) in relation to catecholamines in the cat. Acta. Physiol. (Scand.) 125: 145-154.

Fukuda, Y., Y. Honda (1983). Effects of hypocapnia on respiratory timing and inspiratory activities of the superiorlaryngeal, hypoglossal, and phrenic nerves in the vagotomized rat. Jap. J. Physiol. 33: 733-742.

Gauthier, P., R. Monteau (1986). Respiratory resetting induced by activation of inspiratory bulbo-spinal neurons. Resp. Physiol. 65: 155-168.

Getting, P.A. (1981). Mechanism of pattern generation underlying

swimming in Tritonia. I. Neuronal network formed by monosynaptic connections. J. Neurophysiol. 46: 65-79.

Getting, P.A. (1983a). Mechanisms of pattern generating underlying swimming in Tritonia. II. Network reconstruction. J. Neurophysiol. 49: 1017-1035.

Getting, P.A. (1983b). Mechanism of pattern generation underlying swimming in Tritonia III. Intrinsic and synaptic mechanism for delayed excitation. J. Neurophysiol. 49: 1036-49.

Getting, P.A., P.R. Lennard, R.I. Jume (1980). Central pattern generator mediating swimming in Tritonia. I. Identification and synaptic interactions. J. Neurophysiol. 44: 151-164.

Gilbey, M.P., D. Jordan, O.W. Richter, K.M. Spyer (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardio-inhibitory neurones in the cat. J. Physiol. 365: 68-78.

Gilbey, M.P., N. Yoshinobu, K.M. Spyer (1986). Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. J. Physiol. (Lond.). 378: 253-266.

Glover, V.A.S., L.T. Potter (1981). Purification and properties of choline acetyltransferase from ox brain striate nuclei. J. Neurochem. 18:571-580.

Gootman, P.M., M.I. Cohen (1974). The interrelationships between sympathetic discharge and central respiratory drive. In: Central Rhythmic and Regulation ed. W. Umbach, H.P. Koepchen,

p. 195-209, Stuttgart: Hippokrates Verlag.

Gootman, P.M., J.L. Feldman, M.I. Cohen (1980). Pulmonary afferent influences on respiratory modulation of sympathetic discharge. In: Central Interaction Between Respiratory and Cardiovascular Control Systems, ed. H.P. Koepchen, S.M. Milton, A. Trzebski, pp. 172-178. Berlin-Springer.

Gromysz, M., W.A. Karczewski (1980). Generation of respiratory pattern in the rabbit-brainstem transections revisited. Acta Neurobiol. Exp. 40: 985-992.

Gromysz, M., W.A. Karczewski (1982). Phrenic motoneuron activity in split-brainstem cats and monkeys. Respir. Physiol. 50: 51-61.

Hanna, B.D., F. Lioy, C. Polosa (1979). The effect of cold blockade of the medullary chemoreceptors on the CO_2 modulation of vascular tone and heart rate. Can J. Physiol. Pharma, 57: 461-468.

Hartzell, M.C. (1981). Mechanisms of slow postsynaptic potentials. Nature 291: 539-544.

Henderson, G.G., A. Ungar (1978). Effect of cholinergic antagonists on sympathetic ganglionic transmission of vasomotor reflexes from carotid baroreceptors and chemoreceptors of the dog. J. Physiol. 277: 379-385.

Hilaire, G., R. Monteau (1976). Connexions entre les neurones inspiratoires bulbaires et les motoneurones phreniques et intercosteaux. J. Physiol. (Paris). 72: 987-1000.

Hilaire, G., R. Monteau, A.L. Bianchi (1984). A cross-correlation study of interactions among respiratory neurons of dorsal, ventral and retrofacial groups in cat medulla. Brain Res. 302: 19-31.

Holz, A.A., K. Dunlap, R.M. Kream (1988). Characterization of the electrically evoked release of sub P from dorsal root ganglion neurons. Methods and Dihydropyridine sensitivity. J. Neurosci. 8: 463-471.

Ip, N., R.E. Zigmond (1984). Pattern of presynaptic nerve activity can determine the type of neurotransmitter regulating a postsynaptic event. Nature 311: 472-474.

Iscoe, S., J. Dankoff, R. Migicovsky, C. Polosa (1976). Recruitment and discharge frequency of phrenic motoneurons during inspiration. Resp. Physiol. 26: 113-128.

Jan, Y.U., L.Y. Jan, S.W. Kuffler (1980). Further evidence for peptidergic transmission in sympathetic ganglia. Proc. Natl. Acad. Sci. 77: 5008-5012.

Jan, L.Y., J.N Jan (1982). Peptidergic transmission in sympathetic ganglia of the frog. J. Physiol. 327: 219-246.

Jänig, W., R. Krauspe, G. Wiedersatz (1983). Reflex activation of postganglionic vasoconstrictor neurones supplying skeletal muscle by stimulation of arterial chemoreceptors via non-nicotinic synaptic mechanism in sympathetic ganglia. Pflügers Arch. 396: 95-100.

Jänig, W., A. Sundolf, B.A. Wallin (1983). Discharge pattern of

sympathetic neurons supplying skeletal muscle and skin in man and cat. J. Auto. Nerv. Syst. 7: 239-256.

Jänig, W.A. (1985). Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. Rev. Physiol. Biochem. Pharmacol. 102: 119-213.

Jordan, D., K.M. Spyer (1987). Brainstem intergration of cardiovascular and pulmonary afferent activity. Prog. Brain Res. 67: 295-314.

Kaneko, C.R.S., M. Merickel, S.B. Kater, (1978). Centrally programmed feeding in Helisoma: identification and characteristics of an electrically coupled pre-motor neuron network. Brain Res. 146: 1-22.

Karczewski, W.A., J.A. Widdicombe (1973). Neural Control of Breathing. Acta. Neurobiol. Exp. 33: 1-432.

Knox, C.K. (1973). Characteristics of inflation and deflation reflexes during expiration in the cat. J. Neurophysiol. 36: 284-295.

Koepchen, H.P. (1983). Respiratory and cardiovascular "centres", functional entirety or separate structures? In: Central Neurone Environment, Schläfke, H.P. Kopchen, See, W.R. (eds.). Springer Verlag, Berlin Heidelberg, pp. 221-237.

Koizumi, K., M. Kollai (1987). Cardiorespiratory relationships: correlation between the activity of cardiac vagus, sympathetic and phrenic nerves. In: Organization of the Autonomic Nervous System. Central and Peripheral Mechanisms. Ed. J.

Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa, pp. 153-167. Alan R. Liss Inc.

Krukoff, T.L., J. Ciriello, F.R. Calaresu (1985). Segmental distribution of peptide-like immunoreactivity in cell bodies of the thoracolumbar sympathetic nuclei of the cat. J. Comp. Neurol. 240: 90-102.

Kuba, K., S. Minota (1986). General characteristics and mechanisms of nicotinic transmission in sympathetic ganglia. In: Autonomic and Enteric Ganglia. Ed. by A.G. Karczmar, K. Koketsu, S. Nishi, Princeton Press. N.Y. pp. 107-132.

Kuffler, S.W. (1980). Slow synaptic responses in autonomic ganglia and the pursuit of a peptidergic transmitter. J. Exp. Biol. 89: 257-286.

Larrabee, M.A., D.W. Bronk (1947). Prolonged facilitation of synaptic excitation in sympathetic ganglia. J. Neurophysiol. 10: 139-154.

Lebedev, V.P., A.V. Syromyatnikov, V.I. Skok (1977). The convergence of sympathetic preganglionic fibers on vasomotor neurones in the ganglion in lumbar sympathetic chain. Neurosci. Lett. 4: 243-247.

Ledsome, J.R., R.J. Linden (1968). The role of left atrial receptors in the diuretic response to left atrial distension. J. Physiol. 198: 487-503.

Lev-Tov, A., R. Rahamimoff (1980). A study of tetanic and post-tetanic potentiation of miniture end-plate potentials at

the frog neuromuscular junction. J. Physiol. 309: 247-273.

Lewis, G.J., J. Porte, M.J. Purves (1980). Fluctuations of Pa Co_2 with the same period as respiration in the cat. J. Physiol. 298: 1-11.

Libet, B. (1964). Slow synaptic responses and excitatory changes in sympathetic ganglia. J. Physiol. 174: 1-25.

Linden, R.J. (1979). Atrial receptors and heart rate. In: Cardiac Receptors. Eds. H. Hainsworth, C. Kidd, R.J. Linden. Cambridge University Press, London.

Lindsay, B.G., L.S. Segers, R. Shannon (1987). Functional associations among lateral medullary respiratory neurons in the cat. II. Evidence for inhibitory actions of expiratory neurons. J. Neurophysiol. 57: 1101-1117.

Lioy, F., B.D. Hanna, C. Polosa (1978). CO_2 dependent component of the neurogenic vascular tone in the cat. Pflugers Arch. 374: 187-191.

Lipski, J. (1981). Antidromic activation of neurons as an analytical tool in the study of the central nervous system. J. Neurosci. Meth. 4: 1-32 (1981).

Lipski, J., E.A. Merrill (1979). Electrophysiological demonstration of projection from expiratory neurones in rostral medulla to contralateral dorsal respiratory group. Brain Res. 197: 521-524.

Long, S., J. Duffin (1986). The neuronal determinants of respiratory rhythm. Prog. Neurobiol. 27: 101-182.

Lundberg, J.M. (1981). Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurones in cat exocrine glands. Morphological, biochemical and functional studies. Acta Physiol. Scand. Suppl. 496: 1-57.

Lundberg, J.M., A. Anggard, J. Parnow, T. Hokfelt, (1985). Neuropeptide Y, substance P and VIP immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. Cell Tiss. Res. 239: 9-18.

Lundberg, J.M., A. Rudehill, A. Solleri, E. Theodorsson-Norheim, B. Hamberger (1986). Frequency and reserpine-dependent chemical coding of sympathetic transmission: differential release of noradrenaline and neuropeptide Y from pig spleen. Neuroscience Letts. 63: 96-100.

Lunteren, E.K., J. Mitra, N.R. Prabhakar, M.A. Haxhiu, W.S. Cherniack (1987). Ventral medullary surface inputs to cervical sympathetic neurons produce respiratory oscillations Am. J. Physiol. 252: R1032-R1038.

MacIntosh, F.C., B. Collier (1976). Neurochemistry of cholinergic terminals. In: Handbook of Experimental Pharmacology. Ed. Born, G.V.R., Eichles, O., A. Farah, H. Herken, A. Welch Vol. 42: 99-228. Springer Verlag.

Magleby, K.L. (1973). The effect of tetanic and post-tetanic potentiation on facilitation of transmitter release at the frog neuromuscular junction. J. Physiol. 234: 353-371.

Magleby, K.L., J.E. Zengel (1975a). A dual effect of repetitive stimulation on post-tetanic potentiation of transmitter release at the frog neuromuscular junction. J. Physiol. 245: 163-182.

Magleby, K.L., J.E. Zengel (1975b). A quantitative description of tetanic and post-tetanic potentiation of transmitter release at the frog neuromuscular junction. J. Physiol. 245: 183-208.

McAllen, R.M. (1987). Central respiratory modulation of subretrofacial bulbospinal neurones in the cat. J. Physiol. (Lond.) 388: 533-545.

Merrill, E.A. (1970). The lateral respiratory neurons of the medulla: their association with nucleus ambiguus, nucleus retroambigualis, the spinal accessory nucleus and the spinal cord. Brain Pes. 24: 11-28.

Merrill, E.A. (1974). Finding a respiratory function for the medullory respiratory neurons. In: essays on the Nervous System, Ed. by R. Bellairs, E.A. Gray. Oxford. Oxford Univ. Press. pp. 451-486.

Merrill, E.G. (1981). Where are the real respiratory neurones? Fed. Proc. 40: 2389-2394.

Merrill, E.G. (1982). One source of expiratory inhibition of phrenic motoneurons in the cat. J. Physiol. 332: 779-789.

Merrill, E.G., L. Fedorko (1984). Monosynaptic inhibition of phrenic motoneurons; a long descending projection from

Bötzinger neurons, J. Neurosci. 4: 2350-2353.

Merrill, E.G., J. Lipski, L. Kubin, L. Fedorko (1983). Origin of the expiratory inhibition of nucleus tractus solitarius inspiratory neurones. Brain Res. 263: 43-50.

Miles, R., R.K.S. Wong (1983). Single neurones can initiate synchronised population discharge in the hippocampus in vitro. Nature 306: 371-373.

Miller, J.P., A.I. Selverston (1982). Mechanisms underlying pattern generation in lobster somatogastric ganglion as determined by selective inactivation of identified neurons. IV. Network properties of the pyloric system. J. Neurophysiol. 48: 1416-1432.

Mirgorodsky, V.N., V.I. Skok (1969). Intracellular potentials recorded from a tonically active mammalian sympathetic ganglion. Brain Res. 15: 570-572.

Mirgorodsky, V.N., V.I. Skok (1970). The role of different pregnanglionic fibers in tonic activity of the mammalian sympathetic ganglion. Brain Res. 22: 262-263.

Mitchell, R.A. (1977). Location and function of medullory respiratory neurons. Am. Rev. Resp. Dis. 115: 207-216, 1977.

Mitchell, R.A., D.A. Herbert (1974a). The effect of carbon dioxide on the membrane potential of medullary respiratory neurons. Brain Res. 75: 345-349.

Mitchell, R.A. D.A. Herbert (1974b). Synchronized high frequency

synaptic potentials in medullary respiratory neurons. Brain Res. 75: 350-355.

Mochida, S., B. Libet (1985). Synaptic long-term enhancement (LTE) induced by a hetrosynaptic neural input. Brain Res. 329: 360-363.

Netick, A., J. Orem (1981). Erroneous classification of neuronal activity by the respiratory modulation index. Neurosci. Lett. 21: 301-306.

Nield, T.O. (1978). Slowly developing depolarisation of neurones in the guinea pig inferior mesenteric ganglion following repetitive stimulation of the preganglionic nerves. Brain Res. 140: 231-239.

Nilsson, U., B. Ljung, N. Sjoblom, B.A. Wallin (1985). The influence of sympathetic impulse pattern on the contractile responses of rat mesenteric arteries and vein. Acta. Physiol. (Scand.). 123: 303-309.

Nishi, S., K. Koketsu (1968). Early and late afterdischarges of amphibian sympathetic ganglion cells. J. Neurophysiol. 31: 109-121.

Nishino, T., Y. Honda (1986). Time-dependent responses of respiration reflex in cats. J. Appl. Physiol. 61: 430-435.

Nja, A., D. Purves (1977). Specific innervation of guinea-pig superior cervical ganglion cells by preganglionic fibers arising from different levels of the spinal cord. J. Physiol. 264: 565-583.

North, R.A., T. Tokimassa (1984). The time course of muscarinic depolarisation of guinea-pig myenteric neurons. Brit. J. Pharmacol. 82: 85-92.

Olsen, M., G.S. Tyson, G.W. Maier, J.M. Davies, J.S. Rankin (1985) Diminished cardiac stroke volume during inspiration: a reverse thoracic pump. Circulation 72: 668-679.

Pantaleo, T., M. Corda (1985). Expiration-related neurons in the region of the retrofacial nucleus: vagal and laryngeal inhibitory influences. Brain Res. 359: 343-346.
Res. 359: 343-346.

Percy, W.H., J. Krier (1987). Cholinergic excitatory synaptic potentials of neurones in mammalian lumbar paravertebral ganglia. J. Auto. Nerv. Syst. 18: 195-205.

Perri, V., O. Sacchi, C. Casella (1970). Electrical properties and synaptic connections of the sympathetic neurons in the rat and guinea pig superior cervical ganglion. Pflugers Arch. 314: 40-54.

Perri, V., O. Sacchi, C. Casella (1970). Synaptically mediated potentials elicited by the stimulation of post-ganglionic trunks in the Guinea-Pig superior cervical ganglion. Pflügers Arch. 314: 55-67.

Peterson, E.L. (1983). Generation and coordination of heartbeat timing oscillation in the medicinal leech. I. Oscillation in isolated ganglia. J. Neurophysiol. 49: 611-626.

Pinsker, H.M., J. Ayers. (1983). Neuronal oscillators. In:

Rosenberg. R.N (ed): The Clinical Neurosciences. New York: Churchill Livingston. Vol. 5. pp. 203-266.

Polosa, C. (1968). Spontaneous activity of sympathetic preganglionic neurons. Can. J. Physiol. Pharm. 46: 887-896.

Polosa, C., U. Gerber, R. Schondorf (1980). Central mechanisms of interactions between sympathetic preganglionic neurons and the respiratory oscillator, In: Central Interactions Between Respiratory and Cardiovascular Control Systems. Ed. H.P. Koepchen, S.M. Milton, A. Trzebski. Springer Verlag, (Berlin). pp. 137-143.

Polosa, C., F. Lioy, B.D. Hanna (1983). The role of the ventral medulla in the control of sympathetic activity by systemic arterial CO_2. In: Central Neurone Environment. Ed. M.E. Schlaefke.

Preiss, G., F. Kirchner, C. Polosa (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. Brain Res. 87: 363-374.

Preiss, A., C. Polosa (1977). The relation between end-tidal CO_2 and the discharge pattern of sympathetic preganglionic neurons. Brain Res. 122: 255-267.

Purves, D., D.J. Wigston (1983). Neural units in the superior cervical ganglion of the guinea pig. J. Physiol. 334: 169-178.

Purves, D., J.W. Lichtman. (1978). Formation and maintenance of synaptic connections in autonomic ganglia. Physiol. Rev.

58: 821-862.

Rahamimoff, H., E. Aramovitz, D. Papazian, S.M. Goldin, R. Spanier (1980). Calcium transport systems in nerve terminals. Studies on membrane vesicles. J. Physiol. (Paris) 76: 487-495.

Redman, S., B. Walmsley (1983). Amplitude fluctuations in synaptic potentials evoked in cat spinal motoneurones at identified group Ia synapses. J. Physiol. 343: 135-145.

Remmers, J.E., D.W. Richter, D. Ballantyne, C.R. Bainton, J.P. Klein (1986). Reflex prolongation of the stage I of expiration. Pflügers Arch. 407: 190-198.

Richardson, C.A., R.A. Mitchell (1982). Power spectral analysis of spectral analysis of inspiratory nerve activity in the decerebrate cat. Brain Res. 233: 317-336.

Richter, D.W. (1982). Generation and maintenance of the respiratory rhythm. J. Exp. Biol. 100: 93-107.

Richter, D.W., D. Ballantyne (1983). A three phase theory about the basic respiratory pattern generator. In: M.E. Schlaefke, H.P. Koepchen, W.R. See (Eds). Central Neurone Environment and the Control Systems of Breathing and Circulation, Springer, Berlin-New York, 1983 pp. 164-174.

Richter, D.W., Ballantyne, D., Remmers, J.E. (1986). How is the respiratory rhythm generated? A model. NIPS 1: 109-112.

Richter, D.W., Ballantyne, D., Remmers, J.E. (1987). The differential organization of medullary post-inspiratory

activities. Pflûgers Arch. 410: 420-427.

Richter, D.W., H. Cameron, M. Meesman, N. Roehrig (1979). Studies on synaptic interconnections between bulbar respiratory neurons of cats. Pflügers Arch. 380: 245-257.

Richter, D.W., F. Heyde (1975). Accommodative reactions of medullary respiratory neurons of the cat. J. Neurophysiol. 38: 1162-1171.

Richter, D.W., F. Heyde, M. Gabriel. (1975). Intracellular recordings from different types of medullary respiratory neurons of the cat. J. Neurophysiol. 38: 1162-1171.

Robertson, R.M., M. Moulins (1984). Oscillatory command input to the motor pattern generators of the crustacean somatogastric ganglion. II. Gastric rhythm. J. Comp. Physiol. 154: 473-492.

Rosenbaum, M., D. Race (1968). Frequency-response characteristics of vascular resistance vessels. Am. J. Physiol. 215: 1397-1402.

Sacchi, O., V. Perri (1971). Quantal release of acetylcholine from the nerve endings of the Guinea Pig superior cervical ganglion. Plûgers Arch. 329: 207-219.

Salmoiraghi, A.C., B.D. Burns (1960). Notes on mechanisms of rhythmic respiration. J. Neurophysiol. 23: 14-26.

Satchell, G.H., (1960). The reflex coordination of the heart beat with respiration of teleost fish. J. Exp. Biol. 37: 719-731.

Satterlie, R.A. (1985). Reciprocal inhibition and postinhibitory rebound produce reverberation in a locomotor pattern generator. Science 229: 402-404.

Sears, T.A., A.J. Berger, E.A. Phillipson (1982). Reciprocal tonic activation of inspiratory and expiratory motoneurones by chemical drives. Nature (Lond.) 299: 728-730.

See, W.R., M.I. Cohen, R, Barnhardt, C.N. Christakos (1987). Intracellular potentials and discharge patterns of expiratory neurons in the caudal ventral respiratory group: influence of phasic pulmonary afferent input. Brain Res. 421: 363-366.

Segers, L.S., Shannon., Saporta, S., Lindsay, B.G. (1987). Functional associations among lateral medullary respiratory neurons in the cat. I. Evidence for excitatory and inhibitory actions of inspiratory neurons. J. Neurophysiol. 57: 1078-1100.

Selverston, A.I., J.P. Miller (1980). Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. I. Phyloric system. J. Neurophysiol. 44: 1102-1121.

Selyanko, A.A., V.A. Derkach, V.I. Skok (1979). Fast excitatory postsynaptic currents in voltage-clamped mammalian sympathetic ganglion neurons. J. Auto Nerv. Syst. 1: 127-137.

Shelton, G., D.J. Randall (1962). The relationship between heart beat and respiration in teleost fish. Comp. Biochem. Physiol. 7: 237-250.

Short, S.P., J. Butler, E.W. Taylor (1977). The relative importance of nervous, humoral, and intrinsic mechanisms in the regulation of heart rate and stroke volume in the dogfish, schliorhinus canicula. J. Exp. Biol. 70: 77-92.

Shulman, J.A., F.F. Weight (1976). Synaptic transmission: long lasting potentiation by a postsynaptic mechanism. Science. 194: 1437-1439.

Skok, V.I. (1986). Spontaneous and Reflex activities: General characteristics. In: Autonomic and Enteric Ganglia. Ed. by A.G. Karczmar, K. Koketsu, S. Nishi pp. 137-160.

Speck, D.F., J.L. Feldman (1982). The effect of microstimulation and microlesions in the ventral and dorsal respiratory groups in the medulla of the cat. J. Neurosci. 2: 744-757.

St.John, W.M., D. Bartlett, K.V. Knuth, J.C. Hwong (1981). Brain stem genesis of automatic ventilatory patterns independent of spinal mechanisms. J. Appl. Physiol. 51: 204-210.

Summer, W.R., S. Permutt, K. Sagawa, A. Shoukas, B. Bromberger-Barnea (1979). Effects of respiration on spontaneous respiration on canine left ventricular function. Circ. Res. 45: 719-725.

Suzue, T. (1984). Respiratory rhythm generation in the in-vitro brain stem-spinal cord preparation of the neonatal rat. J. Physiol. 354: 173-183.

Taylor, E.W., P.J. Butler (1982). Nervous control of heart rate: activity in the cardiac vagus of the dogfish. J. Appl. Physiol. 53: 1330-1335.

Taylor, E.K., J. Duffin, B.R. Vachon, D.M. McCracken (1978). The recruitment timer and firing patterns of the medullary respiratory neurons of the cat. Respir. Physiol. 34: 247-266.

Tazaki, K., I.M. Cooke (1979). Spontaneous electrical activity and interactions of large and small cells in cardiac ganglion of the crab, Portunus sanguinolentus. J. Neurophysiol. 42: 975-999.

Trimby, R.M., H.C. Nicholson (1924). Some observations on the nature of the respiratory waves in arterial blood pressure. Am. J. Physiol. 70: 289-291.

Vassalle, M. (1977). The relationship among cardiac pacemakers: overdrive suppression. Circ. Res. 41: 269-277.

Wallin, B.G., J. Fagius (1986). The sympathetic nervous system in man - aspects derived from microelectrode recordings. TINS (Feb) 63-67.

Wigston, D.J. (1983). Innervation of individual guinea pig superior cervical ganglion cells by axons with similar conduction velocity. J. Physiol. 334: 179-187.

Wojtowicz, J.M., H.L. Atwood (1985). Correlation of presynaptic and postsynaptic events during establishment of long term facilitation at crayfish neuromuscular junction. J.

Neurophysiol. 54: 220-230.

Wojtewicz, J.W., H.L. Atwood (1986). Long-term facilitation alters transmitter releasing properties at the crayfish neuromuscular junction. J. Neurophysiol. 55: 484-498.

Wurster, R.D., C.A. Connelly (1987). Phase changes of sympathetic activity with respiration before and after pontine lesions. In: Organization of the Autonomic nervous system: Central and Peripheral Mechanisms. Ed. J. Ciriello, F.R. Calaresu, L.P. Renaud, C. Polosa, pp. 169-178. Alan Liss Inc.

Yoshimura, M., C. Polosa, S. Nishi (1986). Afterhyperpolorisation mechanisms in the cat sympathetic preganglionic neuron in vitro. J. Neurophysiol. 55: 1234-1246.

Yoshimura, M., C. Polosa, S. Nishi (1987). Noradrenaline induces rhythmic bursting in sympathetic preganglionic neurons. Brain Res. 420: 147-151.

Zengel, J.E., K.L. Magleby, J.P. Horn, D.A. McAfee, P.J. Yarowsky (1980). Facilitation, augmentation, and the potentiation of synaptic transmission at the superior cervical ganglion of the rabbit. J. Gen. Physiol. 76: 213-233.