# PROPERTIES OF SYMPATHETIC NEURON RESPONSES TO CEREBRAL ISCHEMIA AND TO SYSTEMIC HYPOXIA OR HYPERCAPNIA WHICH SUGGEST MEDIATION BY CENTRAL CHEMOSENSITIVE MECHANISMS

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## ABSTRACT

This thesis concerns the possible existence of central nervous system (CNS) chemosensitive mechanisms influencing sympathetic activity. The thesis is based on observations of sympathetic neuron and cardiovascular responses to CNS ischemia, systemic hypoxia and systemic hypercapnia. Investigation of the pressor response to cerebral ischemia in the cat indicates that it is mediated by superficial regions of the ventral medulla also involved in the pressor response to central hypercapnia. Experiments concerning the sympathetic response to systemic hypoxia in the CNS-intact sino-aortic denervated cat revealed a two-component response of the firing rates of single sympathetic preganglionic neurons (SPN) , the mass activity of the cervical sympathetic trunk, and the neurogenic component of hindlimb vascular resistance (N-HLVR). The response consisted of: i) an increase of all three variables during extreme hypoxia, and ii) a decrease during moderate hypoxia. The hypoxic sympatho-depression resulted from loss of central respiratory input to SPNs as well as of respiration-independent input. The hypoxic sympatho-excitation involved only the latter input. Investigation of the sympathetic response to systemic hypercapnia in the acute $C_1$ spinal cat demonstrated a direct relationship between SPN firing rate or N-HLVR and arterial $PCO_2$ between normocapnia and severe hypercapnia. N-HLVR also increased in this preparation during systemic hypoxia.

## RESUME

Cette thèse concerne les mécanisme chimiosensibles du système nerveux central (SNC) qui peut influencent l'activité du système sympathique durant d'ischémie cérébrale, d'hypoxie systémique ou d'hypercapnie systémique. L'étude de la réponse pressive, suite à une ischémie cérébrale chez le chat, démontre que cette réponse est produite par l'intermédiare des régions superficielle de la portion ventrale du bulbe rachidien. Ces régions participent aussi à la réponse pressive observée lors d'une hypercapnie centrale. Des expériences relatives à la réponse sympathique résultant des suites d'une hypoxie systémique pratiquée sur des chats possédant un SNC intact, à l'exception d'une ennervation sino-aortique, révèlent que les changements de la fréquence de décharge des neurones préganglionnaires sympathiques individuel (NPS), de l'action de masse du tronc sympathique cervical et de la composante neurogène de la résistance vasculaire des pattes postérieures (M-RVPP) sont formés de deux composantes: i) une augmentation lors d'hypoxie aigüe et ii) une réduction lors d'hypoxie modérée. L'abaissement de la fonction sympathique est produite par la perte d'influx nerveux provenant des centres respiratoires sur les NPS et d'influx indépendants de la respiration. Pour sa part, l'excitation de la fonction sympathique est due à des influx indépendants de la respiration. L'étude de la réponse sympathique lors d'une hypercapnie systémique chez un chat avec la moelle épinière complètement sectionée au niveau de la première vertèbre cervicale, démontre une relation directe entre la fréquence de décharge des NPSs, ainsi que la N-RVPP et le $CO_2$ artériele entre une normocapnie et une hypercapnie aigüe. La N-RVPP augmente aussi dans ce type de préparation lors d'une hypoxie systémique.

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It is acceptable for theses to include as chapters authentic copies of papers already published, provided these are duplicated clearly on regulation thesis stationery and bound as an integral part of the thesis. Photographs or other materials which do not duplicate well must be included in their original form. In such instances, connecting texts are mandatory and supplementary explanatory material is almost always necessary.

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## Authorship

The Candidate has taken advantage of the option to submit as part of this thesis the text of 7 published papers (see previous page regarding McGill thesis guidelines). These papers are principally the work of the Candidate in collaboration with the thesis supervisor Dr. Canio Polosa of McGill University. Individuals other than the Candidate and the thesis supervisor were involved in two of the seven papers. Dr. T. Hakim of McGill University assisted in the performance of the experiments described in the paper entitled: "Neural effects of systemic hypoxia on hindlimb vascular resistance in sino-aortic denervated cats" (Rohlicek, Hakim, & Polosa, 1986) by bringing expertise in vascular perfusion and isolation to the project. The experimental work described in the paper entitled; "Responses of sympathetic preganglionic neurons to systemic hypercapnia in the acute spinal cat" (Zhang, Rohlicek, & Polosa, 1982) was performed in collaboration with Dr. T.X. Zhang while the latter was a visiting scholar from Tianjin Institute of Materia Medica, Tianjin, Peoples Republic of China. This work was based on preliminary results obtained by the Candidate during preparation of a previous publication (Rohlicek & Polosa, 1981). The Candidate was intimately involved in the planning and performance of all the experiments as well as in the preparation of the manuscript.

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## ABBREVIATIONS

| | |
|---|---|
| ATP | Adenosine tri-phosphate |
| CBF | Cerebral blood flow |
| CIR | Cerebral ischemic response |
| CNS | Central nervous system |
| CPP | Cerebral perfusion pressure |
| CST | Cervical sympathetic trunk |
| EPP | End-plate potential |
| EPSP | Excitatory post-synaptic potential |
| ISSA | Inspiration-synchronous sympathetic activity |
| $P_ACO_2$ | Alveolar carbon dioxide tension |
| $P_AO_2$ | Alveolar oxygen tension |
| $PaCO_2$ | Arterial carbon dioxide tension |
| $PaO_2$ | Arterial oxygen tension |
| $P_{insp}CO_2$ | Inspired carbon dioxide tension |
| $P_{insp}O_2$ | Inspired oxygen tension |
| PP | Perfusion pressure |
| SAD | Sino-aortic denervated |
| SAP | Systemic arterial pressure |
| SPN | Sympathetic preganglionic neuron |
| TSA | Tonic sympathetic activity |
| VLM | Ventrolateral medulla |

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# CHAPTER 1

## PREFACE

The component of cardiovascular tone generated by the sympathetic system is important not only for the maintenance of a normal arterial blood pressure but also for a number of adaptive responses of the cardiovascular system. Changes in sympathetic activity are largely the result of input from various sensory and central neuronal systems which provide the sympathetic preganglionic neurons with information regarding both the internal and external environment of the organism (Polosa et al., 1979). The observation that cerebral ischemia causes a large neurogenic pressor response (McDowall, 1933; Guyton, 1948; Sagawa et al., 1961; Downing et al., 1963; Levy et al., 1968; Takeuchi et al., 1969; Dampney et al., 1979; Dampney & Moon, 1980) suggests that one input carrying information about the internal environment to sympathetic neurons is generated intracranially. One hypothesis is that the pressor response to cerebral ischemia is a manifestation of central chemosensitivity influencing the sympathetic system. The presence of such central chemosensitivity is suggested by the observation that cephalic perfusion with hypoxic or hypercapnic blood has a marked excitatory effect upon sympathetic activity (Kao et al., 1962; Downing et al., 1963; DeGeest et al., 1965a; McGillicudy et al.,1978; Hainsworth et al., 1984; Ford et al., 1985). By initiating various cardiovascular adjustments such central chemosensitivity may be considered as a line of defence for protecting the "normal" chemical composition of the environment of the central nervous system (CNS). Such central chemosensitivity may also influence sympathetic neurons over a range of conditions wider than just cerebral ischemia .

This thesis deals with the problem of chemosensitive mechanisms within the CNS which influence sympathetic preganglionic neurons under experimental situations which decrease perfusion, cause $O_2$ lack , or produce $CO_2$ excess in the CNS. The principal questions which are addressed are: i) the location of these mechanisms, and ii) their properties and the extent of control over sympathetic neuron activity that they exert.

The main original contributions of this thesis are:

i) The discovery that the pressor response to cerebral ischemia is greatly attenuated by inactivation of superficial regions of the ventral medulla which include those important in mediating central respiratory and sympathetic $CO_2$ sensitivity.

ii) The definition of the relationship between systemic hypoxia and sympathetic neuron activity in the CNS-intact, sino-aortic denervated (SAD) animal as well as the determination of the importance of systemic hypoxia in this preparation and in the acute $C_1$ spinal animal in controlling the activity of a large population of sympathetic neurons which innervate hindlimb vascular smooth muscle.

iii) The discovery of a supra-spinal sympatho-depressant effect of systemic hypoxia in the CNS-intact SAD animal. This effect appears to be due in part to loss of the excitatory input to sympathetic preganglionic neurons from brainstem inspiratory neurons and in part to withdrawal of excitatory input not related to central respiratory activity.

iv) The discovery of a relationship between systemic hypercapnia and sympathetic neuron activity in the acute $C_1$ spinal animal as well as the determination of the importance of systemic hypercapnia in this preparation in controlling the activity of a large population of sympathetic neurons innervating hindlimb vascular smooth muscle.

# CHAPTER 2

## INTRODUCTION

The mammalian central nervous system (CNS) is one of the tissues in the body the function of which is most dependent on an adequate supply of blood. This requirement is a consequence of the absence in the CNS of significant energy stores or $O_2$ reserves and of the inability of nervous tissue to produce adequate amounts of high energy intermediates such as ATP or phosphocreatine by anaerobic mechanisms (McIlwain & Bachelard, 1985). The critical dependence of brain function on cerebral blood flow (CBF) is dramatically demonstrated by observations in various species that following interruption of CBF loss of consciousness occurs within 10 seconds (Rossen et al., 1943), respiratory activity ceases within less than one minute (Schmidt, 1928), and necrotic changes in some CNS regions are initiated after as little as five minutes (Ito et al., 1975).

Several rapidly acting cardiovascular reflexes contribute to ensure the maintenance of an adequate CBF. One such reflex is that evoked by the baroreceptors of the carotid sinus and aortic arch. During conditions of decreased systemic arterial pressure (SAP) baroreceptor unloading can cause an increase in total peripheral resistance and heart rate which tends to restore SAP and CBF (Heymans & Neil, 1958). Similar reflex effects may also occur as a result of arterial chemoreceptor excitation (Landgren & Neil, 1951; Lee et al., 1964; Paintal, 1967; Biscoe et al., 1970; Lahiri et al., 1980) or unloading of cardiopulmonary mechanoreceptors (Gupta et al., 1966; Öberg & White, 1970; Pelletier et al., 1971; Clement et al., 1972; Chen et al., 1978) during systemic hypotension. Another reflex mechanism, located intracranially, is revealed by

the observation that when CBF in the sino-aortic denervated (SAD) preparation is reduced below a critical value SAP begins to rise (Sagawa et al., 1961). This pressor response to cerebral ischemia has been termed the cerebral ischemic response (CIR) (Dampney et al., 1979). While it has been established that the CIR is neurogenic (e.g. Dampney et al., 1979) and of brainstem origin (Takeuchi et al., 1969) little is known about its trigger mechanisms.

The CIR could be the result of a sensitivity of the sympathetic system to changes in the chemical composition of the extracellular and/or intracellular fluid of the CNS. It is known that CNS ischemia results in nervous tissue hypoxia (Wadouh et al., 1985) and hypercapnia (Siesjö & Wieloch, 1985). Both CNS hypoxia and hypercapnia have been shown to lead to increased sympathetic activity (Kao et al., 1962; Downing et al., 1963; DeGeest et al., 1965a; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985). Thus it is conceivable that changes in nervous tissue $PO_2$ and $PCO_2$ are involved in generating the CIR. The observation of Sagawa et al. (1961) that below a critical value of CBF SAP increases as a continuous , inverse, function of decreasing CBF suggests that the postulated chemosensitive mechanisms of CIR production are likely to have relevance even outside the extreme conditions of complete interruption of CBF. Thus the CIR might be the expression of chemosensitive mechanisms which provide a link between the central neuronal environment and sympathetic activity over a wide range of conditions and thus exert a graded control over the cardiovascular system. The operation of such mechanisms, as a result of chronic disturbances of the blood supply to the brain, has been suggested as a possible cause of certain clinically observed cases of neurogenic hypertension (Guyton, 1948; Dickinson, 1964). Central chemosensitive mechanisms affecting sympathetic activity may not be restricted to the brainstem. In the same manner that chemosensitive brain mechanisms may be thought to be responsible for the CIR, spinal chemosensitive mechanisms activated by spinal cord ischemia

(Rohlicek & Polosa, 1981) may be responsible for the pressor responses to spinal cord compression (Groat 1945, Hoff & Reis 1970, Meyer & Winter 1970, Eidelberg 1973).

Support for the hypothesis that central chemosensitive mechanisms are involved in generating the CIR remains circumstantial. Furthermore, the response of the sympathetic system to conditions which may cause changes in the chemical composition of the CNS environment, such as decreased $PO_2$ or increased $PCO_2$, is not entirely known. The experiments presented in this thesis concern the problem of chemosensitive mechanisms within the CNS which influence sympathetic preganglionic neurons under experimental situations which decrease perfusion, cause $O_2$ lack or produce $CO_2$ excess in the CNS. The INTRODUCTION of this thesis reviews present knowledge concerning CNS ischemia and the CIR as well as concerning central sympathetic sensitivity to hypoxia and hypercapnia.

In the first part of the INTRODUCTION various issues related to CNS ischemia are considered. The metabolic constraints dictating the need for an uninterrupted blood flow to the CNS are outlined. The two main compensatory mechanisms for the maintenance of adequate tissue perfusion, decrease in vascular resistance, and increase in perfusion pressure, are discussed next. The limitations of the autoregulatory mechanisms acting on CNS vascular resistance are examined. The literature on the neurogenic increase in SAP seen during impaired CBF is reviewed. The phenomenon of an increase in SAP during decreased spinal cord perfusion is also discussed. Present knowledge concerning the regions of the brain which may be initiating the pressor response to cerebral ischemia and the nature of the stimuli involved are reviewed. Existing evidence suggesting that the CIR may be the expression of a chemoreflex of central origin is reviewed.

In the second part of the INTRODUCTION present knowledge concerning sympathetic responses to systemic hypoxia and hypercapnia is reviewed. As a start, the role of the arterial chemoreceptors in these responses is reviewed. The sympatho-

excitatory effects of systemic hypoxia and hypercapnia following arterial chemoreceptor denervation are described next. The evidence for spinal and supra-spinal components of such responses is examined. Finally, the evidence that the supra-spinal component of the sympathetic response to changes in arterial $PCO_2$ is mediated by superficial regions of the ventrolateral medulla is reviewed.

## I) CARDIOVASCULAR RESPONSE TO CNS ISCHEMIA

### CNS energy metabolism

The metabolic characteristics of mammalian nervous tissue create the necessity for continuous vascular perfusion. The mammalian CNS has a very high metabolic rate (3.5 ml $O_2$/100 g/min (Altman & Dittmar, 1974)) in comparison to the body as a whole (0.33 ml $O_2$/100g/min (Altman & Dittmar, 1971)) and, in contrast to other body tissues, it is almost completely dependent on the oxidation of glucose as a source of energy (McIlwain & Bachelard, 1985)[1]. Since glycogen, glucose and $O_2$ reserves in the CNS are quite limited in relation to metabolic demand, energy production by the CNS is dependent on a continuous supply of glucose and $O_2$ by the arterial blood (Patton et al., 1976). The high metabolic rate and the dependence on exogenous glucose supply is reflected in the fact that while the CNS accounts for only 3% of body weight it is responsible for 25% of total body consumption of glucose (Bachelard, 1970). More than 90% of the glucose used is utilized for energy production by way of glycolysis and the tricarboxylic acid cycle, while the remainder is used in the synthesis of amino acids,

[1] Nervous tissue can also metabolize circulating β-hydroxybutyrate and acetoacetate. However, periods of prolonged fasting are required for appreciable levels of these compounds to appear in the circulation and for these ketoacids to displace glucose oxidation as the primary energy source in the CNS (Cahill, 1970; McIlwain & Bachelard, 1985).

proteins, lipids and nucleic acids (McIlwain & Bachelard, 1985). The almost exclusive use of oxidation of glucose as energy source by the CNS is indicated by the fact that the brain has a respiratory quotient near unity and a ratio of $O_2$ uptake to glucose consumption of 5.5 . The latter value is quite close to the stoichiometric ratio of 6 expected from the complete oxidation of glucose (Balazs, 1970)[2]:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Moreover, cerebral $O_2$ uptake is significantly decreased if arterial glucose content is reduced from the normal level of 4.5 - 5.5 mmol/l to 1.0 mmol/l (McIlwain, 1985). The effects of cerebral ischemia on carbohydrate metabolism and energy state have been studied in some detail (see reviews by Maker & Lehrer, 1971; Siesjö & Wieloch 1985). In the awake rat one minute of cerebral ischemia resulted in a decrease in brain glucose content of 97%, a tissue pH decrease from 7.04 to 6.60 as a result of lactate accumulation, and an average increase in tissue $PCO_2$ from 45 mm Hg to 99 mm Hg largely due to the reaction of lactate with the bicarbonate buffer system (Ljunggren et al., 1974).

**Compensatory mechanisms for the maintenance of CNS blood flow**

When blood flow becomes inadequate to meet the metabolic demands of an organ there are only two compensatory mechanisms available (Dickinson, 1964). One consists of decreasing the resistance of the vascular bed of the organ by dilation of blood vessels already in use as well as by opening previously closed ones. The other

[2]Cerebral energy metabolism under normal conditions and the role of carbohydrate metabolism in CNS energy supply is dealt with extensively in numerous texts (White et al., 1978; Orten et al., 1982; McIlwain & Bachelard, 1985;) and reviews (Bachelard, 1970; Balazs, 1970; Smith & Sokoloff, 1981; Hawkins & Mans, 1983) to which the reader is referred for more detailed information.

mechanism is by increasing SAP, i.e. the perfusion pressure of the organ. Both mechanisms are brought into play during impaired brain blood flow. As mentioned above SAP increases markedly during cerebral ischemia. In addition the cerebral circulation possesses strong autoregulatory properties which tend to maintain CBF in the face of changing cerebral perfusion pressure (CPP). However, in contrast to other organs, such as those of the gastrointestinal tract, the potential for the opening of collateral channels of blood flow to the brain are extremely limited since the blood supply to the mammalian brain is by way of only two pairs of arteries; the carotid and vertebral arteries (see APPENDIX A). The autoregulatory properties of the CNS circulation, as well as the cardiovascular response to CNS ischemia, are considered below in greater detail.

**Autoregulation of CNS blood flow**

The term "autoregulation" is commonly used to refer to a relative independence of blood flow from vascular perfusion pressure. One manner in which adequate CBF is ensured is through the autoregulatory properties of the cerebral circulation. A number of reviews concerning the intrinsic regulation of the cerebral circulation have been published (Lassen, 1976; Kuschinsky, 1978; Kontos, 1981; Mchedlishvili, 1986). It is well established that CBF remains relatively constant over a range of SAP values from 40 to 150 mm Hg (Kuschinsky, 1978; Mchedlishvili, 1986). In addition, it is becoming increasingly evident that a tight coupling exists regionally between cerebral function, metabolism, and blood flow (Kuschinsky, 1978; Mchedlishvili, 1986). Experimentally induced changes in the functional activity of specific brain regions are associated with changes in local glucose consumption and blood flow (Sokoloff, 1961; Des Rosiers et al., 1974; Sokoloff, 1978; Smith & Sokoloff, 1981). The autoregulation of CBF and the close association of cerebral metabolism to blood flow are thought to be mediated largely by local chemical factors. It is known that an increase in arterial $PCO_2$ or a decrease in arterial

$PO_2$ produce significant cerebral vasodilation (Wolff, 1936). The cerebral vasodilation during arterial hypercapnia may be due mainly to the action of extracellular $H^+$ on cerebral vascular smooth muscle (Kontos, 1981; Mchedlishvili, 1986). Increases in the extracellular concentrations of adenosine, $K^+$, and $H^+$ have been implicated in the cerebral vascular response to arterial hypoxia (Kuschinsky, 1978). A myogenic mechanism such as that postulated by Bayliss (1902) may also play a role in the autoregulation of CBF (Kontos, 1981). Bayliss (1902) proposed that vascular smooth muscle reacts to an increase in vessel wall tension by contraction and to a decrease in such tension by relaxation thus increasing or decreasing vascular resistance respectively. This mechanism may be viewed as helping to maintain a constant blood flow through tissues despite changes in perfusion pressure. However, involvement of such a mechanism in the control of the cerebral circulation has not been clearly demonstrated (Mchedlishvili, 1986). Whether the innervation of the cerebral vessels has a significant role in the regulation of CBF remains controversial (c.f. Heistad, 1978; Purves, 1978; Siesjö & Ingvar, 1983; Mchedlishvili, 1986).

Studies of the spinal circulation are not as extensive as those of the cerebral circulation. However, there are indications that marked similarities between the two exist (see review by Sandler & Tator, 1976). For instance, autoregulation of spinal cord blood flow over a range of SAPs from 50 to 160 mm Hg has been described by a number of authors (Flohr et al., 1970; Griffiths, 1973; Kobrine et al., 1976; Hickey et al., 1986). Investigations of the relation between spinal cord metabolic activity and spinal cord blood flow have not been conducted. It is known, however, that an increase in $PaCO_2$ or a large decrease in $PaO_2$ causes significant increases in spinal cord blood flow (Sandler & Tator, 1976).

**Cardiovascular effects of acute CNS ischemia**

A second manner by which inadequate CBF may be corrected is through an increase in SAP and hence CPP. Interruption of blood flow to the brain results in a stereotyped response, in a number of different animal species, consisting of an increase in SAP, total peripheral resistance and ventricular contractility, of bradycardia, and of a decrease in cardiac output as well as of hyperpnea followed by apnea[3] (McDowall, 1933; Guyton, 1948; Sagawa et al., 1961; Downing et al., 1963; Levy et al., 1968; Takeuchi et al., 1969; Dampney et al., 1979; Dampney & Moon, 1980). The cardiovascular effects of cerebral ischemia have been termed the cerebral ischemic response (CIR) (Dampney et al., 1979). Some of the properties of the CIR are illustrated in Fig. 1. The pressor response to cerebral ischemia begins within 3 to 4 seconds of the interruption of cerebral blood flow and reaches a peak within 30 seconds (Dampney et al., 1979). The increase in SAP during cerebral ischemia is due mainly to neurally mediated peripheral vasoconstriction (Downing et al., 1963; Takeuchi et al., 1969; Dampney et al., 1979). Although adreno-medullary catecholamine release increases during cerebral ischemia (Anrep, 1925) this does not appear to be a significant factor in the peripheral vasoconstriction (Dampney et al., 1979). A mechanical increase in total peripheral resistance caused by occlusion of the carotid and vertebral arteries can account for an increase in SAP of less than 25% (see APPENDIX B) which is considerably less than that which is usually seen during cerebral ischemia (i.e. Dampney et al., 1979). The increase in ventricular contractility and the bradycardia , seen during cerebral ischemia , are the net result of enhanced cardiac sympathetic and parasympathetic tone (Downing et al., 1963;

[3]Occlusion of the carotid and vertebral arteries, in the dog, produces an initial respiratory stimulation followed by respiratory arrest(Hill, 1896). The respiratory effects of reduction or interruption of CBF were studied in some detail by Schmidt (1928). Severe reduction or interruption of CBF for 1 to 2 min. in anaesthetized cats produced a transient hyperpnea, lasting for about 20 seconds, followed by apnea. The effect was reversible. More recent experiments in the unanaesthetized goat have confirmed Schmidt's observations (Chapman et al., 1979a, 1979b).

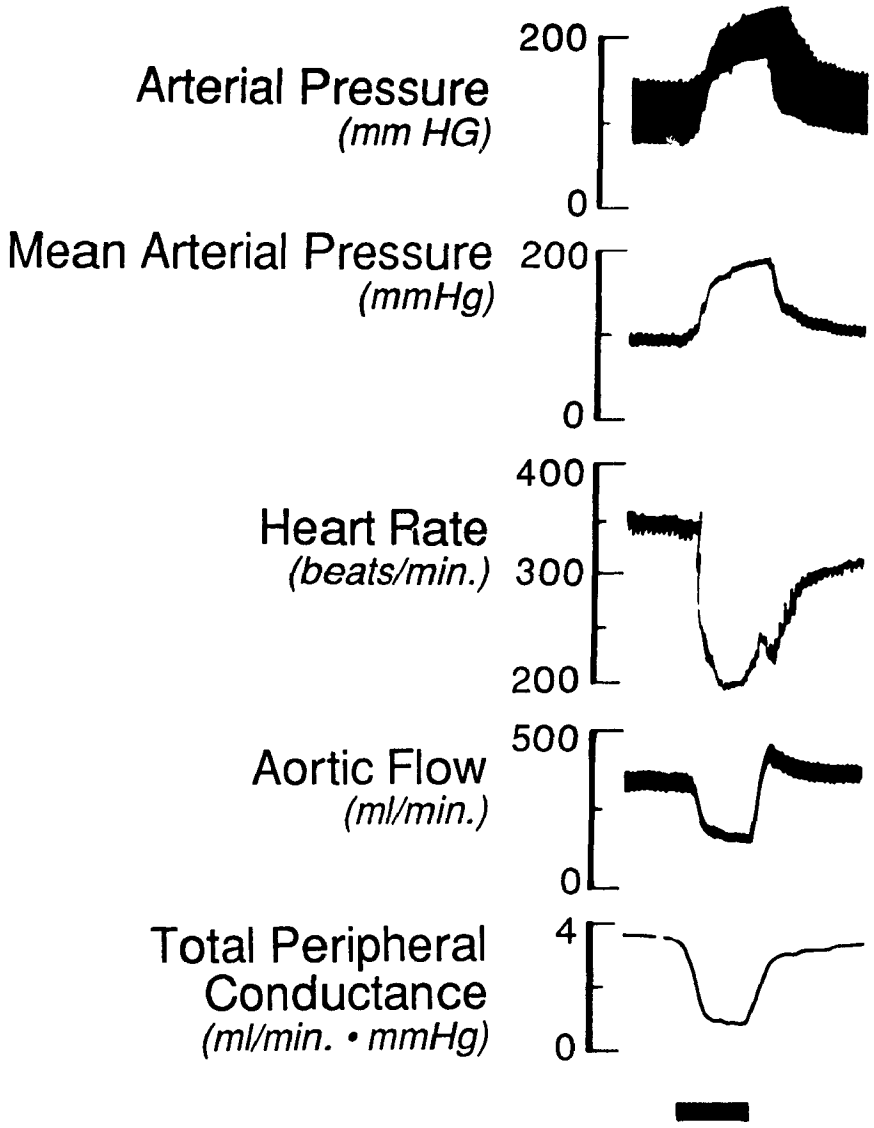


*Fig. 1.* Cardiovascular effects of acute cerebral ischemia in the rabbit produced by occlusion of he vertebral and common carotid arteries. Solid horizontal bar at the bottom represents the 35 second period of cerebral ischemia (Dampney et al.,1979).

Levy et al., 1968). Following cervical vagotomy, tachycardia and a greater increase in ventricular contractility are seen during cerebral ischemia (Levy et al., 1968). The net effect of the neurogenic vascular and cardiac effects of cerebral ischemia on cardiac output is a decrease (Dampney et al., 1979). It is of interest that raised intracranial pressure is associated with cardiovascular and respiratory responses similar to the CIR[4]. It has long been suggested that the pressor response to raised intracranial pressure is a result of nervous tissue ischemia (Cushing 1901,1903).

Sagawa et al. (1961) studied in detail the relationships between cerebral perfusion pressure (CPP) or CBF and SAP in sino-aortic denervated anaesthetized dogs with an isolated, artificially perfused, cerebral circulation in which CPP could be controlled independently of SAP. In the range of CPP from 140 to 0 mmHg and of CBF from 115 to 0 ml/100g/min. these relationships were approximately hyperbolic (Fig. 2). SAP did not change until CPP had decreased to 40-60 mmHg and CBF had fallen to 20-40 ml/100g/min. Below these levels SAP increased markedly in response to decreases in CPP or CBF. The maximum increase in SAP was 100 - 180 mmHg, from a control level of 86 mmHg, and occurred during complete ischemia.

Of interest is that during conditions of severely impaired CPP and CBF, SAP may oscillate (Sagawa et al., 1962; Miyakawa et al., 1984). Sagawa et al. (1962) performed

[4]Cushing (1901,1903) found that when intracranial pressure (ICP) was raised above SAP hypertension, bradycardia and apnea ensued. Cushing (1901) attributed these effects to cerebral ischemia, since when ICP exceeded SAP CBF would be interrupted. This remains a popular but as yet unproven hypothesis. The cardiovascular effects of intracranial hypertension are mediated primarily by an excitation of the sympathetic nervous system (Brashear et al., 1970). An increase in sympathetic activity (Tanaka et al., 1976) and peripheral resistance (Brown 1956; Richardson, 1965; Ducker et al., 1968) have been observed during raised intracranial pressure. The vagally mediated bradycardia reported by Cushing (1901) has been confirmed by more recent investigators (Evans, 1967; Doba & Reis, 1972) although others have observed an initial bradycardia followed by a return of heart rate to control level (Richardson, 1965) or have seen an increase in heart rate (Brashear, 1970; Graf, 1978; Roozerkans, 1979). Both decreases and increases in cardiac output have been observed during increased ICP (Richardson, 1965; Ducker, 1968; Brashear, 1978). Investigation of the respiratory effects of increased ICP has shown that there is an initial increase in respiratory rate and tidal volume followed by apnea (Jennett & North, 1975).

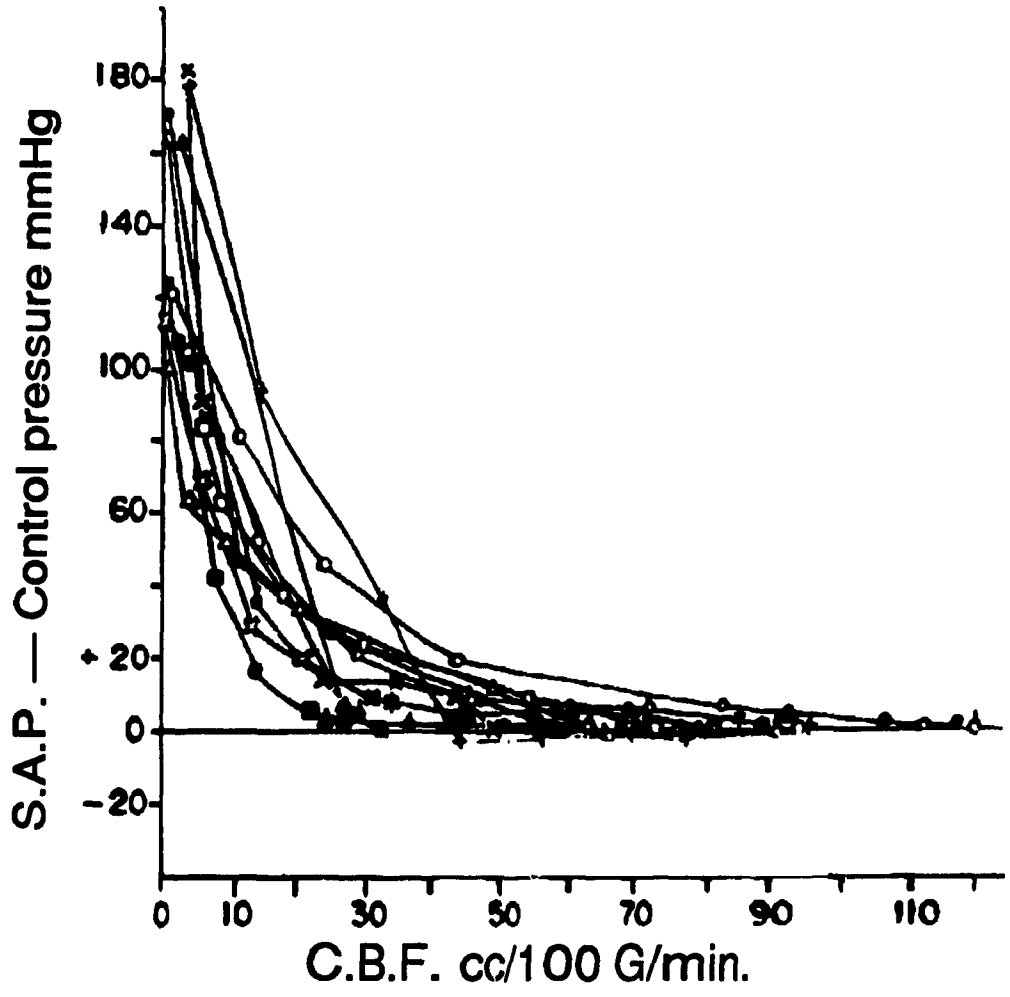


*Fig. 2.* Effect on systemic arterial pressure (SAP) in the anaesthetized dog of varying cerebral blood flow (CBF). The effect is plotted as the change in SAP over the control SAP. The control mean SAP at a CBF of 100 ml/min/100g was 86mmHg. (Sagawa et al.,1961).

experiments in sino-aortic denervated dogs in which CPP could be adjusted to be equal to SAP minus some constant value. In this manner CPP was "biased" to levels below SAP and changes in SAP continued to be reflected in changes in CPP. When CPP was reduced in such a manner from a value equal to SAP (= 150 mmHg) to a value of 10-30 mmHg sustained oscillations of SAP having an amplitude of 40 - 110 mmHg and a period of 30 - 85 seconds were observed. Similar observations were made by Miyakawa et al. (1984) in anaesthetized rabbits in which CPP and CBF were reduced by graded occlusion of the common carotid arteries while the vertebral arteries were completely occluded. The amplitude of the SAP oscillations is directly related to the magnitude of the reduction of CPP and CBF (Miyakawa et al., 1984). The mechanism of generation of these SAP oscillations during impaired CPP and CBF is controversial (Polosa 1984).

A decrease in spinal cord blood flow may also be corrected by an increase in SAP. Because of the anatomy of the spinal circulation (see APPENDIX A) it is difficult to produce selective spinal cord ischemia by arterial occlusions. Therefore, maneuvers which indirectly cause a decrease in spinal cord blood flow have been used. As mentioned above, systemic hypotension leads to a decrease in spinal cord blood flow when SAP falls below the autoregulatory range of 50 to 160 mmHg (Flohr et al., 1970; Griffiths, 1973, Kobrine et al., 1976; Hickey et al., 1986). An increase in the pressure of the cerebrospinal fluid (CSF) surrounding the CNS can also decrease nervous tissue blood flow (Johnston et al., 1972; Grubb et al., 1975). Brooks (1933) showed that chronic spinal animals were able to compensate for a fall in SAP produced by rapid hemorrhage by a neurally mediated peripheral vasoconstriction and tachycardia. Brooks (1933) concluded that these responses to systemic hypotension in the spinal animal were generated within the spinal cord since they were abolished by excision of the sympathetic chains but persisted after dorsal rhizotomy . A number of authors have also found that increased intradural pressure in the spinal animal causes an increase in the

firing rate of sympathetic neurons (Meyer & Winter 1970) and in SAP (Groat 1945, Hoff & Reis 1970, Meyer & Winter 1970, Eidelberg 1973) possibly as a result of decreased nervous tissue blood flow associated with increased CSF pressure.

**Mechanisms generating the neurogenic component of the cerebral ischemic response**

Following the description of the arterial baroreceptor reflex (Hering, 1924) the pressor response to occlusion of the arteries supplying the brain was attributed to this reflex (Heymans, 1931). However, subsequent experiments by McDowall (1933) and Guyton (1948) demonstrated that the pressor response was not diminished after denervation of the arterial baroreceptors. The vagal bradycardia produced by cerebral ischemia also persisted after arterial baroreceptor denervation (Guyton, 1948; Dampney et al., 1979). Present knowledge indicates that the neurogenic component of the cardiovascular response to cerebral ischemia is mainly generated in the medulla oblongata. In the rabbit serial transections of the brainstem showed that the peripheral vasoconstriction and increase in SAP during cerebral ischemia were unaffected by transection of the brainstem more than 8 mm rostral to the obex but were progressively attenuated as transections were made more caudally and were completely abolished by transection of the brainstem 3 mm rostral to the obex (Fig. 3) (Takeuchi et al.,1969) . More recently Dampney et al. (1979) have indirectly confirmed these findings in the rabbit by showing that following transection of the brainstem at the ponto-medullary junction (approximately 12 mm rostral to the obex) the pressor response to cerebral ischemia persists while it is abolished by transection of the spinal cord at $C_1$ . It was also demonstrated that lesions placed in the dorsomedial or ventrolateral medullary reticular formation (Kumada et al., 1979; Dampney & Moon, 1980) greatly attenuated the pressor response to cerebral ischemia. Unfortunately, these lesions were relatively large and

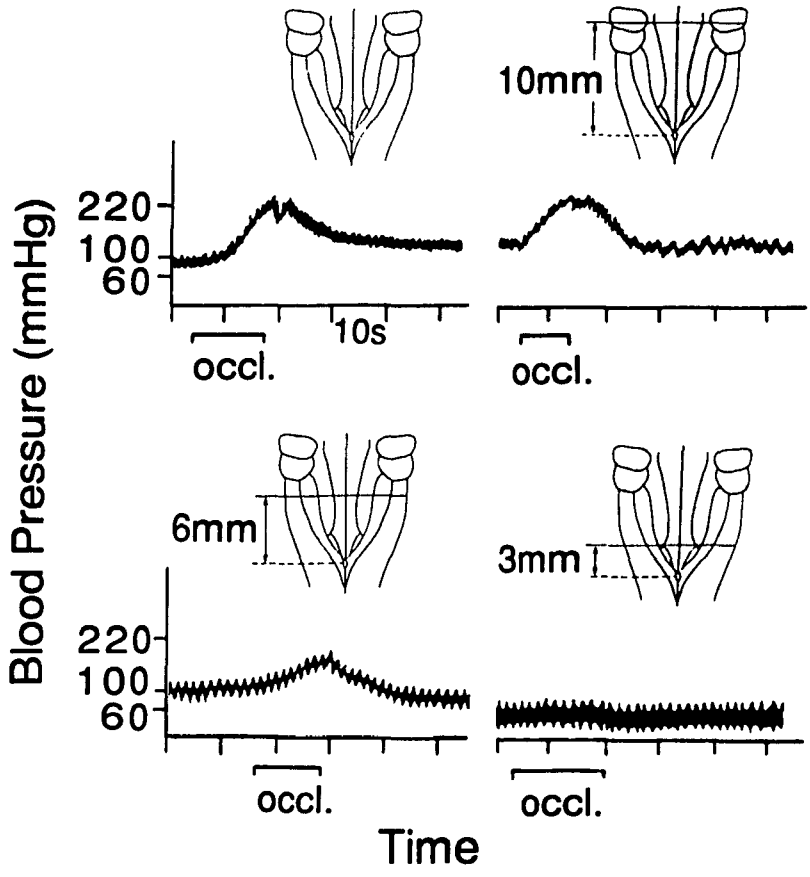


*Fig. 3.* The effect of successive transections of the medulla oblongata of the rabbit on the pressor response to cerebral ischemia. The levels of the transections, relative to the obex , are indicated in the diagrams of the dorsal view of the medulla oblongata above each panel. (Takeuchi et al.,1969).

caused a large decrease in SAP. It is difficult therefore to assess the extent to which these lesions interrupted descending sympatho-excitatory paths from the brain rather than specifically eliminated regions generating the pressor response to cerebral ischemia.

Concerning the mechanisms generating the neurogenic component of the CIR at least two hypotheses can be considered. Mechanoreceptors within the cranium may be affected by the decrease in cerebral perfusion pressure during cerebral ischemia resulting in a sympatho-excitation. Alternatively the CIR may be initiated by changes in the chemical environment of the CNS which occur during cerebral ischemia.

In regard to the first hypothesis, intracranial receptors sensing vascular pressures, similar in function to the baroreceptors of the aortic arch and carotid sinus, may exist. Alternatively the decrease in extracellular volume and increase in intracellular volume of the nervous tissue during cerebral ischemia (Hansen, 1985) might cause sufficient tissue distortion to activate as yet unidentified extravascular, intracranial mechanoreceptors. However, the existence of intravascular or extravascular pressoreceptors within the cranium has never been substantiated. Hoff and Reis (1970) were able to elicit pressor responses when they applied, with small metal probes, pressures of 2.5 - 3.0 g/mm$^2$ (approximately 180 - 220 mmHg) to small regions of the floor of the fourth ventricle or to the dorsal surface of the cervical and thoracic spinal cord . Doba and Reis (1972) found in addition that injection of small volumes of saline (1-3 µl) into the brainstem in the region of the nucleus paragiganto-cellularis dorsalis also elicited a pressor response. However, the connection between the hypothesis that specific mechanoreceptors are excited during cerebral ischemia, resulting in the CIR, and these findings remains somewhat tenuous.

Concerning the second hypothesis a number of investigators have postulated that the stimulus for the pressor response to cerebral ischemia is a chemical or metabolic consequence of the ischemia (Hill, 1896; McDowall,1933; Guyton,1948; Guyton &

Satterfield,1952; Heymans & Neil, 1958; Sagawa et al., 1961; Dickinson, 1965). As reviewed above, interruption of CNS blood flow leads rapidly to nervous tissue hypoxia, hypoglycemia, hypercapnia and acidosis (Wadouh et al., 1985; Siesjö & Wieloch, 1985). Both cerebral hypoxia and hypercapnia have been previously suggested as the stimulus for the CIR (Hill, 1896; McDowall, 1933; Guyton, 1948; Dickinson, 1965). These suggestions are supported by the finding that cephalic perfusion with hypoxic or hypercapnic blood can cause sympathetic excitation (Kao et al., 1962; Downing et al., 1963; DeGeest et al., 1965a; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985) [This material is reviewed in greater detail in the next section of the INTRODUCTION]. A further observation suggesting a role for $CO_2$ in generating the CIR was made by McDowall (1933). In the dog this investigator found that reducing arterial $PCO_2$ by hyperventilation prior to occlusion of the vertebral and common carotid arteries significantly delayed the onset of the pressor response to cerebral ischemia (see Fig. 4).

**Summary**

The mammalian CNS requires an uninterrupted and sufficient flow of arterial blood in order to function. This is a result of the high metabolic rate of nervous tissue, its dependence on the oxidation of glucose as an energy source and the lack of significant reserves of $O_2$ , glucose or glycogen within the CNS. Compensation for inadequate cerebral blood flow can be initiated either by decreasing cerebrovascular resistance or by increasing cerebral perfusion pressure. In regard to the former compensatory mechanism autoregulation of the cerebral circulation is known to maintain CBF relatively constant in the range of CPP from 150 to 40 mmHg as a result of adjustments in cerebrovascular resistance. A second intracranially generated compensation for decreased CBF is the neurogenic increase in SAP which is observed during cerebral ischemia.

*Fig. 4.* The effect of cerebral ischemia on systemic arterial pressure in the dial anaesthetized cat before (A) and after hyperventilation resulting in decreased arterial $PCO_2$ (B). Time markers on the bottom trace are one minute apart. (McDowall, 1933)

This increase in SAP is initiated as CPP falls below 40 - 60 mmHg with progressively greater increases in SAP as CPP decreases further. This SAP response is generated within the medulla. The fact that cerebral ischemia leads to CNS hypoxia as well as hypercapnia and the observation that perfusion of the brain with hypoxic or hypercapnic blood causes sympathetic excitation suggests that the basis for the neurogenic increase in SAP during cerebral ischemia is a central sympathetic chemosensitivity to $O_2$ lack or $CO_2$ excess. Similar spinal cord phenomena may be responsible for the neurogenic increase in SAP seen during decreased spinal cord blood flow. Present knowledge concerning the central sympathetic chemosensitivity to hypoxia and hypercapnia is reviewed in the next section.

## II) SYMPATHETIC RESPONSES TO HYPOXIA AND HYPERCAPNIA

### Sympathetic effects of systemic hypoxia in the CNS-intact animal

Systemic hypoxia in the CNS-intact animal has a predominantly sympatho-excitatory effect. Work by a number of investigators has shown that in spite of the direct cardiodepressant (Katz, 1977) and vasodilator (Somlyo & Somlyo, 1970) effects of $O_2$ lack the overall effects of systemic hypoxia in the anaesthetized dog or cat are tachycardia, increased cardiac output, increased ventricular contractility, increased SAP, venoconstriction and increased resistance in some vascular beds (for refs. see Eyzaguirre et al., 1983). Similar effects have been seen in awake man, dogs, and rabbits (Korner, 1959; Korner, 1971; Krasney & Koehler, 1980). A number of these effects are neurogenic. Greenfield et al. (1963) showed that the increase in heart rate and cardiac contractility during ventilation of the anaesthetized dog with 10% $O_2$ in $N_2$ is abolished by cardiac denervation. Kontos and Lower (1969) found that the increase in heart rate and

cardiac output caused in the awake dog by inhalation of 7% $O_2$ in $N_2$ persisted after adrenalectomy but was abolished by β-adrenergic blockade with propranolol. The vasoconstrictor response of the renal and portal vascular beds in the awake rabbit breathing 7% $O_2$ in $N_2$ was abolished by chemical sympathectomy with guanethidine (Chalmers et al., 1967). The demonstration of an increase in the firing rate of sympathetic postganglionic neurons innervating heart, skeletal muscle, kidney and other abdominal viscera during systemic hypoxia in anaesthetized cats and rabbits provides direct evidence of sympathetic excitation (von Kehrel et al., 1962; Downing & Siegel, 1963; Iriki & Kozawa, 1975; Gregor & Jänig, 1977).

The importance of the arterial chemoreceptors in the cardiovascular response to systemic hypoxia was demonstrated by the observation that the pressor response to systemic hypoxia in the anaesthetized dog was abolished by section of the sino-aortic nerves (Heymans & Neil, 1958). These findings were subsequently confirmed by several investigators (Litwin, 1960; Kontos et al., 1970; Sylvester et al., 1979). Sino-aortic denervation has also been shown to markedly attenuate or abolish the increase in the resistance of hindlimb, renal and portal vascular beds as well as in myocardial contractility which occurs during systemic hypoxia (Litwin, 1960; Kahler et al., 1962; Chalmers et al., 1967). Perfusion of the isolated carotid sinus at constant pressure with hypoxic blood in the dog causes an increase in aortic pressure and in the resistance of forelimb, hindlimb, splanchnic , and renal vascular beds (Bernthal 1938; de Burgh-Daly & Scott, 1962; Pelletier, 1972; Mancia, 1974; Heistad et al., 1975). The increase in aortic pressure and hindlimb vascular resistance is in proportion to the degree of hypoxic stimulation of the arterial chemoreceptors (Pelletier,1972).

There is some evidence that systemic hypoxia decreases the activity of sympathetic neurons innervating cutaneous vascular beds. During systemic hypoxia

blood flow to the ear increases in the rabbit (Chalmers et al., 1967). This increase in blood flow seems to be due to a decrease in sympathetic vasoconstrictor activity since it is abolished following chemical sympathectomy with guanethidine (Chalmers et al., 1967). A decrease in the activity of sympathetic postganglionic neurons innervating the rabbit ear (Iriki & Kozawa, 1975) or cutaneous regions of the cat hindlimb (Gregor & Jänig, 1977; Blumberg et al.,1980) has also been reported during systemic hypoxia. Gregor and Jänig (1977) suggested that this decrease in sympathetic activity was mediated by the arterial chemoreceptors since only increases in firing rate of these neurons were seen after sino-aortic denervation. This conclusion is supported by the findings of Heistad et al. (1975) that blood flow to the mainly cutaneous vascular bed of the dog hindpaw, perfused at constant pressure with normoxic and normocapnic blood, increased during perfusion of the isolated carotid sinus at constant pressure with hypoxic blood.

That other mechanisms, in addition to the arterial chemoreceptors, mediate the sympathetic response to systemic hypoxia is suggested by the observation by a number of investigators of an increase in heart rate, cardiac output and ventricular contractility during systemic hypoxia in CNS-intact animals with sectioned arterial chemoreceptor afferents (Penna et al., 1962; Kontos et al. 1970; Achtel et al. 1970; Krasney et al. 1977; Sylvester et al., 1979; Koehler et al. 1980). The work of Koehler et al. (1980) indicates that such cardiovascular responses occur over a considerable range of arterial $PO_2$ ($PaO_2$) in the awake dog. An increase in sympathetic neural activity to various effector organs during systemic hypoxia has also been reported in CNS-intact animals with denervated arterial chemoreceptors (von Kehrel et al., 1962; Downing & Siegel, 1963; Iriki & Kozawa, 1975; Gregor & Jänig, 1977). Since after sino-aortic denervation there are no known peripheral structures sensitive to $O_2$ lack which have reflex effects upon sympathetic neurons it can be suggested that a central action of hypoxia is responsible for this increase in sympathetic activity.

### Cephalic hypoxia

Experiments of cephalic perfusion with hypoxic blood suggest an intracranial component of the sympathetic sensitivity to systemic hypoxia. Downing et al. (1963) perfused the head of the dog with hypoxic blood through the brachiocephalic artery following ligation of the left subclavian artery and found that cephalic hypoxia was associated with an increase in SAP, total peripheral resistance, heart rate and atrial as well as ventricular contractility. In a similar preparation DeGeest et al. (1965a) demonstrated that cephalic hypoxia produces a large increase in systolic pressure of the innervated, paced, isovolumetric left ventricle (Fig. 5). More recent experiments by McGillicudy et al. (1978) in the rhesus monkey have shown that, following ligation of the vertebral arteries, perfusion of the head through the carotid arteries with hypoxic blood caused a significant pressor response.

### Systemic hypoxia in the spinal animal

There is also evidence that a spinal chemosensitivity to hypoxia exists. In the acute $C_1$ spinal cat Kaya and Starling (1908) and Mathison (1910) demonstrated a large pressor response to ventilation with $N_2$. The experiments of Alexander (1945) in the cat suggest that such a sympathetic excitation is generated by an action of hypoxia within the spinal cord. After acute spinal transection at low cervical and mid-thoracic levels as well as section of the intervening dorsal roots ventilation with $N_2$ still caused a marked increase in inferior cardiac nerve activity. In chronic spinal cats Jänig and Spilok (1978) recorded sympathetic postganglionic neurons in hindlimb skin nerves whose firing rate increased during ventilation with 8% $O_2$ in $N_2$. Rohlicek and Polosa (1981) described the relationship between the firing rate of thoracic sympathetic preganglionic neurons and arterial $\dot{P}O_2$ in acute spinal cats. The firing rate of these neurons started to increase when

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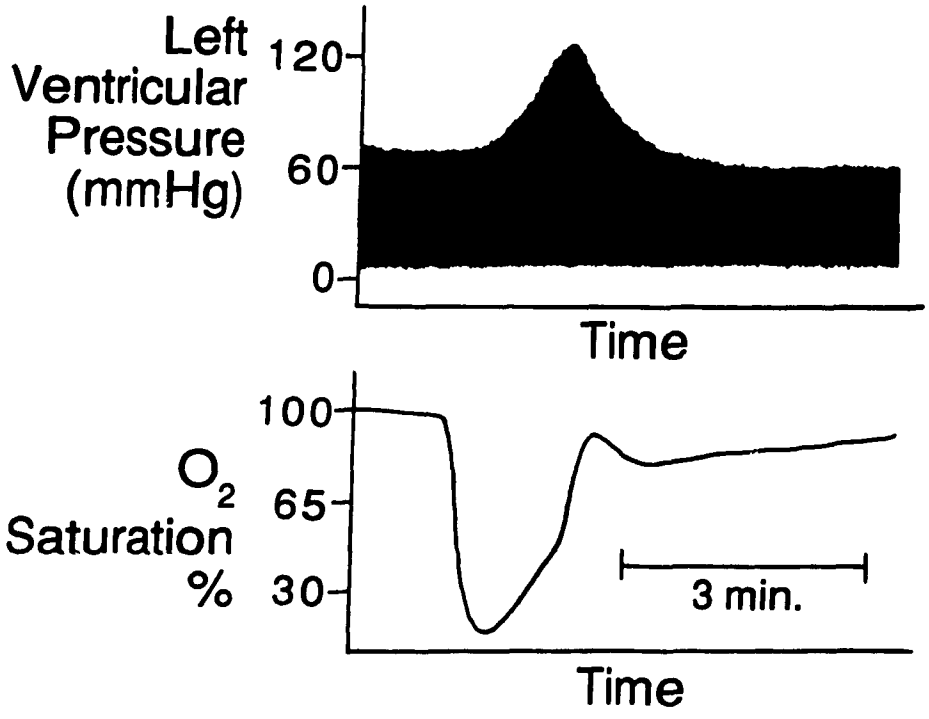


*Fig.5.* Effect of perfusion of the head of the dog with hypoxic blood on left ventricular pressure in the innervated, paced, isovolumetric left ventricle (DeGeest et al., 1965a).

$PaO_2$ fell below 40 mmHg. The firing rate increased further with additional reduction in $PaO_2$ and reached a value of ten times the control level at a $PaO_2$ of 20 mmHg (Fig. 6).

**Sympathetic effects of systemic hypercapnia in the CNS-intact animal**

Systemic hypercapnia has cardiodepressant and vasodilator effects as a result of actions of $CO_2$ directly on the myocardium and vascular smooth muscle (Suutarinen, 1966; Somlyo & Somlyo, 1970; Katz, 1977). Nevertheless, ventilation of the anaesthetized dog with normoxic or hyperoxic gas mixtures containing elevated concentrations of $CO_2$ can cause an increase in SAP (e.g. Hill & Flack, 1908; Kaya & Starling, 1909). Later work implicated the sympathetic nervous system in such a pressor response since adrenergic block with ergotamine (Heymans & Bouckaert 1933; McDowall 1933) or surgical sympathectomy (Bacq et al., 1933) abolished the increase in SAP seen during systemic hypercapnia. Investigations in awake man and anaesthetized dogs have shown that in addition to an increase in SAP systemic hypercapnia causes an increase in total peripheral resistance, heart rate, cardiac output, and stroke volume (see reviews by Price, 1960; and Suutarinen, 1966). The sympatho-excitatory action of systemic hypercapnia is demonstrated by the observation of increased firing in various sympathetic nerves of anaesthetized cats, dogs and rabbits during inhalation of gas mixtures containing concentrations of $CO_2$ of up to 10% (Gernandt et al., 1946; Downing & Segal, 1963; Millar & Biscoe, 1965; 1966; Cohen & Gootman, 1970; Bower, 1975; Preiss & Polosa, 1977). In the anaesthetized cat the vascular resistance of the vascularly isolated, innervated hindlimb perfused with normocapnic and normoxic blood was found to be a continuous function of arterial $PCO_2$ ($PaCO_2$) between 14 and 58 mmHg $PaCO_2$ (Lloy et al.,1978). Recording from sympathetic preganglionic axons in the cervical sympathetic trunk of the cat Preiss and Polosa (1977) found that the firing rate of most of the

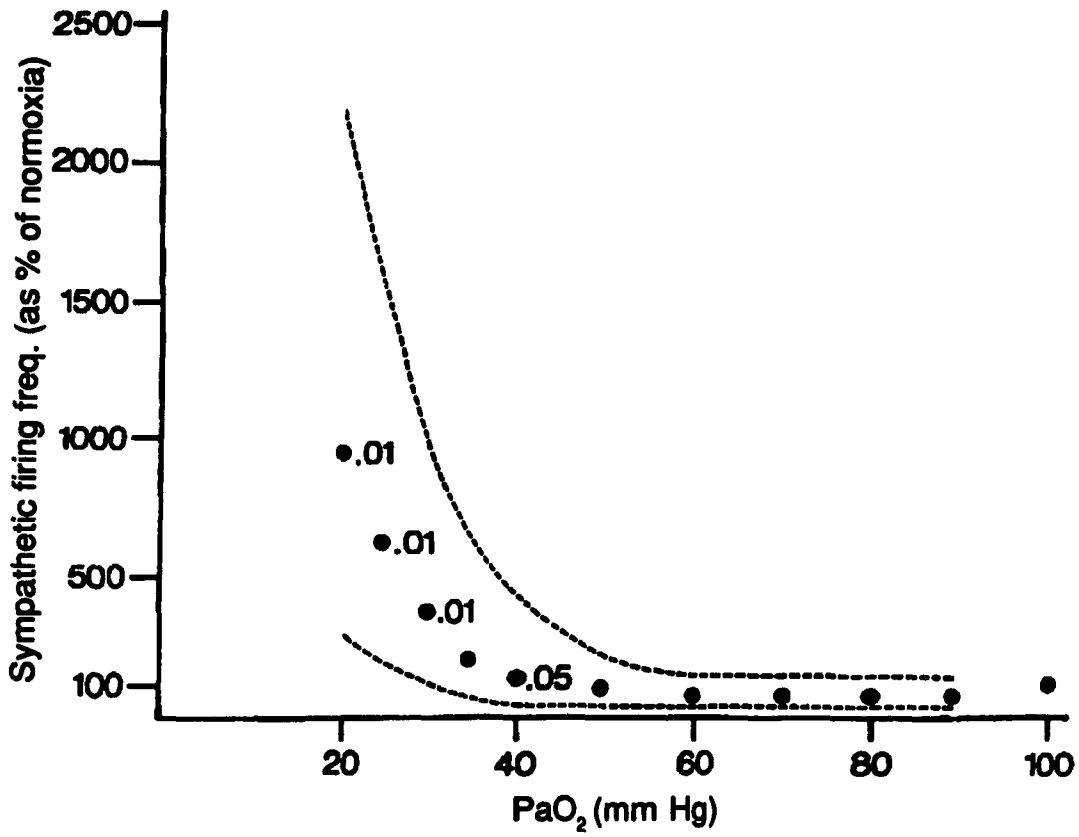


*Fig. 6.* Sympathetic preganglionic neuron response to graded isocapnic arterial hypoxia in the acute $C_1$ spinal cat. Points are averages based on data recorded from 13 strands of the cervical sympathetic trunks of 8 acute $C_1$ spinal cats. A significant difference between a point and the next on the right is indicated by the P values. The dashed lines indicate the range (Rohlicek & Polosa, 1981).

sympathetic neurons studied was directly related to inspired $CO_2$ levels over a range of 2% to 7% inspired $CO_2$. In the same preparation Hanna et al. (1981) described a sigmoid relationship between sympathetic preganglionic neuron firing rate and $PaCO_2$ in the range of $PaCO_2$ from 20 to 90 mmHg with the maximum slope of the relationship being between 50-70 mmHg $PaCO_2$.

Arterial chemoreceptors may be responsible, at least in part, for the relation existing between $PaCO_2$ and sympathetic activity since they are sensitive to changes in arterial $PCO_2$ and pH over a wide range of values (Eyzaguirre et al., 1983) and they exert reflex actions on cardiovascular effectors (Heymans and Neil ,1958). Bernthal (1938) found that forelimb vascular resistance increased as the $PaCO_2$ of blood perfusing the isolated carotid sinus of the anaesthetized dog was increased from 44 to 98 mmHg. Later Pelletier (1972), in a similar preparation in the cat found that hindlimb vascular resistance was directly related to the $PaCO_2$ of the blood perfusing the isolated carotid sinus between 39 and 71 mmHg $PaCO_2$. Blumberg et al. (1980) demonstrated an increase in sympathetic neuron activity in a skeletal muscle nerve of the cat during injection of small volumes of $CO_2$ saturated saline into the carotid sinus.

A role of additional mechanisms besides the arterial chemoreceptors in the sympathetic excitation by systemic hypercapnia was suggested by the finding of Downing & Segal (1963) that in the sino-aortic denervated dog inferior cardiac nerve activity increased during ventilation with a hypercapnic gas mixture even after arterial chemoreceptor denervation. Similar results were reported by Bower (1975) in the rabbit during recording from an intestinal nerve. Lioy et al. (1978) showed that a qualitatively similar relationship between $PaCO_2$ and hindlimb vascular resistance persisted after arterial chemoreceptor denervation. Experiments conducted by Lioy et al. (1984) in the sino aortic denervated (SAD) rat have shown that a direct relationship exists between

$PaCO_2$ and vascular resistance in skeletal muscle, skin, pancreas, large intestine and kidney. Hanna et al. (1981) demonstrated in the SAD cat that a direct relationship exists between sympathetic preganglionic neuron firing rate and $PaCO_2$ over the range of $PaCO_2$ values from 40 to 90 mmHg (Fig. 7). Since after sino-aortic denervation there are no known peripheral structures sensitive to $CO_2$ which have reflex effects upon sympathetic neurons it can be suggested that a central action of $CO_2$ is responsible for the increase in sympathetic activity observed in hypercapnic SAD animals.

**Cephalic hypercapnia**

Experiments of cephalic perfusion with hypercapnic blood suggest that a component of the sympathetic response to increases in $PaCO_2$ is intracranially mediated. Kao et al. (1962) perfused the head of the dog with hypercapnic blood through the vertebral arteries following ligation of the common carotid arteries. Pulmonary ventilation and SAP increased during cephalic hypercapnic perfusion. A more detailed investigation by Downing et al. (1963) revealed that perfusion of hypercapnic blood through the brachiocephalic artery of the dog, following ligation of the left subclavian artery, was associated with increases in SAP, total peripheral resistance, heart rate and atrial as well as ventricular contractility. In a similar preparation DeGeest et al. (1965a) found that cephalic hypercapnia caused an increase in ventricular systolic pressure in the innervated, paced, isovolumetric canine left ventricle. In rhesus monkeys McGillicudy et al. (1978) found that cephalic perfusion with hypercapnic blood causes a pressor response. More recently Hainsworth et al. (1984) and Ford et al. (1985) have shown that in the dog a direct relationship exists between ventricular contractility, abdominal vascular resistance and the $PaCO_2$ of the blood perfused through the brachiocephalic and left subclavian arteries

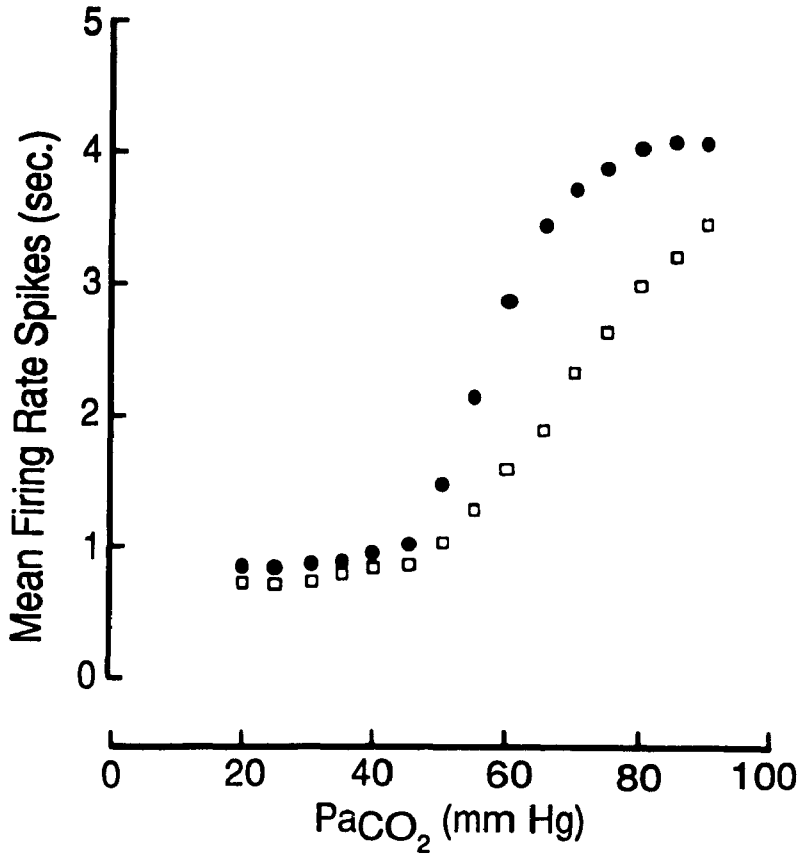


*Fig. 7.* The relation between $PaCO_2$ and thoracic sympathetic preganglionic neuron firing rate before (●) and after (□) bilateral carotid sinus nerve section in anaesthetized cats with previously sectioned vagus and aortic nerves. Each point is the average of the observations in 7 cats. (Hanna et al., 1981).

following ligation of the common carotid arteries over a range of $PaCO_2$ values between 28 and 60 mmHg.

It is well known that in the animal with denervated arterial chemoreceptors a large part of the sensitivity of brainstem respiratory neurons to changes in $PaCO_2$ or arterial pH remains (Loeschke 1974). This respiratory chemosensitivity has been shown to be largely mediated intracranially (e.g. Kao et al., 1962). The mechanisms mediating this respiratory chemosensitivity appear to be closely related to those responsible for the sympathetic chemosensitivity to $CO_2$ and $H^+$ generated intracranially (Trzebski et al., 1970; Szulczyk & Trzebski, 1976; Hanna et al., 1979; Lioy et al., 1981).

Work by Leusen (1954) first suggested that superficial regions of the brain might be responsible for the central respiratory chemosensitivity. Subsequent work indicates that there are three bilateral regions of the superficial ventral medulla involved in central respiratory chemosensitivity (see reviews: Loeschke, 1974; Bledsoe & Hornbein, 1981; Schlaefke,1981; Loeschke, 1982; Millhorn & Eldridge, 1986). One such region was described by Mitchell et al. (1963) in the cat and was later designated as area "M" (Schlaefke 1981). This region is bounded medially by the pyramidal tracts, laterally by the VII and XI nerve roots, rostrally by the pons and extends 5-6 mm caudal to the ponto-medullary junction. A chemoreceptor function was attributed to this region on the basis of the finding in the spontaneously breathing cat that application to this region of artificial CSF of high $PCO_2$ and $[H^+]$ resulted in increased tidal volume while application of artificial CSF of low $PCO_2$ and $[H^+]$ resulted in decreased tidal volume (Mitchell et al., 1963). Schlaefke et al. (1970) subsequently confirmed these observations and also described a second chemosensitive region, caudal to area "M", extending between 9 and 12 mm caudal to the ponto-medullary junction and between 2 and 5 mm laterally from the midline. Respiratory effects could be initiated by the application of artificial CSF of varying $PCO_2$

and [$H^+$] to this area which was later designated as area "L" (Schlaefke, 1981). A third area of the ventral medullary surface involved in central respiratory chemosensitivity was found between areas "M" and "L" lateral to the pyramidal tracts, medial and rostral to the XII nerve roots (Schlaefke & Loeschke,1967). This area was designated as the area "S" (Schlaefke 1981). Schlaefke and Loeschke (1967) attributed a role in central respiratory chemosensitivity to this region on the basis of the observation that cold blockade of this area in the spontaneously breathing cat resulted in a decreased tidal volume. Cherniak et al. (1979) have since shown that cold blockade of this region considerably decreases the $CO_2$ sensitivity of respiratory neurons over a wide range of $PaCO_2$ values. It appears that the structures of the ventral medulla which are involved in central respiratory chemosensitivity are very superficially located. Trouth et al. (1973a) found that the strongest respiratory responses to electrical stimulation in regions corresponding to areas "M" and "L" occurred within a depth of 200 μm from the ventral medullary surface. The responses decreased with increasing depth and disappeared at a depth of 600 - 800 μm. Schwanghart et al. (1974) have estimated with histochemical techniques that the depth to which procaine applied to the ventral medullary surface must penetrate in order to eliminate central respiratory chemosensitivity is less than 100 μm. The results presented above have been taken to indicate the presence of superficially located chemosensitive structures within areas "M" and "L" which have excitatory input to respiratory neurons (Loeschke,1974; Bledsoe & Hornbein, 1981; Schlaefke,1981; Loeschke, 1982; Millhorn & Eldridge, 1986). The afferent pathways from these structures may pass through or make synaptic connections in area "S" (Schlaefke, 1981). Alternatively it has been suggested that area "S" may not contain such pathways or synaptic connections but rather that neurons of area "S" provide facilitatory input to second order neurons which

receive projections from the chemosensitive structures of areas "M" and "L" (Millhorn & Eldridge, 1986).

A number of experimental observations in the cat suggest that superficial regions of the ventrolateral medulla are also involved in the intracranial sympathetic chemosensitivity to $CO_2$ and $H^+$. Trzebski et al. (1974) and Szulczyk and Trzebski (1976) found that injection of acidic artificial CSF into the subdural space overlying the ventrolateral medulla produced an increase in SAP, hindlimb vascular resistance, sympathetic neuron firing rate, as well as in phrenic nerve activity. Experiments by Lioy et al. (1981) showed that superfusion of the ventral medullary surface with acidic and hypercapnic artificial CSF caused an increase in SAP, heart rate, hindlimb vascular resistance and phrenic nerve activity while superfusion with alkaline and hypocapnic artificial CSF led to a decrease in these variables. The work of Hanna et al. (1979) demonstrated that ventral medullary structures play a role in the sympathetic response to systemic hypercapnia by showing that bilateral cold blockade of the area "S" significantly decreased the slope of the relationship between $PaCO_2$ and hindquarter vascular resistance (Fig. 8). The structures responsible for this effect were less than 2 mm from the ventral medullary surface since pressor responses evoked by electrical stimulation within the medulla at depths of 2 mm or greater were unaffected by the cold blockade. It has been estimated that in the anaesthetized cat one third of the neurogenic vasoconstrictor tone of the hindlimb in normocapnia is maintained by the $CO_2$ dependent input to sympathetic neurons from this area (Polosa et al., 1983).

Of interest is the observation in sino-aortic denervated cats and rats (Trzebski & Kubin, 1981; Lioy & Trzebski, 1984) that the threshold for sympathetic activation by $CO_2$ is lower than that for initiation of respiratory motoneuron discharge. Whether this is due to the existence of separate central chemosensory structures with different properties

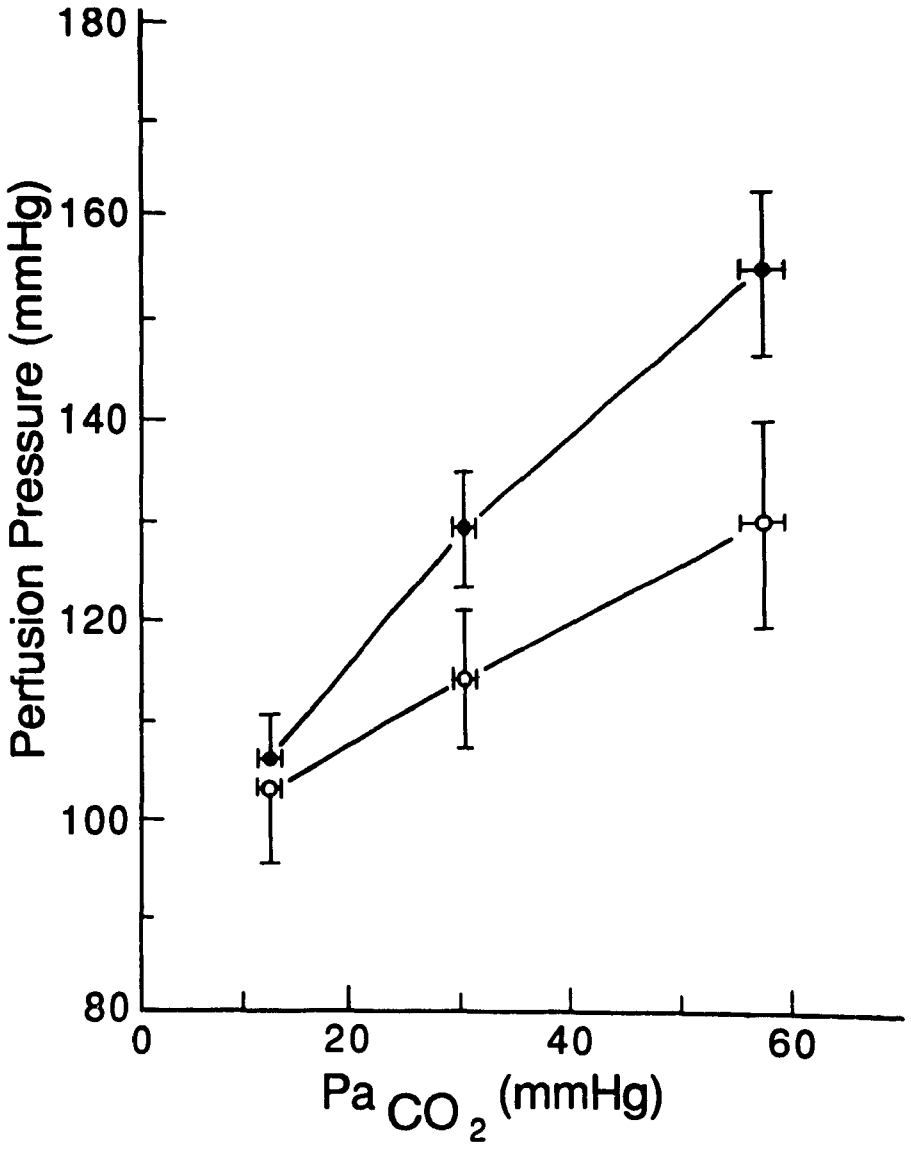


*Fig. 8.* The relation between $PaCO_2$ and perfusion pressure of the vascularly isolated hindlimb of the cat perfused at constant flow before (•) and after (o) cooling area "S" of the ventrolateral medulla (Hanna et al., 1979).

subserving sympathetic and respiratory neurons or rather to substantial differences in pathways and connections from a common set of central chemoreceptors is not clear.

**Systemic hypercapnia in the spinal animal**

In the experiments of Mathison (1910) SAP of the acute $C_1$ spinal cat increased when the animal was ventilated with gas mixtures containing very high concentrations of $CO_2$ (i.e. 16 - 50% $CO_2$ in $O_2$). Kaya and Starling (1909) failed to see any increases in SAP of this preparation during ventilation with gas mixtures containing less than 16% $CO_2$. Recording the activity of sympathetic neurons from the inferior cardiac nerve in the anaesthetized cat following acute spinal transections at $C_5$ and $T_6$ as well as section of the intervening dorsal roots Alexander (1945) found that ventilation with 10% $CO_2$ in $O_2$ did not increase sympathetic activity over the level observed during ventilation with room air. More recently Lioy et al. (1978) failed to see any change in the vascular resistance of the hindlimb, perfused at constant flow with normocapnic blood, during systemic hypercapnia following spinal transection at a mid-thoracic level in the anaesthetized cat. In contrast, Johnson et al. (1969) have shown that ventilation with 10% $CO_2$ in air of spinal man or ventilation with 10% $CO_2$ in $O_2$ of cats acutely spinalized at a mid cervical level leads to an increase in SAP. In the latter preparation the pressor response to systemic hypercapnia was abolished by sympathetic ganglionic blockade with hexamethonium. Szulczyk and Trzebski (1976) have reported that injection of acidic and hypercapnic artificial CSF subdurally over the dorsal surface of the thoracic spinal cord in cats with the spinal cord transected at the level of $T_1$ increased sympathetic neuron activity in the vertebral and cardiac nerves and increased the SAP.

## Summary

Both systemic hypoxia and hypercapnia produce mainly a sympathetic excitation in the CNS-intact animal. While the arterial chemoreceptors play a role in the sympathetic response to these stimuli it is also apparent that excitatory effects of $CO_2$ excess or $O_2$ lack occur also after arterial chemoreceptor denervation. The latter sympatho-excitatory effects may be attributed to a central sympathetic chemosensitivity. In this regard cephalic perfusion with hypoxic or hypercapnic blood has been shown to produce a sympathetic excitation. It appears that an important component of the supra-spinal central sympathetic chemosensitivity to changes in $PaCO_2$ is mediated by a restricted region of the ventrolateral medulla over a considerable range of $PaCO_2$. Little information exists concerning the characteristics of the supra-spinal sympatho-excitatory effects of $O_2$ lack or of the location of the mechanisms involved. The observation in the spinal preparation that sympathetic neuron firing is increased during severe hypoxia even after spinal cord de-afferentation indicates a spinal sympathetic sensitivity to hypoxia. The existence of a similar spinal sympathetic chemosensitivity to $CO_2$ excess remains controversial. Thus while sufficient evidence exists concerning the existence of central sympathetic chemosensory mechanisms to implicate such mechanisms in the sympathetic response to CNS ischemia knowledge of these central chemosensory mechanisms remains incomplete.

# CHAPTER 3

## RESULTS I

As reviewed in the INTRODUCTION (pp. 3 - 4, 15 - 18) the pressor response to cerebral ischemia suggests that the sympathetic system responds to changes in the chemical composition of the brain environment. The mechanism involved may be a central sympathetic chemosensitivity to changes in $PO_2$, $PCO_2$ and pH of the brain environment. This chapter describes the results of experiments aimed at the question of where mechanisms mediating such a chemosensitivity of the sympathetic system may be located.

The sympathetic responses to cerebral ischemia, hypoxia and hypercapnia can result from the operation of either diffuse or localized mechanisms. The hypothesis of a diffuse mechanism could explain the responses as a consequence of a general property of neurons responding to changes in $PO_2$, $PCO_2$ and pH of their environment with changes in excitability. According to this hypothesis the sympathetic responses could be the result of a generalized excitation of brain neurons. However, the brain, and in particular the brainstem, contain sets of neurons which provide both excitatory and inhibitory input to sympathetic neurons. Thus the hypothesis of a diffuse mechanism should include provisions to account for the fact that the overall effect on sympathetic activity of cerebral ischemia, hypoxia and hypercapnia are excitatory. A difference in the number of facilitatory and inhibitory neurons providing input to sympathetic neurons, the extent of their connections, or a difference in the effect of changes in the brain environment on these two sets of neurons might provide an adequate explanation. However, no evidence for these possibilities exists at present. The hypothesis of a

localized mechanism could explain the sympathetic responses to cerebral ischemia, hypoxia and hypercapnia as the consequence of activation of specific sets of neurons which are sensitive to chemical stimuli such as changes in $PO_2$, $PCO_2$ or pH to a much greater extent than other neurons. An analogous mechanism in the peripheral nervous system is that of the arterial chemoreceptors which are more sensitive to such chemical stimuli than other sensory structures (Eyzaguirre et al., 1983). Experimental evidence is available to make the second hypothesis preferable. The main piece of evidence is the demonstration of circumscribed sites of sensitivity to $CO_2$ and $H^+$ in the medulla capable of translating the local concentration of these chemicals into a graded facilitatory input to respiratory neurons as described in the INTRODUCTION (pp. 30 - 32) (Loeschke,1974; Bledsoe & Hornbein, 1981; Schlaefke,1981; Loeschke, 1982; Millhorn & Eldridge, 1986). As also reviewed in the INTRODUCTION (pp.32 - 34) there is an indication that an analogous mechanism serves the sympathetic system (Trzebski et al., 1970; Szulczyk & Trzebski, 1976; Hanna et al., 1979; Lioy et al., 1981). The working hypothesis underlying the studies presented in this chapter is that the sympathetic responses to cerebral ischemia, systemic hypoxia and systemic hypercapnia are the result of activation of specific neurons which monitor the CNS environment.

The first paper in this chapter describes an attempt to localize the mechanism responsible for the sympathetic excitation seen during cerebral ischemia by using a local anaesthetic topically applied to the ventral medullary surface. This procedure led to a marked and reversible attenuation of the pressor response to cerebral ischemia. By relating the latency of the blocking effect to the pattern of distribution of the local anaesthetic in the nervous tissue an estimate was obtained of the maximum depth at which the blocking effect occurred and, by inference, of the maximum depth at which some key component of the CIR generating mechanism is located. The justification of exploring the ventral medullary surface in this fashion was provided by previous results

which indicated that a marked sympatho-excitation could be obtained by superfusion of the ventral medullary surface with artificial CSF of high $PCO_2$ and $H^+$ ion concentration (Lioy et al., 1981) and the observation that cold blockade of certain superficial regions of the ventral medullary surface markedly attenuated the sympathetic sensitivity to systemic hypercapnia in the sino-aortic denervated animal (Hanna et al., 1979). The second paper in this chapter reviews evidence relating the pressor response to cerebral ischemia to the mechanism causing the sympathetic excitation seen during systemic hypercapnia in the sino-aortic denervated cat. In addition the second paper reviews indirect evidence suggesting that within the ventrolateral medulla there is a mechanism generating sympathetic tone which is distinct from the superficial chemosensitive mechanisms.

# MEDIATION OF PRESSOR RESPONSES TO CEREBRAL ISCHEMIA BY SUPERFICIAL VENTRAL MEDULLARY AREAS

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## ABSTRACT

The effects on the pressor response to cerebral ischemia (CIR) of superfusion of the ventral medullary surface with artificial cerebrospinal fluid (CSF) containing local anaesthetic was investigated in 10 sino-aortic denervated, anaesthetized cats. Prior to application of the local anaesthetic, occlusion of the common carotid and vertebral arteries caused an increase in mean systemic arterial pressure (SAP) of 58 ± 7 mmHg (± SEM) from an initial level of 98 ± 6 mmHg. Following 108 ± 15 s of superfusion with artificial CSF containing 2% procaine, the CIR decreased to 19 ± 3 mmHg. At this time phrenic nerve activity had been eliminated but basal SAP had only decreased by 14 ± 2 mmHg, and significant neurogenic vasomotor tone remained. The residual CIR can be accounted for by the passive increase in systemic resistance due to occlusion of the cerebral vascular bed. The effects of procaine were reversible. On attenuation of the CIR, electrical stimulation of the pressor points 2-4 mm from the ventral medullary surface was still effective. Autoradiographic analysis following application of $^{14}$C-labeled lidocaine showed that attenuation of the CIR occurred when estimated concentrations of the anaesthetic sufficient to block nerve conduction extended 85 μm from the ventral medullary surface. These results indicate that the CIR is mediated by superficial structures in the ventral medulla that are not involved in the generation of a major fraction of basal vasomotor tone.

## INTRODUCTION

Brain ischemia causes a marked sympathetic excitation and increase in systemic arterial pressure (SAP) often referred to as the cerebral ischemic response (CIR) (Downing et al., 1963; Guyton, 1948; Hill, 1896; McDowall, 1933; Sagawa et al., 1961). The CIR is initiated in the medulla (Takeuchi et al., 1969) and can be abolished by lesions of the dorsal medulla (Kumada et al., 1979) or of the ventrolateral medulla (Dampney & Moon, 1980), which also eliminate basal vasomotor tone. There seems to be agreement that changes in the chemical environment of medullary neurons, resulting from the cerebral ischemia, are the likely cause of the CIR. It has been suggested that the relevant stimulus may be cerebral hypoxia, hypercapnia, or acidosis, or a combination of these factors (Downing et al., 1963; Guyton, 1948; McDowall, 1933; Takeuchi et al., 1969). However, these hypotheses have not been subjected yet to experimental tests. It is also unknown which medullary neurons may translate changes in the chemical environment of the brain during cerebral ischemia into an excitatory drive for sympathetic neurons.

Previous work (Hanna et al., 1979; Lioy et al., 1981; Schlaefke et al., 1970; Trzebski et al., 1974) has shown that superficial regions of the ventral medulla, in addition to mediating the central chemosensitivity of the brain stem respiratory neurons (Schlaefke 1981), also mediate $CO_2$- or $H^+$- dependent excitation of sympathetic neurons. The ability of lesions of the ventrolateral medulla to abolish the CIR (Dampney & Moon, 1980) as well as the evidence outlined above for the existence of sympatho-excitatory chemosensors in this region suggests the hypothesis that increased $CO_2$ partial pressure ($PCO_2$) or $H^+$ concentration ($[H^+]$) in brain tissue, resulting from cerebral ischemia, could be the stimulus causing the CIR by acting on these ventral medullary chemosensors. This hypothesis leads to the expectation that procedures that eliminate the central $CO_2$ sensitivity of sympathetic neurons should also eliminate the CIR. Since the central $CO_2$ sensitivity of sympathetic neurons seems to be due to receptors superficially located on

the ventrolateral aspect of the medulla (Hanna et al., 1979), it would be expected that inactivation of only superficial layers of the ventral medulla should be required to eliminate the CIR. Moreover, since these central chemoreceptors are not responsible for a large fraction of the basal vasomotor tone in normocapnia, i.e., their elimination does not dramatically lower vascular resistance (Polosa et al., 1983), it would further be expected that the CIR should be eliminated by such procedures without a dramatic drop in SAP.

The present experiments represent a test of this hypothesis. They were directed to determine 1) whether the CIR can be eliminated by inactivation of superficial layers of the ventral medulla to a depth from the surface consistent with the apparent depth of the ventral medullary chemosensitive structures and 2) whether the CIR can be eliminated without a concomitant major loss of vasomotor tone.

Past workers have found that application of local anaesthetic solutions to the ventral medullary surface reversibly blocks central respiratory chemosensitivity when the anaesthetic has penetrated within 30-350 μm of the surface (Berndt et al., 1970; Mitchell et al., 1963; Schwanghart et al., 1974). Since the $CO_2$- or $H^+$-sensitive structures with sympatho-excitatory action appear to be located in similarly superficial regions of the ventrolateral medulla (Hanna et al., 1979), local anaesthetic solutions applied to the ventral medullary surface should also abolish the CIR after a relatively short penetration, if the hypothesis outlined above is correct.

We have found that local anaesthetic applied to the ventral medullary surface greatly attenuates the CIR at a time when it has penetrated to an estimated depth of 85 μm in a concentration sufficient to block nerve conduction (Truant & Takman, 1959). Inactivation of neurons in this superficial layer by the local anaesthetic neither significantly reduced vasomotor tone in a number of experiments nor impaired pressor responses to electrical stimulation within the medulla at depths 2-4 mm from the ventral surface. Respiratory activity was eliminated in all experiments by the application of the local

anaesthetic. These results are consistent with the hypothesis that the superficially located ventral medullary chemosensors, which exert an excitatory influence on sympathetic neurons, are an important component of the mechanism of the pressor response to cerebral ischemia. Activation of these chemosensors would presumably be due to the accumulation of $CO_2$ or $H^+$ centrally as a result of cerebral ischemia.

## METHODS

Experiments were conducted on 12 adult cats of either sex weighing between 3 and 4.5 kg. Ten animals were anaesthetized with pentobarbital sodium (35 mg/kg ip followed by supplementary iv doses as required). Two cats were decerebrated at a midcollicular level under ether anaesthesia The rostral portion of the midbrain and the whole forebrain were removed by suction. After completion of the decerebration, anaesthesia was discontinued. All the cats were paralyzed with pancuronium bromide (0.5 mg/kg iv) and ventilated with positive pressure. Ventilation was adjusted to maintain end-tidal $PCO_2$ between 25 and 35 mmHg. Rectal temperature was maintained between 36 and 37° C by means of infrared heat lamps. Tidal $PCO_2$ and femoral arterial pressure were monitored with a Beckman LB-2 gas analyzer and a Statham pressure transducer, respectively, and displayed on a Grass model 7 polygraph. A phrenic nerve was sectioned peripherally, desheathed, and submerged in a pool of paraffin oil made of the skin flaps. The nerve was placed on bipolar silver hook electrodes, its electrical activity recorded using a Grass P511 preamplifier, and displayed on a Tektronix storage oscilloscope. Phrenic nerve activity was also half-wave rectified and integrated using a "leaky" RC circuit with a time constant of 100 ms. This integrated signal was continuously displayed on the polygraph.

In all cats the arterial chemoreceptors and baroreceptors were denervated by bilateral section of the carotid sinus, aortic, and vagus nerves. Completeness of

peripheral chemoreceptor denervation was tested by observing the respiratory effects of systemic hypoxia and of bolus intravenous injections of KCN (50 µg/kg) (Dumke et al., 1941). Both stimuli produced respiratory stimulation prior to denervation but had no excitatory effect following sino-aortic denervation.

In all the cats the ventral surface of the medulla oblongata was exposed by removal of a portion of the occipital bone and underlying dura. The exposed area extended from the caudal extent of the XII nerve rootlets to a level just caudal of the exit of the VI nerves and laterally about 5 mm from the midline on both sides. Once exposed the surface of the medulla was superfused with artificial cerebrospinal fluid (CSF) at a rate of between 10 and 30 ml/min (Berndt et al., 1969). The depth of the superfusing pool was kept at approximately 2 mm. The composition of the artificial CSF was (in mM) NaCl 128, $NaHCO_3$ 17, $CaCl_2$ 0.75, KCl 5. The pH and $PCO_2$ of the solution were adjusted to 7.4 and 35 mmHg, respectively, by bubbling a gas mixture composed of 5% $CO_2$ + 95%$O_2$ through the solution during the experiment. The temperature of the superfusing solution was maintained constant at between 37 and 39 ° C.

The vertebral arteries were permanently ligated in all the preparations just distal to their confluence to form the basilar artery. As a result, the blood flow to the basilar artery and thus to the brain stem was supplied by the common carotid arteries by way of the circle of Willis (Davis & Story, 1943; Holmes et al., 1958). Thus the brain could be made ischemic by occlusion of the common carotid arteries. A similar preparation has been used previously in the study of the effects of cerebral ischemia in the rabbit (Dampney et al., 1979; Dampney & Moon, 1980; Kumada et al., 1979; Takeuchi et al., 1969). Only those preparations that did not show respiratory depression and/or a fall in mean systemic arterial pressure (SAP) to levels below 70 mmHg following the surgical preparation, including the ligation of the vertebral arteries, were used in the present experiments. In this manner preparations in which the brain stem may not have been viable as a

consequence of severely compromised blood flow were excluded. Mean SAP in the animals studied averaged 98± 6 (±SE) mmHg. The completeness of cerebral ischemia above the vertebral ligatures following occlusion of the common carotid arteries was confirmed in initial experiments by a lack of india ink in the cerebral vasculature following perfusion of the ink through the left ventricle at pressures of 150-175 mmHg while the common carotid arteries were occluded.

After the effects of bilateral carotid occlusion were observed while the ventral medullary surface was superfused with artificial CSF, procaine HCl (20 g/l, Sigma) or lidocaine HCl (2 g/l, Astra) was added to the superfusing solution for 2-3 min at a time, and the carotid occlusion was repeated. To assess the depth to which the local anaesthetic had penetrated the medulla $^{14}$C-labeled lidocaine HCl (0.45 μCi/ml, 48.3 mCi/mmol in physiological saline, New England Nuclear) was added to the superfusing solution in four of the experiments. At the end of the period of superfusion with artificial CSF containing $^{14}$C-labeled lidocaine HCl, the cats were quickly guillotined, and the head was immediately submerged in liquid nitrogen (-196 ° C). The brain was subsequently removed, and transverse sections of the medulla were cut at a thickness of 16 μm in a Harris cryostat at -16 ° C. The sections were picked up on cover glass kept at room temperature, rapidly dried on a hot plate at 70° C, assembled on pieces of cardboard, and placed in contact with β-particle-sensitive film (Ultrofilm, LKB) in an X-ray cassette at -4° C for 2 weeks. The autoradiographs were analyzed by a computer-based interactive image processing system that digitized the images on the basis of optical densities (Ciriello et al., 1983; Poulsen et al., 1975). The data were displayed on a cathode-ray tube as well as in numerical form for further analysis. The optical densities of various areas on the autoradiographs were taken to be directly related to the concentration of $^{14}$C-labeled lidocaine at the corresponding locations in the tissue sections. The optical density of the autoradiographs at positions corresponding to the ventral medullary surface was taken to

represent the concentration of $^{14}C$-labeled lidocaine applied to the surface of the medulla. The lidocaine concentrations at other locations were estimated relative to the latter value.

In one experiment the possibility of absorption into the systemic circulation of the $^{14}C$-labeled lidocaine, which had been applied to the ventral medullary surface, was assessed by measurement of radioactivity in plasma samples. Blood was withdrawn from the femoral artery and centrifuged. One milliliter of plasma was then added to 9 ml of Aquasol (New England Nuclear) and counted in a Hewlett-Packard scintillation counter.

In three experiments electrical stimulation within the medulla was performed. This was done using tungsten microelectrodes that had a resistance between 500 kΩ and 1 M Ω when an alternating current of 1,000 Hz was applied. The stimulus parameters were 2-5 V, 0.2 ms, and 40 Hz. The stimulus sites were marked by passing a DC current through the stimulating electrode to ground. The animals were subsequently perfused through the left ventricle with phosphate-buffered 10% formaldehyde and sucrose. The brains were removed, and paraffin-embedded sections of the medulla oblongata were cut at 16-μm thickness and stained with neutral red.

## RESULTS

Bilateral occlusion of the common carotid arteries, following sino-aortic denervation and ligation of the vertebral arteries, caused a marked increase in SAP. In 14 trials in 10 cats mean SAP increased on occlusion of the common carotid arteries by 58 ± 7 (±SEM) mmHg from a control level of 98 ± 6 to a peak value of 156 ± 7 mmHg. Peak SAP levels were attained in 20 ± 10 s and remained constant thereafter. The occlusion was maintained for 35 ± 3 s. In all the experiments SAP began to fall immediately on release of the carotid occlusion, reaching the control level in 23 ± 4 s. In a few trials the

return to control levels was preceded by a transient undershoot of SAP of 10-15 mmHg lasting less than 20 s. The SAP response to occlusion of the carotid and vertebral arteries appeared to be largely neurogenic since it could be abolished, or markedly reduced, by the administration of hexamethonium (10 mg/kg iv) even when SAP was maintained at control level by a continuous intravenous infusion of norepinephrine. The component of the response due to the passive increase in total systemic resistance resulting from occlusion of the carotid arteries was small as judged from the effects of common carotid occlusion on SAP when the vertebral arteries were not occluded (mean 16 mmHg, range 5-20 mmHg in 4 cats) (see also Borgdorff & van den Horn, 1980; Iriuchijima, 1972).

Associated with the SAP response there were also marked changes in phrenic nerve activity in all the cats. Phrenic nerve activity was eliminated or became apneustic after about 25 s of occlusion of the cerebral circulation. Apnea or apneusis was preceded by a progressive decline in peak integrated phrenic nerve amplitude in two cats, by no change in amplitude in three other cats, and by an initial increase in amplitude of up to 2.5 times the control value followed by a progressive decline in amplitude in five additional cats. In two of the latter cats the transient increase in peak integrated phrenic nerve amplitude was accompanied by an increase in respiratory frequency.

When the ventral surface of the medulla was superfused with artificial CSF containing 2% procaine for a period of 108 ±15 a prior to occlusion of the cerebral circulation, the SAP response to occlusion was attenuated by 65 ± 7%. In 14 trials in 10 cats SAP increased by only 19 ± 3 mmHg from an initial level of 84 ± 7 following the application of the local anaesthetic (compared with an increase of 58 ± 7 prior to superfusion of the ventral medullary surface with artificial CSF containing procaine). At this time basal SAP had decreased by 14 ± 2 mmHg, and there was still a considerable amount of neurogenic cardiovascular tone since continued application of procaine, iv administration of hexamethonium (10 mg/kg), or section of the spinal cord at $C_1$ all led to a

large further decrease in SAP to levels of approximately 40 mmHg. The magnitude of the SAP response to common carotid artery occlusion during superfusion with procaine was similar to that observed in the absence of procaine when the vertebral arteries were not ligated. Since the latter SAP response appears to be due to a passive increase in total peripheral resistance (Borgdorff & van den Horn, 1980; Iriuchijima, 1972) the former response may also be due to this mechanism. Superfusion of the ventral medullary surface with artificial CSF containing 2% procaine was also accompanied by a gradual decline in integrated phrenic nerve amplitude leading to its elimination after 58 ± 9 s.

In the two unanaesthetized midcollicular decerebrate preparations, bilateral occlusion of the vertebral and carotid arteries was accompanied by pressor responses of 105 and 95 mmHg. In one animal cerebral ischemia was accompanied by apneusis, whereas in the other cerebral ischemia was accompanied by a slowing of respiratory frequency with no change in integrated phrenic nerve amplitude. Superfusion of the ventral medullary surface with artificial CSF containing 2% procaine in these animals attenuated the pressor response to occlusion of the cerebral circulation by 77 and 75%, respectively.

The attenuation of the pressor response to cerebral ischemia did not appear to be due to the decrease in SAP seen during superfusion of the ventral medullary surface with artificial CSF containing procaine, inasmuch as in three trials in three CNS-intact preparations in which SAP did not change during application of procaine the SAP response to occlusion of the cerebral circulation was also substantially attenuated (by 37, 74, and 78%). Following application of the local anaesthetic to the ventral medullary surface, the ability of deeper brain stem structures to continue to influence sympathetic activity in our experiments was indicated by the fact that spinal section at $C_1$ caused a further reduction in SAP as well as the observation that electrical stimulation at a depth of 2 - 4 mm from the ventral medullary surface was still effective in causing a pressor

response (i.e., Fig. 9). Absorption of significant amounts of local anaesthetic into the circulation in our experiments did not occur because when $^{14}C$-labeled lidocaine, in concentrations producing the effects described above for procaine, was applied to the ventral medullary surface for a similar period of time no appreciable radioactivity over the background level was detectable in plasma samples. In addition, as mentioned above, electrical stimulation of brain stem sites continued to be effective in causing a significant pressor response.

Examples of the SAP and phrenic nerve response to cerebral ischemia before and after superfusion of the ventral medullary surface with artificial CSF containing 2% procaine are shown in Figs. 9 and 10. Figure 9 also shows the effects of procaine on the SAP response to electrical stimulation in the medulla and on basal SAP following prolonged application.

The effects of ventral medullary superfusion with procaine described above on the pressor response to cerebral ischemia, SAP levels, and phrenic nerve activity were all reversible. Within 20 - 30 min of reverting to procaine-free artificial CSF, the pressor response to cerebral ischemia, SAP level, and phrenic nerve activity all returned to close to control levels. The return to superfusion with procaine-free artificial CSF was followed by a gradual recovery of SAP, in turn followed by a recovery of phrenic nerve activity. In two experiments in which carotid occlusion was performed periodically during the recovery phase the CIR recovered after the recovery of the SAP but prior to the onset of phrenic nerve activity.

The depth of penetration of local anaesthetic into the brain stem was assessed from autoradiographs of brain stem sections obtained from four animals whose brains had been quickly frozen following attenuation of the CIR by $^{14}C$-labeled lidocaine applied to the ventral medullary surface (see METHODS) . The concentration of lidocaine applied (0.2%) produced effects similar to those described above for procaine with a somewhat

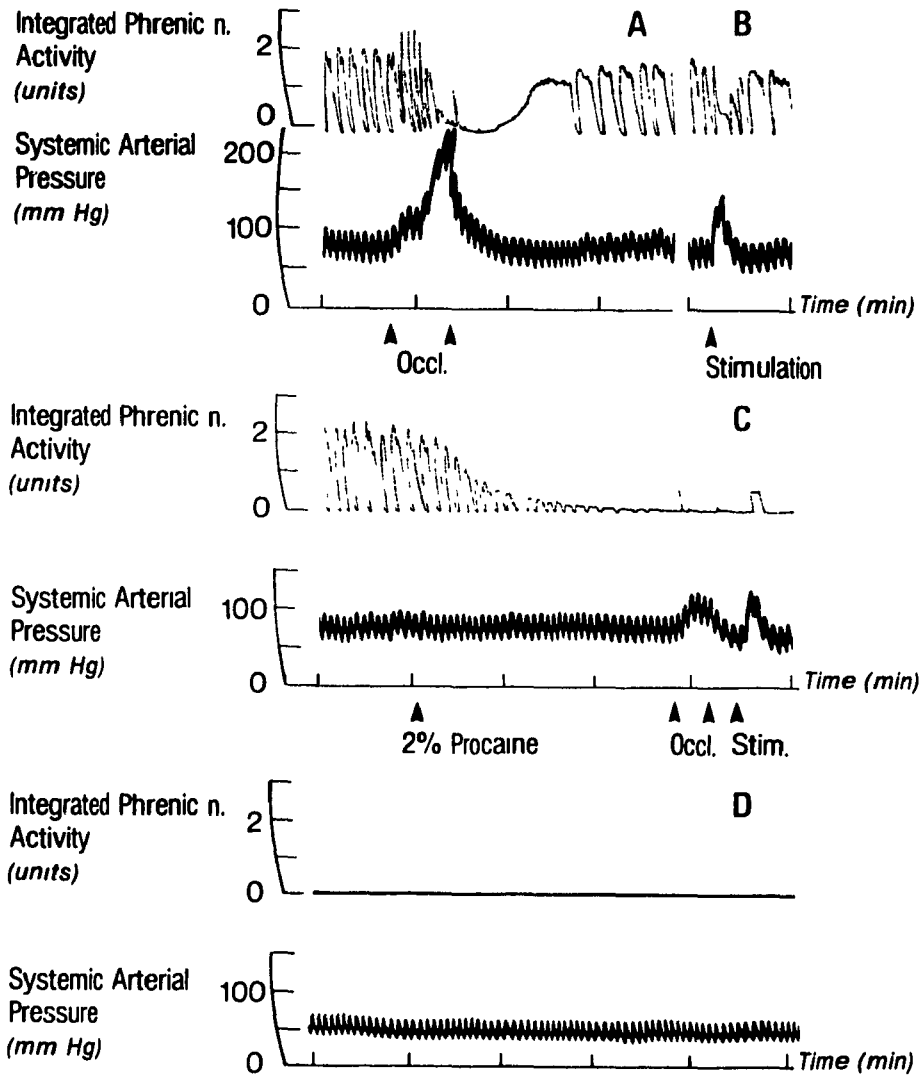


*Fig. 9.* **Attenuation of the pressor response to cerebral ischemia (CIR) by superfusion of the ventral medullary surface with artificial cerebrospinal fluid (CSF) containing 2% procaine. A: systemic arterial pressure (SAP) and phrenic nerve response to occlusion (Occl) of stimulation (Stim) (3V, 0.2 ms, 40 Hz, for 5s at the level of the XII nerve rootlets, 3.6 mm lateral from midline and 3 mm from ventral medullary surface) (1 min of record omitted between A and B). C: effects of superfusion of ventral medullary surface with artificial CSF containing 2% procaine on the CIR, phrenic nerve activity, and electrical stimulation within medulla as in B (1 min of record omitted between B and C). D: SAP 15 min following application of procaine.**

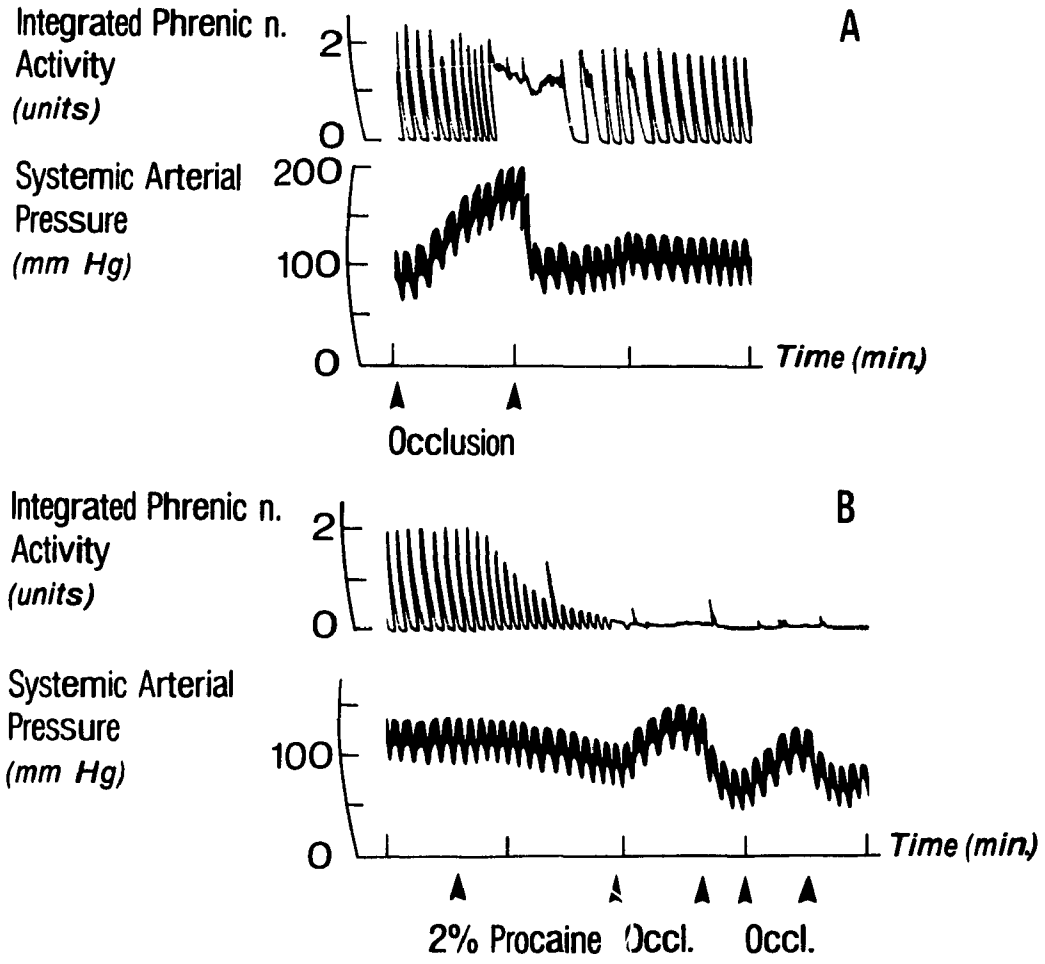


*Fig. 10.* Progressive attenuation of pressor response to cerebral ischemia following application of 2% procaine to the ventral medullary surface. A: systemic arterial pressure ( SAP) and phrenic nerve response to occlusion (Occl) of common carotid and vertebral arteries. B:SAP and phrenic nerve response to occlusion of common carotid and vertebral arteries following 1.25 and 2.33 min of superfusion of ventral medullary surface with artificial cerebrospinal fluid containing 2% procaine (1.42 min of record omitted between A and B).

faster time course (the CIR was virtually eliminated 30 s after the application of lidocaine). Inspection of the autoradiographs showed increased density of photographic emulsion, i.e., presence of $^{14}C$-labeled lidocaine, in relatively superficial regions of the ventral medulla. Detailed analysis of autoradiographs from one experiment was conducted (see METHODS) to obtain estimates of the concentration of lidocaine at various depths from the ventral medullary surface. This analysis indicated a rapid "exponential" decay of concentration with increasing depth from the surface. Concentrations one-tenth of those at the surface were observed at depths of about 550 μm from the ventral medullary surface and previously cited minimum concentrations of lidocaine necessary for nerve conduction block (0.07% lidocaine) (Truant & Takman, 1959) at a depth of 325 μm. The relationship between estimated lidocaine concentration ($N$) and depth ($x$) was well fit, using the least-squared difference method, by an equation describing simple diffusion with time ($t$) in one dimension through an isotropic medium from an infinite source ($N_o$) at the surface (Crank, 1956)

$$(1) \qquad N(x, t) = N_o \operatorname{erfc}\left(2\frac{x}{\sqrt{Dt}}\right)$$

where erfc is the complement of the error function and $D$ is the diffusion constant. The CIR was virtually eliminated after about 30 s of application of the local anaesthetic , whereas it was not possible to freeze the brain in this case until 3.75 min later. Substitution for $t$ = 30 s produced the concentration profile existing at the time at which the CIR was eliminated subject to the assumptions for Eq. 1 outlined above. Such extrapolation back in time indicated that minimum nerve conduction blocking

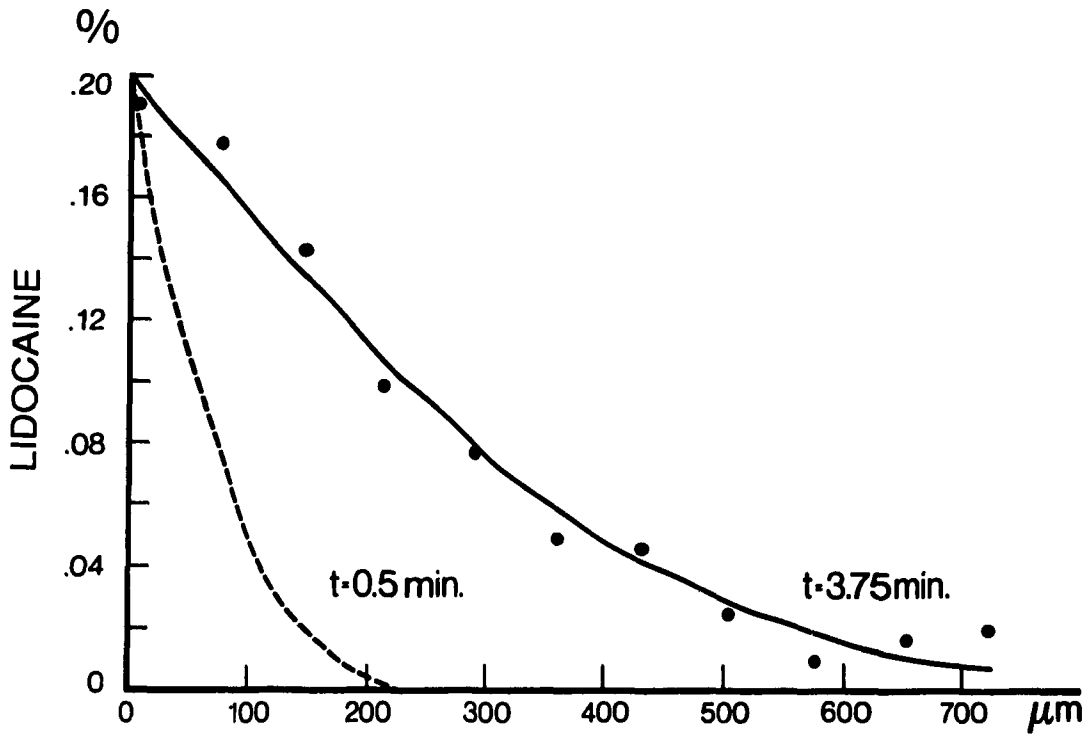


*Fig. 11.* Estimated [$^{14}$C] lidocaine concentration at various depths from the ventral medullary surface. Vertical axis is estimated [$^{14}$C]-lidocaine concentration (%); horizontal axis is depth from ventral medullary surface (μm). Points are estimates of lidocaine concentration in brain tissue based on autoradiographic analysis (see METHODS) following 3.75 min of application of [$^{14}$C] lidocaine to the ventral medullary surface. Solid line is the relation fit to these estimates of [$^{14}$C]-lidocaine concentration assuming simple diffusion in one dimension through an isotropic medium from an infinite source at the surface (see RESULTS). Diffusion constant ($D$) obtained was $2.6 \times 10^{-6}$ cm$^2$/s. Broken line is concentration of profile of lidocaine expected at 0.5 min, the time at which the pressor response to cerebral ischemia was attenuated in the experiment, using Eq. 1 (see RESULTS) and the value of $D$ cited above.

concentrations of lidocaine (Truant & Takman, 1959) would have been observed at a depth of 85 μm from the ventral medullary surface. The concentrations of lidocaine observed at various depths from the ventral medullary surface, the relation fit to this data, as well as the relation between lidocaine concentration and depth from the ventral medullary surface thought to have existed at the time that the CIR was eliminated, are shown in Fig. 11.

## DISCUSSION

The main finding of this investigation is that application of local anaesthetic to the ventral surface of the medulla in the sino-aortic denervated cat causes marked attenuation of the CIR. A number of factors indicate a relatively superficial site of action of the local anaesthetic. The latency with which the CIR is attenuated is of the same order of magnitude as that for the arrest of phrenic nerve activity, which is known to occur on inactivation of structures located between 30 and 350 μm from the ventral medullary surface (Berndt et al., 1970; Mitchell et al., 1963; Schwanghart et al., 1974). Pressor responses to electrical stimulation of medullary pressor points situated as close as 2 mm from the ventral medullary surface were not significantly decreased at the time when the CIR had been attenuated by the local anaesthetic. The experiments with radioactive lidocaine indicated a superficial distribution in the ventral medulla. This distribution was well fit by a relation describing simple diffusion (see RESULTS), and extrapolation back in time, subject to the assumptions outlined in RESULTS, showed that attenuation of the CIR occurred at a time when the local anaesthetic had reached a nerve conduction blocking concentration (Truant & Takman, 1959) at an estimated depth of 85 μm from the ventral medullary surface. Thus these experiments confirm the conclusion of Dampney and Moon (1980), based on lesion experiments, that the mechanism of the CIR includes

an essential component in the ventral medulla and add evidence that this mechanism is superficially located.

In contrast to past work (Dampney and Moon, 1980), our experiments indicate that attenuation of the CIR can be obtained without a large decrease in SAP and, by inference, in neurogenic vasoconstrictor tone. Attenuation of the CIR, following application of local anaesthetic to the ventral medullary surface, occurred at a time when there was a modest decrease in SAP (14 ± 2 mmHg), and a significant amount of neurogenic vascular tone remained as evidenced by large further falls in SAP on spinal section at $C_1$, ganglionic blockade, or more prolonged application of local anaesthetic to the ventral medullary surface. Furthermore, following prolonged application of the local anaesthetic, which caused a large fall in SAP comparable in magnitude to that seen by Dampney and Moon (1980), as well as attenuation of the CIR, recovery of SAP to control levels occurred prior to recovery of the CIR. This suggests that the medullary mechanisms generating the CIR and those responsible for the major component of neurogenic vasoconstrictor tone are separate. As a result, if changes in the chemical environment of medullary neurons are the stimulus for the CIR, then it would appear that the ability to sense these changes is not a general property of the neurons that generate the supraspinal component of neurogenic vascular tone but rather a property of a separate, and as discussed above, superficially located set of neurons that do not usually contribute a large component of neurogenic vascular tone.

Previous work, summarized in the INTRODUCTION, has suggested the presence of $CO_2$- or $H^+$-sensitive structures, with excitatory effects on the sympathetic and respiratory systems, superficially located in the ventrolateral medulla (Hanna et al., 1979; Lioy et al., 1981; Schlaefke et al., 1970; Trzebski et al., 1974). These structures can produce a powerful excitation of sympathetic neurons when stimulated by large increases in arterial $CO_2$ or in CSF $CO_2$ or $H^+$ but are not responsible for a major fraction of the basal

neurogenic vascular tone when arterial $PCO_2$ ($PaCO_2$) and pH are within the normal range (Polosa et al.,1983). Since cerebral acidosis is a likely outcome of cerebral ischemia (Cohen, 1973), the hypothesis that these structures are responsible for initiating the CIR seems consistent with our experimental findings as well as with the inferences made above.

Although we have no direct evidence for this hypothesis there is evidence in our experiments suggesting activation of ventral medullary chemoreceptors during cerebral ischemia as well as inactivation on attenuation of the CIR. Previous work has shown that both phrenic nerve activity and sympathetic preganglionic neuron (SPN) activity respond similarly to changes in $PaCO_2$ in the sino-aortic-denervated cat (Lioy et al., 1981) and that both responses are greatly attenuated or eliminated by inactivation of the same superficial layers of the ventral medulla (Hanna et al., 1979; Schlaefke et al., 1970). Activation of ventral medullary chemoreceptors during cerebral ischemia is suggested by the pattern of phrenic nerve response observed in some of the present experiments. These responses consisted, in a number of cases, of an initial increase in integrated phrenic nerve amplitude followed by a progressive decline in amplitude. Since in the absence of arterial chemoreceptor input respiratory rhythm generation depends critically on input from ventral medullary chemosensitive structures (Loeschke, 1974), it seems possible that the increase in tissue $PCO_2$ or $H^+$, consequent to cerebral ischemia, acting on these receptors produces an increased excitatory input to the respiratory system that is subsequently countered by the depressant effect of nervous tissue hypoxia (Morrill et al., 1975) also resulting from cerebral ischemia. The fact that the ventral medullary chemoreceptors are inactivated during superfusion with procaine, at the time when the CIR is greatly attenuated, is suggested by the close relation in time between the loss of phrenic nerve activity and the attenuation of the CIR. The former is attributed to loss of

virtually all centrally mediated $CO_2$ sensitivity of the respiratory system [i.e., all tonic excitatory input to the respiratory system in the sino-aortic-denervated cat (Loeschke, 1974)] and on the basis of previous work (Hanna et al., 1979) is presumably associated with loss of sympathetic $CO_2$ sensitivity.

Of considerable interest is the dramatic difference in the response of sympathetic and respiratory neurons to cerebral ischemia. Although cerebral ischemia caused a large excitation of sympathetic neurons, the predominant response of respiratory neurons was depression. This difference in the way brain stem respiratory neurons and brain stem neurons antecedent to the SPNs respond to the changes in the brain tissue chemical environment resulting from ischemia suggests the question of whether this difference has to be attributed to the existence of central chemoreceptors with different properties for the two systems (Lioy & Trzebski, 1981; Trzebski & Kubin, 1981) or to substantial differences in pathways and connections from a common set of central chemoreceptors.

# MECHANISMS IN VENTROLATERAL MEDULLA FOR CONTROL OF SYSTEMIC ARTERIAL PRESSURE AND VASCULAR RESISTANCE

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## ABSTRACT

This paper describes properties of a ventrolateral medullary mechanism mediating chemoreflex responses of sympathetic preganglionic neurons in the sino-aortic denervated cat. This mechanism mediates the sympathetic responses to brain hypercapnia and ischemia as well as, by inference, to brain hypoxia and must be distinguished from other sympatho-excitatory mechanisms in the same region. This mechanism is responsible for a significant but not large fraction of basal sympathetic tone and is situated as superficially as the chemoreceptor mechanism which maintains the activity of the brain stem respiratory pattern generator in sino-aortic denervated animals. Autoradiography after superfusion with radioactive local anaesthetic shows that this mechanism is situated within 85 μm from the surface. Still close to the surface but deeper there appear to be other mechanisms for generation and/or transmission of sympathetic tone, since by the time the local anaesthetic has reached 200 μm from the ventral surface, the basal sympathetic tone has decreased to a level similar to that of the acute $C_1$ spinal animal.


## INTRODUCTION AND METHODS

In the course of investigations leading to the identification of chemosensitive sites on the surface of the ventrolateral medulla (VLM) mediating the respiratory response to $H^+$ and $CO_2$, cardiovascular effects were also observed (Schlaefke, 1981). In

studies involving the application of various drugs to, or lesion of, sites on the VLM surface dramatic cardiovascular effects were often seen (Schlaefke, 1981). One role of the VLM seems to be that of influencing cardiovascular functions through chemosensitive mechanisms. Intracranial chemosensitive mechanisms for cardiovascular control were demonstrated by the fact that cephalic perfusion with hypoxic or hypercapnia blood (deGeest et al., 1965a; Downing et al., 1963) or brainstem ischemia (Takeuchi et al., 1969) increased sympathetic output to heart and blood vessels. Similar effects were obtained by superfusion of the VLM surface with acid and/or hypercapnic artificial CSF (Polosa et al., 1983; Schlaefke, 1981). An important chemosensitive site for reflex control of the circulation was found in or around area 'S' (Schlaefke, 1981): reflex sensitivity to $CO_2$ of vascular resistance in sino-aortic denervated (SAD) cats decreased by half upon cooling this area to 12° C (Polosa et al., 1983). One third of neurogenic vasoconstrictor tone of the hindlimb in normocapnia is maintained by the $CO_2$ - dependent input from this area (Polosa et al., 1983). However, inactivation of this chemosensitive mechanism cannot account for the large systemic arterial pressure (SAP) decreases observed in some of the previous experiments. This suggests that chemosensitive structures may not be the only mechanisms in the VLM involved in the generation of neurogenic vasoconstrictor tone.

This paper shows that chemosensitive and non-chemosensitive mechanisms of the VLM surface, involved in generation of vascular tone, may be separated by appropriate experimental techniques. The observations reported here were made during superfusion of the exposed VLM surface with artificial CSF containing procaine while monitoring SAP, phrenic nerve activity, and the pressor response to systemic hypercapnia as well as to brain ischemia in anaesthetized, SAD, vagotomized, paralyzed and artificially ventilated cats. Systemic hypercapnia was produced by ventilation with 10% $CO_2$ in $O_2$. Cerebral ischemia was produced by occlusion of the common carotid

and vertebral arteries. Details of the methods are given in another publication (Rohlicek & Polosa, 1983).

## RESULTS

*1. Effect on the pressor response to systemic hypercapnia.* In SAD cats systemic hypercapnia reflexly increases sympathetic neuron activity and hindlimb vascular resistance by central mechanisms (Polosa et al., 1983). One of these mechanisms is close to the VLM surface (Polosa et al., 1983). Systemic arterial pressure (SAP) increases during systemic hypercapnia unless prevented by the direct vasodilator and negative chronotropic effects of $CO_2$. This pressor effect can be used as an index of the reflex sympatho-excitation and vasoconstriction elicited by systemic hypercapnia via the VLM mechanism. Procaine attenuated this response. An example is shown in Fig. 12a,b. In this case the attenuation occurred at a time when the peak amplitude of the phrenic neurogram had decreased by 70% and mean SAP had decreased from 123 to 100 mmHg. The residual response may be attributed , at least in part, to a sympatho-excitatory chemoreflex of the spinal cord (Zhang et al., 1982 [this paper appears in CHAPTER 4]).

*2. Effect on the pressor response to cerebral ischemia.* The intracranial chemosensitive mechanism involved in cardiovascular control is activated by hypercapnia, hypoxia and ischemia (deGeest et al., 1965a; Downing et al., 1963). One working hypothesis is that these stimuli all act on structures close to the VLM surface in a manner similar to their action on the arterial chemoreceptors (Heymans & Neils, 1958). The magnitude of the pressor response to brainstem ischemia may then be taken as an indication of the responsiveness of this intracranial chemosensitive mechanism. During procaine superfusion the cerebral ischemic pressor response was attenuated by 65% with a time-course similar to that of the attenuation of the pressor response to systemic

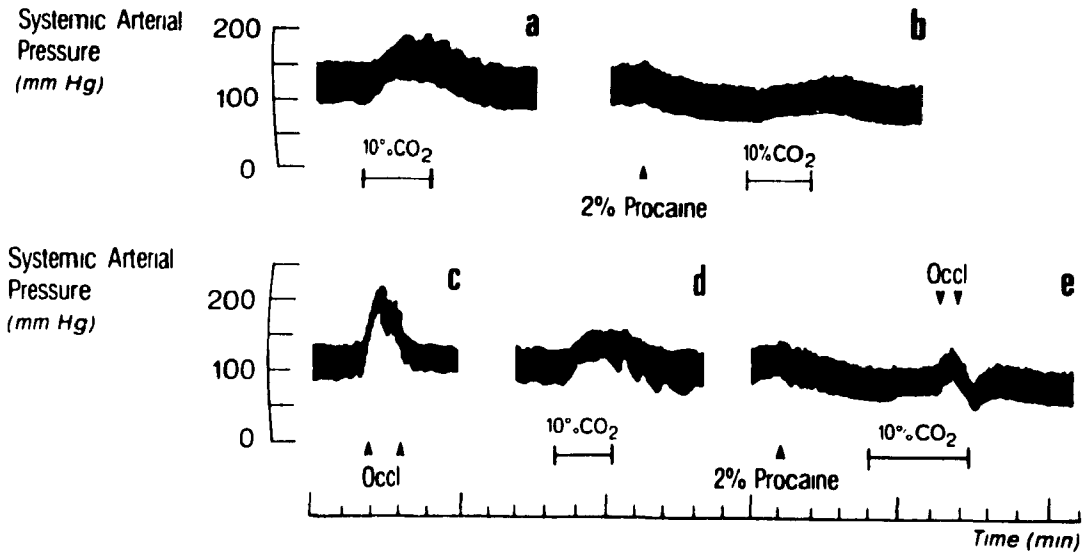


*Fig. 12.* **Sino-aortic denervated cat. Superfusion of the ventrolateral medulla with artificial CSF. a: control pressor response to systemic hypercapnia; b: during procaine superfusion; c d: control pressor responses to common carotid and vertebral artery occlusion and to systemic hypercapnia; e: during procaine superfusion.**

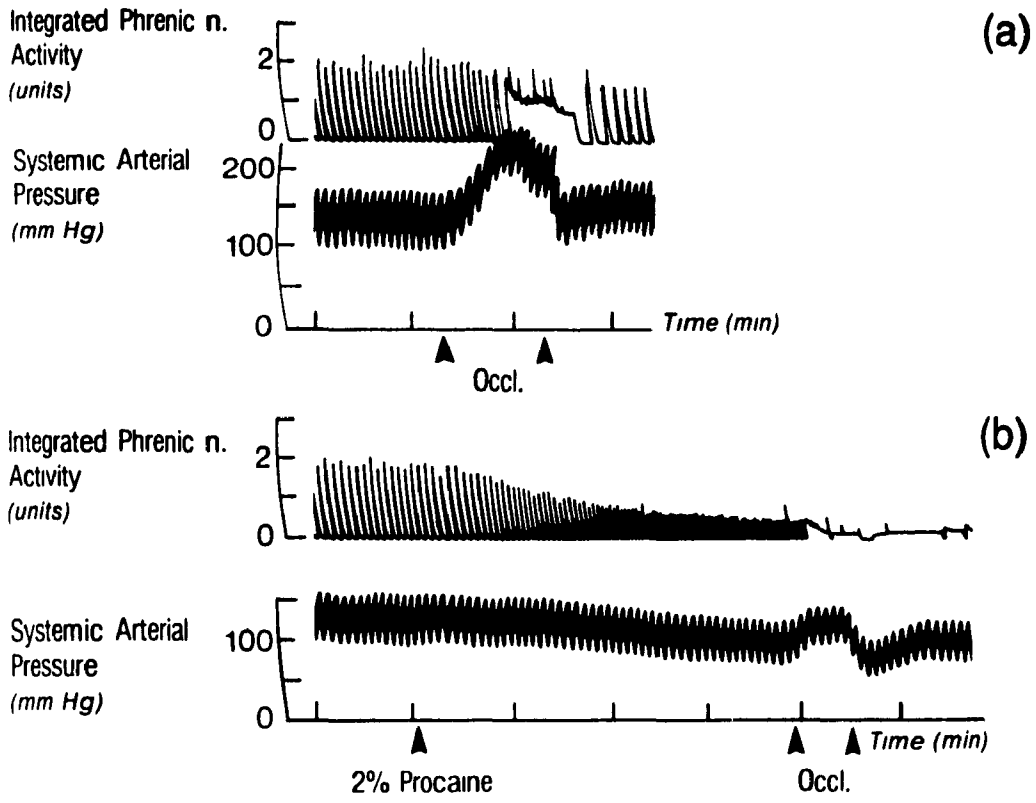


*Fig.13.* Sino-aortic denervated cat. Superfusion of the ventrolateral medulla with artificial CSF. a: control pressor response to common carotid and vertebral artery occlusion; b: during procaine superfusion.

hypercapnia (Fig. 12c,d,e) and of peak amplitude of the phrenic burst (Fig. 13). The magnitude of the observed attenuation of the ischemic response is consistent with the abolition of its neurogenic component (Rohlicek & Polosa, 1983).

*3. Effect on phrenic nerve activity* Phrenic nerve activity was also suppressed by superfusion of the VLM surface with procaine (Figs. 13 and 14). The disappearance of phrenic nerve activity occurred close in time to the attenuation of the pressor responses to systemic hypercapnia and to cerebral ischemia. This suggests that the medullary mechanisms involved in the generation or transmission of central chemoreceptor input to neurons controlling respiration and circulation are located at similar depths from the ventral medullary surface. Often there was an obvious parallelism between the time course of the fall of peak phrenic burst amplitude and that of a concomitant SAP fall. This decrease in SAP was not large (see below) and in some cases there were no changes in SAP during the phase of decline of phrenic nerve burst amplitude.

*4. Changes in SAP.* In a series of 10 cats mean SAP, measured at the time of phrenic nerve activity disappearance and of maximum attenuation of both ischemic and hypercapnic response, was 84 mmHg, down from the control value of 98 mmHg. This pressure drop is only a fraction of that caused by spinal cord transection at $C_1$ or by administration of hexamethonium. This SAP drop can be thought to result from withdrawal of the component of neurogenic cardiovascular tone generated by the VLM chemoreceptor mechanisms. The magnitude of the drop fits with prior estimates that in normocapnia the $CO_2$-dependent component of hindlimb vascular resistance, in the SAD preparation, is approximately one-third of the total neurogenic vascular resistance (Polosa et al., 1983). This fraction is nearly completely accounted for by the influence of area S of the VLM (Polosa et al., 1983). At the time when phrenic nerve activity and the pressor responses to hypercapnia and to cerebral ischemia reached maximum attenuation, following application of the local anaesthetic, the ability of deeper brainstem structures to

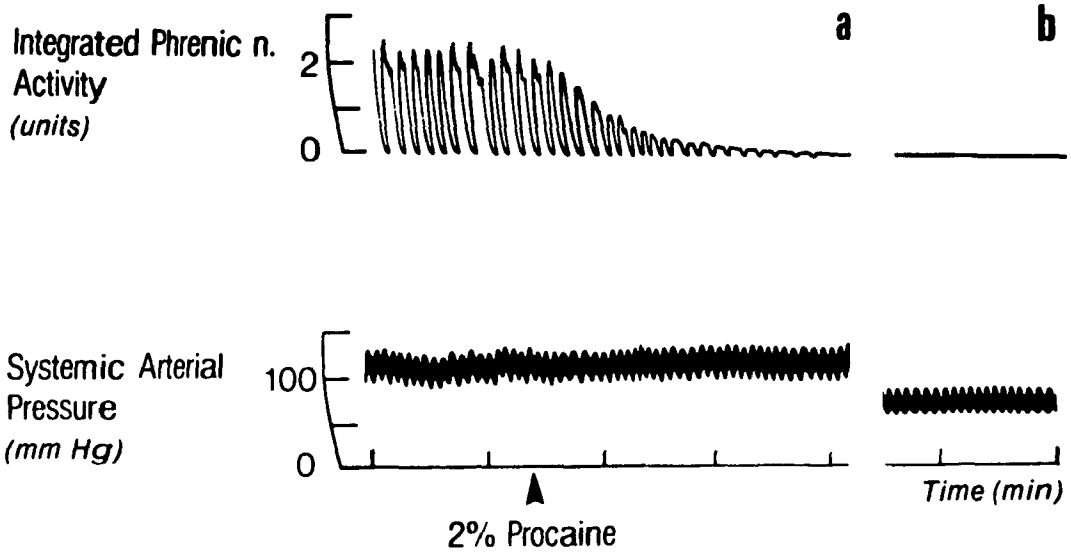


*Fig. 14.* Sino-aortic denervated cat. Superfusion of the ventrolateral medulla with artificial CSF. a: onset of procaine superfusion; b: after 15 min of procaine superfusion.

influence sympathetic activity was maintained. This was indicated by the fact that spinal section at $C_1$ caused a further reduction in SAP and the electrical stimulation of pressor sites at depths of 2-4 mm from the ventral surface was still effective in causing a pressor response. When the superfusion was continued beyond the time at which the phenomena described above were observed, there was a progressive further decline in SAP, which eventually attained levels comparable to those obtained after administration of hexamethonium or spinal transection at $C_1$. In a series of 10 cats, continuous superfusion with procaine resulted, after 5.5 min, in a drop in mean SAP from 98 mmHg to a level of 52 mmHg.

*5. Anatomical correlates* Autoradiography of the brainstem was performed after superfusion with [$^{14}$C]lidocaine. Details of these experiments are described elsewhere (Rohlicek & Polosa, 1983). The results show that 85 μm is the maximum distance from the surface at which the drug attains a concentration, blocking nerve conduction at the time of suppression of phrenic nerve activity, as well as maximum attenuation of the pressor response to systemic hypercapnia and cerebral ischemia first occur. These results fit well with those obtained in a study (Ciriello et al., 1985) in which the uptake of [$^{3}$H]2-deoxyglucose by the CNS of the SAD rat during systemic normocapnia and hypercapnia was compared. In hypercapnia, a zone of increased uptake was found along the ventral surface of the medulla, extending from the surface to a depth of less than 100 μm from the surface, in the region occupied by the superficial arcuate fibers and the hypoglossal rootlets. On the other hand it was estimated that SAP had reached its lowest level at a time when the anaesthetic had diffused, in concentrations just sufficient to cause nerve conduction block, about 200μm from the ventral medullary surface.

## DISCUSSION AND CONCLUSIONS

Some tentative conclusions, concerning cardiovascular control by VLM mechanisms can be drawn from the experimental data reviewed in this paper.

The abolition of phrenic nerve activity by procaine after a very short diffusion from the ventral surface is consistent with the view that VLM surface chemoreceptors, in the peripherally chemodenervated cat, provide the main excitatory input to the brainstem respiratory pattern generator, which in turn drives rhythmically the respiratory motoneurons. This view is based on experiments in awake chronic cats with lesions of area S (Schlaefke, 1981). It may be expected that procaine would block these same structures or their output connections.

The fall in SAP, associated with the disappearance of phrenic nerve activity, can be attributed to the abolition of the inspiration-synchronous activity of the sympathetic neurons, which, like phrenic nerve activity, depends on input from the respiratory pattern generator (Polosa et al., 1980). Estimates of the fraction of the neurogenic component of hindlimb vascular resistance due to this component of sympathetic discharge range between 20 and 30% (Bachoo & Polosa, 1985). This value is similar to that obtained for the fraction of the neurogenic component of resistance which is $CO_2$-dependent and derives from the VLM in normocapnia (Polosa et al., 1983), strongly suggesting the possibility that the main pathway by which the VLM chemoreceptors influence sympathetic neurons is by way of the brainstem respiratory neurons

That this is not the only pathway is shown by the different effects of cerebral ischemia on sympathetic neuron and phrenic motoneuron activity. This procedure excites the former but depresses the latter (Rohlicek & Polosa, 1983). Similar results are obtained with hypoxic perfusion of the brainstem (unpublished results). These dissimilarities suggest that there are also connections between VLM surface

chemoreceptors and sympathetic neurons which are independent of the respiratory neurons. These connections do not provide appreciable input to the sympathetic neuron under normal conditions, as shown by the fact that their inactivation does not cause a SAP fall in excess of that which can be explained by the suppression of inspiratory-synchronous neuron activity alone. The latter observation also indicates that some of the large decreases in SAP elicited by experimental manipulations of the VLM regions may not be related to their chemosensitive functions.

# CHAPTER 4

## RESULTS II

As is reviewed in the INTRODUCTION (pp. 20 - 22, 25 - 28) there is evidence to indicate that in the CNS-intact and peripherally chemodenervated animal both systemic hypoxia and hypercapnia exert sympatho-excitatory effects as a result of activation of chemosensory mechanisms within the CNS. This chapter describes the results of experiments aimed at further characterizing the properties of a central sympathetic sensitivity to changes in $PO_2$ as well as $PCO_2$ in CNS-intact or acute spinal cats and the extent of control which such sensitivity exerts over sympathetic neurons.

Previous investigations in the CNS-intact and sino-aortic denervated (SAD) animal indicate that there is a direct relationship between sympathetic neuron firing rate as well as the neurogenic component of peripheral vascular resistance and $PaCO_2$ over a wide range of values between normocapnia and hypercapnia (Lioy et al., 1978; Hanna et al., 1981). However, the relationship between $PaO_2$ and sympathetic activity in the CNS-intact and SAD animal has not been as well characterized. At least a part of the sympathetic sensitivity to $O_2$ lack as well as $CO_2$ excess is supra-spinal in origin (Kao et al., 1962; Downing et al., 1963; DeGeest et al., 1965; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985). However, the possibility that sympathetic chemosensitive mechanisms also exist at a spinal level must be considered. In this regard the sympatho-excitatory action of systemic hypoxia in the spinal animal is known (Alexander, 1945) and the relationship between $PaO_2$ and sympathetic neuron firing in the spinal animal has been described (Rohlicek & Polosa, 1981). However, the importance of changes in $PaO_2$ in the spinal animal in initiating neurally mediated peripheral effector responses is not known. In addition the effects of systemic hypercapnia on sympathetic activity in the

spinal animal remain unclear. The work presented in this chapter deals with these unresolved issues.

The first paper in this chapter describes an attempt to determine the types of SPN responses to CNS hypoxia by determining the relation between SPN firing rate and $PaO_2$ in CNS-intact SAD cats. The results of these experiments indicate; i) a marked increase in SPN firing rate at severe levels of hypoxia, and ii) a significant decrease in SPN firing rate at less extreme levels of hypoxia. In the second paper an effort was made to determine whether the excitatory and depressant sympathetic responses to hypoxia described in the first paper are shared by a significant number sympathetic neurons involved in cardiovascular control. These experiments were conducted by observing the neurogenic changes in hindlimb vascular resistance in CNS-intact SAD cats which were exposed to varying degrees of systemic hypoxia. The results of this work indicate a similar biphasic response of the neurogenic component of hindlimb vascular resistance to systemic hypoxia as recorded from individual SPNs. In the third paper experiments are described which were aimed at determining whether the inspiration synchronous component and the component not related to respiration of the mass activity recorded from the CST in the CNS-intact SAD cat (Bachoo & Polosa, 1985) exhibit different responses to hypoxia. The results show that the sympatho-depressant effect of hypoxia is exerted on both components of sympathetic discharge. In contrast the sympatho-excitatory effect of extreme hypoxia is only exerted on the component of sympathetic activity which is independent of respiration. The fourth paper describes the relation between $PaCO_2$ and SPN firing rate in the acute $C_1$ spinal cat. The results of this work indicate that a large fraction of SPNs are excited during hypercapnia and increase their firing rate in proportion to the increase in $PaCO_2$. In the last paper of this chapter experiments were conducted on the neurogenic component of hindlimb vascular resistance in acute $C_1$ spinal cats to determine whether the spinal sympatho-excitatory

effects of $O_2$ lack and $CO_2$ excess are exerted on a significant number of sympathetic neurons involved in cardiovascular control. The results of this work indicate that similar changes in the neurogenic component of hindlimb vascular resistance as in the SPN firing rate occur during systemic hypoxia and hypercapnia in the acute spinal animal.

# HYPOXIC RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONS IN SINO-AORTIC DENERVATED CATS

Charles V. Rohlicek and Canio Polosa

American Journal of Physiology (1983) 244: H681-H686.


## ABSTRACT

The relation between arterial $O_2$ tension ($PaO_2$) and the firing rate of sympathetic preganglionic neurons (SPN) was studied in 16 strands of the cervical sympathetic trunk (CST) during graded isocapnic hypoxia in 11 sino-aortic denervated central nervous system (CNS)-intact, anaesthetized cats. SPN firing rate was independent of $PaO_2$ from normoxia down to a $PaO_2$ of 40 mmHg. Below this $PaO_2$ level three response patterns were observed, i.e., an excitatory response (n = 8), a depressant response (n = 3), and a mixed response consisting of a depression of firing at less severe hypoxic levels and an increase in firing rate at more extreme hypoxic levels (n = 5). Similar response patterns were also observed in four strands of the CST in three unanaesthetized, sino-aortic denervated, mid-collicular decerebrate preparations. Systemic arterial pressure decreased in all cats as $PaO_2$ decreased. Phrenic nerve activity also decreased in all cats with a course resembling that of the depression of sympathetic firing and disappeared at $PaO_2$ of 20 mmHg. The data suggest that systemic hypoxia in the sino-aortic denervated, CNS-intact or decerebrate animal activates both excitatory and depressant mechanisms acting on SPNs.


## INTRODUCTION

Systemic hypoxia causes complex changes in a number of cardiovascular parameters, the most important among which are heart rate, myocardial contractility,

cardiac output, peripheral resistance, and systemic arterial pressure (SAP) (Korner, 1959; Korner, 1971; Krasney & Koehler, 1977). These changes seem to have the net effect of increasing the fraction of the cardiac output delivered to the brain, heart, and liver while decreasing that delivered to other vascular beds (Adachi et al., 1976).

These circulatory adjustments are the result of both a direct action of systemic hypoxia on cardiovascular effectors (Katz, 1977; Somlyo & Somlyo, 1970) and actions mediated by the central nervous system (CNS). The arterial chemoreceptors have been shown to initiate some of the latter. For instance, stimulation of the carotid sinus chemoreceptors, either by systemic hypoxia or by perfusion of the carotid sinus with hypoxic blood, has been shown to cause peripheral vasoconstriction (Chalmers et al., 1967; Pelletier, 1972), whereas systemic hypoxia following section of the peripheral chemoreceptor afferents is accompanied by peripheral vasodilation (Chalmers et al., 1967). However, some of the cardiovascular changes of systemic hypoxia appear to be also mediated by neural mechanisms that do not involve arterial chemoreceptors. A number of investigators have observed increases in heart rate and ventricular contractility, during systemic hypoxia, in animals lacking arterial chemoreceptors (Achtel & Downing, 1972; deBurgh-Daly & Scott, 1964; Koehler et al., 1980; Kontos & Lower, 1969; Krasney & Koehler 1977). These responses can be eliminated by $\alpha$-adrenergic blockade (Kontos & Lower, 1969). In addition, an increase in sympathetic activity to various effector organs during systemic hypoxia has been reported in animal preparations with denervated arterial chemoreceptors (Gregor & Jänig, 1977; Iriki & Kozawa, 1975; Von Kehrel et al., 1962).

The problem of how these CNS-mediated but peripheral chemoreceptor-independent hypoxic responses are generated is far from being completely solved. A component of the response is of spinal origin, as shown initially by Alexander (1945) and later described in more detail by Rohlicek and Polosa (1981). Another component, of supraspinal origin, has been suggested by the results of experiments involving perfusion

of the head with hypoxic blood (DeGeest et al., 1965a; Downing et al., 1963; Gömori et al., 1960; McGillicudy et al., 1978). How these two central components interact with each other and with the peripheral chemoreceptor-mediated CNS effect are questions that must be answered to explain the overall response of the intact animal to systemic hypoxia.

The present study, which follows a previous one on the cat made acutely spinal by transection of the neuraxis at $C_1$ (Rohlicek & Polosa 1981), was undertaken to explore the neural mechanisms underlying the CNS-mediated, peripheral chemoreceptor-independent response to systemic hypoxia in the CNS-intact or midcollicular decerebrate cat. This was done by studying the types and properties of sympathetic neuron responses to graded systemic hypoxia in these preparations and by comparing these responses with those seen in the previous study in the spinal cat.

The main results of the present investigation are that both excitatory and inhibitory effects of systemic hypoxia on the firing rate of sympathetic preganglionic neurons (SPN) are found in the CNS-intact or midcollicular decerebrate chemodeafferented cat. These findings contrast with those obtained in the acute $C_1$ spinal preparation in which excitation only was observed (Rohlicek & Polosa 1981).

## METHODS

Experiments were conducted on adult cats of either sex weighing between 3 and 5 kg. Eleven cats were anaesthetized with pentobarbital sodium (35 mg/kg ip followed by supplementary doses of 4 mg/kg iv as required). Three cats were decerebrated at a midcollicular level under ether anaesthesia. The rostral portion of the midbrain and the whole forebrain were removed by suction. After completion of the decerebration, anaesthesia was discontinued. All the cats were paralyzed with pancuronium bromide (0.5 mg/kg iv) and ventilated with positive pressure. Ventilation was adjusted to maintain

an end-tidal $CO_2$ tension between 30 and 40 mmHg. Rectal temperature was maintained between 36 and 37° C by means of infrared lamps.

In all the cats the arterial chemoreceptors and baroreceptors were denervated by section of the carotid sinus, aortic and vagus nerves. Completeness of arterial chemodenervation was tested by observing the respiratory effects of systemic hypoxia and bolus intravenous injections of KCN (50 µg/kg) (Dumke et al., 1941). Both stimuli produced respiratory stimulation prior to denervation but had no excitatory effect following sino-aortic denervation. One cervical sympathetic trunk ( CST) and one phrenic nerve were desheathed and submerged in paraffin oil in a pool made of the skin flaps. The electrical activity of single or multiunit strands of the CST as well as the electrical activity of the whole phrenic nerve were recorded using bipolar silver hook electrodes and Grass P511 preamplifiers. This neural activity was displayed on a storage oscilloscope. Sympathetic unit firing frequency was measured with an amplitude discriminator and ratemeter and displayed on a polygraph. Phrenic nerve discharge was half-wave rectified and integrated using a "leaky" RC circuit with a time constant 100ms. The integrated signal was also continuously displayed on the polygraph.

Inspired $O_2$ tension ($PO_2$), as well as inspired and expired $CO_2$ tension ($PCO_2$) levels, were monitored with a Beckman OM-15 and LB-2 gas analyzer, respectively. End-tidal $CO_2$ was taken as a measure of alveolar $CO_2$ tension ($P_ACO_2$). Arterial blood pressure was monitored from a cannula in the femoral artery using a Statham pressure transducer. Arterial $PO_2$ and $PCO_2$ ($PaO_2$ and $PaCO_2$, respectively) and pH were periodically determined in duplicate with a Radiometer PHM-72 MK-2 digital acid-base analyzer and a BMS-3 MK-2 blood microsystem. Inspired $O_2$, inspired/expired $CO_2$ , and arterial blood pressure were also displayed on the polygraph. Sympathetic activity, phrenic nerve activity, and arterial blood pressure signals were stored on magnetic tape.

$PaO_2$ was gradually decreased from a mean normoxic level of 95.0 ± 3.0 (±SEM) mmHg to a mean maximally hypoxic level of 20.1 ± 1.3 mmHg at a mean rate of 4.5 ± 0.6 mmHg/min. This was accomplished by gradually decreasing the proportion of inspired $O_2$ to $N_2$. A period of at least 20 min was allowed to elapse between experimental hypoxic runs. $PaO_2$ was determined from blood samples taken at the beginning, the end, and at one or two intermediate points during each hypoxic run. For plots such as those in Figs. 1-4, $PaO_2$ values that were not measured directly were estimated by interpolation with respect to time between the measured $PaO_2$ values. When $PaO_2$ was estimated as a function of $PAO_2$ from an equation determined by the least-squares linear regression method using simultaneously measured $PaO_2$ and $PAO_2$ values, analogous results were obtained.

Differences between means were tested for statistical significance with the Wilcoxon signed-rank test (Rumke & DeJonge, 1964). P values of less than 0.01 were considered to be significant. Means listed in the text are given with their accompanying standard error.

## RESULTS

The relation between $PaO_2$ and SPN firing rate was studied in 8 single-unit and 12 multiunit strands of the CST in the 14 sino-aortic denervated cats as $PaO_2$ was gradually lowered from normoxia to extreme hypoxia while maintaining $PaCO_2$ constant. The firing rate of the strands studied did not change with time under control conditions. In normoxia ($PaO_2$ 95.0 ± 3.0 mmHg), $PaCO_2$ and mean SAP were 37.9 ± 1.7 and 135 ± 9 mmHg, respectively.

Three types of SPN response to graded hypoxia were observed in the 11 CNS-intact animals. Eight of the sixteen strands in these animals showed an increase in firing

rate only, three exhibited a decrease in firing rate, and five displayed a mixed response that was characterized by an initial decrease in firing rate followed by an increase as $PaO_2$ was gradually lowered. All three response patterns were observed in both single-unit and multi-unit strands. A general property of all three types of responses was that they appeared only at very low $PaO_2$ values. A typical example of a strand showing the excitatory response is presented in Fig. 15A. The firing rate of this strand did not change appreciably as $PaO_2$ was decreased from 100 to 25 mmHg. At the latter value the firing rate began to increase, reaching a level six times the normoxic level at a $PaO_2$ of 20 mmHg. An example of a mixed response is displayed in Fig. 15B. The firing rate of this strand did not change as $PaO_2$ was lowered from 100 to about 40 mmHg. As $PaO_2$ fell below 40 mmHg, the firing rate of this strand decreased abruptly to approximately 90% of the control rate. As $PaO_2$ was decreased further the firing rate of this strand remained at this low level until a $PaO_2$ of about 30 mmHg was reached, at which point the firing rate began to increase, reaching a level 4.8 times the normoxic level at a $PaO_2$ of 23 mmHg. The increase in the strands showing these two types of response pattern had an average threshold of 27 ± 2 mmHg and reached an average peak value of 3.6 ± 0.4 times the normoxic firing rate. A constant feature of the hypoxic excitation of these strands was the steep increase in firing rate as $PaO_2$ was lowered past the threshold $PaO_2$. A typical example of the response pattern characterized by a decrease in SPN firing rate alone is shown in Fig. 15C. This strand showed no change in firing rate as $PaO_2$ was lowered from 100 to 40 mmHg. However, as $PaO_2$ was decreased to below 40 mmHg the firing rate fell abruptly by approximately 90% of the normoxic firing rate. The hypoxic decrease in firing frequency in the strands that showed this response, either alone or in combination with an increase in firing rate, had a threshold of 34 ± 5 mmHg $PaO_2$ and at the maximum decrease in firing rate represented, on average, an 83 ± 9% reduction of the normoxic firing rate. When preparations were reoxygenated following a period of

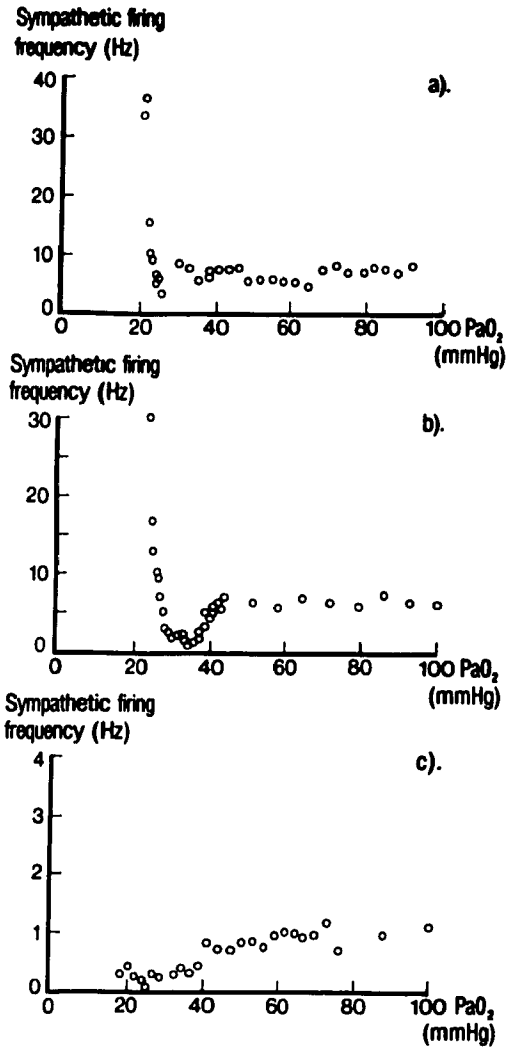


*Fig. 15.* Types of sympathetic preganglionic neuron responses to graded, isocapnic, systemic hypoxia recorded from strands of the cervical sympathetic trunk. In all panels vertical axis is mean firing rate of single nerve strands in spikes/s; horizontal axis is arterial $O_2$ tension ($PaO_2$) in mmHg. A: purely excitatory response; B: mixed response; C: purely depressant response.

graded hypoxia, SPN firing rates returned close to the control levels over a period of 1-3 min.

The excitatory and depressant responses to hypoxia had in common a sudden onset as a critical $PaO_2$ level was crossed. While the depressant response clearly reached a steady level in a number of cases, there was no evidence of saturation of the hypoxic excitation. However, the range of $PaO_2$ values that could be explored once the threshold $PaO_2$ for the excitatory effect was crossed was often limited by the onset of cardiac complications.

In all 11 CNS-intact cats studied, the response of SAP to graded systemic hypoxia was a decrease. The average response is shown in Fig. 16. Normoxic mean SAP was $138 \pm 11$ mmHg. There was significant change in mean SAP in the $PaO_2$ range from 100 to 60 mmHg. At 60 mmHg $PaO_2$ mean SAP began to decrease reaching a level of 45% of the normoxic pressure at the mean maximal hypoxic $PaO_2$ of 20 mmHg.

Systemic hypoxia was also accompanied by a depression of phrenic nerve activity in all the trials conducted. On average, there was little effect until $PaO_2$ decreased below 55 mmHg, at which value integrated phrenic nerve amplitude began to decrease with all phrenic nerve activity disappearing at a $PaO_2$ of 20 mmHg. Depression of respiratory activity by hypoxia is a well-known phenomena (Dumke et al., 1941), and similar results have been reported previously (e.g., Morrill et al., 1975). In those experiments in which a hypoxic depression of SPN firing rate was observed, the depression of phrenic nerve activity was seen to follow a similar course over the same range of $PaO_2$ values . This is shown in Fig. 17 in which both SPN firing frequency and integrated phrenic nerve amplitude, from a single experiment, are plotted against $PaO_2$.

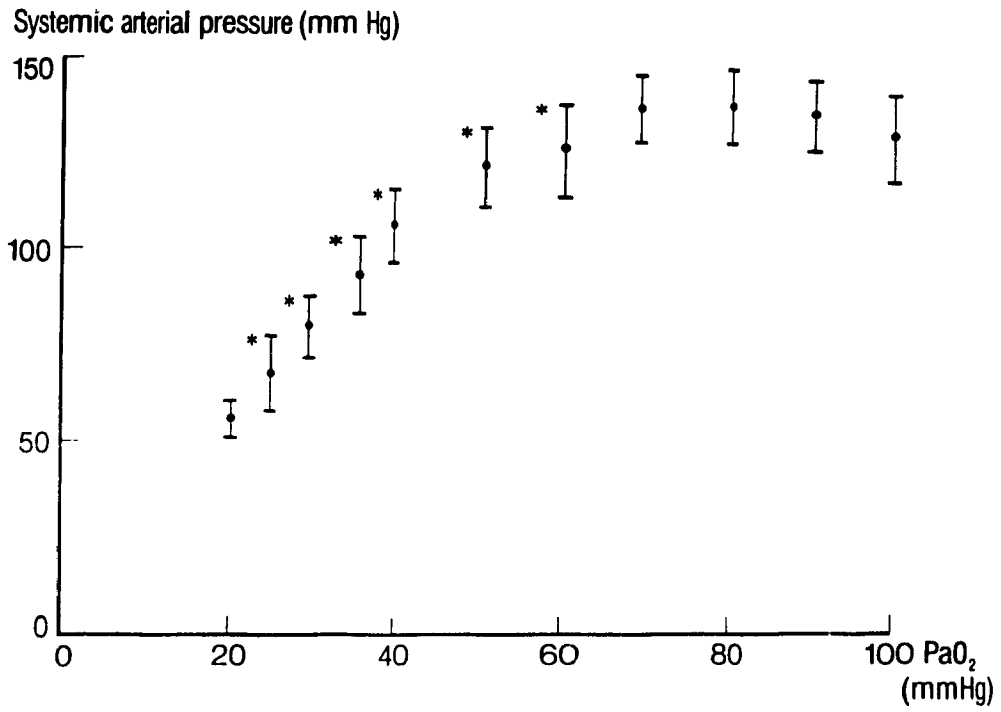


*Fig. 16.* Mean systemic arterial pressure response to graded, isocapnic, systemic hypoxia. Points are averages based on 16 trials in 11 sino-aortic-denervated, CNS-intact cats. Bars indicate ±SE. Asterisks indicate a significant difference ($P<0.01$) between a point and next point on right.

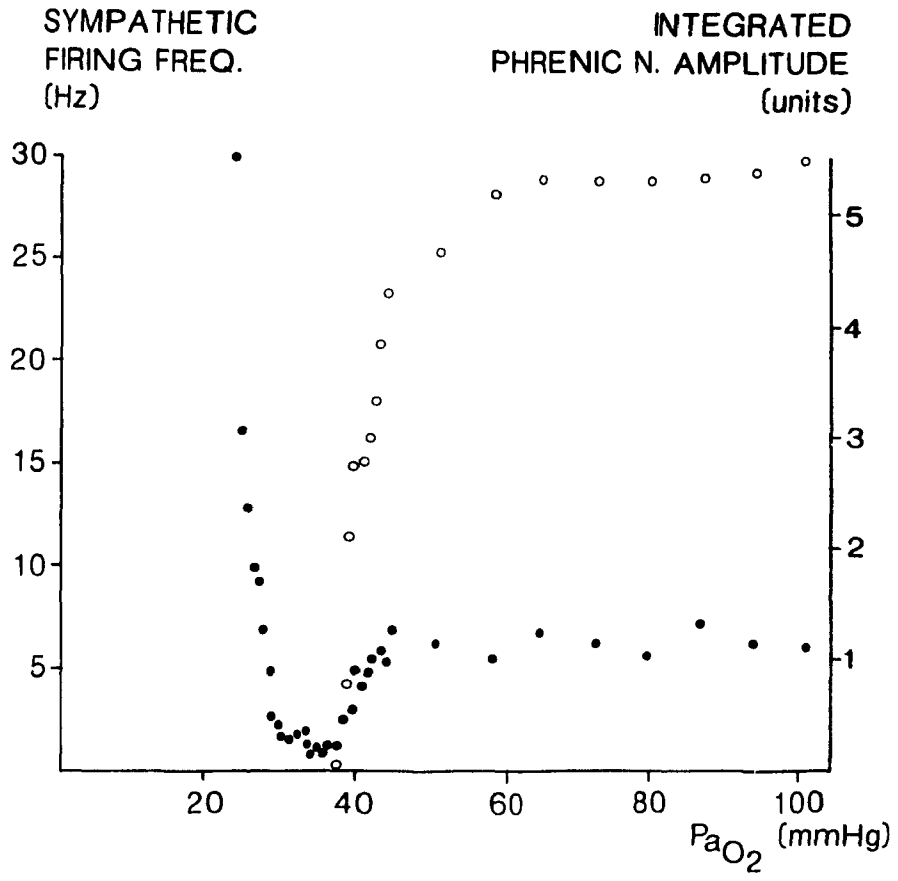


*Fig. 17.* Plot of peak amplitude of integrated phrenic neurogram and firing rate of a sympathetic strand simultaneously recorded in a sino-aortic denervated, CNS-intact cat during graded isocapnic hypoxia.

The response to hypoxia of SPN firing rate, mean SAP, and phrenic nerve activity observed in the three unanaesthetized decerebrate animals was qualitatively similar to those seen in the CNS-intact animals. Of the four strands studied in these preparations, two showed only an excitation, one a depressant response, and another a mixed response.

## DISCUSSION

This paper describes the responses of sympathetic preganglionic neurons (SPN) of the cervical sympathetic trunk (CST) to graded, systemic, isocapnic hypoxia in sino-aortic denervated, CNS-intact or midcollicular decerebrate cats. Three response patterns have been described, i.e., an excitatory response, a depressant response, and a combination of these two, consisting of a depression at less severe levels of hypoxia followed by excitation at more extreme levels. All three types of responses occurred at $PaO_2$ values lower than 40 mmHg. This suggests that, as in the acute spinal cat (Rohlicek & Polosa, 1981), nervous tissue $PO_2$ does not play a role in the generation of the tonic activity of these neurons in the sino-aortic denervated, CNS-intact or midcollicular decerebrate preparations when $PaO_2$ is within the physiological range. Although excitatory responses of sympathetic neurons to steady-state levels of systemic hypoxia had been previously observed in preparations lacking the arterial chemoreceptors (Gregor & Jänig, 1977; Iriki & Kozawa, 1975; Von Kehrel et al., 1962), to our knowledge it had not previously been demonstrated that systemic hypoxia causes depression of sympathetic activity in such preparations. The latter finding contrasts with the results of a previous study of acute spinal cats (Rohlicek & Polosa, 1981) in which only an increase in the firing frequency of CST SPNs was observed at low $PaO_2$ levels.

In agreement with previous results (Bernthal & Woodcock, 1951; Bouckaert et al., 1941; Gellhorn & Lambert, 1939) we found that SAP decreased with increasing degrees of hypoxia in SAD animals. The SAP response to hypoxia results from the interaction of peripheral and CNS- mediated effects (Chalmers et al., 1967). Because in the acute spinal animals studied previously (Rohlicek & Polosa, 1981) equally severe levels of hypoxia were associated with a neurogenic increase in SAP to levels greater than those observed at the same $PaO_2$ in the present experiments (Fig. 18), it must be concluded that in contrast to the sympathetic excitation elicited during hypoxia in the spinal animal the sympathetic excitation elicited in the sino-aortic-denervated, CNS-intact or midcollicular decerebrated preparation is insufficient to overcome the peripheral effects of $O_2$ lack (Skinner & Costin, 1969). The different SAP response to graded hypoxia of the present preparations (decrease) from that of the acute spinal preparation (increase) cannot be attributed to exclusion of the cephalic circulation in the latter preparation because SAP also went down in the decerebrate preparation in which the forebrain circulation was excluded. Neither can it be attributed to the influence of anaesthesia because SAP decreased in both unanaesthetized, decerebrate and the CNS-intact anaesthetized preparations. The discrepancy between the SAP response to hypoxia of the spinal animal and the sino-aortic-denervated, CNS-intact preparation is also evident in the literature (Bernthal & Woodcock, 1951; Bouckaert et al., 1941; Gellhorn & Lambert, 1939; Kaya & Starling, 1909; Mathison, 1910). Whereas hypoxia appears to have only an excitatory effect on sympathetic neurons in the spinal animal (Alexander, 1945; Rohlicek & Polosa, 1981), antagonistic excitatory and inhibitory processes have been suggested to be acting on sympathetic neurons in the sino-aortic denervated, CNS-intact preparation (Bernthal & Woodcock, 1951). It is possible that the depressant response to hypoxia , observed by ourselves in some SPNs, is a manifestation of such an inhibitory

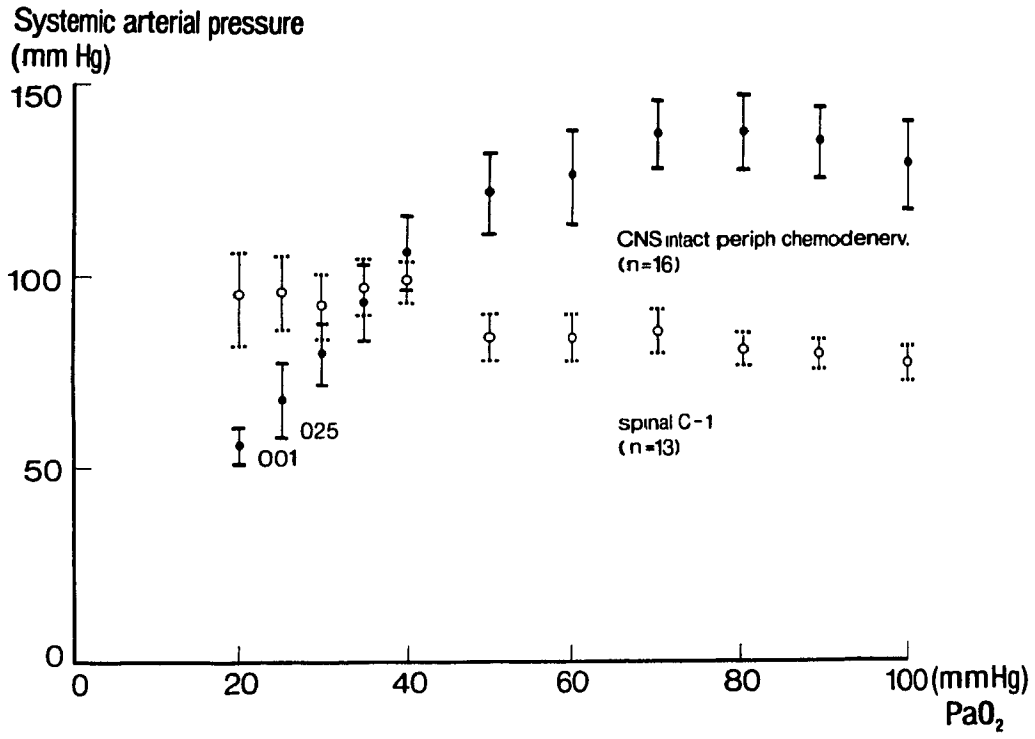


*Fig. 18.* Mean systemic arterial pressure response to graded, isocapnic systemic hypoxia in acute spinal cats (Rohlicek & Polosa, 1981) and CNS-intact, sino-aortic denervated cats. Points are averages based on 13 and 16 trials, respectively. Bars indicate ±SE. At arterial $O_2$ tension ($PaO_2$) levels of 20 and 25 mmHg mean systemic arterial pressure in the acute spinal animals was significantly greater [$P<0.001$ and $P<0.025$, respectively; Wilcoxon two-sample test] than in the CNS-intact, sino-aortic denervated preparations.

effect and forms a significant component of the response of vasomotor sympathetic neurons to hypoxia in the sino-aortic-denervated, CNS-intact animal that is not present in the spinal animal.

In view of the lack of evidence that systemic hypoxia evokes reflex effects on sympathetic neurons in the absence of arterial chemoreceptors (Korner, 1971) and in view of the extensive evidence for direct effects on anoxia on CNS neurons (Krnjevic, 1975), these responses may be attributed to a direct effect of hypoxia on the SPN and/or on antecedent neurons. The SPN excitation observed in the acute spinal cat in a previous study was attributed to a depolarization of the SPN membrane or of that of antecedent excitatory neurons, presumably as a result of decreased activity of the sodium pump consequent to oxygen lack (Rohlicek & Polosa, 1981). The similar shape of the $PaO_2$-SPN firing frequency relation of the excitatory responses observed in the present study to those observed in the spinal preparations suggests either that the former are due largely to the spinal effect described previously or that similar excitatory phenomena, caused by an analogous mechanism, are initiated supraspinally in neurons antecedent and excitatory to the SPN. The hypoxic depression of SPN firing observed in the present study, in both the CNS-intact and the midcollicular-decerebrate preparations, had not been observed in the acute spinal preparation (Rohlicek & Polosa, 1981). Therefore, this response appears to be generated in the brain stem. Excitation, resulting from decreased activity of the sodium pump, of a sympatho-inhibitory pathway originating in the brain stem could be the mechanism of these depressant responses. Hypoxic activation of this hypothetical inhibitory system could antagonize, at the membrane of the SPN or of antecedent, excitatory interneurons, the concurrent depolarizing effect of the passive inward $Na^+$ current resulting from the hypoxic depression of the sodium pump by virtue of the increase in $K^+$ or $Cl^-$ conductance that is likely to be associated with the inhibition. It is possible that in some SPNs this hypothetical inhibition of brain stem origin

may be overcome by the spinally and supraspinally generated excitation, whereas in others it may not, and in still others it may be overcome only at certain $PaO_2$ values. Thus the three response patterns observed would result. In addition, interaction of inhibitory and excitatory effects of systemic hypoxia, at the level of the SPN or of antecedent excitatory neurons might result in reduced sympathetic output to the cardiovascular system in comparison with the spinal animal under certain situations. This is in fact suggested by the present results. As mentioned above, the possibility that hypoxia activates antagonistic excitatory and inhibitory processes acting on sympathetic neurons in CNS-intact animals lacking arterial chemoreceptor afferents has been previously suggested by Bernthal and Woodcock (1951).

A further point of interest, in connection with the hypoxic depression, is the apparent similarity in time course between the hypoxic depression of SPN firing and that of phrenic motoneuron burst firing. The latter is the neurophysiological event underlying the central respiratory depressant action of hypoxia and has been described previously (e.g., Morrill et al., 1975). This observation suggests the possibility of a common mechanism for the generation of the two phenomena and adds to the growing list of analogies in behaviour that have been shown to exist between SPNs and phrenic motoneurons (Polosa et al., 1980).

# NEURAL EFFECTS OF SYSTEMIC HYPOXIA ON HINDLIMB VASCULAR RESISTANCE IN SINO-AORTIC DENERVATED CATS

C.V. Rohlicek, T. Hakim, and C. Polosa

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## ABSTRACT

The effects of systemic hypoxia on the neurogenic component of hindlimb vascular resistance were studied in six sino-aortic denervated, anesthetized cats with intact central nervous system. Hindlimb perfusion pressure (PP) was measured under conditions of constant flow of normoxic blood from a donor cat. The neural component of the PP was estimated from the change in PP upon administration of hexamethonium (10 mg/kg i.v.). Ventilation of the recipient cat with 12.5% and 10% $O_2$ was associated with an average 35 ± 15% (SEM) and 13 ±10% decrease, respectively, of the estimated neural component of the PP. In contrast ventilation with 7.5% and 5% $O_2$ produced increases of 27 ± 7% and 58 ± 10%, respectively, of the estimated neural component of the PP. Both the vasodilator and vasoconstrictor responses were abolished by hexamethonium (10 mg/kg i.v.). The vasodilator response appeared to be due to withdrawal of α-adrenergic tone since it was eliminated by constant infusion of phentolamine to the hindlimb (45 μg/ml blood/min). We conclude that, in addition to the already known sympatho-excitation seen in the sino-aortic denervated cat during severe systemic hypoxia, there is also a sympath-depressant effect which dominates at more moderate levels of systemic hypoxia.

## INTRODUCTION

Some components of the cardiovascular response to systemic hypoxia appear to be mediated by neural mechanisms independent of the arterial chemoreceptors. For instance, increases in heart rate and ventricular contractility (Achtel & Downing, 1972; de Burgh-Daly & Scott,1964; Koehler et al., 1980; Kontos & Lower, 1969; Krasney & Koehler, 1977), which could be eliminated by β-adrenergic blockade (Kontos & Lower, 1969), have been observed during systemic hypoxia in sino-aortic denervated (SAD) animals. In addition, increases in sympathetic activity to various effector organs during systemic hypoxia have also been reported in such preparations (Gregor & Jänig,1977; Iriki & Kozawa, 1975; von Kehrel et al., 1962). These effects have been interpreted to be the manifestation of purely excitatory actions of systemic hypoxia upon sympathetic structures in the central nervous system (CNS). However, there is evidence that the central actions of hypoxia upon sympathetic structures are more complex and include a sympatho-depressant component mediated supraspinally. For example, in a recent study on the effect of systemic hypoxia on the activity of sympathetic preganglionic neurons (SPNs) of the cervical sympathetic trunk in CNS intact or mid-collicular decerebrate SAD cats we found that while many increased their firing rate during extreme systemic hypoxia many of these SPNs also exhibited a decrease in firing rate at less extreme hypoxic levels (Rohlicek & Polosa, 1983). By contrast, in the acute $C_1$ spinal preparation all SPN studied were only excited by severe systemic hypoxia (Rohlicek & Polosa, 1981). It is not clear, however, whether or not the observed depression influenced sympathetic neurons innervating cardiovascular effectors. Some data in the literature suggest that this is the case. During systemic hypoxia systemic arterial pressure (SAP) falls in anesthetized, CNS-intact, SAD preparations (Bernthal & Woodcock, 1951; Bouckaert et al., 1941; Gellhorn & Lambert, 1939; Rohlicek & Polosa, 1983) whereas it increases in acute high

spinal preparations (Kaya & Starling, 1909; Mathison 1910; Rohlicek & Polosa, 1981). However, the fact that these data were obtained in preparations in which hypoxia acted not only on the CNS but also on the vascular smooth muscle and on the myocardium, both of which are known to be very sensitive to $O_2$ lack (Katz, 1977; Somlyo & Solmlyo, 1970), justifies some reservation in accepting this data as conclusive evidence of sympatho-depression.

The aim of the present study was to establish whether the hypoxic sympatho-depression observed in SAD cats (Rohlicek & Polosa, 1983) effects sympathetic neurons innervating vascular smooth muscle. Therefore we studied the effect of various levels of systemic hypoxia, in the SAD cat, on the vascular resistance of the hindlimb perfused with normoxic blood. If our findings with the SPNs of the cervical sympathetic trunk apply also to the SPNs controlling vascular smooth muscle tone of the hindlimb, then a decrease in vascular resistance should be observed at some levels of systemic hypoxia.

The results of this work show that during systemic hypoxia in SAD cats, in addition to a vasoconstrictor effect observed at very low $PaO_2$ values, a vasodilator effect also occurs at less severe hypoxic levels. Both response patterns are abolished by ganglionic blockade. The vasodilator effect of systemic hypoxia seems to be due to a decrease in sympathetic vasoconstrictor activity since it is abolished by $\alpha$-adrenergic block.

## METHODS

Experiments were conducted on 6 pairs of adult cats of either sex anesthetized with sodium pentobarbital (35 mg/kg i.p. followed by supplemental doses of 12 mg i.v. as required). All the cats were paralyzed (pancuronium bromide 0.5 mg/kg i.v.) and ventilated with positive pressure to maintain end-tidal $PCO_2$ constant at between 25 and 35 mm Hg. Core temperature was maintained constant at between 36 and 38°C by means of infrared heat lamps.

Each experiment required 2 cats. One cat (the recipient) was sino-aortic denervated (SAD) by bilateral section of the carotid sinus, aortic, and vagus nerves. Both cats were give 500 - 700 units/kg of heparin i.v. After laparotomy, one external iliac artery of the recipient cat was peripherally cannulated and the abdominal aorta ligated just rostral to the bifurcation. Using a dual head Masterflex roller type pump and Tygon tubing (I.D. 1.65 mm, O.D. 4.93 mm, volume 1.5 ml) blood was pumped at a constant flow (5 - 18 ml/min) from a common carotid artery of a second cat (the donor) into the peripherally cannulated external iliac artery of the recipient cat and returned to the donor cat by pumping blood at the same rate from a common carotid artery of the recipient into a jugular vein of the donor cat. The absence of shifts in blood volume between the two cats was confirmed by continuously monitoring the body weight of the donor cat with an accuracy of 10 g. The flow of the blood into the external iliac artery was adjusted so that the perfusion pressure (PP) of the hindlimb was between 150 and 200 mmHg. The pressure drop across the perfusion line to the external iliac artery was always less than 5 mm Hg with flows from 1 to 120 ml/min. The venous return from the perfused hindlimb occurred through natural channels. 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 PP fell to an average value of 28 ± 6 mm Hg (mean ±SEM) prior to administration of hexamethonium (10 mg/ kg i.v.) and to a level of less than 10 mm Hg after ganglionic blockade. The absence of significant collateral circulation was also tested by increasing or decreasing SAP of the recipient by administration of noradrenaline i.v. or by acute hemorrhage. No changes in PP were observed for at least 20 s following changes in the recipient's SAP as large as 125 mm Hg.

Systemic arterial pressure (SAP) of both donor and recipient cats was continuously measured through catheters advanced from the femoral artery into the abdominal aorta. Perfusion pressure of the hindlimb was measured less than 5 cm from

the entry of the cannula into the external iliac artery used to perfuse the hindlimb. Central venous pressure of the recipient cat was monitored through a catheter advanced approximately 15 cm from the femoral vein into the vena cava. All pressure measurements were made using Statham pressure transducers and were continuously displayed on a Grass model 7 polygraph. Inspired/expired $CO_2$ and $O_2$ of the recipient cat were continuously monitored using Beckman LB-2 and OM-11 or OM-15 gas analyzers. These values were also continuously displayed on the polygraph.

With constant flow perfusion vasoconstriction and vasodilation were indicated by increases or decreases in PP since central venous pressure did not change during these experiments. The neurogenic component of the perfusion pressure was estimated from the change in PP upon administration of hexamethonium (10 mg/kg i.v.). The fraction of the PP eliminated by hexamethonium in the cats studied was 53 ± 3% (mean ± SEM). Systemic hypoxia of the recipient was produced by ventilation with gas mixtures containing 12.5%, 10%, 7.5% and 5% $O_2$ in $N_2$ while the donor cat continued to be ventilated with room air and the perfused hindlimb of the recipient thus continued to receive normoxic blood. Each hypoxic gas mixture was administered to the recipient cat for a period of 4 - 5 min and an interval of at least 10 min allowed to elapse before another trial was performed. Two trials were performed on each cat with each gas mixture and the results averaged. Typical end-tidal $PO_2$ values under control conditions and during ventilation with these mixtures were 115, 58, 45, 35 and 30 mm Hg respectively. Differences between sets of results were tested for significance using the Mann-Whitney U-test (Siegel, 1956).

**Results**

Ventilation of the recipient animals with hypoxic gas mixtures (5%, 7.5%, 10%, and 12.5% $O_2$ in $N_2$), while their hindlimbs were perfused at constant flow rates with

normoxic blood, was associated in all cases (32 trials in 6 cats) with either an increase or a decrease in PP. Both responses were abolished by ganglionic blockade with hexamethonium (10mg/kg i.v.). Repeated administration of a particular gas mixture in any one preparation always produced the same effects. Ventilation of the recipient cats with 5% $O_2$ and 7.5% $O_2$ in $N_2$ caused increases in hindlimb PP in all the preparations (4 cats tested with both gas mixtures). In contrast when the recipient cats were ventilated with 10% $O_2$ and 12.5% $O_2$ in $N_2$ PP decreased in most of the animals (3 of 4 and 4 of 4 cats respectively). The latter responses were significantly different ($P < 0.001$) from those obtained by ventilation with 5% $O_2$ and 7.5% $O_2$ in $N_2$. Ventilation of the recipient animals with 5% $O_2$ and 7.5% $O_2$ led respectively to a 58 ± 10% (mean ± SEM) and 27 ± 7% peak increase in the neurogenic component of the PP while ventilation with 10% $O_2$ and 12.5% $O_2$ led to decreases of 13 ± 10% and 35 ± 15%. Mean SAP levels fell from control values of 139 ± 13 mm Hg to minimum values of 112 ± 10, 107 ±10, 99 ±10 and 87 ± 6 mm Hg respectively during ventilation with 12.5%, 10%, 7.5% and 5% $O_2$ respectively in these experiments.

An example of vasoconstrictor response is shown in Fig. 19. PP increased from 200 to 265 mm Hg within 1 min from the onset of ventilation of the recipient cat with 5% $O_2$ and remained elevated throughout the period of hypoxemia. Upon reoxygenation PP fell to 95 mm Hg over a period of about 30 s and subsequently returned toward the control PP over the ensuing 2 min.

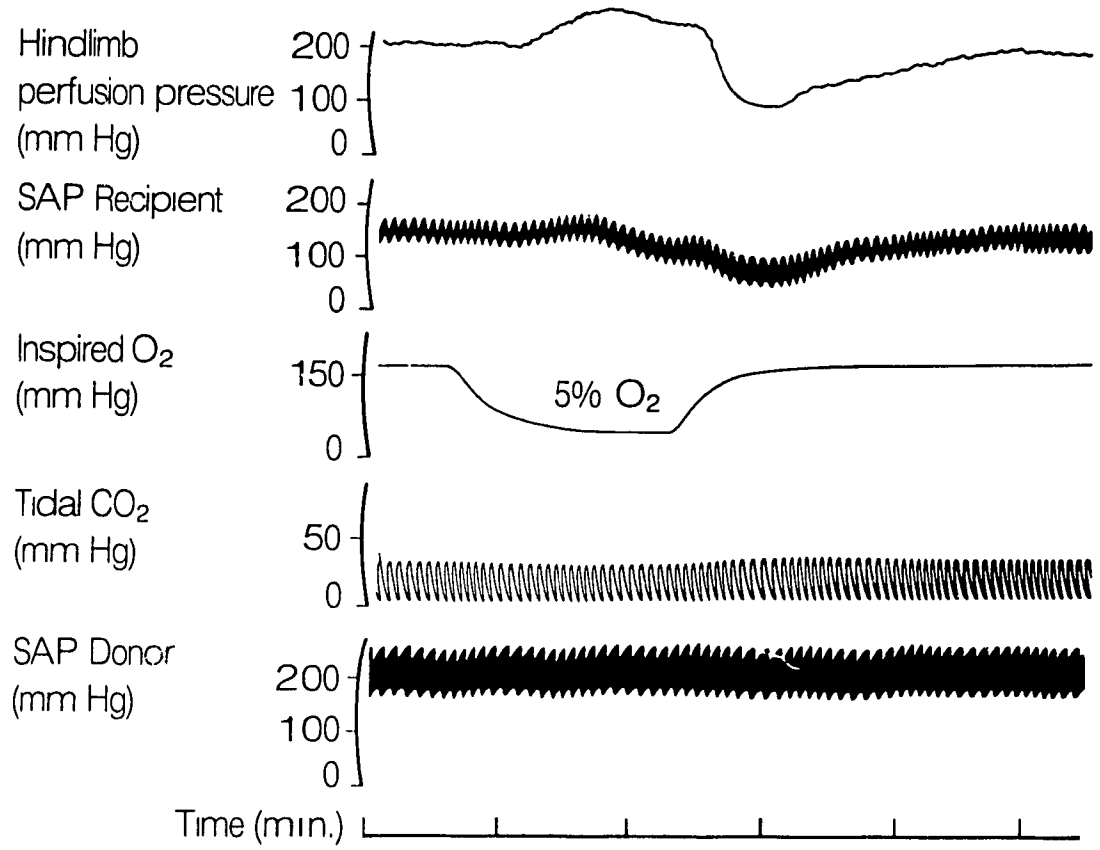


*Fig. 19.* **Hindlimb perfusion pressure (PP) changes during ventilation of a sino-aortic denervated cat with 5% $O_2$ in $N_2$. From top to bottom: recipient's hindlimb PP in mm Hg, recipient's systemic arterial pressure (SAP) in mm Hg, recipient's inspired $PO_2$ in mm Hg, recipient's tidal $PCO_2$ in mm Hg, donor's SAP in mm Hg.**

As in the experiment illustrated in Fig. 19 a transient vasodilation was seen following all the cases of hypoxic vasoconstriction. The peak magnitude of post hypoxic vasodilation ranged from a decrease of 24% to a decrease of 56% of the control PP level and appeared to be related to the amount of vasoconstriction during the period of hypoxemia (see Fig. 20). The time course of the post-hypoxic vasodilations ranged from 40 s to 120 s and appeared to be directly related to the magnitude of the dilation. Such post-hypoxic vasodilations were not seen following elimination of the hypoxic vasoconstrictor response by ganglionic block.

An example of a vasodilator response to systemic hypoxia is shown in Fig. 21. PP fell from 215 to 155 mm Hg upon ventilation with 12.5% $O_2$ and remained at this depressed level during the period of hypoxemia. Upon re-oxygenation of the recipient PP returned to the control level. The vasodilator response appeared to be due to a withdrawal of $\alpha$-adrenergic tone since it was abolished by a continuous infusion of the $\alpha$-adrenergic blocker phentolamine (45 µg/ml blood infused to the hindlimb/min). Vasodilation was still possible at this time since the $\beta$-adrenergic agonist isoproterenol (0.02 µg to the hindlimb) was still capable of eliciting a substantial vasodilation. The fraction of the PP eliminated by administration of phentolamine to the hindlimb was approximately the same as following the administration of the ganglionic blocker hexamethonium intravenously (see Methods) . The vasodilator response was unaffected by administration of the muscarinic antagonist atropine (0.5 mg/kg i.v.).

In 2 trials in 2 cats in which administration of 10% $O_2$ had induced vasodilation a further decrease in PP was observed upon return to ventilation with room air. In both cases the post-hypoxic vasodilations were of similar peak magnitude (decrease of 31 % and 19% of the control PP level) and duration (45 s and 40 s) as the post hypoxic vasodilations following hypoxic vasoconstriction described above.

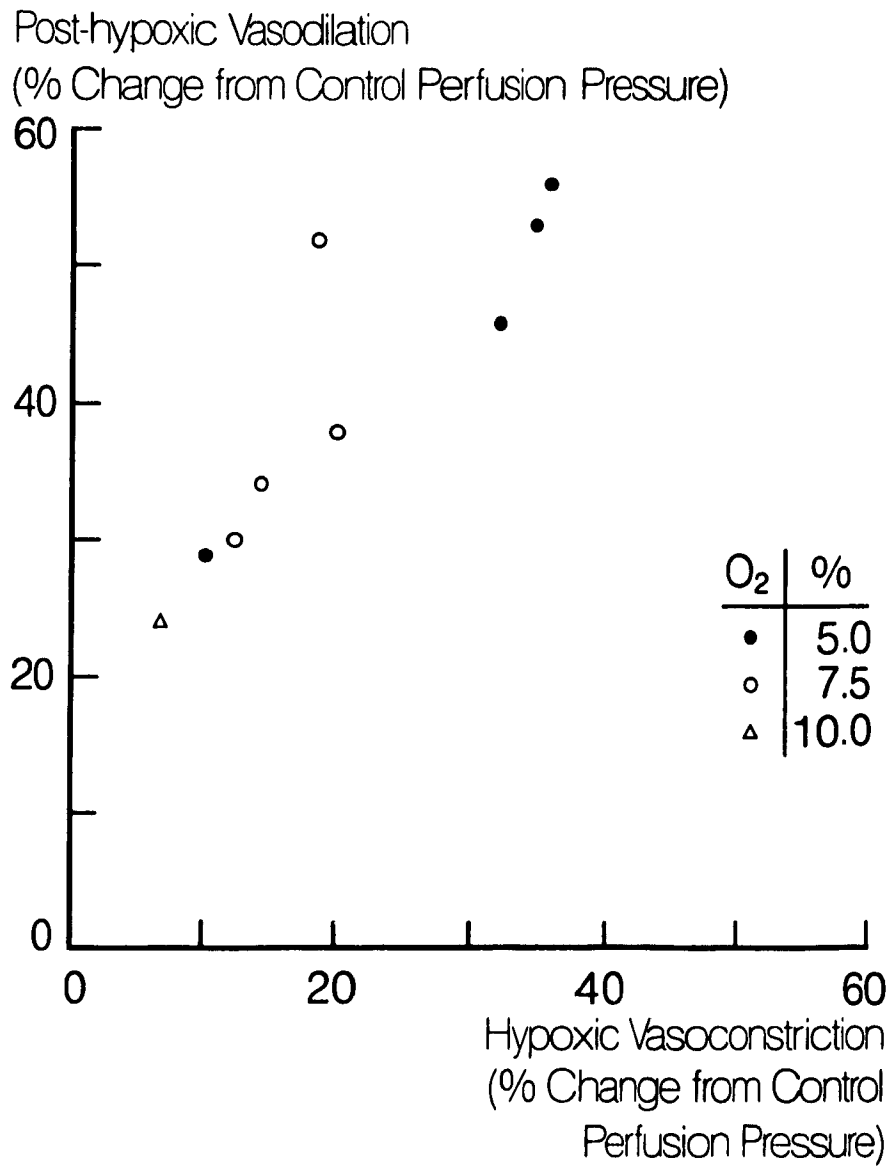


*Fig. 20.* **Average post-hypoxic vasodilation [percent change from control perfusion pressure (PP)] versus hypoxic vasoconstriction (percent change from control PP) in cats showing a vasoconstrictor response to ventilation with 5% $O_2$ ( • ), 7.5% $O_2$ ( o ), and 10% $O_2$ ( Δ )**

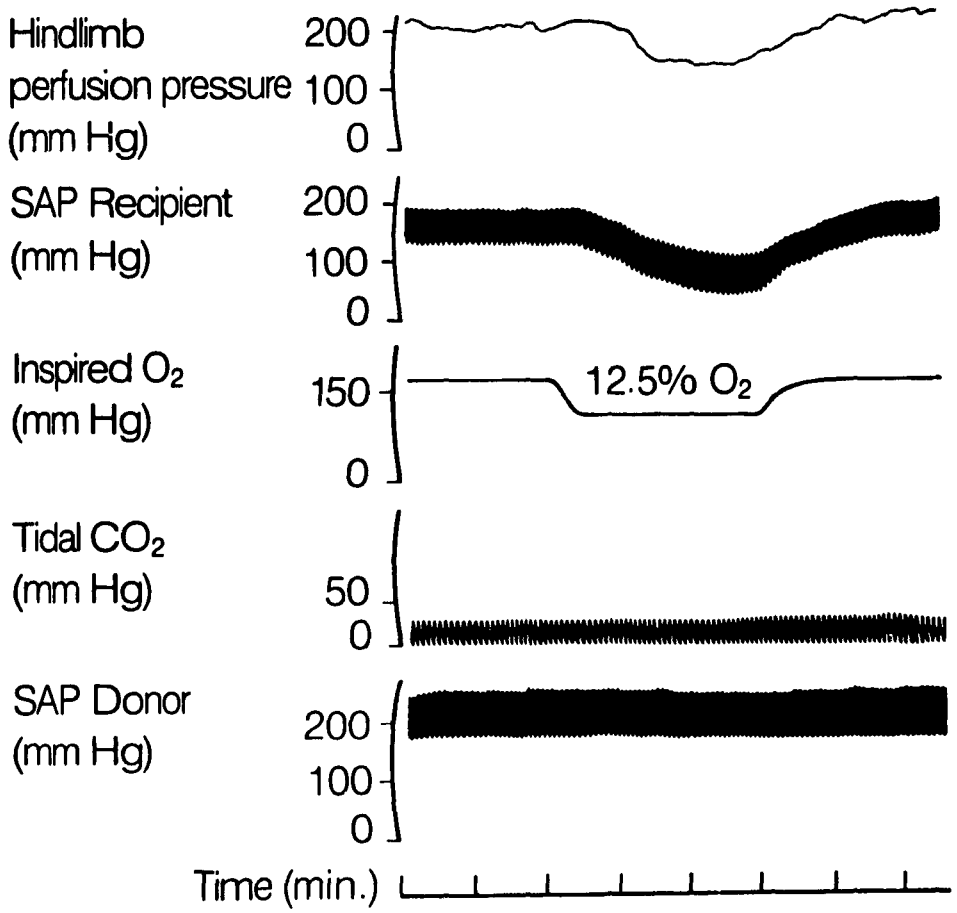


*Fig. 21.* **Hindlimb perfusion pressure (PP) changes during ventilation of a sino-aortic denervated cat with 12.5% $O_2$ in $N_2$. From top to bottom: recipient's hindlimb PP in mm Hg, recipient's systemic arterial pressure (SAP) in mm Hg, recipient's inspired $PO_2$ in mm Hg, recipient's tidal $PCO_2$ in mm Hg, donor's SAP in mm Hg**

## DISCUSSION

This investigation has shown that systemic hypoxia in the sino-aortic denervated (SAD) preparation causes vasoconstriction or vasodilation of the hindlimb vasculature perfused at a constant flow with normoxic blood. The vasoconstrictor response was dominant at the more severe levels of hypoxemia (5% and 7.5% inspired $O_2$) while the vasodilator response was dominant at the less severe levels of hypoxemia (10% and 12.5% inspired $O_2$). The latter response has not been previously described in SAD preparation. Both of these vascular responses could be eliminated by ganglionic blockade.

It seems unlikely that the observed PP responses were the result of variations in collateral blood flow from the recipient, resulting from the hypoxic decreases in the recipients' SAP, since SAP changes of comparable magnitude under control conditions had no immediate effect on hindlimb PP (see METHODS). Also unlikely is that the observed PP changes were caused by an effect on the hindlimb vascular smooth muscle of hypoxic arterial blood (Somlyo & Solmlyo, 1970), supplied to the hindlimb by such collateral channels, since the PP responses disappeared after ganglionic or $\alpha$-adrenergic block. Central venous pressure in the recipient animals did not change during systemic hypoxia and therefore did not contribute to the PP changes. Finally, it seems unlikely that the observed PP changes were due to vasoactive humoral substances released by the recipient during systemic hypoxia since the return of hypoxic blood from the recipient to the donor was without effect on the SAP of the latter animal. On the basis of these considerations and of the observation that both the vasoconstrictor and vasodilator effects were eliminated by the ganglionic blockade it would appear that the observed PP changes during systemic hypoxia were neurogenic. While the neurogenic vasoconstrictor effect of systemic hypoxia indicated an increase in activity of adrenergic

vasoconstrictor fibres innervating the hindlimb vasculature the neurogenic vasodilator response seemed to be due to a withdrawal of sympathetic vasoconstrictor tone since the response was eliminated by α-adrenergic block with phentolamine. The observation that large decreases, or increases, in the recipient animal's SAP had no significant effect on hindlimb PP (see Methods) suggests that the observed neurogenic changes in hindlimb PP were not initiated reflexly by the decrease in SAP occurring in the hypoxic animal.

In the absence of evidence for peripheral $PO_2$-sensitive chemoreceptive structures with reflex actions on sympathetic neurons outside the sino-aortic regions and of evidence that systemic hypoxia excites other peripheral sensory systems with reflex action on sympathetic neurons, it may be assumed that the vasomotor responses observed in the present study were generated in the CNS as a result of changes in nervous tissue $PO_2$. It seems likely that the central mechanisms underlying these vascular responses are the excitation and inhibition of sympathetic preganglionic neuron activity which have been previously described in the CNS-intact SAD cat during severe and more moderate systemic hypoxia respectively (Rohlicek & Polosa, 1983). Since neurogenic changes in hindlimb vascular resistance must represent changes in activity of large populations of sympathetic neurons our present results indicate that during systemic hypoxia in the SAD, CNS intact, preparation such phenomena influence a significant number of sympathetic neurons innervating vascular smooth muscle. To our knowledge this is the first demonstration of a decrease in the tonic activity of a large population of sympathetic neurons during systemic hypoxia in a preparation lacking arterial chemoreceptor afferents.

In the animal with intact sino-aortic chemoreceptor innervation it is likely that during systemic hypoxia the central hypoxic effects on sympathetic activity described here would interact with those initiated by the arterial chemoreceptors. Thus it is conceivable that at certain $PaO_2$ values the central depressant effect observed here

would attenuate the chemoreflex influence on the sympathetic neurons. This could be the mechanism underlying the failure of the compensatory sympathetic vasoconstrictor response to hemorrhagic shock observed by various authors (Corazza et al., 1963; Gootman & Cohen, 1970; Lundgren et al., 1964; Rothe et al., 1963). In these cases CNS $PO_2$ might have fallen because of the reduced cerebral blood flow accompanying the hemorrhagic shock into the range in which the central sympatho-depressant effect is dominant.

An incidental observation of some interest is that the vasoconstrictor responses to systemic hypoxia (i.e. Fig. 19) were invariably followed by a transient but marked decrease in vascular resistance below the control level when the preparations were re-oxygenated. The most marked "off" responses were associated with the most marked vasoconstrictor responses (Fig. 20). Similar responses of the hindlimb vasculature have been reported following vasoconstriction elicited by electrical stimulation of sympathetic axons in the upper lumbar ventral roots (Lioy & Polosa, 1971) or in the lumbar sympathetic chain (Khayutin, 1964). These post-constriction dilations have been attributed to mechanisms operating within the ganglia (Lioy & Polosa, 1971) or at the effector cell level (Khayutin, 1964). A post-hypoxic silence of sympathetic preganglionic neurons which might contribute to the observed post-constriction dilation has also been observed (Rohlicek & Polosa, 1981).

# OBSERVATIONS ON THE HYPOXIC DEPRESSION OF SYMPATHETIC DISCHARGE IN SINO-AORTIC DENERVATED CATS

Charles V. Rohlicek and Canio Polosa

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## ABSTRACT

The effect of graded isocapnic hypoxia on the mass activity of the cervical sympathetic trunk was studied in sino-aortic denervated (SAD) barbiturate-anaesthetized cats. Under control conditions (normoxia, normocapnia) sympathetic discharge showed i) a burst of action potentials synchronous with the phrenic nerve burst, which was selectively abolished by procedures suppressing inspiratory neuron activity (inspiration synchronous sympathetic activity, ISSA); and ii) a lower level of sympathetic activity during expiration (tonic sympathetic activity, TSA). The effects of graded hypoxia on these two components of sympathetic discharge were different. ISSA showed depression only which began at an inspired $PO_2$ ($P_{insp}O_2$) of 58 ± 10 mmHg (SEM), became progressively more marked as $P_{insp}O_2$ decreased further, and was paralleled by depression of phrenic nerve activity. Both ISSA and phrenic nerve activity were suppressed at $P_{insp}O_2$ of 46 ± 9 mmHg. TSA increased progressively with the lowering of $P_{insp}O_2$, beginning at a $P_{insp}O_2$ significantly lower than that at which ISSA depression began (50 ± 13 mmHg, $P < .01$). In the range of $P_{insp}O_2$ values intermediate between the thresholds for ISSA depression and for TSA increase, some animals showed a depression of TSA that reversed to an increase as $P_{insp}O_2$ decreased further. During brief (1.5 ± 0.2 min) episodes of cerebral ischemia produced by occlusion of the brachiocephalic and left subclavian arteries the two components of sympathetic discharge showed responses similar to those observed in hypoxia, namely depression of ISSA as

well as depression and enhancement of TSA. These findings show that CNS hypoxia influences sympathetic activity be several mechanisms and that all components of the response can be generated supraspinally. The hypoxic depression of ISSA is presumably due to withdrawal of facilitatory input to sympathetic preganglionic neurons from brainstem inspiratory neurons.

## INTRODUCTION

In the sino-aortic denervated (SAD) animal with intact CNS, systemic hypoxia can cause a neurogenic increase in cardiac output, ventricular contractility and heart rate (Korner, 1959, 1971; Krasney & Koehler, 1980) as well as in the firing rate of sympathetic postganglionic neurons innervating myocardium, skeletal muscle, kidney, abdominal viscera and skin (Gregor & Jänig, 1977; Iriki & Kozawa, 1975; von Kehrel et al., 1962). However, we have previously shown (Rohlicek et al., 1984; Rohlicek & Polosa, 1983a) that during graded isocapnic hypoxia in SAD animals sympathetic preganglionic neuron (SPN) firing as well as the neurogenic component of hindlimb vascular resistance decrease at moderate levels of hypoxia ($PaO_2$ in the range of 60 to 35 mmHg) and increase only at more severe levels of hypoxia ($PaO_2$ in the range of 35 to 20 mmHg). The finding that the hypoxic sympatho-depression occurs in the range of $PaO_2$ values in which depression of phrenic nerve activity also occurs (Rohlicek & Polosa, 1983a) suggests the possibility of a relation between these two phenomena. Since hypoxia has a depressant effect on the activity of brainstem inspiratory neurons (van Beek et al., 1984) and these neurons provide a considerable fraction of the input generating the background discharge of SPNs (Bachoo & Polosa, 1985), the hypoxic depression of sympathetic activity may be tentatively explained by the withdrawal of this input. However,

from the data available it is not possible to eliminate the possibility of depression by hypoxia of the SPNs themselves or of synaptic input to the SPNs unrelated to respiration. The purpose of the present investigation was to further analyze the properties of the hypoxic depression of sympathetic activity. Since it is possible to distinguish in the discharge of a sympathetic nerve a component which is inspiration-related and another which is not (Bachoo & Polosa, 1985), it was of interest to determine whether or not both components were affected by hypoxia, what was the temporal relation between hypoxic changes in sympathetic and phrenic nerve activity, and whether or not there was depression of sympathetic activity when the inspiration-related activity was absent, e.g., in hypocapnia. To determine whether or not the hypoxic depression of sympathetic activity is due to a supraspinal mechanism, we examined the response of sympathetic discharge to cerebral ischemia. Since it is known that in the SAD cat, systemic hypercapnia/acidosis has an excitatory effect mediated by the ventrolateral medulla on sympathetic neurons (Hanna et al., 1981), any depression of sympathetic activity that might be observed during cerebral ischemia would presumably be the result of brain hypoxia.

## METHODS

Experiments were conducted on adult cats of either sex (3 - 4.5 kg) under pentobarbital anaesthesia (35 mg/kg i.p. followed by a maintenance dose of 9 mg/kg i.v. every 3 hours). The animals were paralyzed with pancuronium bromide (initial dose 0.25 mg/kg i.v. followed by maintenance doses of 0.10 mg/kg i.v. 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 the level of anaesthesia) and ventilated with positive pressure to maintain end-tidal $PCO_2$ constant between 30 and 40

mmHg. Core temperature was maintained constant between 36 and 38°C by means of infrared heat lamps.

Arterial chemoreceptors and baroreceptors were denervated by bilateral section of the carotid sinus, aortic and vagus nerves. Effective denervation of the arterial chemoreceptors was demonstrated by the loss, after nerve section, of the increase in phrenic nerve activity caused by systemic hypoxia (ventilation with 10% $O_2$ in $N_2$) or by i.v. injection of a bolus of KCN (50 µg/kg Dumke et al., 1941). A cervical sympathetic trunk and phrenic nerve were desheathed and kept submerged in paraffin oil in a pool made of the skin flaps. The electrical activity of both nerves was recorded monophasically with silver hook electrodes, amplified (Grass P511 pre-amplifier, 1/2 amplitude bandpass 30-3,000 Hz) and displayed on a storage oscilloscope. The amplified signals were also integrated, after half-wave rectification, using a RC circuit with a 100 ms time constant and displayed on a Grass model 7 polygraph. Sympathetic discharge was characterized, in control conditions, by two components; a burst of action potentials, synchronous with the phrenic nerve burst, and a lower level of discharge during expiration (Bachoo & Polosa, 1985). These two components are referred to in this paper as the inspiration synchronous component of sympathetic activity (ISSA) and the tonic component of sympathetic activity (TSA), respectively. The ISSA can be selectively eliminated by hypocapnia or superior laryngeal nerve stimulation (Bachoo & Polosa, 1985). Both components shows fluctuations at frequencies faster than the respiratory frequency (Polosa, 1984). The TSA and ISSA can be separated by procedures which selectively block the ISSA (Bachoo & Polosa, 1985). The zero level of activity in the cervical sympathetic trunk was determined by cutting the nerve proximal to the recording electrodes.

Systemic arterial blood pressure was monitored from a cannula in the femoral artery using a Statham pressure transducer. End-tidal $PCO_2$ and inspired $PO_2$ were monitored with a Beckman LB-2 and OM-15 gas analyzer respectively. These variables were continuously displayed on the polygraph.

Systemic hypoxia was produced by gradually decreasing inspired $PO_2$ ($P_{insp}O_2$) from normoxia (160 mmHg) to a maximally hypoxic level of 26 ± 3 (mean ± SE) mmHg at a mean rate of 19 ± 1 mmHg/min. This was accomplished by gradually decreasing the proportion of inspired $O_2$ to $N_2$. Cerebral ischemia was produced by occlusion of the brachiocephalic and left subclavian arteries for periods of 1 to 2.75 minutes (1.5 ± 0.2 minutes). An interval of at least 10 minutes elapsed between successive hypoxic or ischemic trials.

Differences between means were tested for statistical significance with the Wilcoxon signed-rank test (Rumke & DeJonge, 1964). P values of less than 0.01 were considered to be significant. Means listed in the text are given with their accompanying standard error.

## RESULTS

The effect of progressive systemic hypoxia on cervical sympathetic trunk (CST) and phrenic nerve activity was observed in 17 trials in 8 animals. Three different effects on sympathetic activity, which appeared at different levels of hypoxia, could be identified: i) a depression of ISSA closely associated with depression of phrenic nerve activity, ii) a decrease in TSA , and iii) an increase in TSA.

In all the experiments the effect which appeared first as $P_{insp}O_2$ began to decrease was the depression of phrenic nerve activity and of ISSA (Figure 22 record at $P_{insp}O_2$ of 50 mmHg). On average, the ISSA was first noticeably depressed at an inspired $PO_2$ of 58±10 mmHg. With a further decrease in inspired $PO_2$ complete

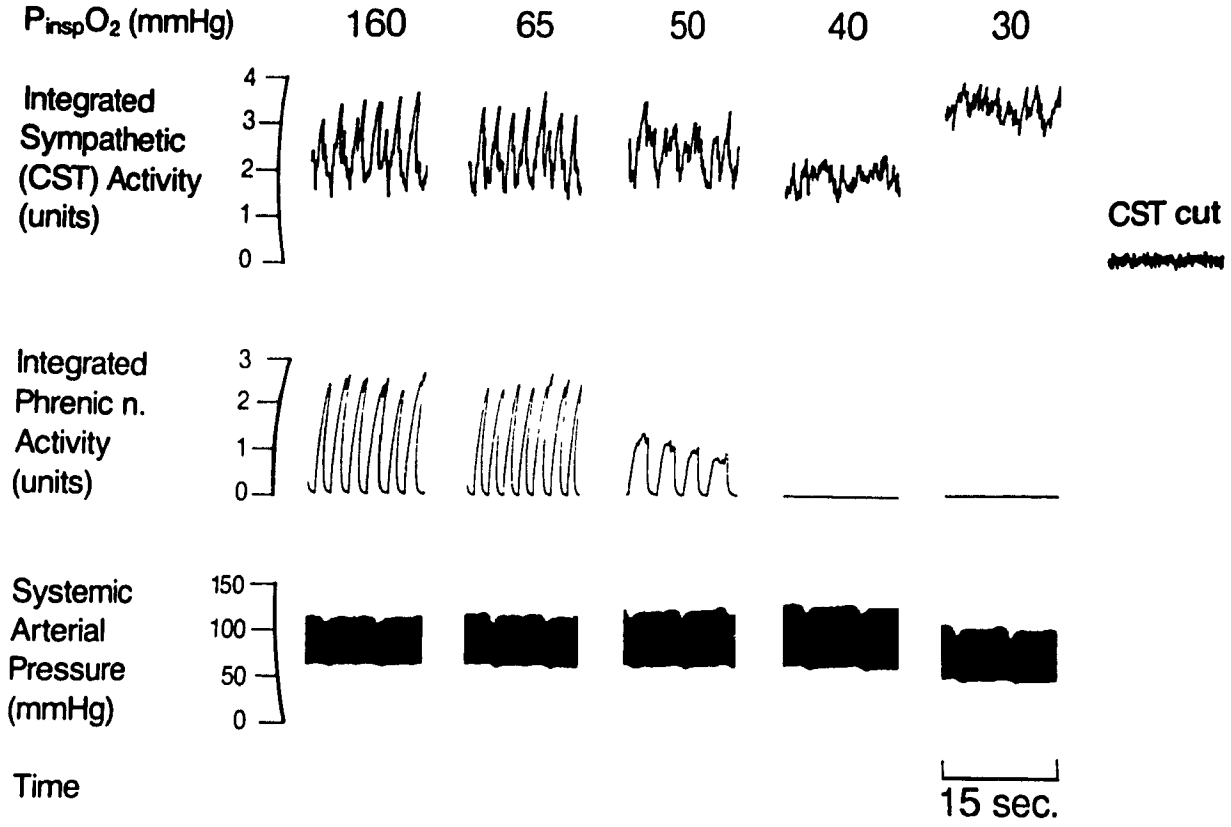


*Fig.22.* Sino-aortic denervated cat. End-tidal $PCO_2$, 37 mmHg. Response of cervical sympathetic trunk (CST) and phrenic nerve discharge to progressive systemic hypoxia (average $P_{insp}O_2$ for the 15 s record shown in each panel is indicated on top of the panel). Notice parallel depression of inspiration-synchronous sympathetic discharge and phrenic nerve discharge. At $P_{insp}O_2$ of 40 mmHg, both are suppressed. Tonic sympathetic activity increases at 30 mmHg. Record at extreme right shows cut-nerve base line for cervical sympathetic trunk record.

suppression of phrenic nerve activity and ISSA occurred at an average inspired $PO_2$ of 46 ± 9 mmHg (Figure 22, record at $P_{insp}O_2$ of 40 mmHg).

In all experiments TSA started to increase when $P_{insp}O_2$ reached a level lower than that at which the depression of ISSA began (50 ± 13 mmHg compared with 58 ± 10 mmHg; $P < 0.01$). As inspired $PO_2$ was lowered further, TSA progressively increased reaching a maximum level at the lowest $PO_2$ tested (26 ± 3 mmHg) (Figure 22, record at $P_{insp}O_2$ of 30 mmHg). At the lowest $P_{insp}O_2$ the TSA level averaged 200 ± 28 % of the level in normoxia.

In 5 of the 8 animals only the two responses described above were observed. In 3 of these 5 animals TSA remained approximately constant over a small range of decreasing $P_{insp}O_2$ values after the disappearance of ISSA and prior to the increase in TSA (Figure 22 record at $P_{insp}O_2$ of 40 mmHg). Thus, in these animals there was a clear separation in time, and therefore $PO_2$, of the two responses. In the 2 remaining animals TSA started to increase while ISSA was still present and becoming progressively smaller.

An additional form of depression of sympathetic discharge during systemic hypoxia was observed in the remaining 3 animals. In these preparations a depression of TSA occurred at levels of hypoxia sufficient to depress or abolish phrenic nerve discharge and ISSA but less severe than those associated with the increase in TSA. The threshold for this depression was in the $P_{insp}O_2$ range of 50-40 mmHg and the peak (at 20-50% of the control TSA level) in the $P_{insp}O_2$ range of 35-20 mmHg . This decrease in TSA during systemic hypoxia was also present when phrenic nerve discharge and ISSA were first eliminated as a result of hypocapnia produced by hyperventilation (Figure 23).

The effect of cerebral ischemia on the activity of the CST and phrenic nerve was recorded in 6 animals. In all the animals there was depression of phrenic nerve activity and of ISSA. In some preparations this depression was preceded by a transient increase in

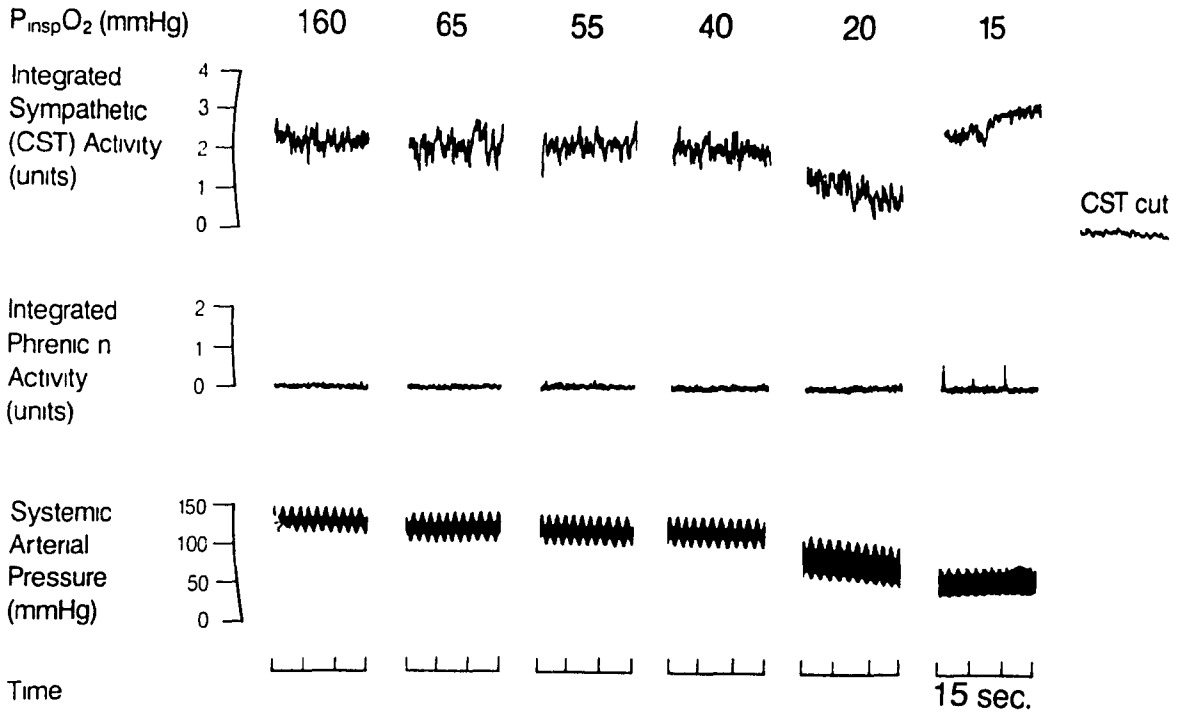


*Fig.23.* Sino-aortic denervated cat. Response of sympathetic activity to progressive systemic hypoxia. End-tidal $PCO_2$ ,15 mmHg. At this $PCO_2$ level inspiration synchronous sympathetic discharge and phrenic nerve discharge are absent. Notice depression of tonic sympathetic activity at $P_{insp}O_2$ of 20 mmHg, followed by increase at 15 mmHg. Record at extreme right shows cut-nerve base line for cervical sympathetic trunk record.

phrenic nerve discharge and ISSA (2 animals). In 5 of the 6 animals an increase in TSA was observed concomitantly with or following the depression of ISSA while in the remaining animal TSA did not change. In two animals a decrease in TSA was also observed following the initial increase in TSA. As in the experiments involving systemic hypoxia this decrease in TSA was also present following elimination of phrenic nerve discharge and ISSA, prior to cerebral ischemia, by hyperventilation producing hypocapnia. Examples of different response patterns of CST and phrenic nerve discharge to cerebral ischemia are shown in Figure 24.

During systemic hypoxia SAP decreased in all the animals from a control value of 130±8 mmHg to a value of 53±3 mmHg at the peak hypoxic level ($P_{insp}O_2$ of 26±3 mmHg). Similar results have been observed in previous studies (Bernthal & Woodcock, 1951; Bouckaert et al., 1941; Gellhorn & Lambert, 1939; Rohlicek & Polosa, 1983a; Rohlicek et al., 1984). The fall in SAP during hypoxia was smaller in some animals compared with others (c.f. Figure 1 & 2). This may have been due to a greater level of circulating vasopressor agents during hypoxia in some preparations (Ashack et al., 1985; Bayliss et al., 1977; Rose et al., 1983). During occlusion of the brachiocephalic and left subclavian arteries there was a marked pressor response (67±9 mmHg increase from a control level of 124±10 mmHg). Similar results have been obtained previously (Guyton, 1948; Sagawa et al., 1961; Downing et al., 1963; Takeuchi et al., 1969; Dampney & Moon, 1980; Rohlicek & Polosa 1983b). It does not appear that the changes in SAP associated with systemic hypoxia or cerebral ischemia were primarily responsible for the observed effects on sympathetic activity, as a decrease in mean SAP to 43 ± 7 (n = 3) mmHg produced by rapid hemorrhage or an increase in mean SAP to 180 ± 15 (n = 3) mmHg as a result of bolus i.v. injection of noradrenaline (5 - 10 μg) did not have an appreciable effect on sympathetic activity.

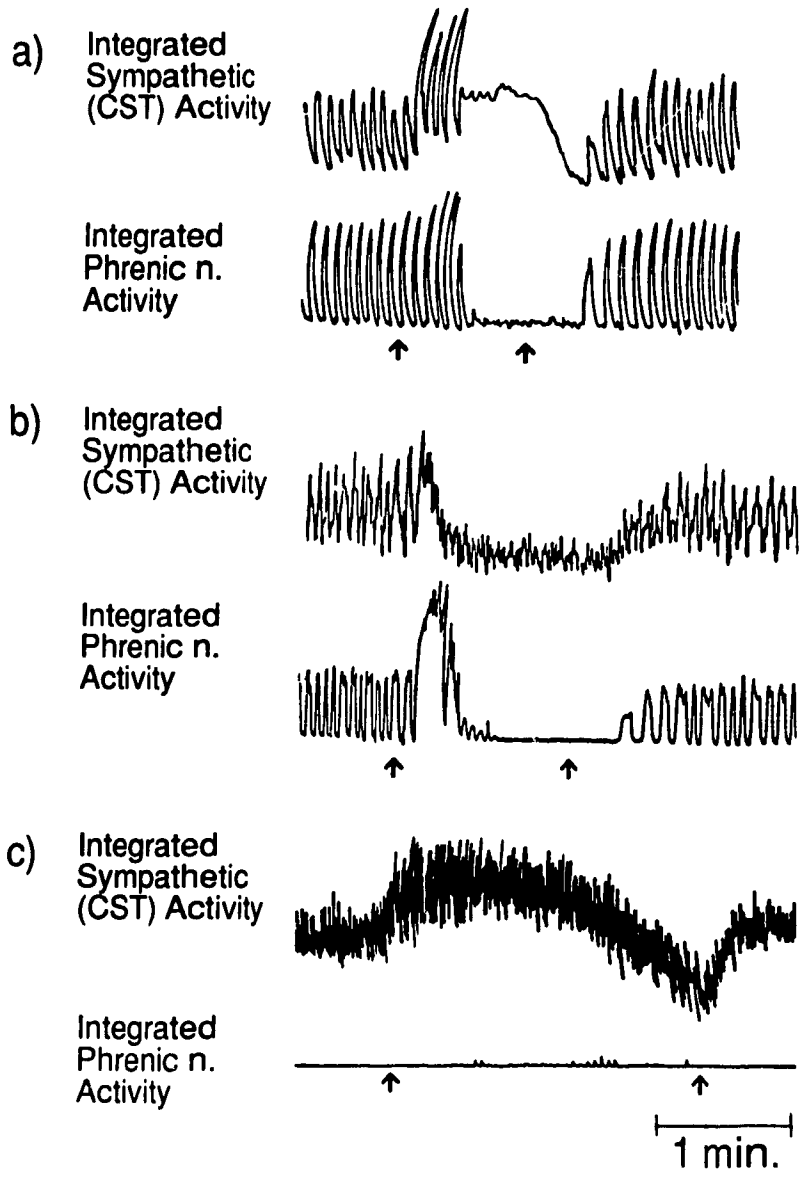


*Fig.24.* **Effects of cerebral ischemia on cervical sympathetic trunk and phrenic nerve discharge. (*a - c*) Records from three different sino-aortic denervated cats. The cats of panels *a* and *b* were normocapnic. In the cat of panel *c*, phrenic nerve and inspiration-synchronous sympathetic discharges were eliminated by hyperventilation in air (end-tidal $PCO_2$, 20 mmHg). Between arrows: occlusion of the brachiocephalic and left subclavian artery.**

## DISCUSSION

The SPN response to systemic hypoxia in the CNS-intact SAD preparation is more complex than previously thought (Rohlicek & Polosa, 1983a). We have identified three components of this response with different $PO_2$ thresholds: a decrease in the ISSA, an increase in TSA and a decrease in TSA.

The loss of ISSA during systemic hypoxia can be attributed to withdrawal of the phasic facilitatory input to SPNs provided by brainstem inspiratory neurons (Bachoo & Polosa, 1985), which are depressed by hypoxia (Van Beek et al., 1984) because: i) the time course of the depression of ISSA paralleled the time course of the depression of phrenic nerve discharge as inspired $PO_2$ was gradually decreased; and ii) ISSA was depressed independently of changes in TSA. The latter observation rules out the possibility that the depression of ISSA was due to a depressant action of systemic hypoxia on SPNs or on tonic facilitatory inputs to these neurons because if this was the case TSA also would be depressed over the same range of inspired $PO_2$. Instead, in a number of experiments the level of TSA was unchanged or increasing when ISSA was depressed. Further support for the view that the depression of ISSA during hypoxia is a result of the withdrawal of excitatory input to sympathetic preganglionic neurons from brainstem inspiratory neurons is provided by the observation that ISSA is also depressed during cerebral ischemia. As during systemic hypoxia, during brain ischemia ISSA was depressed concomitantly with the phrenic nerve discharge, while TSA showed either no change or an increase. This effect of cerebral ischemia can be attributed to an action of brain hypoxia because CNS hypercapnia and acidosis, which also result from cerebral ischemia (Ljunggren et al.,1974), appear to have only excitatory effects on sympathetic and respiratory activity (Hanna et al., 1981; Berkenbosch et al., 1979a) while brain hypoxia is known to depress respiration regardless of these simultaneously occurring excitatory effects (Morrill et al., 1975).

A second component of the CST response to systemic hypoxia was an increase in TSA. This effect is likely of both spinal and supraspinal origin since previous work has shown that severe hypoxia in the spinal animal has sympatho-excitatory effects (Alexander, 1945; Rohlicek & Polosa, 1981; 1986) as does cephalic perfusion with hypoxic blood (Downing et al., 1963; De Geest et al., 1965a; McGillicudy et al., 1978). The increase in TSA during hypoxia may be due to increased excitability of SPNs or of antecedent spinal and supraspinal excitatory neurons as well as to a decrease in tonic sympatho-inhibitory influences.

A third component of the CST response to systemic hypoxia was a decrease in TSA. A reason why this component was not seen in all the animals may be that the range of $PO_2$ over which this effect appears overlaps with that producing the increase in TSA. The hypoxic depression of TSA seems to be of supraspinal origin since it is absent in the $C_1$ spinal animal (Rohlicek & Polosa, 1981; 1986) but it was seen during cerebral ischemia. In the latter case, for the reasons given above, the depression can be attributed to the effects of cerebral hypoxia. The hypoxic depression of TSA does not appear to be related to the hypoxic decrease in central respiratory activity since it was also seen in the absence of phrenic motoneuron discharge resulting from hypocapnia. The hypoxic decrease in TSA may result from the depression by hypoxia of supraspinal mechanisms involved in the generation of tonic sympathetic activity or the activation of sympatho-inhibitory pathways.

Following denervation of the arterial chemoreceptors there are no known remaining peripheral chemosensory structures sensitive to $O_2$ lack with reflex action on sympathetic neurons. Therefore, changes in sympathetic preganglionic neuron activity during systemic hypoxia in the SAD preparation can be attributed to central actions of $O_2$ lack. CNS hypoxia has depolarizing (Lorenté de No, 1947; Lundberg & Oscarsson, 1953; Kolmodin & Skoglund, 1959; Collewijn & Van Harreveld, 1966; Eccles et al., 1966;

Chalazonitis, 1963; Segal, 1970) or hyperpolarizing (Hansen et al., 1982; Glotzner, 1967) effects on neuron membrane as well as a depressant effect on synaptic transmission (Hansen, 1985). Depolarization of sympathetic preganglionic neurons or of antecedent excitatory neurons, decreased efficacy of tonically active sympatho-inhibitory pathways as a result of depressed synaptic transmission or hyperpolarization of neurons in such a pathway could lead to increased sympathetic activity. Similarly depolarization of inhibitory neurons antecedent to sympathetic preganglionic neurons or decreased efficacy of tonically active sympatho-excitatory pathways could lead to decreased sympathetic activity.

The mechanism of the hypoxic depression of brainstem inspiratory neurons, which is suggested as the cause of the depression of ISSA during hypoxia, is not known. A direct effect on central respiratory neurons has been suggested (Cherniack et al., 1971). A mediation by adenosine (Millhorn et al., 1984) or GABA (Yamada et al., 1981) has also been proposed.

These central sympatho-depressant and sympatho-excitatory effects of systemic hypoxia may be physiologically significant. It is likely that these excitatory and depressant effects interact with the input to sympathetic neurons from the arterial chemoreceptors (Trzebski, 1983) to produce the overall sympathetic response to systemic hypoxia in the intact animal. Krasney and coworkers have suggested that central sympathetic effects of hypoxia are particularly important in neurogenic cardiovascular adjustments during acute systemic hypoxia (Koehler et al., 1980; Krasney & Koehler 1977). Changes in sympathetic activity, resulting from CNS hypoxia, may modulate arterial chemoreceptor output as a result of the sympathetic innervation of the carotid sinus (O'Regan & Majcherczyk 1983). The central sympatho-excitatory effects of hypoxia may explain the increased pressor effect of carotid baroreceptor unloading during systemic hypoxia (Bagshaw et al. 1986). Finally central hypoxic sympatho-depressant effects may explain

the decrease in neurogenic vasoconstrictor tone sometimes seen during systemic hypotension produced by hemorrhage (Corazza et al., 1963; Gootman & Cohen, 1970; Lundgren et al., 1964; Rothe et al., 1963).

# RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONS TO SYSTEMIC HYPERCAPNIA IN THE ACUTE SPINAL CAT

Ting-Xin Zhang, Charles V. Rohlicek and Canio Polosa

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## ABSTRACT

The relation between end-tidal $PCO_2$ ($P_ACO_2$) and firing rate of sympathetic preganglionic neurons (SPN) of the cervical sympathetic trunk was studied during hyperoxic hypercapnia in acute $C_1$ or $C_4$ spinal cats. The cats were under barbiturate anaesthesia or anemically decerebrate. The firing rate of the majority of the tonically active strands (18/22) increased in hypercapnia and showed a continuous relation to $P_ACO_2$ within the range studied. The firing rate of the remaining 4 strands was uneffected. The maximum increase in firing rate of the responsive strands was 3.7 times the control value on average (range 2.5-14.0). Recruitment of units which were silent in control conditions also occurred. These data demonstrate the existence of a spinal mechanism responsible for excitation of SPN during systemic hypercapnia.

## INTRODUCTION

In cats with afferents from arterial chemoreceptors cut there is a significant neurogenic vasoconstrictor response to systemic hypercapnia (Lioy et al., 1978). This response is greatly attenuated, but not abolished, by cold-block of superficial areas of the ventral medulla which are known to mediate central chemosensitivity (Hanna et al., 1979). The source of the residual $CO_2$-sensitivity of sympathetic neurons, which persists after section of the buffer nerves and cold-block of the ventral surface of the medulla, is not

known. In the experiments to be described we tested the possibility that some of this residual component could be generated in the spinal cord.

Previous observations are conflicting. Alexander (1945) found no effect of systemic hypercapnia on the discharge of the inferior cardiac nerve in the acute high spinal cat. A lack of influence of $CO_2$ on the activity of spinal cardiovascular centers had also been suggested by Kaya and Starling (1909) on the basis of systemic arterial pressure (SAP) measurements. In contrast, Johnson et al. (1969) found that systemic hypercapnia often resulted in an increased SAP in midcervical acute spinal cats. This increase was considered neurogenic since systemic hypercapnia, following administration of hexamethonium, caused a fall in SAP. Consistent with the latter results is the observation by Szulczyk and Trzebski (1976) that superfusion of the dorsal surface of the thoracic spinal cord with acid artificial CSF in cats with spinal cord transected at the level of the first thoracic segment, caused an increase in SAP and in the electrical activity of the inferior cardiac and vertebral nerves. Earlier papers (Dale & Evans, 1922; Mathison, 1910) had also suggested some $CO_2$ effects on the spinal cardiovascular centers on the basis of indirect evidence.

In the present study we have examined the response of sympathetic preganglionic neurons (SPNs) of the cervical sympathetic trunk (CST) to systemic hypercapnia in cats made acutely spinal by transection of the cord at $C_1$ or at a midcervical level. Systemic hypercapnia was produced by administration of an inspiratory gas mixture containing 10% $CO_2$ and 90% $O_2$. We found that a significant number of SPNs were excited by hypercapnia.

## MATERIALS AND METHODS

Adult cats of either sex (2.5-4.5 kg body weight) were anesthetized with sodium pentobarbital (35 mg/kg i.p.) or anemically decerebrated by permanent occlusion of the

common carotid and vertebral arteries. They were made spinal by transection of the cord at $C_1$ or $C_4$. Ventilation was maintained with 100% $O_2$ using a pump adjusted to maintain end-tidal $PCO_2$ ($P_ACO_2$) at 20 mm Hg. Muscle paralysis was produced with pancuronium bromide (0.5 mg/kg i.v.). Systemic arterial pressure, $P_ACO_2$ and rectal temperature were continuously monitored. Infrared lamps were used to maintain rectal temperature between 36 and 37°C.

The cervical sympathetic trunk was desheathed and split, under a dissecting microscope, into small filaments containing a single active unit or a few active units. Both trunk and filaments were kept under paraffin oil in a pool made of skin flaps. The electrical activity of the filaments was recorded using silver hook electrodes, amplified, displayed on an oscilloscope and stored on magnetic tape. In addition, a gated pulse counter gave a continuous record of the firing frequency of the sympathetic units.

Systemic hypercapnia was produced by administering an inspiratory gas mixture of 10% $CO_2$ and 90% $O_2$. The mixture was delivered to the pump inlet through an anesthesia bag initially filled with 100% $O_2$. End-tidal $PCO_2$ increased gradually and reached a steady plateau of 69 ± 8 (mean ±S.D.) mm Hg in 2-3 min when a 1 liter bag was used, or in 5-8 min when a 5 liter bag was used. When hypercapnic runs were repeated, at least 20 min were allowed between successive runs. Statistical significance of differences between means was treated with a paired t-test (Snedcor & Cochran, 1980).

## RESULTS

The data presented below were obtained from 22 CST strands (18 single unit, 4 multiunit) in 17 cats. Recording of unit activity began at least 8h after spinal transection. Control (ET $PCO_2$ 20 mm Hg) mean SAP was 69 ± 21 mm Hg. Control mean firing rate for the single units was 0.44 ±0.58 spikes/s (range 0.05-2.50). These very low rates are

typical of SPN firing in acute high spinal animals (Mannard & Polosa, 1978; Wyszogrodski & Polosa, 1973).

During systemic hypercapnia the mean firing rate of most strands (16 single unit, 2 multi-unit) increased. At the peak $P_ACO_2$ attained, the mean firing rate of the 16 single units was 1.61 ±1.50 (range 0.33-6.40) spikes/s, representing an average 3.7 times increase over the control value (range 2.5-14.0 times, $P<0.001$). The firing rates of the remaining 2 single units and 2 multiunit strands did not change during hypercapnia. A typical unit response during hypercapnia is shown in Fig. 25. The response of a given unit was reproducible on repetition of the stimulus. When $P_ACO_2$ returned to control values, so did the firing rate of the unit. A record of the actual firing of the responsive unit in normocapnia and during ventilation with 10% $CO_2$ and 90% $O_2$ is shown in Fig. 26. As outlined in MATERIALS and METHODS, inspired $CO_2$ could be increased more slowly in the cases in which a 5 l anesthesia bag was used. The relationship between $P_ACO_2$ and mean firing rate of the responsive units was plotted in these cases using $P_ACO_2$ values from the phase of increasing $P_ACO_2$. This relationship was sigmoid, with a flat slope in the low $CO_2$ range including normocapnia, followed by a steeper portion and a plateau at the highest $PCO_2$ values attained. An example of this relationship is shown in Fig. 27. The figure shows the existence of a relation between $P_ACO_2$ and SPN firing rate over a wide range of $PCO_2$ values, although the actual shape and position of the relation might have been influenced by the non-steady-state conditions under which the measurements were made. The $CO_2$ sensitivity of the SPNs in these preparations expressed as the ratio of the firing rate change over the $P_ACO_2$ change, was on average 0.016 spikes/s/mm Hg (0.8 spikes/s/50 mm Hg).

During hypercapnia, in addition to the previously described increase in firing rate of units which were already active in control conditions, there was also recruitment of

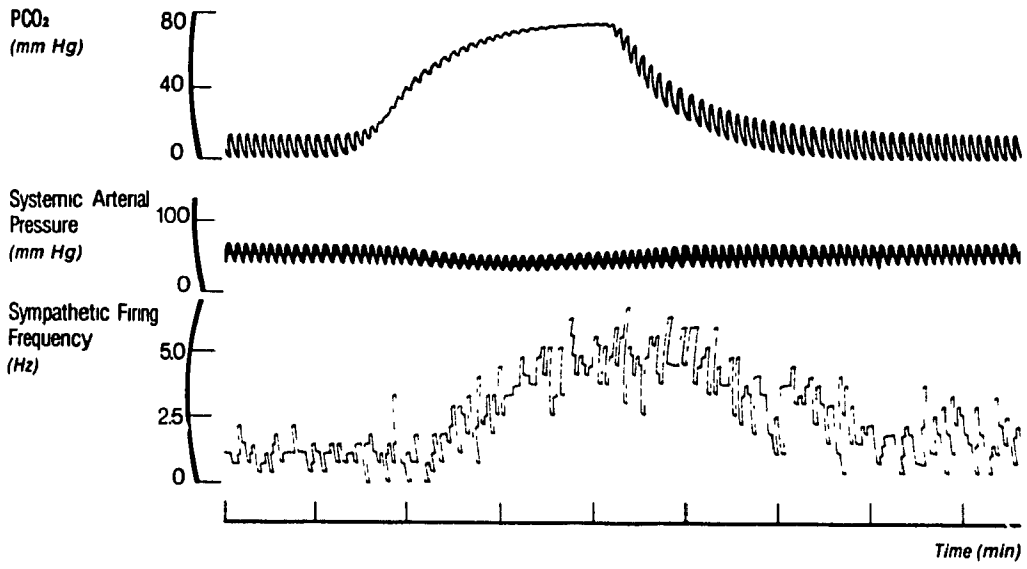


*Fig. 25.* **Response of a sympathetic preganglionic neuron in an acute $C_4$ spinal cat to ventilation with 10% $CO_2$ and 90% $O_2$. From top: tidal $CO_2$, systemic arterial pressure, ratemeter record of unit activity shown in Fig. 26.**

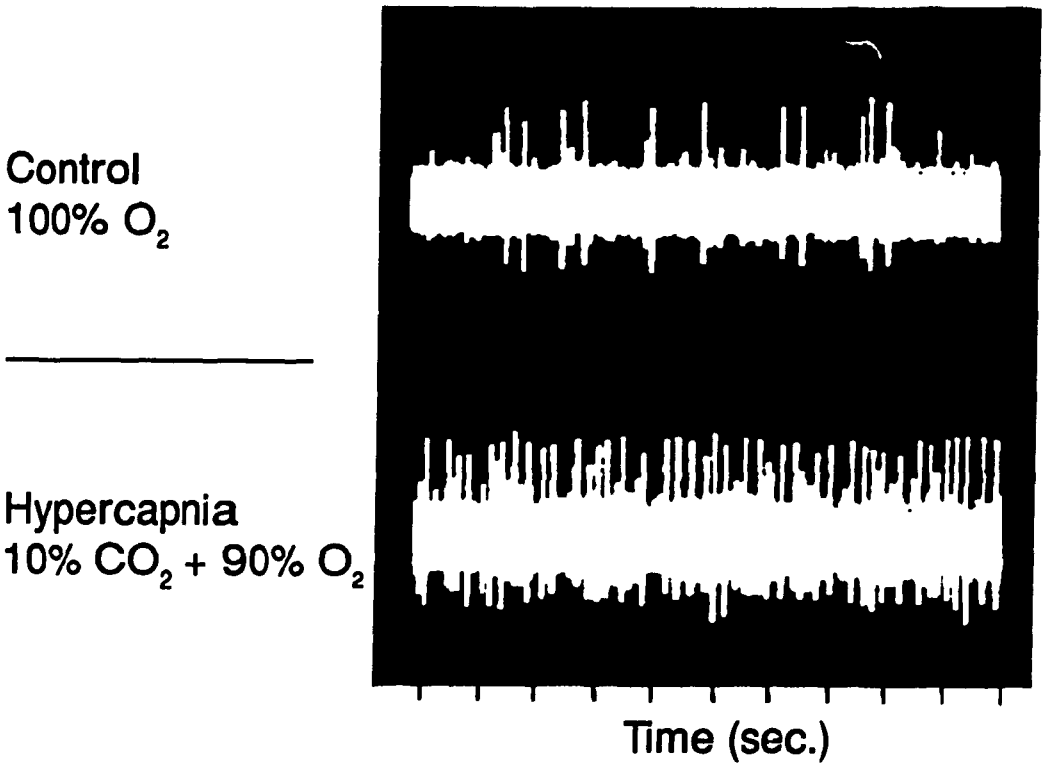


*Fig. 26.* Record of the spike activity of the unit shown in Fig. 25. Top: sample of unit spike activity at end-tidal $PCO_2$ of 20 mm Hg. Bottom: sample of unit activity at end-tidal $PCO_2$ of 60 mm Hg.

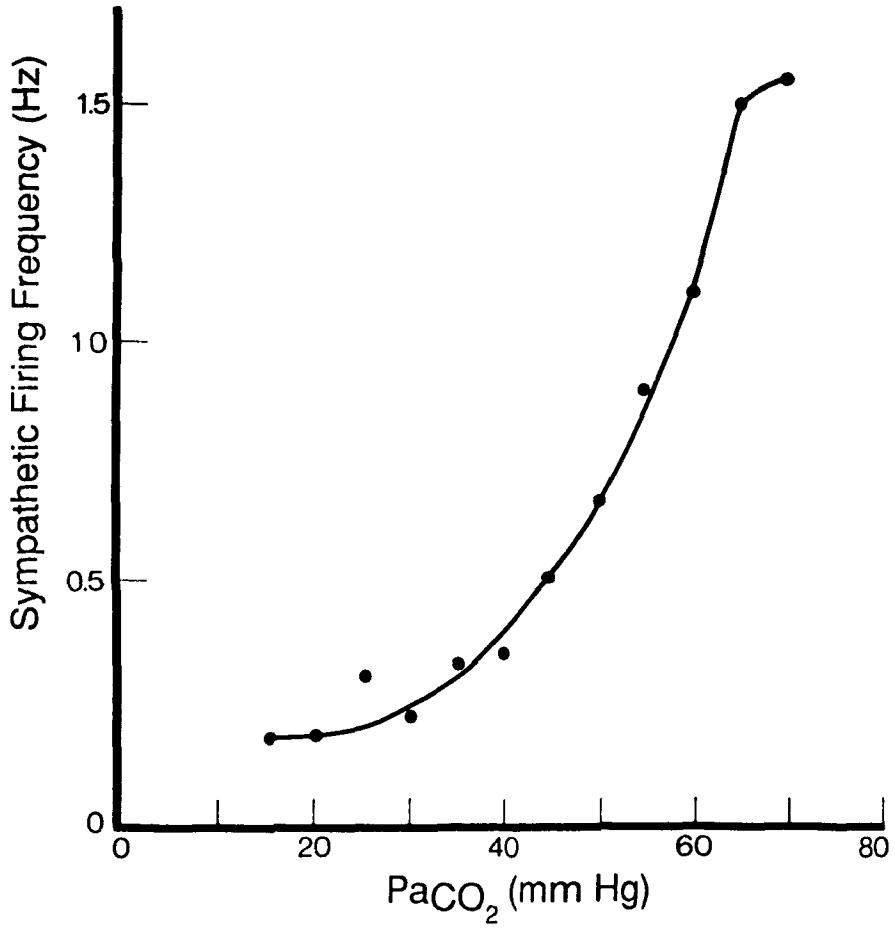


*Fig. 27.* Relation between firing rate and end-tidal $PCO_2$ for a single unit. In this experiment end-tidal $PCO_2$ was changing at a rate of 8 mm Hg/ min. Acute $C_1$ spinal cat.

previously silent units. Recruitment was observed in 13 of the 22 strands studied (Fig. 28).

In contrast to the consistent increase in SPN firing rate during systemic hypercapnia , SAP showed no change in 4 experiments, decrease in 7, increase in 1 and in 6 showed a biphasic response, consisting of a decrease followed by an increase. These various SAP responses can be explained as resulting from the combination of the observed excitatory effect of $CO_2$ on SPNs with the known direct inhibitory effect of $CO_2$ on vascular smooth muscle and on cardiac muscle (Jerusalem & Starling, 1910; Somlyo & Somlyo, 1970). The fact that the same SPN response to hypercapnia (increase in firing) was associated with all 4 SAP response patterns suggests that the SAP change was not the cause of the neuron response. During the post-hypercapnic period SAP was always higher than control for a variable period of time (range 5-10 min), presumably reflecting the release of adrenal medullary catecholamines (Tenney, 1960) or other pressor agents during the preceding hypercapnic period.

In 2 units in 2 cats we tested the response to hypercapnia before and after i.v. doses of pentobarbital from 5 to 30 mg/kg had been given. Although the mean firing rate of the units decreased as a result of the administration of the drug, the response to ventilation with 10% $CO_2$ was not appreciably changed. An example is shown in Fig. 29.

## DISCUSSION

These experiments have shown that a large fraction of spontaneously active units of the CST are excited by systemic hypercapnia in the acute $C_1$ or $C_4$ spinal cat. In addition, a significant number of silent units are recruited into activity by hypercapnia. This excitation could be due to an action of $CO_2$ or $H^+$ on the SPN, or on spinal interneurons or hypothetical chemoreceptor cells, specialized for $CO_2$ or $H^+$ detection, antecedent and excitatory to the SPN. Thus, the SPN could be responding to changes in $CO_2$ or $H^+$ in its

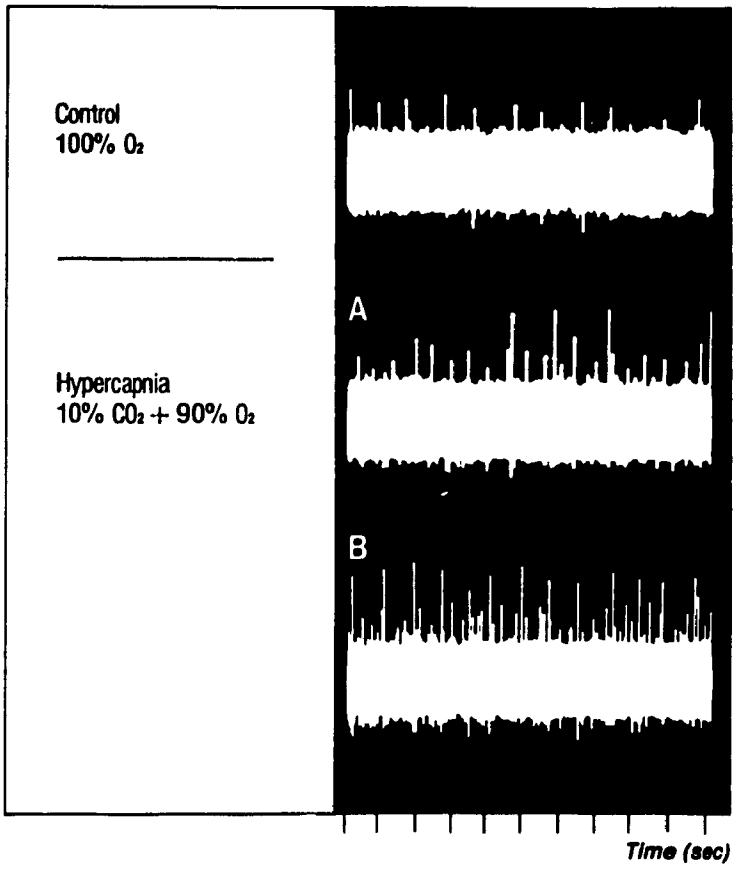


*Fig. 28.* Recruitment of sympathetic unit in hypercapnia. Top: spike activity in the strand in control conditions. Bottom: A, in hypercapnia, a larger spike is recruited; and B, activity of strand at end-tidal $PCO_2$ level higher than in A. Acute $C_1$ spinal cat.

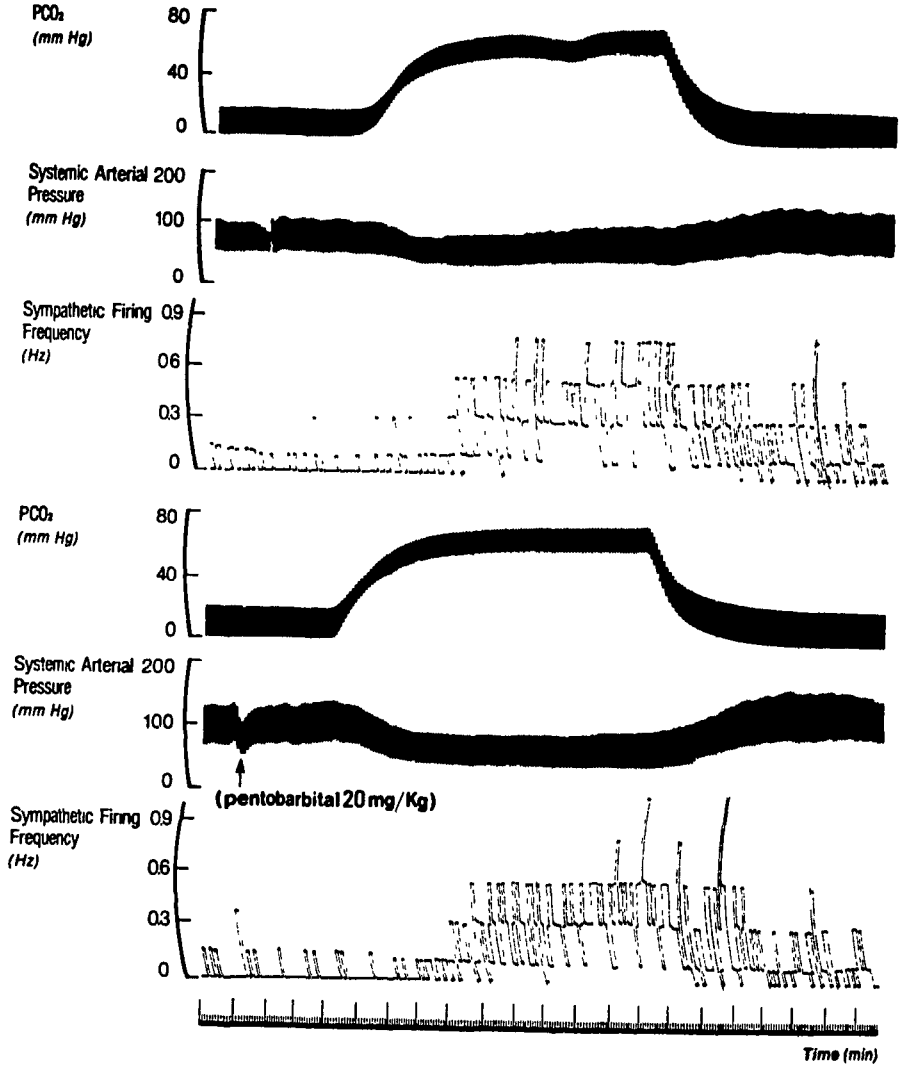


*Fig. 29.* **Effect of pentobarbital on the response of a sympathetic unit to hypercapnia in an anemic decerebrate, spinal cat. Top: control. Bottom: after 20 mg/kg pentobarbital i.v. In each panel records from top to bottom are tidal $CO_2$, systemic arterial pressure and firing rate.**

environment with excitation like peripheral and central chemoreceptor cells of mammals, some Aplysia neurons (Chalazonitis, 1963) and some alpha-motoneurons (Papajewski et al., 1969), but unlike cortical neurons (Krnjevic et al., 1965), brainstem respiratory neurons (Marino & Lamb, 1975; Mitchell & Herbert, 1974) and the majority of $\alpha$-motoneurons (Papajewski et al., 1969) which are depressed by systemic hypercapnia.

Our results are consistent with the results of Szulczyk and Trzebski (1976) who found increases of SAP on superfusion of the dorsal aspect of the thoracic spinal cord with hypercapnic, acid-artificial CSF. In these experiments the SPNs could have been excited by changes in $PCO_2$ or pH of their environment. The failure of pentobarbital to alter the SPN response to systemic hypercapnia (Fig. 29) allows a comparison of the SPN responses of the spinal preparations, obtained in the present study, with those of the CNS-intact, pentobarbital anesthetized, preparations, obtained in two previous studies (Hanna et al., 1981; Preiss & Polosa, 1977). The $CO_2$ sensitivity of the SPN in the spinal preparation is one order of magnitude smaller than that of the CNS-intact preparation (Hanna et al., 1981; Preiss & Polosa, 1977). Peak firing rates attained by the SPNs during hypercapnia were lower in the acute spinal animals than in the CNS-intact animals. In addition, no units in the spinal preparation showed an inverse relation between firing rate and ET $PCO_2$ as was the case in some CNS-intact animals (Papajewski et al., 1969). We have as yet no data concerning how the supraspinal $CO_2$-sensitive input from peripheral and central chemoreceptors (Hanna et al., 1979; Lioy et al., 1978) may interact with the spinal response seen here. It is possible that such an interaction might take the form of a simple summation. If this were the case the residual $CO_2$-sensitivity, observed in experiments involving cold block of the ventral surface of the medulla (Hanna et al., 1979), might well represent the contribution of the spinal component.

Since spinal cord tissue hypercapnia and acidosis will develop when perfusion pressure falls to low values (e.g. as a result of hemorrhage) the SPN excitation by

hypercapnia, described here, could be a mechanism involved in the partial compensation of SAP described in the chronic spinal animal (Brooks, 1935) after hemorrhage.

In a previous study (Rohlicek & Polosa, 1981) we found that severe systemic hypoxia (arterial $PO_2$ less that 40 mmHg) is also associated with excitation of SPNs in the acute $C_1$ spinal animal. There are, however, differences between the two responses. All SPNs studied responded to hypoxia, whereas some of the SPNs did not respond to hypercapnia. The firing rate, on average, increased much more in hypoxia (10 times the normoxic value) than in hypercapnia (4 times the control value). This was not due to the fact that the maximum response to hypercapnia was not attained, since in many cases the shape of the stimulus-response curve suggested saturation (e.g. Fig. 27). The hypoxic excitation, on the other hand, did not appear to saturate. The hypoxic response was followed, in some cases, by a long silent period, i.e. on reoxygenation the firing of several cells ceased for up to 7 min before returning to control values, whereas for the hypercapnic response the firing rate returned gradually to control. In spite of these differences it seems reasonable to ask whether both responses may not be mediated, at least in part, in the same way, i.e. by an increase in $H^+$ concentration caused by accumulation of $CO_2$ in one case and by accumulation of metabolically produced $H^+$ in the other, as postulated, for instance, for the response of peripheral chemoreceptors to low $O_2$ and high $H^+$ and $CO_2$ (Torrance, 1974).

# NEURAL EFFECTS OF SYSTEMIC HYPOXIA AND HYPERCAPNIA ON HINDLIMB VASCULAR RESISTANCE IN ACUTE SPINAL CATS

Charles V. Rohlicek and Canio Polosa

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## ABSTRACT

The effects of systemic hypoxia and hypercapnia on the neurogenic component of hindlimb vascular resistance were studied in 10 unanesthetized acute $C_1$ spinal cats. Hindlimb perfusion pressure (PP) was measured under conditions of constant flow of normoxic and normocapnic blood from a donor cat. Ventilation with 5% $CO_2$ and 10% $CO_2$ in $O_2$ caused increases in PP of 15 ± 2 (mean ± SE) mm Hg and 27 ± 3 mm Hg from a control level of 106 ± 6 mm Hg during ventilation with 100% $O_2$. Changing the inspired gas mixture from 95% $O_2$ plus 5% $CO_2$ to 12.5%, 10%, 7.5%, or 5% $O_2$ plus 5% $CO_2$ in $N_2$ caused increases in PP of 1.5 ± 1, 14 ± 2, 38 ± 6, and 69 ± 15 mm Hg respectively from a control level of 121 ± 9 mm Hg. These vasoconstrictor effects were abolished by ganglionic blockade with hexamethonium (10 mg/kg i.v.). We conclude that in the acute $C_1$ spinal cat a large part of the population of sympathetic preganglionic neurons in the lumbar spinal cord, controlling vascular smooth muscle of the hindlimb, is excited by systemic hypoxia or hypercapnia over a considerable range of $PaO_2$ and $PaCO_2$ values.


## INTRODUCTION

Systemic hypoxia or hypercapnia cause considerable changes in many cardiovascular variables (Korner, 1959; Suutarinen, 1966). These changes are in part neurogenic. The arterial chemoreceptors are one source of these neurogenic responses. However, recording of sympathetic neuron firing (von Keherel et al., 1962;

Downing & Siegel, 1963; Bower, 1975; Iriki & Kozawa, 1975; Gregor & Jänig, 1977; Preiss & Polosa, 1977; Hanna et al., 1981; Rohlicek & Polosa, 1983a) or peripheral vascular resistance (Hanna et al., 1979; Rohlicek et al., 1984) indicates that the sympathetic nervous system retains a significant ability to respond to systemic hypoxia and hypercapnia following denervation of the arterial chemoreceptors.

Work by a number of investigators over the last ten years has led to the conclusion that a large part of the sympathetic neuron excitation by systemic hypercapnia is mediated by superficial regions of the ventrolateral medulla (Trezbski et al., 1974; Szulczyk & Trezbski, 1976; Hanna et al., 1979; Lioy et al., 1981). However, following inactivation of these regions in sino-aortic denervated animals a considerable sympathetic sensitivity to $PaCO_2$ remains (Hanna et al., 1979). An excitatory effect of systemic hypercapnia on sympathetic neuron firing in the acute $C_1$ spinal cat has been demonstrated (Zhang et al., 1982) showing that part of this residual chemosensitivity is of spinal origin. However, the importance of this spinal chemosensitivity in terms of cardiovascular control is not clear.

Both sympatho-excitatory and sympatho-depressant effects on sympathetic neuron firing (Rohlicek Polosa ,1983a) and on neurogenic vascular resistance (Rohlicek et al., 1984) in response to various levels of systemic hypoxia have been observed in CNS-intact animals with denervated arterial chemoreceptors. The anatomical substrate of these responses to systemic hypoxia has not been completely identified. A spinal component has been demonstrated by the finding of a strong excitatory effect of systemic hypoxia on the firing rate of sympathetic neurons in the acute $C_1$ spinal cat (Alexander, 1945; Rohlicek & Polosa, 1981) and in the chronic low thoracic spinal cat (Jänig & Spilok, 1978). The importance of this spinal response to hypoxia in terms of cardiovascular control has not been demonstrated.

The present study was undertaken to determine the role of $PaCO_2$ and $PaO_2$ in controlling vasoconstrictor tone by means of spinal mechanisms. This was done by perfusing a vascularly isolated hindlimb in an acute $C_1$ spinal cat with normoxic and normocapnic blood at a constant flow-rate while monitoring perfusion pressure as the remainder of the animal was made hypoxic or hypercapnic to varying degrees.

The results of the study indicate that while the proportion of the hindlimb vascular resistance which is neurogenic in the acute $C_1$ spinal cat is considerably less than that in the CNS-intact and sino-aortic denervated animal (Rohlicek et al., 1984) both systemic hypoxia and hypercapnia can elicit significant vasoconstrictor responses. The relation between $PaCO_2$ or $PaO_2$ values and vasoconstrictor tone extends over a considerable range of values.

## METHODS

Experiments were conducted on 10 pairs of adult cats of either sex weighing between 3 and 4 kg. The animals were spinalized at $C_1$ and anemically decerebrated by occlusion of the carotid and vertebral arteries under ether anaesthesia.

After ligation of these arteries, anaesthesia was discontinued. Disappearance of the corneal and other reflexes involving muscles of the head together with a fixed and dilated pupil unreactive to light were considered evidence of effective decerebration. All the cats were paralyzed (pancuronium bromide 0.5 mg/kg i.v.) and ventilated with positive pressure. Core temperature was maintained constant at between 36°C and 38°C by means of infrared heat lamps.

Each experiment required two cats both prepared as described above. Both cats were given 500 - 700 units/kg of heparin i.v. After laparotomy, one external iliac artery of the recipient cat was peripherally cannulated and the abdominal aorta ligated just rostral to

the bifurcation. Using a dual head Masterflex roller type pump and Tygon tubing (ID 1.65 mm, OD 4.93 mm, volume 1.5 ml) blood was pumped at constant flow from a common carotid artery of a second cat (the donor) into the peripherally cannulated external iliac artery of the recipient cat and returned to the donor's jugular vein by pumping blood at the same rate from a common carotid artery of the recipient. The absence of shifts in blood volume between the two cats was established by continuously monitoring the body weight of the donor cat with an accuracy of 10g. The flow of blood into the external iliac artery (23 ± 2 ml/min, mean ± SEM) was set at the beginning of the experiment so that the perfusion pressure (PP) of the hindlimb was somewhat greater (110 ± 9 mm Hg) than the systemic arterial pressure of the recipient (80 ± 7 mm Hg). The pressure drop across the perfusion line of the external iliac artery was less than 5 mm Hg with flows up to 120 ml/min. The venous return from the perfused hindlimb occurred through natural channels. 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 PP fell to a value of less than 15 mm Hg. The absence of significant collateral circulation was also tested by increasing or decreasing the systemic arterial pressure of the recipient by the administration of noradrenaline i.v. (10μg/kg) or by acute hemorrhage. No changes in PP were observed for at least 20 s following changes in the recipient's systemic arterial pressure, which in the case of noradrenaline administration were as large as 125 mm Hg.

Systemic arterial pressure (SAP) of both donor and recipient cats was continuously measured through catheters advanced from the femoral artery into the abdominal aorta. Perfusion pressure of the hindlimb was measured less than 5 cm from the entry of the cannula into the external iliac artery used to perfuse the hindlimb. Central venous pressure of the recipient cat was monitored through a catheter advanced approximately 15 cm from the femoral vein into the inferior vena cava. All pressure measurements were made using Statham pressure transducers and were continuously

displayed on a Grass model 7 polygraph. Inspired/expired $PCO_2$ and $PO_2$ of the recipient and donor cat were monitored using Beckman LB-2 and OM-11 gas analyzers. These values were also continuously displayed on the polygraph.

With constant flow perfusion vasoconstriction and vasodilation were indicated by increases or decreases in PP since central venous pressure did not change during these experiments. At the beginning of an experiment the respiratory pump rate was adjusted to obtain hypocapnia (17 ± 1 mm Hg $PaCO_2$) during ventilation with 100% $O_2$. The respiratory pump settings were then maintained unchanged and the desired levels of $PaCO_2$ and $PaO_2$ obtained with the appropriate gas mixtures. $PaCO_2$ values of 40 ± 2 mm Hg and 64 ± 2 mm Hg were obtained with gas mixtures containing 5 and 10% $CO_2$ in $O_2$. $PaO_2$ values of >650, 87 ± 3, 66 ± 3, 49 ± 2, and 36 ± 3 mm Hg were obtained with gas mixtures containing 95, 12.5, 10, 7.5 or 5% $O_2$ plus 5% $CO_2$ and balance $N_2$. $PaCO_2$ during ventilation with the latter gas mixtures averaged 40 ± 2 mm Hg. Each gas mixture was administered to the recipient cat for a period of 4 -6 min and the same interval of time allowed to elapse before another trial was performed. The donor cat was ventilated with room air throughout the experiment. The hindlimb PP response to each gas mixture was measured as the average PP level over a period of 30 s after 2 - 3 min of application of the gas mixture when a steady response was seen. $PaO_2$ and $PaCO_2$ were also measured at this time. In control conditions ($PaO_2$>650, $PaCO_2$ 17 ± 2 mm Hg) hexamethonium (10 mg/kg i.v.) given to the recipient caused a fall in PP from 115 ± 11 mm Hg to 87 ± 10 mm Hg (26 ± 6%, n=7). The fraction of PP thus eliminated was taken as an approximation of the neurogenic component of the perfusion pressure. Differences between sets of results were tested for significance ($P<0.01$) using the Wilcoxon signed-ranks test (Siegel 1956).

## RESULTS

Ventilation of the recipient animals with 5% $CO_2$ in $O_2$ caused an increase in hindlimb perfusion pressure (PP) in 22 of 25 trials and no change in 3 trials of 10 animals. The average increase in PP was 15 ± 2 mm Hg from a control level of 106 ± 6 mm Hg. On average there was a small increase in SAP from 73 ± 7 mm Hg to 77 ± 5 mm Hg. Ventilation of the recipient cats with 10% $CO_2$ in $O_2$ caused an increase in hindlimb perfusion pressure in all 14 trials in 8 animals. The average increase in PP was 27 ± 3 mm Hg from a control level of 110 ± 9 mm Hg. At this level of hypercapnia there was on average a small decrease in SAP from 80 ± 6 mm Hg to 77 ± 6 mm Hg. The PP values during ventilation with 5 and 10% $CO_2$ were significantly different from those during ventilation with 100% $O_2$ ($P<0.01$). These hindlimb vasoconstrictor responses were eliminated by ganglionic blockade of the recipient cat with hexamethonium (10 mg/kg i.v.). These results are summarized in Fig. 30. Sample records of the vasoconstrictor responses to ventilation with hypercapnic gas mixtures are shown in Fig. 31. In this experiment, upon ventilation of the recipient animal with 5% $CO_2$ in $O_2$ hindlimb perfusion pressure increased from the control value of 100 mm Hg to a peak level of 115 mm Hg. The increase in hindlimb PP was accompanied by an increase in mean SAP from a control level of 66 mm Hg to a peak value of 83 mm Hg. Upon return to ventilation with 100% $O_2$ both hindlimb perfusion pressure and mean SAP also returned to the control level. Subsequent ventilation with 10% $CO_2$ and 90% $O_2$ led to an increase in perfusion pressure from the control level of 100 mm Hg to a peak level of 125 mm Hg. Mean SAP increased from 60 to 77 mm Hg. Following the return to ventilation with 100% $O_2$ perfusion pressure and SAP again returned to the control values.

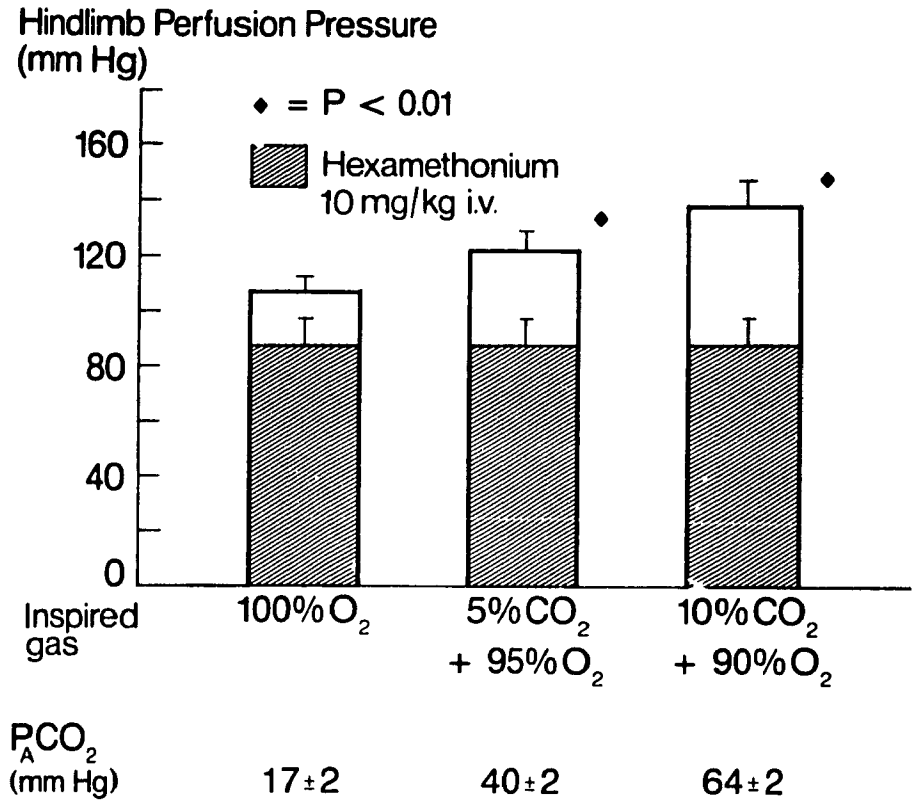


*Fig. 30.* Mean hindlimb perfusion pressure (PP) during ventilation of acute $C_1$ spinal cats with 100% $O_2$, 5% $CO_2$ in $O_2$, and 10% $CO_2$ in $O_2$. Data from 25, 25 and 14 trials in 10, 10 and 8 animals respectively. $PaCO_2$ values are indicated. Hatched areas indicate PP measured after administration of hexamethonium 10 mg/kg i.v. during ventilation with 100% $O_2$ (87 ± 10 mm Hg, n=7). Bars indicate 1 SEM. Diamonds indicate a significant difference ($P<0.01$) in PP from that during ventilation with 100% $O_2$

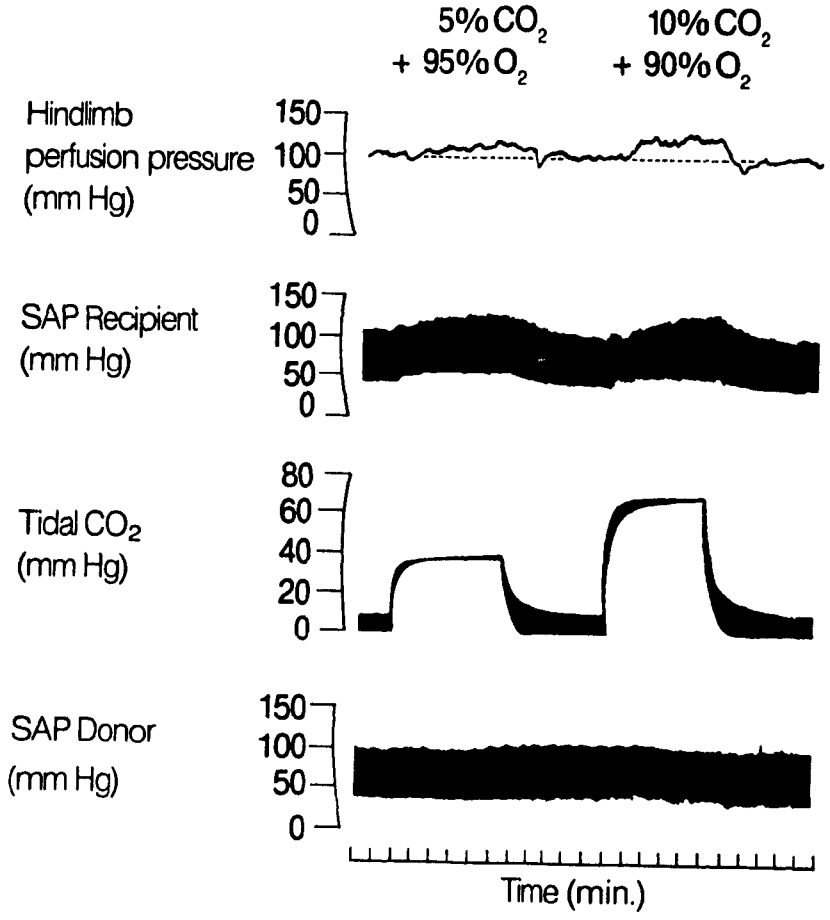


*Fig. 31.* Hindlimb perfusion pressure (PP) changes during ventilation of an acute $C_1$ spinal cat with 5% $CO_2$ in $O_2$ and 10% $CO_2$ in $O_2$. From top to bottom: recipient's hindlimb PP in mm Hg, recipient's systemic arterial pressure (SAP) in mm Hg, recipient's tidal $CO_2$ in mm Hg, and donor's SAP in mm Hg. Time is indicated along the horizontal axis in minutes. When not ventilated with hypercapnic gas mixtures the animal was ventilated with 100% $O_2$.

Ventilation of the recipient cats with the hypoxic gas mixtures was associated with increases in hindlimb PP. During ventilation with 12.5% $O_2$ mixture (10 trials in 8 cats) there was an increase in hindlimb PP in 6 trials and no change in 4. With the other mixtures an increase was observed in all trials (12, 11 and 8 trials in 9, 9 and 6 cats with the 10, 7.5 and 5% $O_2$ gas mixtures). The average increase in PP over the control level of 121 ± 9 mm Hg was 1.5 ± 1 (12.5% $O_2$), 14 ± 2 (10% $O_2$), 38 ± 6 (7.5% $O_2$) and 69 ± 15 (5% $O_2$) mm Hg (Fig. 32). These responses were eliminated by ganglionic blockade with hexamethonium (10 mg/kg i.v.). Mean systemic arterial pressure, which in control conditions was 71 ± 8 mm Hg, decreased during ventilation with 12.5 and 10% $O_2$ to 66 ± 6 and 65 ± 7 mm Hg, and increased during ventilation with 7.5 and 5% $O_2$ to 73 ± 7 and 83 ± 11 mm Hg. The hindlimb PP values during ventilation with 10% $O_2$, 7.5% $O_2$ and 5 % $O_2$+5% $CO_2$ were significantly greater than those during ventilation with 95% $O_2$ + 5% $CO_2$ ($P<0.01$). The magnitude of the hindlimb vasoconstrictor responses to ventilation with 5% $O_2$+5% $CO_2$ were also significantly greater than those observed on ventilation with 10% $CO_2$+90% $O_2$ ($P<0.01$). Sample records of the vasoconstrictor responses to ventilation with hypoxic gas mixtures in one experiment are shown in Fig. 33. Hindlimb perfusion pressure during ventilation with 95% $O_2$+5% $CO_2$ was 125 mm Hg. Subsequent ventilation with 12.5%, 10%, 7.5% and 5% $O_2$ plus 5% $CO_2$ in $N_2$ was associated with increases in hindlimb perfusion pressure to levels of 133, 147, 156 and 182 mm Hg respectively. Upon return to ventilation with 95% $O_2$ + 5% $CO_2$ hindlimb PP returned to close to the control value.

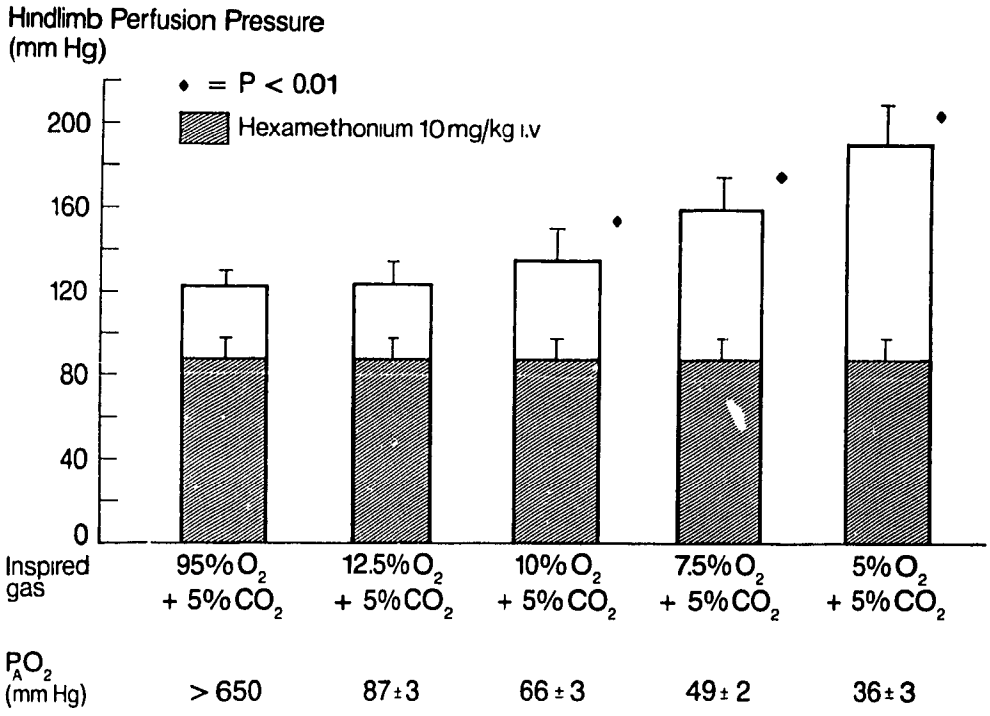


*Fig. 32.* Mean hindlimb perfusion pressure (PP) during ventilation of acute $C_1$ spinal cats with 5% $CO_2$ in $O_2$ (control) and with 12.5% $O_2$ + 5% $CO_2$, 10% $O_2$ + 5% $CO_2$, 7.5% $O_2$ + 5% $CO_2$ and 5% $O_2$ + 5% $CO_2$ in $N_2$. Data from 10, 12, 11 and 8 trials in 9, 8, 9, 9 and 6 animals respectively. $PaO_2$ values are indicated. $PaCO_2$ was 40 ± 2 mm Hg. Hatched areas indicate PP after the administration of hexamethonium 10 mg/kg i.v. (87 10 mm Hg, n=7). Bars indicate 1 SEM. Diamonds indicate a significant difference ($P<0.01$) in PP from that during ventilation with 5% $CO_2$+95% $O_2$

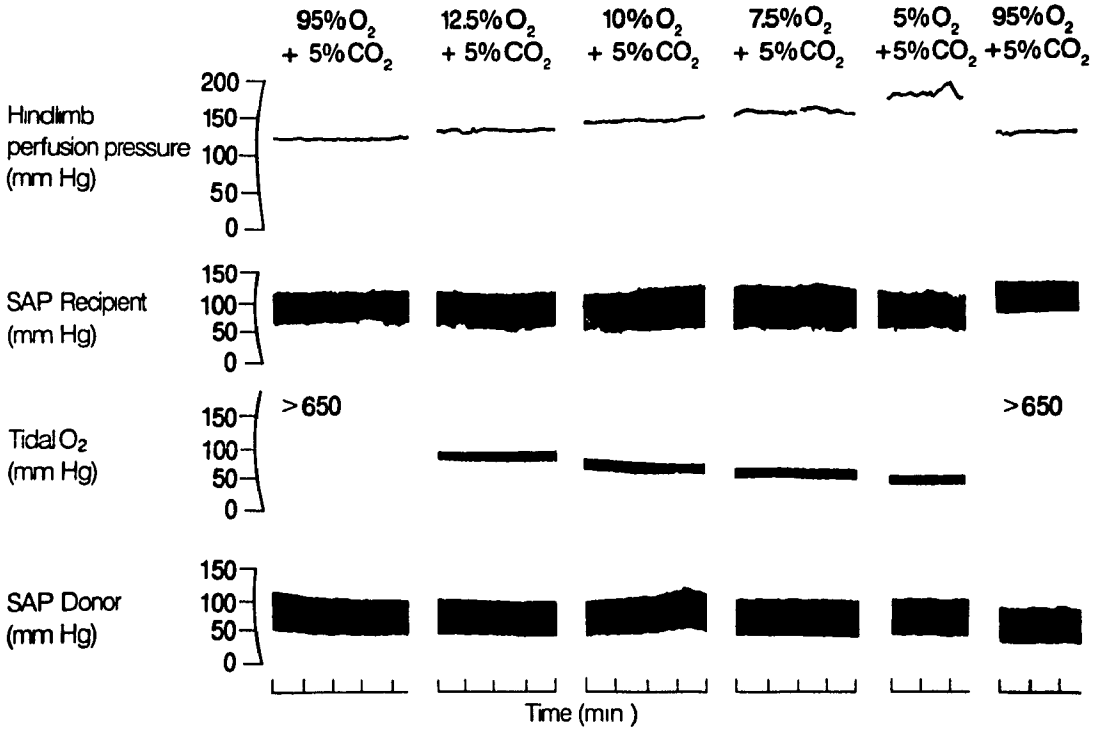


*Fig. 33.* Hindlimb perfusion pressure (PP) during ventilation of an acute $C_1$ spinal cat with 95 % $O_2$+5% $CO_2$ (control), with 12.5% $O_2$ + 5% $CO_2$, 10% $O_2$ + 5% $CO_2$, 7.5% $O_2$ + 5% $CO_2$, 5% $O_2$ + 5% $CO_2$ in $N_2$ and with 95% $O_2$ + 5% $CO_2$ (control). From top to bottom: recipient's hindlimb PP in mm Hg, recipient's systemic arterial pressure (SAP) in mm Hg, recipient's tidal $PO_2$ in mm Hg, donor's SAP in mm Hg. Time is indicated along the horizontal axis in minutes. The initial 1-2 min of ventilation with each gas mixtures are not shown

## DISCUSSION

These experiments have shown that systemic hypercapnia or hypoxia cause increases in vascular resistance in the normocapnic and normoxic hindlimb of the unanesthetized acute $C_1$ spinal animal. This result suggests that a significant number of vasoconstrictor sympathetic preganglionic neurons in the lumbar spinal cord are excited by these chemical stimuli.

It is improbable that the observed PP responses were caused by changes in collateral blood flow from the recipient, consequent to changes in the recipient's SAP during systemic hypoxia and hypercapnia, since the SAP changes in these experiments were small. In addition, under METHODS, experimental evidence was given for the absence of significant collateral circulation in this preparation. Also unlikely is that the observed PP changes were caused by an effect on the hindlimb vascular smooth muscle of hypoxic or hypercapnic arterial blood (Somlyo & Somlyo, 1970), supplied to the hindlimb by collateral channels, since the PP responses disappeared after ganglionic block. Central venous pressure in the recipient animals did not change during systemic hypoxia or hypercapnia and therefore did not contribute to the PP changes. Finally, it seems unlikely that the observed PP changes were due to vasoactive humoral substances released by the recipient animal during systemic hypoxia or hypercapnia since the return of hypoxic or hypercapnic blood from the recipient to the donor was without effect on the SAP of the latter animal. On the basis of these considerations and of the observation that the vasoconstrictor effects were eliminated by ganglionic blockade it would appear that the observed PP changes during systemic hypoxia or hypercapnia were neurogenic.

The mechanism of these spinal sympatho-excitatory responses is unknown. Both the afferent fibres from the carotid sinus-aortic arch chemoreceptors (Heymans &

Neil, 1958) and the more recently demonstrated abdominal chemoreceptors of Howe et al. (1981) travel in cranial nerves and do not enter the spinal cord to make reflex connections with sympathetic neurons. The observed sympatho-excitatory effects may alternatively be attributed to a direct action of oxygen lack and carbon dioxide excess on the excitability of sympathetic preganglionic neurons or of antecedent excitatory or inhibitory spinal interneurons. In this regard both depolarizing and hyperpolarizing actions of hypoxia and hypercapnia have been observed in various neuron types (Kolmodin & Skoglund, 1959; Chalazonitis, 1963; Krnjevic et al., 1965; Collweijn & Van Harreveld, 1966; Glotzner & Grusser, 1967; Papajewski et al., 1969; Brown & Berman, 1970; Hansen et al., 1982). Postulated mechanisms for the latter changes in membrane potential include effects on Na-K pump activity (Ritchie, 1973), generalized increases in membrane permeability (Segal, 1970), increased $K^+$ conductance (Hansen et al., 1982) and increased $Cl^-$ conductance (Brown & Berman,1970).

There are three reasons for our findings to be of physiological significance. First, the spinal mechanism described may have a role in arterial pressure homeostasis. During periods of inadequate spinal cord perfusion the ensuing tissue hypoxia and hypercapnia would generate an increase in sympathetic activity leading to an increase in systemic arterial pressure, and thus improved spinal cord perfusion. Thus, in low systemic arterial pressure states as after hemorrhage there may be a spinally-generated component in the sympathetic vasoconstrictor response. Second, such a sensitivity to nervous tissue hypercapnia and hypoxia may be responsible for the tonic activity of sympathetic preganglionic neurons observed after cervical spinal cord section and complete dorsal rhizotomy (Polosa, 1968; Mannard & Polosa, 1973). Third, this work indicates that the neurogenic cardiovascular response of the CNS-intact animal to systemic hypoxia or hypercapnia is likely to have a component of spinal origin.

# CHAPTER 5

## DISCUSSION OF METHODS

In the course of the experiments described in this thesis three types of preparation were used: anaesthetized CNS-intact preparations, unanaesthetized mid-collicular decerebrate preparations, and anaesthetized as well as unanaesthetized acute spinal preparations. Pentobarbital was used as an anaesthetic in the experiments on CNS-intact animals as well as in some of the acute spinal animals. This drug has been shown to decrease the magnitude of sympathetic reflex responses (Sato et al.,1965; Cox & Bagshaw, 1979; Zimpfer & Vatner ,1981) as well as the firing rate of sympathetic preganglionic neurons (Mannard & Polosa, 1973). However, the essential characteristics of both reflex sympathetic responses and background sympathetic activity does not appear to be changed by this anaesthetic (Sato et al .,1965; Mannard & Polosa, 1973; Cox & Bagshaw, 1979; Zimpfer & Vatner ,1981). In the experiments presented in this thesis the sympathetic preganglionic neuron (SPN) responses to hypoxia (CHAPTER 4, pp. 70 - 84) as well as the systemic arterial pressure (SAP) response to cerebral ischemia (CHAPTER 3, pp. 39 - 56) were similar in the pentobarbital anaesthetized CNS-intact animal and in the unanaesthetized mid-collicular decerebrate preparation. Also similar were the responses of SPNs in the spinal animal to hypoxia (Rohlicek & Polosa, 1981) and hypercapnia (CHAPTER 4, pp.112 - 123) in the presence or absence of pentobarbital. Thus it would appear that the use of pentobarbital did not play a major role in determining the type of sympathetic responses observed. Concerning the experiments performed on acute spinal animals, it is known that following spinal cord

transection there ensues a period of hypo-reflexia loosely defined as "spinal shock" (Guttman, 1976). The intensity and duration of spinal shock appears to vary with the animal species studied. Sherrington (1911) found that after spinal cord transection in the monkey the quadriceps stretch reflex could not be elicited for about one month whereas in the rabbit it was absent for only about 10 - 15 minutes. Chambers et al. (1966) have shown in the cat that ipsilateral flexion and crossed extension in response to pinching of the skin of the hindlimb skin can be elicited 3 - 4 hours after spinal cord transection. Reflex responses of sympathetic neurons to somatic and visceral afferent stimulation have been recorded in acutely spinalized cats after such an interval (Laskey et al., 1979). In the present experiments at least 3 to 4 hours elapsed between spinal cord transection and the onset of the experiment. Therefore it seems unlikely that spinal shock played a role in determining the results of experiments described here. In any case there is evidence that spinal sympathetic reflexes are less prone to depression following spinalization than somatic reflexes. In man, in whom spinal shock as judged from somatic reflexes may last several weeks (Guttmann 1976), certain autonomic reflexes such as inspiratory vasoconstriction in the upper extremities as well as increased SAP and bradycardia during urinary bladder distension have been demonstrated shortly after spinal cord injury at a time when no withdrawal or tendon reflexes were present (Silver, 1971).

The activity of the sympathetic system was observed using a variety of techniques. The variables recorded were systemic arterial pressure (SAP), hindlimb vascular resistance, firing rate of single or few unit strands of the cervical sympathetic trunk (CST), as well as mass activity of the CST. Various aspects of these methods of studying sympathetic activity are discussed below.

Changes in SAP under particular experimental conditions can be attributed to direct local actions, humoral effects, and the influence of changes in autonomic efferent

activity on the myocardium and peripheral vasculature. If direct and humoral actions prove not to be important then changes in SAP represent the sum of changes in autonomic efferent activity to many different cardiovascular effectors. Thus neurogenic changes in SAP give an overall measure of changes in autonomic nervous activity concerned with cardiovascular control.

In the study of ventral medullary mechanisms mediating the CIR (CHAPTER 3, pp. 39 - 66) the magnitude of the pressor response to cerebral ischemia was used as an index of sympathetic activation. As reviewed in the INTRODUCTION (pp. 10 - 14) previous work has shown that cerebral ischemia leads to sympathetic activation and to a pressor response (Downing et al., 1963; Levy et al., 1968; Takeuchi et al., 1969; Dampney et al., 1979). In the present experiments recording of SPN activity demonstrated increases in sympathetic neuron firing during cerebral ischemia (Fig. 24, p. 107). In addition the pressor response to cerebral ischemia could be markedly reduced or abolished by ganglionic blockade. A significant adrenal effect seems unlikely as previous work has shown that the pressor response to cerebral ischemia is not appreciably altered by adrenalectomy (Dampney et al., 1979). Occlusion of the common carotid and vertebral arteries to produce cerebral ischemia produces a mechanical increase in peripheral resistance. As discussed in APPENDIX B this passive effect may theoretically lead to an increase in SAP of up to 25%. However, the observation that in a number of cases the pressor response to occlusion of the common carotid and vertebral arteries was abolished by the application of local anaesthetic to the ventral medullary surface without any change in basal SAP suggests that the passive increase in peripheral resistance resulting from occlusion of the cerebral circulation may play less of a role in the pressor response to cerebral ischemia than theoretically predicted.

In the experiments described in the second paper of CHAPTER 3 (pp. 57 - 66) the increase in SAP during systemic hypercapnia in the CNS-intact and sino-aortic

denervated (SAD) animal was used as an indication of sympathetic activation by central chemosensitive mechanisms. As reviewed in the INTRODUCTION (pp. 27 - 28) previous work has shown that systemic hypercapnia in the SAD animal causes increases in SPN firing rate (Downing & Segal, 1963; Bower, 1975; Hanna et al., 1981) as well as neurogenic increases in hindlimb vascular resistance (Lioy et al., 1978). A significant part of the sympathetic excitation during systemic hypercapnia in the SAD animal is mediated by superficial chemosensory structures in the ventral medulla (Polosa 1983). The observation in the present experiments that superficial application of local anaesthetic to the ventral medulla significantly attenuated the increase in SAP during hypercapnia suggests that the increase was largely a result of activation of these ventral medullary chemosensory structures. The increase in SAP during systemic hypercapnia is not likely to have been the result of adrenal or direct actions on cardiovascular effectors resulting from systemic hypercapnia. Systemic hypercapnia can lead to release of adrenal catecholamines in various species (Tenney, 1956; 1960; Millar, 1960; Sechzer,1960; Cantu et al., 1966; Bloom et al., 1977; Rose et al. 1983). However previous work (Hanna et al., 1979) has shown that the peripheral vasoconstriction during systemic hypercapnia in the SAD cat is not decreased after adrenalectomy. It is well known that $CO_2$ has a direct depressant action on vascular smooth muscle and myocardium (Somlyo & Somlyo, 1970; Suutarninnen, 1966; Katz, 1977). However, such actions would tend to decrease SAP and lead only to underestimation of sympathetic activation by systemic hypercapnia.

More specific information about changes in autonomic nervous activity concerned with cardiovascular control than can be found from observing neurogenic changes in SAP can be obtained from monitoring neurogenic changes in performance of particular cardiovascular effector organs. As with changes in SAP, changes in the performance of cardiovascular effectors under particular experimental conditions can be thought of as being due to local, humoral, or neural actions on the effector organ. For

example measurement of perfusion pressure of a vascular bed under conditions of constant flow gives an indication of vascular smooth muscle activity in resistance vessels resulting from the combined effect of influences such as inherent myogenic tone, circulating humoral factors, local $PO_2$ and $PCO_2$, as well as sympathetic activity. By isolating the vascular supply and maintaining the composition of the perfusing blood constant the effects of changes in $PaO_2$ and $PaCO_2$ as well as the effects of changes in circulating humoral factors can be prevented. Changes in perfusion pressure eliminated by sympathetic ganglion blockade can be taken to represent mainly changes in the activity of the population of sympathetic neurons innervating a particular vascular bed although myogenic mechanisms may have a role in determining the magnitude of the change. This approach was used in the experiments described in CHAPTER 4 in which the neurogenic component of hindlimb vascular resistance was measured during systemic hypoxia and hypercapnia in CNS-intact SAD (pp. 85 - 97) or acute spinal cats (pp. 124 - 136). The details of the methods used and potential complicating factors, in particular the adequacy of arterial isolation of the hindlimb, the neurogenic nature of the responses seen, and the apparent absence of humoral effects are discussed in detail in CHAPTER 4 (pp. 87 - 89, 95 - 96).

Information concerning the types of neural responses generated by the sympathetic system under particular conditions and the neural substrate involved can be gained by recording efferent sympathetic activity. This approach was used in CHAPTER 4 (pp. 70 - 84, 98 - 123) where activity from the CST was recorded during systemic hypoxia, hypercapnia and cerebral ischemia. The identity of the fibres recorded from is discussed below.

It is likely that the activity recorded from the CST was that of SPNs for several reasons. The activity had characteristics similar to that described in previous studies. It decreased during arterial baroreceptor stimulation (Gerber & Polosa 1978), increased

during arterial chemoreceptor stimulation (Hanna et al., 1981), and persisted after sympathetic ganglion blockade with hexamethonium (Hanna et al., 1981; Rohlicek & Polosa, 1981). Experiments involving various nerve transections and subsequent axonal degeneration in the rat (Brooks-Fournier & Coggeshall, 1981) and cat (Foley & DuBois,1940; Foley, 1945) indicate that approximately 65-85% of CST fibres are SPN axons travelling rostrally. The remaining fibres, which travel caudally, consist mainly of sympathetic postganglionic axons with cell bodies in the superior cervical ganglion as well as of some sensory fibres with cell bodies in thoracic dorsal root ganglia. These sympathetic postganglionic axons and sensory fibres are not likely to be tonically active if, as in the experiments described in this thesis, the CST is sectioned proximal to the superior cervical ganglion and activity recorded from the central portion of the CST thus separating these fibres from their respective cell bodies and receptors.

The cell bodies of the SPN axons of the CST are found in the $T_1$ to $T_6$ spinal segments (Oldfield & MacLachlan, 1981). These SPNs innervate sympathetic postganglionic neurons of the superior cervical ganglion which in turn innervate vascular smooth muscle of the facial and nuchal skin, of the oropharyngeal and nasal mucosa, as well as of the underlying musculature; the piloerector fibres and sweat glands in these regions; the salivary glands; and smooth muscle of the eye. The CST contains both myelinated and unmyelinated SPN axons (Foley & DuBois, 1940; Foley 1945;Brooks-Fournier & Coggeshall, 1981). The proportion of these two fibre types has not been reliably determined anatomically. Jänig and Schmidt (1970) found that in a sample of 500 units recorded from sympathetic preganglionic axons in the CST of CNS-intact anaesthetized cats 72% of the fibres conducted in the myelinated range while 28% of the fibres conducted in the unmyelinated range. These authors found that in both myelinated and unmyelinated groups only 26% of fibres showed spontaneous activity. A similar proportion of tonically active SPNs was reported by Polosa (1968) while recording

from their SPN cell bodies in the thoracic spinal cord of CNS-intact anaesthetized cats. In spinalized animals the proportion of spontaneously active SPNs with axons in the CST is less than half of this value (Polosa, 1968; Mannard & Polosa, 1973).

# CHAPTER 6

# GENERAL DISCUSSION

## Summary of main findings and conclusions

The working hypothesis of this thesis is that there are chemosensitive mechanisms within the CNS which influence the activity of sympathetic neurons during conditions of decreased CNS perfusion and of CNS perfusion with hypoxic or hypercapnic blood. The basis for this hypothesis is the observation that cephalic perfusion of sino-aortic denervated (SAD) animals with hypoxic or hypercapnic blood causes sympathetic excitation (Downing et al., 1963; De Geest et al., 1965a; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985) and that there is a marked increase in sympathetic neuron firing during systemic hypoxia in the spinal preparation even after extensive spinal cord deafferentation (Alexander 1945). A central sympathetic chemosensitivity may further be inferred from the findings of changes in sympathetic activity during systemic hypoxia or hypercapnia in spinal animals as well as in CNS-intact animals after sino-aortic denervation and cervical vagotomy (see CHAPTER 4) since there are no known peripheral structures sensitive to $O_2$ lack or $CO_2$ excess with reflex actions on sympathetic neurons in such preparations. The experiments described in this thesis provide some insight into i) the possible location of central sympathetic chemosensitive mechanisms, and ii) their properties and the extent of their control over sympathetic neuron activity.

In regard to the location of such CNS chemosensitive mechanisms the investigation of the pressor response to cerebral ischemia (CIR) in the cat, described in CHAPTER 3 (pp. 39 - 56), indicates that this response is mediated by superficial regions

of the ventral medulla. Inactivation of the superficial regions of the ventral medulla was also found to abolish the pressor response to systemic hypercapnia in the CNS-intact animal with denervated arterial chemoreceptors (CHAPTER 3, pp. 57 - 66). Previous investigations have provided suggestive evidence that superficial regions of the ventral medulla provide chemosensory input concerning CNS $PCO_2$ and pH to sympathetic and respiratory neurons (Trzebski et al., 1970; Szulczyk & Trzebski, 1976; Hanna et al., 1979; Lioy et al., 1981; Loeschke, 1974; Schlaefke et al., 1981; Bledsoe & Hornbein, 1981; Loeschke, 1982; Millhorn & Eldridge, 1986). The depth from the ventral medullary surface of the structures mediating the CIR is consistent with the depth of the structures presumed to be involved in respiratory (Trouth et al., 1973a; Schwanghart et al., 1974) and in sympathetic ventral medullary chemosensitivity (Hanna et al., 1979). Thus these results support the hypothesis that the CIR is mediated by chemosensitive mechanisms in superficial regions of the ventral medulla. Since ischemia leads to tissue hypoxia in addition to hypercapnia and acidosis, the results described here also suggest that superficial regions of the ventral medulla are involved in the sympathetic excitation in response to brain hypoxia (Downing et al., 1963; De Geest et al., 1965a; McGillicudy et al., 1978) as well as to hypercapnia (Downing et al., 1963; De Geest et al., 1965a; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985). The observation that inactivation with a local anaesthetic of the superficial ventral medullary regions involved in the CIR was not associated with a large fall in SAP indicates that the structures in this region are not involved in generating a major component of vasomotor tone. This is consistent with the suggestion by Polosa et al. (1983) that central chemosensitive mechanisms are responsible for only a fraction of resting sympathetic activity. This observation also implies that the CIR is due to the excitation of specific chemosensitive structures rather than of the brainstem neurons generating sympathetic tone. The observations in CHAPTER 4 (pp. 112 - 136) of sympathetic excitation in the acute $C_1$

spinal cat during systemic hypoxia and hypercapnia suggest also a sympathetic chemosensitivity located within the spinal cord. This conjecture is supported by the findings of Alexander (1945) of marked increases in sympathetic neuron firing during systemic hypoxia in the spinal preparation even after spinal cord deafferentation and by the lack of known spinal cord afferents sensitive to the hypoxic stimulus (Alexander, 1945; Rohlicek & Polosa, 1981). The location and identity of the spinal cord structures mediating such a spinal sympathetic chemosensitivity is not known.

Concerning the properties of the central chemosensory mechanisms responsive to hypoxia and the extent of their control over sympathetic neurons the experiments in CHAPTER 4 (pp. 70 - 111) revealed a two-component response to systemic hypoxia in the CNS-intact SAD cat : i) an excitation at extreme levels of systemic hypoxia, and ii) a depression at less extreme levels of systemic hypoxia. The sympatho-excitatory response is likely to be of spinal as well as of supra-spinal origin. As for the spinal component, sympatho-excitation was previously described in thoracic sympathetic preganglionic neurons (SPN) of acute $C_1$ spinal cats (Rohlicek & Polosa 1981) and evidence of a neurogenic increase in hindlimb vascular resistance in response to hypoxia in the acute $C_1$ spinal animal has been described in this thesis (CHAPTER 4, pp. 124 - 136). A supra-spinal component is suggested by the work of other investigators demonstrating sympathetic activation during purely cephalic hypoxia (Downing et al., 1963; De Geest et al., 1965; McGillicudy et al., 1978) as well as by the pressor response and sympatho-excitation to cerebral ischemia reported in CHAPTER 3 (pp. 39 - 66) and 4 (pp. 98 - 111) which, as discussed above, is likely in part due to cerebral hypoxia. On the other hand depression of sympathetic activity by hypoxia appears to be mediated supra-spinally only and to consist of at least two components. One component has a lower hypoxic threshold (i.e. it appears at a higher $PaO_2$) and seems due to withdrawal of phasic facilitatory input to SPNs from brainstem inspiratory neurons since it parallels the hypoxic

depression of phrenic nerve discharge. A second component has a higher hypoxic threshold (i.e. it appears at a lower $PaO_2$) and is independent of changes in central respiratory activity. The observation that changes in the neurogenic component of hindlimb vascular resistance occurred during systemic hypoxia that were consistent with those found in recording from SPNs indicates not only that a large number of sympathetic neurons respond as those described in CHAPTER 4 (pp. 70 - 84) but also that these responses are significant in terms of cardiovascular control. Since both excitatory and depressant effects on SPN firing rate only occurred at $PaO_2$ values lower than 40 mmHg, nervous tissue $PO_2$ does not appear to play a role in the generation of sympathetic neuron activity when $PaO_2$ is in the physiological range. How the depressant and excitatory components of the sympathetic response to hypoxia interact is not known. The simplest hypothesis is that of an algebraic summation. The observation that systemic hypoxia causes a pressor response in the spinal animal (Rohlicek & Polosa, 1981), in which only excitatory responses are observed, whereas it causes a fall in SAP in the CNS-intact SAD preparation (CHAPTER 4, pp. 70 - 84), in which both excitatory and depressant responses are observed, is consistent with this hypothesis. Of considerable interest is also how these responses interact with the mainly sympatho-excitatory effects of the arterial chemoreceptors (Trzebski, 1983) to produce the overall sympathetic response to systemic hypoxia in the intact animal. In this regard Krasney and coworkers (Koehler et al., 1980; Krasney & Koehler 1977) have suggested that central sympathetic effects of hypoxia are particularly important in neurogenic cardiovascular adjustments during acute systemic hypoxia. Changes in sympathetic activity, resulting from CNS hypoxia, may modulate arterial chemoreceptor output through the sympathetic innervation of the carotid sinus (O'Regan & Majcherczyk,1983; Matsumoto et al., 1987). In addition, central sympatho-excitatory effects of hypoxia may explain the increased

pressor effect of carotid baroreceptor unloading during systemic hypoxia (Bagshaw et al. 1986).

Concerning the properties of the central chemosensory mechanisms responsive to hypercapnia and the extent of their control over sympathetic neuron activity it has been previously shown that systemic hypercapnia in the CNS-intact SAD animal produces sympathetic excitation (Downing & Segal, 1963; Bower, 1975) which is directly related to $PaCO_2$ over a wide range of $PaCO_2$ values (Hanna et al 1981). This excitatory effect has been attributed to supra-spinal actions mediated by superficial ventral medullary structures (Lioy et al., 1981). The experiments described here in the acute $C_1$ spinal cat (CHAPTER 4, pp. 112 - 123) show that a large fraction of SPNs are excited during systemic hypercapnia and increase their firing rate in proportion to the $PaCO_2$. A few SPNs in this preparation are unaffected by systemic hypercapnia. This excitatory response is significant in terms of cardiovascular control since during hypercapnia an increase was observed in the neurogenic component of hindlimb vascular resistance (CHAPTER 4, pp. 124 - 136). In contrast to the influence of hypoxia on sympathetic activity in the spinal animal the effects of changes in $PaCO_2$ extend over a wide range of values from normocapnia to severe hypercapnia.

### Mechanisms controlling SAP and the role of central sympathetic chemosensitivity

One question which arises is the role of central sympathetic chemosensitivity in the control of SAP. Adequate perfusion of the various body tissues is to a large extent achieved through regulation of SAP. This ensures that an adequate pressure head for tissue perfusion is maintained and that the blood flow needs of one tissue do not interfere with the blood supply to the others. Diverse controls of the circulatory system have evolved each with particular properties. Amongst these control systems are the long term control of extracellular fluid volume by the kidney, the more rapidly acting

vasoconstrictor effects of angiotensin II and the fast responding neural control of the circulation. Central sympathetic chemosensitive mechanisms may be seen as part of the latter control.

To a large extent long term control of SAP is achieved by renal control of extracellular fluid volume. This is primarily the result of changes in both NaCl and water excretion . Information concerning extracellular fluid volume is gathered by various intra-thoracic, arterial, renal, and hepatic receptors as well as CNS "osmoreceptors" (Seifter et al., 1986). Notable amongst these sensors are the atrial mechanoreceptors, the arterial baroreceptors and the juxta-glomerular apparatus of the kidney. These volume sensors affect renal function through humorally as well as neurally mediated actions on renal hemodynamics, glomerular filtration rate, and tubular function (Seifter et al., 1986). Important amongst the humoral mediators are the renin-angiotensin-aldosterone system, the posterior pituitary antidiuretic hormone vasopressin, and the recently described atrial natriuretic factor. The renal control of extracellular fluid volume is a very sensitive and powerful, but slow mechanism for control of SAP. Changes in extracellular fluid volume in response to changes in SAP can take hours to days (Guyton & Coleman 1967). Faster, although more limited, adjustments in circulating blood volume occurring over a period of minutes to hours may result from shifts in volume between the vascular and extra-vascular spaces (Guyton et al., 1951) as a result of interaction of capillary and interstitial hydrostatic and oncotic pressures as suggested by Starling (1896).

A more rapid control on SAP is produced by the vasoconstrictor action of angiotensin II. Formation of this substance is proportional to renin released by the juxta-glomerular apparatus of the kidney as a result of decreased renal perfusion pressure (Brenner et al., 1986). There are two important characteristics of this control of SAP. The first is that the release of renin by the juxta-glomerular apparatus is most sensitive to decreases in renal perfusion pressure in the range from 100 - 65 mmHg with little

additional secretion of renin as renal perfusion pressure falls below 50 mmHg (Cowley et al., 1971). The second is that at least 15 to 20 minutes are required for the vasoconstrictor effects of the renin-angiotensin system to reach a steady-state following a decrease in renal perfusion pressure (Cowley et al., 1971).

A faster acting control of SAP may be exerted by vasopressin through its peripheral vasoconstrictor effects. Vasopressin is released into the circulation from the posterior pituitary gland in response to information from CNS "osmoreceptors", arterial baroreceptors, atrial stretch receptors, and cardiac receptors with vagal afferents (Robertson & Berl, 1986). Plasma vasopressin levels are directly related to changes in plasma osmolality and show an exponential relationship to decreases in blood volume or SAP (Robertson & Berl, 1986). Vasopressin has a marked antidiuretic action (Seifter, 1986). In the absence of other renal, humoral, and neural SAP control mechanisms a control over SAP by vasopressin through its peripheral vasoconstrictor effect is also evident (see reviews: Cowley et al., 1983; McNeil, 1983; Bennett & Gardiner, 1985). For instance, Cowley et al. (1980) found that anaesthetized dogs whose spinal cord had been destroyed and who had been nephrectomized were largely able to compensate for a rapid fall in SAP from 100 to 50 mmHg over a period of 3 - 4 minutes. This compensation was attributed to a peripheral action of vasopressin since it was associated with a large increase in plasma vasopressin concentration and it could be almost completely abolished by prior administration of a synthetic analogue of vasopressin, likely acting as a competitive antagonist to vasopressin, or by decapitation. However, the role of the vasoconstrictor effect of vasopressin in compensating for decreases in SAP in the intact animal is not clear (Bennett & Gardiner, 1985).

The most rapidly acting SAP controls are the cardiovascular reflexes mediated by the autonomic nervous system. Examples of such reflexes are those initiated by the arterial baroreceptors and chemoreceptors of the aortic arch and carotid sinus as well as

by various cardiopulmonary mechanoreceptors. Changes in sympathetic and parasympathetic neuron firing rate as a result of activation or unloading of these receptors occurs within tenths of seconds (Spyer, 1982). Cardiovascular effector organ response to such changes is rapid. For instance the response of vascular smooth muscle to an increase in sympathetic neuron firing rate begins with a delay of about 0.5 seconds and then proceeds with a time constant of about 9 seconds (Rosenbaum & Race, 1968). The best studied cardiovascular reflex involved in SAP control is that initiated by the arterial baroreceptors. These receptors are slowly adapting mechanoreceptors which increase their firing rate with SAP (Milnor, 1980). During conditions of decreased systemic arterial pressure baroreceptor unloading causes an increase in total peripheral resistance and heart rate which tends to restore SAP (Heymans & Neil, 1958). While this reflex is fast and powerful its operating range is limited to the the range of SAP values from 50-200 mmHg (Sagawa,1983). Thus the arterial baroreceptors can produce rapid circulatory compensations for changes in SAP over a wide range of pressures but are of little use if SAP falls to below 50 mmHg. Cardiopulmonary mechanoreceptors have also been shown to initiate reflex increases in heart rate and peripheral resistance during systemic hypotension (Gupta et al., 1966; Öberg & White, 1970; Pelletier et al., 1971; Clement et al., 1972; Chen et al., 1978). However, the role of such reflexes in the control of SAP remains to be established. Arterial chemoreceptor afferents may also participate in SAP control. It has been known for some time that the firing rate of arterial chemoreceptor afferents is markedly increased when SAP or carotid sinus pressure is reduced below 50-60 mmHg (e.g. Landgren & Neil, 1951). Subsequent work has shown that chemoreceptor afferents in the carotid sinus nerves are relatively insensitive to changes in SAP above 60 mmHg but increase their firing rate at lower SAPs (Paintal, 1967; Lahiri et al., 1980). In contrast the firing rate of chemoreceptor afferent fibres in the aortic nerves shows a continuous inverse relation to SAP beginning at 180 - 80 mmHg and continuing

to 0 mmHg (Lee et al., 1964; Biscoe et al., 1970; Lahiri et al., 1980). Activation of the arterial chemoreceptors leads to increases in cardiac output, total peripheral resistance and SAP (Eyzaguirre et al., 1983). Thus, it would seem likely that the arterial chemoreceptors participate directly in SAP control although the importance of their role remains to be established. The pressor response to cerebral ischemia is a further example of a rapid sympathetic response to decreases in SAP. When SAP or cerebral perfusion pressure is reduced below 40 - 60 mmHg intracranial mechanisms are activated which lead to increases in sympathetic vasomotor activity (Sagawa et al., 1961). These pressures are below the autoregulatory range of the cerebral circulation and result in decreased cerebral blood flow and hence in relative CNS ischemia (Kuschinsky, 1978; Mchedlishvili, 1986). These pressures are also below the effective range of the arterial baroreceptor reflex. This suggests that a role for the chemosensory mechanism of the marked pressor response to cerebral ischemia may be that of rapidly compensating for very severe decreases in SAP which compromise CNS perfusion and which are beyond the range of other rapidly acting and powerful SAP control mechanisms such as the arterial baroreceptor reflex. The finding that extreme values of hypoxia or hypercapnia produce significant sympathetic vasomotor activity in the spinal animal suggests that a similar phenomenon may also occur at a spinal level. This was suggested earlier by Brooks (1933) who showed that chronic spinal animals were able to compensate for systemic hypotension produced by hemorrhage through neurogenic mechanisms. Thus the sympatho-excitatory effects mediated by central chemosensitive mechanisms may provide a direct and rapid link between the CNS environment and SAP control, both at a supra-spinal and spinal level, over a range of SAP which compromises CNS perfusion and in which other control systems are ineffective. The central sympatho-depressant effects of hypoxia described in CHAPTER 4 (pp. 70 - 111) of this thesis complicates the picture of such a compensatory role for central chemosensitivity and under some situations may

render it ineffective. An example of such a situation are the observations of failure of the peripheral vasoconstrictor response to hemorrhagic shock reported in the literature (Corazza et al., 1963; Gootman & Cohen, 1970; Lundgren et al., 1964; Rothe et al., 1963) (see CHAPTER 4, pp. 96 - 97).

**Mechanisms involved in central sympathetic chemosensitivity**

Another central question concerns the mechanisms mediating the responses of sympathetic neurons to CNS ischemia, hypoxia and hypercapnia. These responses might be the result of neuronal membrane and synaptic reactions to hypoxia and hypercapnia of SPNs or of neurons and pathways with input to sympathetic neurons. Alternatively a central chemosensitivity may be mediated through some specialized transduction mechanism with input to sympathetic neurons. Although relatively little investigation of this possibility has taken place the arterial chemoreceptors may provide a model for such a specialized mechanism.

**a) neuronal membrane and synaptic reactions to hypoxia and hypercapnia**

CNS hypoxia and hypercapnia have been shown to have depolarizing as well as hyperpolarizing effects on the resting neuron membrane. Similarly there is evidence that synaptic transmission may be depressed or enhanced during $O_2$ lack or $CO_2$ excess. The increased sympathetic activity observed under these conditions could therefore result from: i) depolarization of SPNs, ii) increased efficacy of sympatho-excitatory pathways as a result of enhanced synaptic transmission or depolarization of neurons in such pathways, and iii) decreased efficacy of sympatho-inhibitory pathways as a result of depressed synaptic transmission or hyperpolarization of sympatho-inhibitory neurons. Reciprocal effects could lead to decrease in sympathetic activity.

Intracellular recording from various neuron types, as well as measurements of nerve demarcation potentials in the earlier literature, have indicated depolarization during hypoxia (Lorenté de No, 1947; Lundberg & Oscarsson, 1953; Kolmodin & Skoglund, 1959; Collewijn & Van Harreveld, 1966; Eccles et al., 1966; Chalazonitis, 1963; Segal, 1970). Since the main source of energy for active Na-K pumping in nerve is oxidative phosphorylation (Ritchie, 1973), inhibition of this pumping by hypoxia may explain at least in part the neuronal depolarization during hypoxia although there is also evidence of a generalized increase in membrane permeability (Segal, 1970). In addition to neuronal depolarization, absence of membrane potential change (Nelson & Frank, 1963) as well as membrane hyperpolarization (Hansen et al., 1982; Glotzner, 1967) have been described. The neuronal hyperpolarization seen during hypoxia has been attributed to activation of a $Ca^{++}$ activated $K^+$ conductance (Hansen et al., 1982) as a result of increased free cytoplasmic $Ca^{++}$ during hypoxia (Hansen, 1985). Increased intracellular $Ca^{++}$ activity during hypoxia may result from decreased ATP-dependent $Ca^{++}$ extrusion, decreased $Na^+$-$Ca^{++}$ exchange driven by the $Na^+$ gradient, and decreased $Ca^{++}$ uptake by mitochondria and other cell organelles (Hansen, 1985). Increased free cytoplasmic $Ca^{++}$ may also lead to neuronal depolarization as a result of activation of slow, inward, non-specific cation currents (Yellen, 1982; Kramer & Zucker, 1985; Swandulla & Lux, 1985).

Neuronal depolarization as well as hyperpolarization during elevated $PCO_2$ have been reported in various neuronal cell types (Chalazonitis, 1963; Krnjevic et al., 1965; Papajewski et al., 1969; Brown & Berman, 1970; Carpenter et al., 1974; Mitchell & Herbert, 1974). Such changes in membrane potential may be the consequence of decreased ATP supply acting in similar ways as described above for hypoxia. Rates of ATP production may decrease during hypercapnia since glucose uptake and metabolism are inhibited by hypercapnia and the associated decrease in pH (Nahas,1974; Folbergova et al.,1975). In addition, an increase in $Cl^-$ and $K^+$ conductance may be responsible for

the neuron membrane potential changes during increased $CO_2$ as a result of changes in extracellular [$H^+$] (Brown & Berman, 1970; Brown et al., 1970; Walker & Brown, 1970). Working on *aplysia* abdominal ganglion neurons *in vitro* Brown and Berman (1970) found that the depolarization or hyperpolarization of these cells during hypercapnia was dependent on the associated decrease in pH of the bathing solution. Further investigations showed that elevation of [$H^+$] of the bathing solution led to a large increase in $Cl^-$ conductance and a smaller increase in $K^+$ conductance of these neurons (Brown et al.,1970). An increase in $Cl^-$ conductance would result in depolarization or hyperpolarization of individual neurons depending upon the value of their resting membrane potential relative to the $Cl^-$ equilibrium potential. An increased $K^+$ conductance would tend to hyperpolarize neurons. The effects on $Cl^-$ and $K^+$ conductance were attributed to an extracellular rather than intracellular action of $H^+$ since identical effects were observed when the bathing solution was acidified with $CO_2$ or a strong acid whereas increases in $PCO_2$ of the bathing solution were without effect when the pH of the solution was maintained constant. It is known that an increase in extracellular $CO_2$ has a rapid and marked effect on intracellular pH while an increase in extracellular $H^+$ has much slower and weaker effect on intracellular pH (Roos & Boron, 1981). An increase in intracellular [$H^+$], as a result of $O_2$ lack or $CO_2$ excess (Roos & Boron, 1981), may also be responsible for modifying the activation properties of the delayed rectifier $K^+$ channel as well as the activation and inactivation properties of the voltage-dependent $Na^+$ channel in giant squid axons (Moody, 1984). These effects may contribute to decrease neuronal excitability. The activation of mechanisms for the regulation of intracellular pH by increases in intracellular [$H^+$] may lead directly to neuronal depolarization. While the cytoplasm of most cells has considerable buffering capacity (Roos & Boron,1981) the observation that intracellular pH is more alkaline than predicted

from the equilibrium potential for $H^+$ suggests the existence of mechanisms for active extrusion of $H^+$ (Moody,1984). Investigations in invertebrates and vertebrates indicate that $H^+$ extrusion is due to a combined $Na^+$-$H^+$-$Cl^-$-$HCO_3^-$ exchange in a number of neurons (Thomas, 1977; Boron & Russel, 1983; Moser, 1985). In some neurons a $Na^+$-$H^+$ exchange independent of $Cl^-$ and $HCO_3^-$ is also present (Moody,1981; Schlue & Thomas, 1985; Chesler, 1987) . It is not clear whether ATP or the influx of $Na^+$ down its electrochemical gradient provides the energy for these processes (Moody, 1984) that appear to be electroneutral (Roos & Boron, 1981). Under conditions of restricted energy supply, such as may occur during hypoxia or hypercapnia, failure to extrude $H^+$ and/or to reduce the increased intracellular [$Na^+$] resulting from the extrusion of $H^+$ might result in intracellular accumulation of these cations and thus depolarization. A decrease in intracellular pH may also result in the activation of various ion conductances by $Ca^{++}$. Free intracellular [$Ca^{++}$] may increase during a fall in intracellular pH (Roos & Boron, 1981; Moody, 1984) possibly as a consequence of the exchange of $H^+$ for $Ca^{++}$ by mitochondria and/or other organelles (Roos & Boron, 1981). As outlined above increased free cytoplasmic $Ca^{++}$ may result in activation of outward $K^+$ currents leading to neuronal hyperpolarization as well as of slow inward, non-specific, cation currents leading to neuronal depolarization (Yellen, 1982;Hansen, 1985; Kramer & Zucker, 1985; Swandulla & Lux, 1985).

There is general agreement that severe hypoxia depresses synaptic transmission (Hansen, 1985). However, in some preparations synaptic transmission may be enhanced during hypoxia prior to depression (Eccles et al., 1966; Hubbard & Loyning, 1966). In the rat phrenic nerve-diaphragm preparation *in vitro* hypoxia blocks transmission at the neuromuscular junction by an effect primarily on nerve conduction (Hubbard & Loyning, 1966). Prior to block of transmission at this synapse end-plate

potential (EPP) amplitude and quantal content were seen to either increase or decrease. In the cat the spinal monosynaptic reflex is initially augmented during ventilation with 5% $O_2$ in $N_2$ or 100% $N_2$ and is completely suppressed after 3 - 4 minutes of ventilation with these gas mixtures (Eccles et al., 1966). These changes were associated with an initial increase, followed by a decrease, in excitatory post-synaptic potential (EPSP) amplitude and by increased primary afferent terminal excitability. Since motoneuron resting membrane potential decreased only slightly during hypoxia the depression of the spinal monosynaptic reflex was attributed to a presynaptic effect, possibly inactivation of the spike generating mechanism in the IA afferent terminals by excess depolarization. The initial increase in EPSP amplitude was attributed to an increase in transmitter release from the afferent terminals. Depression of synaptic transmission during hypoxia has also been observed *in vitro* in the rabbit superior cervical ganglion (Lemeignan et al., 1975) and rat hippocampus (Hansen et al., 1982).

Hypercapnia depresses synaptic transmission in a number of preparations while others seem particularly resistant to hypercapnia. In contrast, there is evidence that the increase in $[HCO_3^-]$ which accompanies hypercapnia may enhance synaptic transmission (Fukuda, 1984). Hypercapnia depresses the spinal monosynaptic reflex (Bradley, 1950; Carpenter et al., 1974; Esplin & Rosenstein, 1963). Spinal polysynaptic reflexes and transmission through the superior cervical ganglion are depressed to a lesser extent (Kirstein, 1951; Esplin & Rosenstein, 1963). In contrast, ventilation with 10% $CO_2$ was without effect on submaximal Renshaw cell responses to ventral root stimulation (Krnjevic et al., 1965). Hypercapnia produces simultaneous increases in $PCO_2$, $[H^+]$, and $[HCO_3^-]$. The effects of changes in $PCO_2$, $[H^+]$, and $[HCO_3^-]$ on synaptic transmission through the superior cervical ganglion have recently been investigated *in vitro* by Fukuda (1984). These experiments indicate that increases in $PCO_2$ or $[H^+]$ cause only small decreases in transmission through this ganglion. However,increases in $[HCO_3^-]$, while $PCO_2$ and pH

were kept constant, were found to be directly related to increases in transmission through the superior cervical ganglion. This suggests that during hypercapnia synaptic transmission may be depressed by the increased $PCO_2$ and $[H^+]$ and enhanced by the accompanying increase in $[HCO_3]$. The mechanisms responsible for the effects of hypercapnia, and of the associated changes in $[H^+]$ as well as $[HCO_3^-]$, on synaptic transmission are not known. A presynaptic effect is suggested by the finding of increased afferent terminal excitability in the cuneate nucleus of the cat during ventilation with 10% $CO_2$ in $O_2$ (Morris, 1971). This may reflect depolarization of afferent terminals which has been suggested to be associated with decreased transmitter release (Takeuchi & Takeuchi,1962) and with block of impulse conduction to the nerve terminals (Krnjevic & Miledi,1959)

**b) specialized chemoreceptor mechanisms**

Extensive investigation of the arterial chemoreceptors, whose afferent activity is directly related to decreases in $PO_2$ or increases in $PCO_2$ or $[H^+]$ of their arterial blood supply over a wide range of values, has led to several models of the transduction of changes in $PO_2$,$PCO_2$ and pH by specialized receptors. The anatomy of the arterial chemoreceptors has been well studied (see reviews: Eyzaguirre et al., 1983; Pallot, 1983; Eyzaguirre & Zapata, 1984). These sensory organs, located in the aortic arch and carotid sinus, consist of small groups of glomus cells (also known as Type I cells) surrounded by a few sustentacular cells (also known as Type II cells). Afferent fibre endings of the carotid sinus and aortic nerves lie in close proximity to the glomus cells. An extensive capillary network supplies these cellular elements. The rate of blood flow through these organs is very high (e.g. 45 - 95 ml/min/g in cat carotid body based on carotid body blood flow of 33 - 69 µl/min (Purves, 1970) and carotid body weight of 720 µg (Fidone et al., 1976)). The appearance that the glomus cells synapse onto afferent fibre endings has led to the hypothesis that these cells react to changes in $PO_2$ and

$PCO_2$ or $[H^+]$ by the release of neurotransmitter that leads to depolarization and firing of the afferent fibre endings (Eyzaguirre & Zapata, 1984). On the basis of data on content, synthesis, and turnover of putative neurotransmitter substances in the aortic and carotid bodies as well as of the action of different pharmacological agents on arterial chemoreceptor afferent activity it has been variously suggested that the neurotransmitter involved is acetylcholine, a catecholamine, serotonin, ATP, substance-P, enkephalin, or another neuropeptide (Eyzaguirre et al., 1983; Eyzaguirre & Zapata, 1984). However, despite much work proof of this hypothesis remains elusive. An interesting hypothesis by Paintal (1967) is that the sustentacular cells, which surround the afferent fibre endings and the glomus cells, undergo a conformational change with changes in $PO_2$ which distorts the afferent fibre endings thus initiating a generator potential. A slight modification of this hypothesis is that the release of acetylcholine by glomus cells during hypoxia causes contraction of the sustentacular cells with a resultant distortion of the afferent fibre endings and initiation of a generator potential in those endings (Jones, 1975). Torrance et al. (1977) have suggested that chemoreceptor afferent fibre endings are sensitive to $H^+$ and that they are located in a space between the glomus and sustentacular cells where pH is controlled by an as yet uncharacterized active transport mechanism of the sustentacular cells membrane which raises $[HCO_3^-]$ in that space. Inhibition of this transport mechanism by hypoxia or hypercapnia would result in increased $[H^+]$. The sensitivity of arterial chemoreceptors to uncouplers of oxidative phosphorylation has also led to the suggestion that during hypoxia a decrease in intracellular ATP may lead to a release of metabolites or neurotransmitters leading to activation of afferent fibre endings (Eyzaguirre et al., 1983; Eyzaguirre & Zapata, 1984). One manner in which decreased ATP supply might result in increased neurotransmitter release would be as a result of increased intracellular $Ca^{++}$ activity (Roumy & Leitner,

1977) in turn resulting from decreased ATP-dependent $Ca^{++}$ extrusion, decreased $Na^{+}$-$Ca^{++}$ exchange driven by the $Na^{+}$ gradient, and decreased $Ca^{++}$uptake by mitochondria and other intracellular organelles (Hansen, 1985). A decrease in ATP supply might also occur during increased $PCO_2$ or $[H^{+}]$ as a result of decreased glucose uptake and inhibition of key glycolytic enzymes due to the increased $[H^{+}]$ (Nahas, 1974; Folbergova et al., 1975).

**c) ventral medullary chemosensitivity**

The observation that inactivation of superficial ventral medullary areas blocks much of the central respiratory and sympathetic response to hypercapnia and acidosis in sino-aortic denervated preparations while local application of solutions high in $PCO_2$ or $H^{+}$ increases respiratory and sympathetic activity (Trzebski et al., 1970; Szulczyk & Trzebski, 1976; Hanna et al., 1979; Lioy et al., 1981; Loeschke, 1974; Schlaefke et al., 1981; Bledsoe & Hornbein, 1981; Loeschke, 1982; Millhorn & Eldridge, 1986) suggests a chemosensitive specialization within this region. Whether this presumed chemosensitivity of the ventral medulla is due to a particularly high sensitivity of neurons and synapses with respiratory and/or sympathetic function in this region to changes in tissue $PO_2$, $PCO_2$ or pH or rather to the presence of specialized chemosensory structures and mechanisms, such as those of the aortic and carotid bodies, is not known.

As is discussed in the INTRODUCTION (pp. 30 - 32) central respiratory chemosensitivity appears to be mediated by structures within 100 μm of the ventral medullary surface (Schwanghart et al., 1974) in a region between 2 and 6 mm lateral to the midline bilaterally extending caudally to the level of the XII nerve rootlets and cranially to the level of the foramen caecum (Loeschke, 1974; Schlaefke et al., 1981; Bledsoe & Hornbein, 1981; Loeschke, 1982; Millhorn & Eldridge, 1986). Cold blockade of structures within this region attenuated the sympathetic response to changes in inspired

$CO_2$ (Hanna et al., 1979). On the basis of subdural injections of acid artificial CSF Trzebski et al. (1974) have suggested that the structures mediating the central sympathetic sensitivity to $CO_2$ or $H^+$ are located more laterally than those mediating the central respiratory chemosensitivity to $CO_2$ or $H^+$.

The stimuli to which the ventral medullary chemosensory structures are sensitive remain unsettled. Loeschke (1982) postulates that a change in $H^+$ is the adequate stimulus. However, work by Harada et al. (1985) suggests a separate sensitivity to $H^+$ as well as $CO_2$. A sensitivity to decreases in tissue $PO_2$ may also exist. This is suggested by the observation described in CHAPTER 3 (pp. 39 - 66) of this thesis that inactivation of superficial regions of the ventral medulla with a local anaesthetic drug significantly attenuates the pressor response to cerebral ischemia.

Several investigators have provided evidence of neurons within the ventral medulla whose activity is influenced by $PCO_2$ or $[H^+]$. Extracellular recording from neurons close to the ventral medullary surface of cats has shown increases in firing rate during inhalation of $CO_2$ or during systemic acidosis produced by intravenous administration of HCl as well as when the pH of artificial CSF superfusing the ventral medullary surface was decreased (Pokorski et al., 1975; Pokorski, 1976; Schlaefke et al., 1978;Shimada et al.,1969). Investigations on slices of the rat medulla *in vitro* have demonstrated neurons whose firing increased with increases in $[H^+]$ of the superfusing solution (Fukuda & Loeschke, 1977 ). Whether or not such neurons are themselves sensitive to changes in $[H^+]$ or rather receive input from other chemosensitive structures within the ventral medulla was not established.

There is evidence to suggest that chemosensitive mechanisms of the ventral medulla involve a cholinergic step. This is suggested by the work of Fukuda and Loeschke (1979) who found that neurons close to the ventral medullary surface of the rat

medulla *in vitro* which were excited by decreases in pH of the superfusing medium were also excited by the addition of acetylcholine to this superfusate. In addition the $H^+$ response of these neurons was depressed by the muscarinic antagonist atropine or the nicotinic antagonist mecamylamine. The manner in which cholinergic transmission may be involved in the response of these neurons to changes in $[H^+]$ is not known. Schlaefke (1981) has suggested that changes in tissue pH may alter the sensitivity of certain neurons to acetylcholine, decrease the effectiveness of acetylcholine esterases, or increase the release of acetylcholine from terminals. Cholinergic mechanisms appear also to be involved in the sympathetic and respiratory chemosensitivity mediated by the ventral medulla in the intact animal. Work by Dev and Loeschke (1979a & b) suggests that cholinergic mechanisms are involved in the respiratory response to changes in $PCO_2$ mediated by ventral medullary structures since local application to the ventral medulla of muscarinic agonists increased respiratory activity while local application of atropine reduced this effect as well as the ventilatory response to inhalation of $CO_2$. The finding of Prabhakar et al. (1986) that the pressor response to cerebral ischemia is significantly attenuated by application of hexamethonium to the ventral medullary surface suggests that this response may also involve cholinergic mechanisms located in the ventral medulla

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**Role of the ventral medulla in sympathetic function and cardiovascular control**

The work presented in this thesis implicates regions of the ventral medulla in the supra-spinal chemosensitivity of sympathetic neurons to changes in CNS $PCO_2$ and $PO_2$ or to ischemia. This provides further evidence that the ventral medulla plays an important role in the function of the sympathetic nervous system and in cardiovascular control.

It has been known for some time that the brainstem has a key role in maintaining SAP (Owsjannikow, 1871; Dittmar, 1873; Alexander, 1946). Loeschke and Koepchen

(1958) found that application of the local anaesthetic procaine to the lateral recesses of the fourth ventricle produced a dramatic fall in SAP. On the basis of the close proximity of the lateral openings of these recesses (i.e. the foramen of Luschka) to the ventrolateral medulla this observation was taken to suggest a role of the latter region in SAP control. The involvement of structures in superficial layers of the ventral medulla was later indicated by the finding that cooling specific regions of the ventral medullary surface (Schlaefke & Loeschke, 1967) or the application of electrical (Loeschke et al., 1970) or chemical stimuli (Schlaefke et al., 1970) to these regions could elicit changes in SAP. As outlined in the INTRODUCTION (pp. 32 - 34) subsequent work has shown that a significant portion of the central sympathetic sensitivity to changes $PaCO_2$ is mediated by regions close to the ventral medullary surface. In addition to these chemosensitive regions two distinct bilateral areas of the superficial ventral medulla have been described which appear to exert opposite effects on SAP and which have distinct pharmacological properties. One of these areas is the so-called "glycine-sensitive" area. Feldberg and Guertzenstein (1972) originally demonstrated that application of pentobarbital through perspex rings to a region of the rostral ventral medullary surface located 1 - 2.5 mm caudal to the inferior border of the trapezoid body, lateral to the pyramids in the region of the hypoglossal nerve rootlets and rostral to the caudal extent of the inferior olivary nucleus (an area roughly overlying area "M" and part of area "S" of the chemosensitive zone (see INTRODUCTION, pp. 30 - 32)) produced a marked fall in SAP. Subsequently it has been shown that application of glycine, GABA, cholinomimetic drugs, anticholinesterases, and clonidine to this region as well as bilateral electrolytic lesion of the region also causes a decrease in SAP as well as in peripheral vascular resistance (Ciriello et al., 1986). Electrical stimulation within the "glycine sensitive" region or application of glutamate and kainate to this area increased SAP , peripheral vascular resistance and sympathetic nerve activity (Ciriello et al., 1986). The latter pharmacological stimulation has been taken to

indicate excitation of cell bodies rather than axons of passage since glutamate and kainate excite somadendritic but not axonal membranes (Fries & Zieglgansberger, 1974). A further region of the ventral medullary surface shown to influence SAP is the so-called "nicotine-sensitive" area. Feldberg and Guertzenstein (1976) showed that application of nicotine through perspex rings to a region of the caudal ventral medullary surface located just lateral to the pyramids approximately 6 - 9 mm caudal to the trapezoid bodies (a region roughly overlying area "L" and the caudal aspect of area "S" of the chemosensitive zone (see INTRODUCTION, pp. 30 - 32)) leads to a marked drop in SAP. Application of electrical stimuli or of excitatory amino acids to this region in cats and to corresponding regions in rats and rabbits has been shown to decrease SAP (Ciriello et al., 1986). Lesion of a corresponding region in the rabbit has been shown to result in an increase in SAP (Blessing et al., 1981) and in renal sympathetic nerve activity (Pilowsky et al., 1985). The data concerning these two areas suggest that the "glycine-sensitive" area represents a region providing excitatory input to sympathetic neurons while the "nicotine-sensitive" area represents a region with a depressor effect on sympathetic activity. There is evidence to suggest connections between these two region with the latter exerting an inhibitory effect on the former (Calaresu & Yardley 1988).

Further support for the view that the ventral medulla provides input to sympathetic neurons comes from the work of Barman and Gebber (1983) who have shown that neurons in the ventrolateral medulla located in the region of the nucleus reticularis paragigantocellularis have spontaneous discharge which is time-locked to rhythmic components of sympathetic nerve discharge. A number of studies using various neuroanatomical and electrophysiological methods have also demonstrated connections between the ventral medulla and regions of the spinal cord containing sympathetic neurons as well as between the ventral medulla and other brainstem regions known to be involved in cardiovascular control (Ciriello et al., 1986; Calaresu & Yardley, 1988). There is

also evidence that the ventral medulla contains portions of the baroreceptor, peripheral chemoreceptor, central chemoreceptor and somatosympathetic reflex arcs. Application of kainic acid, bicuculine or picrotoxin to the ventral medullary surface has been shown to attenuate or abolish the sympatho-inhibitory response to baroreceptor afferent activation by carotid sinus distension (McAllen et al., 1982; Yamada et al., 1984). During activation of baroreceptor afferents in the rat the uptake of radioactively labelled 2-deoxyglucose was increased in regions of the ventrolateral medulla indicating an increase in functional activity (Ciriello et al., 1983). Lesion of the lateral reticular nucleus of the ventral medulla has been shown to significantly attenuate the pressor response to electrical stimulation of peripheral chemoreceptor afferents in the carotid sinus nerve (Ciriello & Calaresu, 1977) and abolish the late component of the somatosympathetic reflex (Ciriello & Calaresu, 1977; Lebedev et al., 1984). As outlined in the INTRODUCTION (pp. 32- 34) it has been shown that superficial regions of the ventral medulla mediate a considerable portion of the sensitivity of sympathetic neurons to changes in $PaCO_2$ in SAD cats (Hanna et al., 1979; Lioy et al., 1981). Finally, experiments by Caverson et al. (1983a;1983b;1984) have shown that many of the neurons of the ventrolateral medulla which could be activated antidromically from regions of the thoracic spinal cord containing SPNs could also be activated orthodromically by selective excitation or unloading of the baroreceptors, as well as by electrical stimulation of the aortic and carotid sinus nerves.

### Role of spinal mechanisms in sympathetic function and cardiovascular control

The observations reported in this thesis that systemic hypoxia and hypercapnia have excitatory effects on sympathetic neurons and cause increases in the neurogenic component of hindlimb vascular resistance in the acute $C_1$ spinal animal provide further evidence that spinal mechanisms also play a significant role in sympathetic and cardiovascular control. It has been known for some time that after spinal transection some

sympathetic tone remains and that SAP can be further lowered by destroying the spinal cord (Tigerstedt, 1923; McDowall, 1933). Recording of sympathetic neural activity has also shown that some sympathetic neurons remain tonically active in the acute $C_1$ spinal preparation (Polosa, 1968; Mannard & Polosa, 1973). In addition, a number of reflex responses of sympathetic neurons have been demonstrated in spinal animals. Sherrington (1911) showed that stimulation of the central end of a hindlimb nerve in the chronic spinal dog produces a large rise in SAP. More recently Beacham and Perl (1964) showed that the background firing of SPNs of the upper thoracic and upper lumbar spinal cord could be modified by cutaneous stimuli in the acute spinal animal and that reflex discharge of these neurons could be elicited by electrical stimulation of dorsal roots, spinal nerves or limb nerves. There is also considerable evidence that stimulation of visceral receptors or afferents leads to sympathetic neuron and cardiovascular effector organ responses in the spinal animal. Stimulation of cardiac and vascular receptors or electrical stimulation of the central end of the inferior cardiac nerve in the spinal animal leads to increases in SAP, heart rate, and cardiac contractility as well as to changes in the firing rate of thoracic SPNs (Peterson & Brown, 1971; Malliani,1972; Malliani et al., 1973; Pagani et al., 1974). Mechanical stimulation of abdominal viscera or electrical stimulation of the central end of the splanchnic nerve increases SAP and modifies the firing rates of thoracic and lumbar SPN in acute spinal animals (Downman & McSwiney, 1946; Franz et al.,1966; Beacham & Kunze, 1969; Wyzogrodski & Polosa, 1972). Urinary bladder distension, spontaneous bladder contractions or electrical stimulation of the central end of the hypogastric nerve increases SAP and thoracic SPN firing rate (Mukherjee, 1957; Schondorf et al., 1981).

**Future Investigation**

The work presented in this thesis suggests opportunities for further investigation. Some specific areas which might be pursued are outlined below.

Concerning the localization of central sympathetic chemosensitive mechanisms the work described in this thesis indicates that the pressor response to cerebral ischemia is mediated by superficial regions of the ventral medulla. One question which arises concerns the exact location of structures mediating this response. While the experiments described in CHAPTER 3 (pp. 39 - 56) give information concerning the depth from the ventral medullary surface of such structures precise topographical information concerning their location is not provided since local anaesthetic was applied to the whole ventral medullary surface. Subsequent work by Prabhakar et al., (1986) has shown that inactivation, with local anaesthetic or by cold blockade, of restricted regions of the ventral medulla known to mediate the central respiratory chemosensitivity to $CO_2$ and $H^+$ does not attenuate the pressor response to cerebral ischemia while, as was found here, inactivation of the entire ventral medullary surface does. Further work involving inactivation of regions of the ventral medulla larger than those mediating central respiratory chemosensitivity but smaller than the entire ventral medullary surface are required to resolve this question.

The observation that the pressor response to cerebral ischemia is greatly attenuated by inactivation with procaine of superficial regions of the ventral medulla suggests that chemosensitive structures of the ventral medulla may be sensitive to $O_2$ lack as well as $CO_2/H^+$ excess. As discussed in the INTRODUCTION cerebral ischemia is accompanied by a decrease in tissue $PO_2$ as well as by an increase in $PCO_2/[H^+]$. In addition both cerebral hypoxia and hypercapnia have been shown to cause increases in sympathetic activity (Downing et al., 1963; DeGeest et al., 1965a; McGillicudy et al., 1978; Hainsworth et al., 1984; Ford et al., 1985). The possibility of a ventral medullary mediation

of the sympathetic responses to cerebral hypoxia has not been previously considered or tested. In this regard it would be interesting to determine whether superficial regions of the ventral medulla mediating the CIR also mediate the supra-spinal sympathetic excitation by hypoxia.

The identity and precise location of the ventral medullary chemosensitive structures influencing sympathetic and respiratory neurons is not known. Using the 2-deoxyglucose method of functional neuroanatomical mapping (Sokoloff, 1977) Ciriello, Rohlicek and Polosa (1985) were able to show in the sino-aortic denervated rat that restricted regions of the ventrolateral medullary reticular formation less than 100 μm from the brainstem surface were activated during systemic hypercapnia. It was suggested that these might represent the ventral medullary chemosensitive structures. More specific information might be obtained by studying the uptake of radioactively labelled 2-deoxyglucose during stimulation of the ventral medullary chemosensory structures with acidic and/or hypercapnic artificial CSF. Refinement of the autoradiographic technique to allow identification of individual cells activated might also provide further information concerning the structures involved in central respiratory and sympathetic chemosensitivity (Durham et al., 1981).

The work outlined in CHAPTER 4 (pp. 70 - 111) indicates that CNS hypoxia has both excitatory and depressant effects on sympathetic activity. The relative contribution of the previously demonstrated supra-spinal and spinal sympatho-excitatory effects of hypoxia (Downing et al., 1963; DeGeest et al., 1965a; McGillicudy et al., 1978; Rohlicek & Polosa, 1981) to the sympathetic excitation observed during systemic hypoxia in the CNS intact SAD animal is not clear. This question might be answered by comparing the relationship between $PaO_2$ and sympathetic activity in the SAD animal with that observed during cephalic perfusion with hypoxic blood while the spinal cord and trunk of the animal remain normoxic. The level of the brain at which such effects are mediated might also be

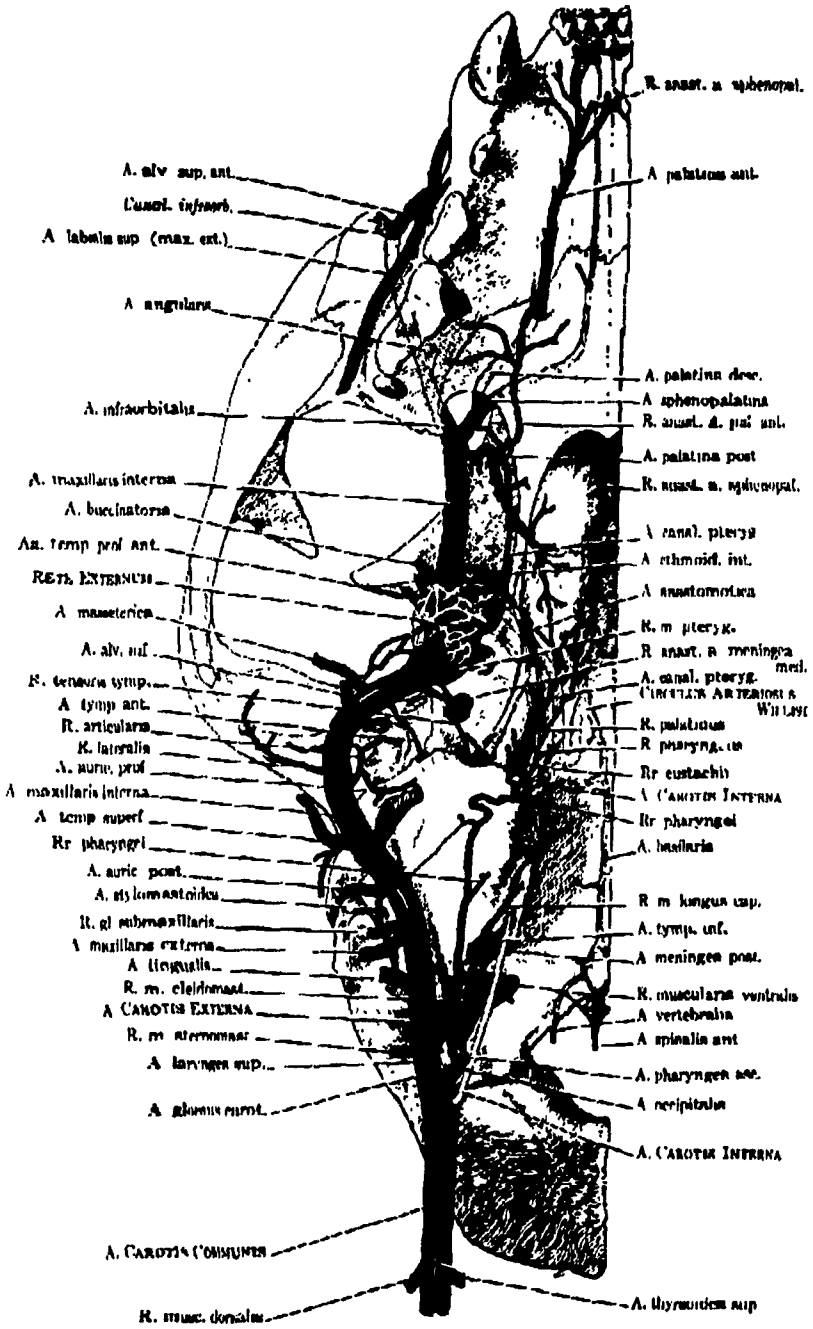
determined by serial transection of the neuraxis. Investigation of the uptake of 2-deoxyglucose during cerebral hypoxia might also suggest regions of the brain taking part in the sympathetic responses to hypoxia.

The observation that sympathetic preganglionic neurons in spinal animals are excited by $O_2$ lack and $CO_2$ excess raises the possibility of a chemosensitivity of these neurons themselves. Recent advances in intracellular recording from SPNs of *in vitro* spinal cord preparations (Yoshimura et al., 1986) suggest the possibility of such recording during hypoxia and hypercapnia. Through ionic or pharmacologic manipulation it might be possible to determine whether the increases in SPN firing rate are due primarily to increases in synaptic input or rather to effects on neuron membrane potential. In the latter case it might also be possible to determine the membrane phenomena, such as changes in various ion conductances, which are responsible.

# APPENDIX A

## ANATOMY OF THE CNS CIRCULATION

A knowledge of the anatomy of the cerebral circulation is important for understanding the limited potential for increases in blood flow through collateral channels during partial or complete occlusion of the main cerebral arteries. The anatomy of the cerebral circulation of the cat is considered here since this was the species used in the experiments described in this thesis. The carotid circulation in the cat has been described in some detail by Davis and Story (1943) (Fig. 34). Unlike in other species the internal carotid artery in the cat is vestigial and is usually not patent. The brain receives blood from the external carotid artery via two large anastomotic vessels connecting the internal maxillary artery (a branch of the external carotid artery) with the circle of Willis (the arterial circle at the base of the brain which joins the vertebral and carotid blood supplies) by way of the orbital fissure, and the foramen ovale, respectively, as well as via the ascending pharyngeal artery. An extensive extracranial and a smaller intracranial rete mirabile exist in connection with the anastomotic vessels through the orbital fissure (Fig. 35). In contrast to the carotid circulation the vertebral circulation of the cat is more similar to that of man and other mammalian species (Gillian, 1976). The vertebral arteries are branches of the subclavian arteries and enter the spinal canal with the first cervical nerves where they join to form the basilar artery on the ventral surface of the brainstem at the level of the XII nerve roots. The basilar artery gives rise to the two posterior cerebral arteries which form the posterior portion of the circle of Willis. On the basis of vessel size Davis and Story (1943) suggested that most of the blood supply to the circle of Willis is by way of the anastomotic vessels arising from the external carotid arteries with relatively less




Ventral view of the arteries of the head in the cat.

*Fig. 34.* Ventral view of the arteries of the head in the cat (Davis & Story 1943).

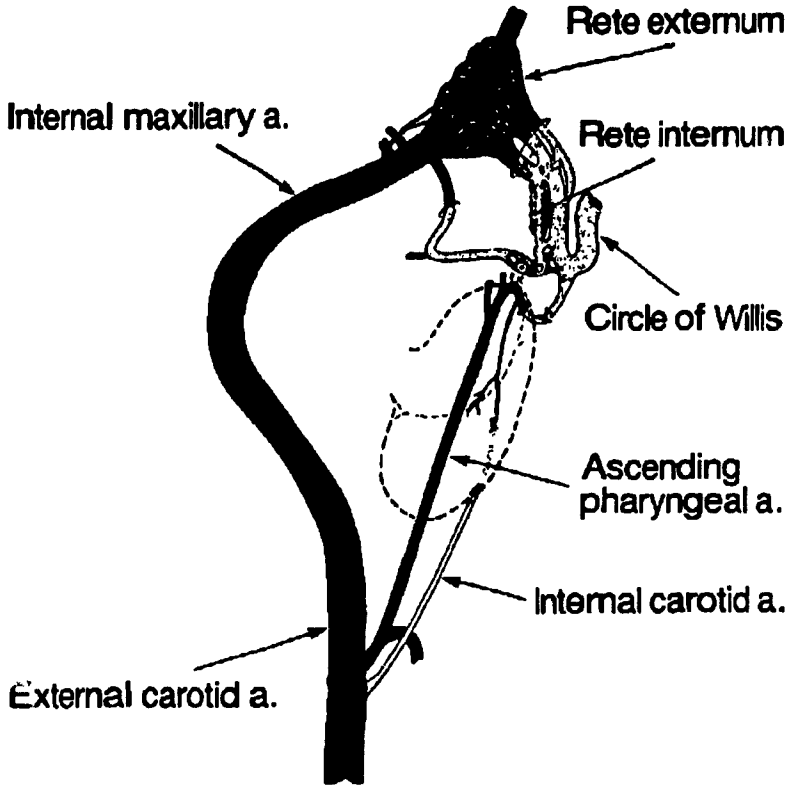



*Fig. 35.* Schematic diagram of the contribution of the external carotid artery to the brain circulation in the cat (Davis & Story 1943).

blood being supplied by the basilar artery. A significant anastomosis between the vertebral and carotid arteries, presumably by way of the circle of Willis, was demonstrated by Chungcharoen et al. (1952). Direct observation of blood flow in the basilar artery has indicated that under normal conditions the point where vertebral and carotid blood mix is usually in the upper basilar artery or in the communicating branches of the circle of Willis close to the basilar artery (Holmes et al., 1958). Upon partial obstruction of the vertebral arteries the point of junction moved caudally (Holmes et al., 1958). Injection of dye into the vertebral and carotid arteries has indicated that vertebral blood perfuses only the medulla oblongata, pons, and cerebellum while the cerebral hemispheres and thalamic regions are perfused primarily by carotid blood (Holmes et al., 1958, Berkenbosch et al., 1979). Injection of radioactive microspheres into the vertebral and carotid arteries has confirmed these findings (Wellens, 1975).

There has been no study of the anatomy of the spinal cord circulation in the cat. However, detailed descriptions of the blood supply to the spinal cord in man exist (e.g. Dommisse, 1975; Crock, 1977). Extensive similarities in the organization of the spinal circulation in rat and man (Woolam, 1955) suggest that similarities may also exist between man and other mammalian species. In man a pair of segmental or radicular arteries enter the spinal canal at every intervertebral level. These radicular arteries are branches of the aorta at lumbar, low, and mid-thoracic levels , branches of the subclavian arteries at cervical and upper thoracic levels and branches of the lateral sacral arteries at sacral spinal levels. In general, one particularly large radicular artery is present at a cervical, mid-thoracic, and lumbar spinal level. Branches of the vertebral arteries also supply blood to the spinal cord through the ventromedial anterior spinal artery and the two dorso-lateral posterior spinal arteries which run longitudinally along the length of the spinal cord. The segmental radicular arteries feed into these longitudinal anterior and posterior spinal arterial trunks.

# APPENDIX B

## PASSIVE HEMODYNAMIC EFFECTS OF OCCLUSION OF THE CAROTID AND VERTEBRAL ARTERIES

Although the pressor response to cerebral ischemia seems to be predominantly due to peripheral vasoconstriction (Downing et al., 1963; Takeuchi et al., 1969; Dampney et al., 1979) a component of the pressor response may be the passive increase in total peripheral resistance resulting from the occlusion of the carotid and vertebrai arteries. This becomes evident if one considers total peripheral resistance ($R_T$) to be comprised of the carotid and vertebral resistances ($R_{CV}$) and the parallel resistance of the remainder of the arterial system ($R_R$):

$$(1) \qquad \frac{1}{R_T} = \frac{1}{R_R} + \frac{1}{R_{CV}}$$

Upon occlusion of the carotid and vertebral arteries the resistance of their vascular beds becomes infinite. If the resistance of the remainder of the arterial system remains unchanged then total peripheral resistance during carotid and vertebral artery occlusion ($R_{T\,occl}$) becomes :

$$(2) \qquad \frac{1}{R_{T_{occl}}} = \frac{1}{R_R} \quad \text{or} \quad R_{T_{occl}} = R_R$$

Under conditions where flow (Q) is directly proportional to pressure (P) :

$$(3) \qquad P = Q \cdot R$$

As a result the passive increase in SAP upon occlusion of the carotid and vertebral arteries ($\Delta P$), assuming no change in total flow or cardiac output (Q) and in resistance of the remainder of the arterial system, is given by:

$$(4) \qquad \Delta P = Q\left(R_{T_{occl}} - R_T\right) = Q\left(R_R - R_T\right)$$

This increase in pressure relative to the original pressure is given by:

$$(5) \qquad \frac{\Delta P}{P} = \frac{Q\left(R_R - R_T\right)}{P}$$

If cardiac output (Q), SAP (P) and the fraction of cardiac output originally flowing through the carotid and vertebral arteries ($\chi$) are known then both $R_T$ and $R_{CV}$ can be obtained :

$$(6) \qquad R_T = \frac{P}{Q}$$

$$(7) \qquad R_{CV} = \frac{P}{\chi \cdot Q}$$

$R_R$ can be obtained from eq. 1 :

$$\frac{1}{R_R} = \frac{1}{R_T} - \frac{1}{R_{CV}}$$

$$= \frac{Q}{P} - \frac{\chi \bullet Q}{P}$$

$$= \frac{(1 - \chi)Q}{P}$$

therefore:

(8) $$R_R = \frac{P}{(1 - \chi)Q}$$

substituting $R_T$ and $R_R$ into eq. 5 :

(9) $$\frac{\Delta P}{P} = \frac{\left(\frac{P}{(1 - \chi)Q} - \frac{P}{Q}\right)Q}{P}$$

$$\frac{\Delta P}{P} = \frac{\frac{P}{(1 - \chi)} - P}{P}$$

$$= \frac{1}{(1 - \chi)} - 1$$

(10) $$= \frac{\chi}{1 - \chi}$$

Carotid flow in the cat has been found to be between 15.6% and 17.2% of cardiac output[1] (Borgdorf & van den Horn, 1980). Blood flow to the regions supplied by the vertebral arteries in the cat (Holmes et al., 1958; Berkenbosch et al., 1979) is between 1 and 3% of cardiac output (Berkenbosch et al., 1979). Thus from eq. 10 an increase in SAP of between 20 and 25% [2] would be expected on occlusion of the carotid and vertebral arteries if cardiac output and the resistance of the other vascular beds remain unchanged. Borgdorf et al. (1979) have compared the increase in peripheral resistance upon occlusion of the carotid arteries alone predicted on the basis of the reasoning outlined above and that observed in the cat with denervated carotid sinus baroreceptors The observed increase in peripheral resistance in four cats (+18%) was within 10% of the predicted value (+20%).

[1] Borgdorf and van den Horn (1980) report an average cardiac output of 260 ± 21 (SEM) ml/min in 7 cats weighing between 3 and 4.8 kg.

[2]

$$20\% = .20 = \frac{(.156 + .01)}{1 - (.156 + .01)} \qquad 25\% = .25 = \frac{(.172 + .03)}{1 - (.172 + .03)}$$

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