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THE ROLE OF DIAPHRAGMATIC AFFERENTS IN THE CONTROL OF BREATHING

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ABSTRACT

This thesis addresses the role of diaphragmatic afferents in the regulation of breathing. The activation of diaphragmatic thin-fiber afferents by bradykinin, a physiological substance produced during muscle contraction, caused a stimulation and a redistribution of inspiratory motor drive, and a decrease in inspiratory time. These afferents are also involved in the regulation of airway smooth muscle tone, since tracheal tension and lung resistance decreased during electrical activation of the phrenic nerve. To mimic *in vivo* activation of mechanoreceptive afferents, phrenic nerve stimulation was performed during inspiration or expiration. Similar increases in inspiratory motor drive and respiratory timing occurred during inspiratory and expiratory phrenic stimulation. In addition, vagal input potentiated the stimulatory effect of inspiratory phrenic, but not tibial, nerve activation. Prolonged activation of diaphragmatic thin-fiber afferents by isc.emia caused inspiratory motor drive to increase and then decrease to baseline values. These findings indicate either a depletion of neurotransmitter substance within afferent pathways, or the development of central inhibition of ventilatory drive. In summary, diaphragmatic thin-fiber afferent activity is an important modulating influence in the control of breathing.

RÉSUMÉ

Cette thèse traite du rôle des afférents diaphragmatiques dans la régulation de la respiration. L'activation de ces fibres minces par la bradykinine, une substance physiologique produite durant la contraction musculaire, a provoqué une stimulation et un redistribution de l'influx moteur inspiratoire, et un diminution de la durée de l'inspiration. Ces afférents sont aussi impliqués dans la régulation du tonus des muscles lisses des voies respiratoires, car la tension trachéale et la résistance pulmonaire s'amoindrissent lors de le stimulation électrique du nerf phrénique. Afin de simuler *in vivo* la stimulation des afférents mécanorécepteurs, le nerf phrénique fut stimulé durant l'inspiration ou l'expiration. Des hausses similaires dans la pulsion motrice inspiratoire et dans la durée respiratoire, se sont produites lors de la stimulation phrénique à l'inspiration et à l'expiration. Toutefois, la vagotomie a attenué quelques unes de ces variations, indiquant que l'influx vagal potentialise la stimulation de l'impulsion ventilatoire causée par l'activité phrénique afférente. L'activation prolongée des fibres minces afférentes du diaphragme par une ischémie, a causé une augmentation de l'influx moteur inspiratoire avant que celui-ci ne revienne à ses valeurs de base, indiquant une possible diminution de la substance neurotransmettrice au sein des voies afférentes. En résumé, l'activité des fibres minces diaphragmatiques afférentes est un important agent modulateur influençant le contrôle de la respiration.

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PREFACE

This thesis is divided into six chapters. Chapter 1 is a review of past and current literature regarding diaphragmatic and limb muscle afferent fibers. Chapters 2-6 are original papers which address the functional significance of diaphragmatic afferents. The bradykinin study described in Chapter 2 has been published in Respiration Physiology. The study in Chapter 3 has been reviewed, and is being revised in accord with the reviewers' suggestions. The studies in Chapters 4 and 5 are being prepared for journal submission. General conclusions drawn from the thesis projects are presented in Chapter 6, and a list of claims to originality follows this chapter. The appendices contain the SI unit equivalents and abbreviations used in the thesis.

Dr. G. Vanelli taught me the methodology involved with the isolated hemi-diaphragm preparation. Dr. S. Gottfried instructed me in the surgical technique used to prepare the isolated tracheal segment, and also provided advice during preparation of the manuscript. My advisors, Drs. Sabah N.A. Hussain and Sheldon Magder, are the senior authors on these studies.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTORY REMARKS

The gas exchange requirements subserving the body's metabolic processes are met by continual breathing movements of the thoracic and abdominal compartments. Ventilation must adapt to changing demands for oxygen delivery and carbon dioxide elimination. Sensory signals provide ongoing feedback to the central nervous system on the status of the organism. By integrating the sensory information from many systems, the central nervous system can modulate ventilatory output to meet the body's requirements.

The diaphragm has an important role as a primary muscle of ventilation. Optimal functioning of the diaphragm should be a key factor in the organization of the efferent signals producing ventilation. Sensory information conveyed by afferent fibers from the diaphragm inform the central nervous system of ongoing thermal, mechanical and metabolic changes in the muscle itself. Given the functional significance of the diaphragm as a ventilatory muscle, it is hypothesized that alterations in diaphragmatic sensory information should alter ventilatory drive. The four studies comprising this thesis address how the activation of diaphragmatic afferent fibers alters the regulation of breathing. To provide a context for this work, the remainder of this introduction will review current information concerning the anatomy and functional significance of diaphragmatic sensation.

1.1. AFFERENT FIBERS FROM SKELETAL MUSCLES

1.1.a. Afferent fiber groups and receptors

Muscular nerves are comprised of somatic efferent nerve fibers, somatic afferent nerve fibers, and visceral efferent and afferent fibers to vascular smooth muscle (145). The somatic afferent fibers found in skeletal muscle nerves are classified into four groups according to fiber diameter and conduction velocity (Table 1). Group I, the largest fibers, are further subdivided into two groups based on receptor endings. Group Ia are primary afferents from muscle spindles, and carry information about muscle length and the rate of change of muscle length from muscle spindles. Group Ib afferents arise from Golgi tendon organs and relay information about muscle tension (6). Group II fibers, the secondary endings of muscle spindles, carry information about muscle length. Group III fibers usually terminate in free nerve endings, but sometimes arise from Pacinian corpuscles, and are activated by mechanical, thermal or chemical stimuli. Group IV fibers are predominately activated by thermal and chemical changes within the muscle (96). Afferent fibers from groups III and IV are frequently referred to jointly as thin-fiber afferents. In addition, group IV afferents are sometimes referred to as C fibers.

Afferent Fiber Type	Myelinated?	Fiber Size (microns)	Conduction Velocity (m/s)
I	yes	12-20	70-120
II	yes	2-16	30-75
III	yes	1-5	3.6-30
IV	no	0.2-2	0.3-2

Table 1.1: Classification of afferent fiber groups (34,86,96)

Afferent nerve fibers may be stimulated by using mechanical perturbations, chemical substances, electrical stimulation, or by changing muscle temperature. Mechanical stimulation of group I and II fibers can be accomplished by vibrating or stretching the muscle (47). In addition, Paintal found that most of the group III fibers studied in the cat responded to strong pressure (101). In the dog, pressure in the receptive field of group III afferent fibers was associated with an initial high frequency dynamic response followed by a sustained lower frequency discharge (70).

Mechanical changes in muscle length and tension occur during muscle contraction, thereby activating large fiber (groups I and II) afferents. However, some thin-fiber (groups III and IV) afferents may be stimulated as well. For example, when static muscular contraction was caused by electrical stimulation of a lumbar ventral root, activity

increased in more than half of the thin-fiber afferents tested. Furthermore, group III afferents tended to fire early in the contraction, indicating a response to mechanical changes. However, an alteration in metabolic activity within the muscle seemed to be the stimulus for group IV afferent activity, since about 4 sec elapsed before group IV afferents began to fire (56). In contrast, Kniffki, Mense and Schmidt (62) were able to demonstrate both contraction sensitive ("ergoceptor") and chemically sensitive ("nociceptor") group IV fibers in the cat. Furthermore, Mense and Stahnke (95) and Kaufman *et al.* (61) have shown that muscle contraction in the presence of ischemia activated some thin-fiber afferents which were not activated by muscle contraction alone. In addition, Kaufman and Rybicki found that group IV afferents were more responsive than group III afferents to ischemic contraction (59,61). A recent study indicates that thin-fiber activity may be altered during mild exercise. In decerebrate cats, Pickar, Hill and Kaufman have demonstrated that group III afferents are activated during dynamic locomotion, elicited by limb swinging or by electrical stimulation of the mesencephalic locomotor region (104).

A search has been conducted for the metabolite produced which either causes or potentiates the activation of thin-fiber afferents during exercise. Mense has shown that bradykinin, serotonin, histamine and potassium activate skeletal muscle thin-fiber afferents while having little

effect on large fiber afferents (91,94). The injection of potassium to levels similar to that found during muscular contraction caused an initial activation of groups III and IV afferents which adapted within seconds (59). Rotto and Kaufman further showed that lactic acid and arachidonic acid will stimulate these afferents, with the latter effect due primarily to cyclooxygenase products such as prostaglandins and thromboxanes (110). In addition, arachidonic acid increases the activation of group III afferents during static contraction (111). Paintal found that the group III fibers which responded to strong pressure were also activated by sodium chloride, but not by asphyxia (101).

Kaufman *et al.* (55) compared the thin-fiber afferent activation profiles of capsaicin (a non-physiological substance derived from red pepper) and bradykinin (a physiological substance produced during muscle contraction). Capsaicin activated 71% of the group IV fibers and only 26% of the group III fibers tested, whereas bradykinin activated about half of the fibers recorded from each group (55). However, Mense found that bradykinin activated 46% of the group IV fibers and 71% of the group III fibers which were monitored (91).

Chemical activation of muscle spindles can be achieved with succinylcholine, which directly affects the spindle itself, while having no effect on the Golgi tendon organ (32).

When electrical stimulation is used to activate fibers, it is difficult to selectively activate only one fiber group. However, preferential activation of fiber groups can be achieved by several methods. Stimulation of fibers at intensities 2-5 times the motor or sensory threshold will activate primarily large fiber afferents, whereas stimulation at 20-40 times threshold will activate small afferent fibers (115). A technique known as anodal blockade can be used to block conduction in group I afferents (52). To block the activation of small afferent fibers, small amounts of procaine can be applied to the nerve (98). Lastly, cooling a nerve to 7 degrees C will block all myelinated axons, while a further reduction in temperature to 2-3 degrees C will block unmyelinated fibers as well (25). However, since there is a peripheral-to-central gradient of cooling or anesthesia, fibers will be differentially affected depending upon the location within the nerve.

Changes in temperature within the muscle will affect the activity of afferent fibers. Decreases in temperature depress large fiber activity, whereas increases in temperature have the opposite effect (92). Some thin-fiber afferents increase activity levels when temperature changes occur within 24-44 degrees C (36). These fibers tend to be unimodal, having no response to mechanical or chemical stimuli. In addition, some group IV fibers respond to large, noxious increases in temperature (97).

1.1.b. Projections of afferent fibers

The majority of somatic afferent nerve fibers enter the dorsal horn of the spinal cord through the dorsal root, the cell bodies being located in the dorsal root ganglion. However, both myelinated and unmyelinated afferent fibers have been demonstrated within the ventral root (9). The dorsal horn and intermediate zones of the grey matter of the cord is transversely divided into lamina. The dorsal horn contains laminae I-V (lamina I is the most dorsally located), and the intermediate zone contains laminae VI-VII.

Large fiber afferents enter the cord medially. Some synapse directly upon alpha-motoneurons in the ventral horn (mediating the monosynaptic stretch reflex), while others may synapse upon interneurons. In addition, collateral branches of large fiber afferents may ascend or descend within the dorsal columns of the white matter. Thin-fiber afferents enter the cord laterally, bifurcate within the white matter, and either ascend or descend one or two segments before terminating in the dorsal horn (87). Mense and Craig (93) used horseradish peroxidase to trace the terminations of afferent fibers originating in the gastrocnemius-soleus muscle. Large fiber afferent projections were located in laminae VI-VII, whereas laminae I-V contained the terminations of small fiber afferents (93).

Large fiber afferents comprise the dorsal column-medial lemniscal sensory system. Collateral branches of large fiber afferents ascend ipsilaterally in the dorsal column of the cord and synapse in the medulla. Afferent fibers originating in cervical and upper thoracic segments synapse in the nucleus cuneatus, and those from the lower thoracic, lumbar and sacral regions synapse in the nucleus gracilus. Secondary afferents then cross the midline and ascend contralaterally (as the medial lemniscal tract) to synapse in either the ventral posterior lateral (VPL) nucleus or the posterior nuclear group of the thalamus. A tertiary neuron projects to the somatosensory cortical region.

Thin-fiber afferents comprise the anterolateral sensory system. After ascending one or two segments, the primary afferent synapses upon an interneuron, which then synapses with a tertiary neuron. After crossing the midline, the tertiary neuron ascends in the anterolateral column of the white matter of the cord, and terminates in either the reticular formation of the lower brain stem or the thalamus. Afferents project from the lateral reticular nucleus in the reticular formation to the anterior lobe of the cerebral cortex, comprising the spinoreticulocerebellar pathway (100). Afferents in the spinothalamic pathway project upon three thalamic regions: the VPL nucleus, the posterior nuclear group and the intralaminar nuclei. Finally, a fourth neuron

projects from the thalamus to the somatosensory region of the cortex (7).

The reticular formation is involved in regulating states of arousal. Information carried by somatic afferents to this region probably play an important role in this function. The afferents projecting to the cerebellar cortex may be involved in the control of postural activity.

1.2 AFFERENT FIBERS FROM THE DIAPHRAGM

1.2.a. Diaphragmatic receptors

Morphologic studies have demonstrated very few (less than ten) muscle spindles in the diaphragm of the cat (18) and rabbit (13). Based on the descriptions of Winckler and Delaloye, the human diaphragm may contain more than ten spindles (144). In contrast, Hinsey (37) was unable to find any spindles in the cat diaphragm. Corda, von Euler and Lennerstrand (11) found that most of the proprioceptors were contained in the crural region of the diaphragm, and that, in contrast to other skeletal muscles, there were more Golgi tendon organs than muscle spindles. In addition, most of the muscle spindles became active during expiration, due to a lack of rhythmic fusimotor activation (which would cause intrafusal muscle fiber shortening during extrafusal fiber contraction). These are termed "passive spindles".

Another method used to determine the nature of diaphragmatic receptors was to measure afferent fiber

activation during mechanical perturbation. Stretching of the diaphragm activated ten fibers from slowly adapting stretch receptors within the left hemi-diaphragm (28). This was a much smaller number of fibers than had been found in other skeletal muscles, a fact also noted in a similar report by Yasargil (146). These fibers arose from both muscle spindles and Golgi tendon organs. Afferent fibers from rapidly adapting end organs were also activated during diaphragmatic stretching (11,28). These receptors, described by Glebovskii as encapsulated bulbous structures, are the pressure-sensitive Pacinian corpuscles. Afferents from these receptors were found to be evenly distributed across the diaphragm (18).

1.2.b. Phrenic nerve anatomy

The phrenic nerve is a muscular nerve, containing both sensory and motor fibers. In addition to diaphragmatic nerve fibers, the phrenic nerve contains fibers from the mediastinum and pericardium (65,112,143). The right phrenic nerve may also contain fibers originating in hepatic and subdiaphragmatic structures (66,143).

Motor fibers in the phrenic nerve provide the exclusive efferent innervation of the diaphragm (120,143). The phrenic nerve is composed of fibers arising from two to four spinal nerves (from the third to eighth cervical levels), depending on the species. In the cat, the costal diaphragmatic regions are innervated by fibers from the fifth cervical nerve, and

the crural diaphragm and areas around the central tendon are innervated by fibers from the sixth cervical nerve (20). Antidromic stimulation of the C5 phrenic root (when the dorsal roots are cut) causes inhibition of C6 phrenic efferent activity in the cat. This action, termed recurrent inhibition, is caused by the activation of Renshaw cells by alpha motoneuron collateral branches within the ventral horn of the spinal grey matter (77). In the rat diaphragm, the muscle fibers belonging to one motor unit are widely scattered, and the innervation ratio is relatively low when compared to other muscles (67).

In 1891, Ferguson (24) presented several observations supporting the notion that the phrenic nerve contained sensory fibers as well as motor fibers. Subsequently, Little and McSwiney (78) showed in the cat that diaphragmatic afferent fibers are carried by the phrenic nerve. Duron and Marlot (19) reported that, in the cat, there were 2.5 times more unmyelinated nerve fibers than myelinated fibers. By causing degeneration of somatic and sympathetic efferent fibers, Hinsey, Hare and Phillips (37) determined that a similar ratio of unmyelinated to myelinated fibers (3:1) existed in the cat diaphragm. The same ratio in other skeletal muscles is about 1:1.

Estimates of the percentage of myelinated sensory fibers in the cat phrenic nerve ranges from 10% (37) to 20-30% (17). This low number is due to the paucity of muscle spindles and

tendon organs in the diaphragm, compared to other skeletal muscles. It has also been estimated that unmyelinated fibers comprise 50-67% of all phrenic afferent fibers in the cat (37,109) and 50% of all phrenic afferent fibers in the rat (73).

1.2.c. Projections of phrenic afferents

Many of the projections of phrenic afferents are similar to the projections of afferents from other skeletal muscles. Using horseradish peroxidase labelling, phrenic afferent projections were found in laminae I-IV of the dorsal horn and in the fasciculus cuneatus in the rat (30). Electrophysiological studies have shown that groups I and II afferents project centrally to the external cuneate nucleus of the medulla (83). This finding was also demonstrated using fluorescent labelling of phrenic afferent neurons (74). In addition, thin-fiber phrenic afferents project to the lateral reticular nucleus of the medulla (80).

Projections have also been described to regions of the medulla associated with respiration, e.g. the Botzinger complex (125), and the dorsal and ventral respiratory groups (80,81,123). In addition, sensory evoked potentials have been recorded in the cerebellar cortex (82), and the sensorimotor cortex of the cat (2,14).

1.2.d. Spontaneous activity of phrenic afferents

Activity in afferent fibers with mechanoreceptive endings occurs in phase with respiration. Group Ib afferents

arising from Golgi tendon organs fire during inspiration (as tension increases) at a rate of 47 Hz. The activity of these fibers have been shown to increase to 61 Hz during abdominal compression (105). Group Ia and II fibers arising from "active" muscle spindles (with fusimotor innervation) fire during inspiration at 150 Hz (17). Fibers from passive spindles become active during expiration.

Thin-fiber afferent activity is minimal during quiet breathing, and tends to occur tonically and sporadically. The frequency ranges from 1-3 Hz in open chested cats (48) to 14 Hz in closed chested cats (31).

1.2.e. Afferent fibers from other ventilatory muscles

The distribution of receptor type and afferent fibers in non-diaphragmatic ventilatory muscles is similar to that of other skeletal muscles. For example, the intercostal muscles have many more spindles than the diaphragm (18), and more spindles than tendon organs (17). In addition, most of the spindles in the intercostal muscles have a fusimotor innervation, enabling the spindle to continue to fire as the extrafusal muscle is contracting. Differences in the number of active spindles has been proposed to be the mechanism underlying the presence of a load compensation (muscle length adjusting) reflex in the intercostal muscles, and the absence of such a reflex in the diaphragm (11).

Lastly, there are fewer spindles in the more caudal intercostal spaces. In addition, there is a decreasing

gradient in the density of spindles from the more superficial rib cage muscle, the external intercostal, to the deepest muscle, the triangularis sterni (18). The increased number of spindles may be related to the postural function of the intercostal muscles (16).

1.3 DIAPHRAGMATIC AFFERENTS: FUNCTIONAL SIGNIFICANCE

1.3.a. Effect on the cardiovascular system

Effects of limb muscle afferent activation

Investigators have long been interested in identifying the types of afferent fibers which are involved in the exercise pressor response. Most studies indicate that group IV fibers have an important role in this reflex. In these reports, afferents have either been activated directly, using electrical or chemical stimulation, or indirectly, by evoking muscle contraction.

In 1895, Hunt (41) suggested that somatic nerves contained depressor and pressor afferent fibers, since increasing the intensity of stimulation of a mixed nerve changed a depressor response into a pressor response. Similar results were also reported by Greene (33). Identification of the afferent fiber types responsible for each response was made by Gordon (29). In this study, anesthetic blockade of thin-fiber afferents during high intensity somatic nerve stimulation converted a pressor

response into a depressor response. This indicated that large fiber afferent activation led to decreases in blood pressure, while thin-fiber afferent activation elevated blood pressure. However, in another study, simultaneous measurements of dorsal root action potentials and blood pressure during limb muscle nerve stimulation revealed that significant depression of blood pressure only occurred when group III fibers were activated (50). Much higher stimulation intensities were required to produce a pressor response, indicating that group IV fibers are responsible for blood pressure elevation.

Several recent studies have used mechanical, chemical or electrical stimulation to investigate the effect of large fiber afferents on the cardiovascular system. Vibration of the triceps surae muscles in anesthetized or decerebrate cats caused no significant changes in mean arterial blood pressure or heart rate (89). Similarly, in paralyzed cats, no cardiovascular changes occurred during chemical activation of gastrocnemius large fiber afferents by succinycholine (139). However, Orani and Decandia (99) found that maximal activation of group I fibers by electrical stimulation of the gastrocnemius-soleus nerve significantly increased mean arterial pressure by 9 mmHg, while having no effect on heart rate or right intraventricular pressure.

To investigate the consequences of selective activation of thin-fiber afferents, chemical activation has been used.

Bradykinin activates groups III and IV afferent fibers, whereas capsaicin will activate predominantly group IV and a few group III fibers. Bradykinin injection into the hindlimb circulation caused a depressor response in anesthetized rabbits (130). These results may be due to the activation of group III afferents (50). Bradykinin activation of gracilis muscle afferents increased mean arterial presure, heart rate, cardiac output, left ventricular end-diastolic pressure and the maximal time derivative of left ventricular pressure (127). These authors subsequently showed that both bradykinin and prostaglandins play an important role in the development of the cardiovascular response to isometric contraction caused by ventral root stimulation (128).

Thin-fiber afferents can be activated by mechanical, thermal and chemical changes, and mechanical alterations alone may contribute to the development of the pressor response. This was demonstrated by Stebbins *et al.* (126), who showed that either passive stretch or external compression of a muscle caused about half of the pressor response observed during isometric contraction. This pressor response was eliminated by dorsal root section of afferent fibers.

The relationship between somatic afferent input and cardiovascular changes has also been investigated when muscle contraction is produced by nerve stimulation. Both blood pressure and heart rate increased during isometric muscle

contraction caused by ventral root stimulation in cats (90). These changes were eliminated by either dorsal root section or anesthetic blockade of thin-fiber afferents. In addition, anodal blockade of large fiber afferents did not alter the blood pressure and heart rate responses to muscle contraction. To determine if neural reflexes are involved in the early cardiovascular responses to exercise, the arterial and venous circulations were occluded both during and after muscle contraction. During occlusion, blood pressure and heart rate increased as soon as stimulation began, eliminating the possibility that cardiovascular changes were caused by humoral substances from the working muscle. When vascular occlusion was maintained after contraction had ceased, blood pressure remained elevated as heart rate fell to baseline levels. These results indicate that muscle chemoreceptor input is important in determining the blood pressure, rather than heart rate, response to exercise. In similar experiments in dogs, Tibes (135) found that cardiovascular changes during both static and dynamic exercise were due to the activation of thin-fiber afferents.

The magnitude of the pressor response which occurs during muscle contraction depends on the amount of contracting muscle mass as well as the tension generated (46). In addition, during both light and intense isometric contractions, ischemia will further augment the pressor response (129).

In addition to modulating blood pressure and heart rate, somatic nerve afferent stimulation also leads to a redistribution of cardiac output to various organs. During sciatic nerve stimulation, total blood flow and flow to the brain and skeletal muscles increased (23), and renal flow decreased (23,50). Similar, although not identical, changes occurred when capsaicin was injected into the hindlimb: resistance of the contralateral hindlimb, gut and renal vasculature increased (141). However, in another study, flow measurements showed that the introduction of capsaicin into a donor perfused hindlimb increased cardiac output and decreased renal flow, but had no effect (despite changes in resistance) on flow to the liver, spleen, brain, heart and skeletal muscles (12). Finally, high intensity sciatic nerve stimulation in vagotomized cats causes an inhibition of the baroreceptor reflex (68).

Thin-fiber afferents can alter sympathetic activity via spinal and supraspinal pathways (118,119). The changes in blood flow distribution which occur during somatic nerve stimulation indicate that supraspinal pathways must be involved in the reflexive changes observed in cardiovascular parameters.

Effects of phrenic afferent activation

Studies addressing the effects of phrenic afferents on the cardiovascular system have used electrical and chemical

stimulation. According to Kohrman, Nolasco and Wiggers (63), electrical stimulation of phrenic afferents dates back to 1868 when Kowaleski and Adamuk found that phrenic nerve stimulation evoked a pressor response. Similar findings were reported by Dingle *et al.* (15), who demonstrated that unilateral phrenic nerve stimulation increased mean arterial pressure by 22-38 mmHg in anesthetized dogs. In contrast, Greene (33) found that changes in blood pressure, heart rate and coronary blood flow during phrenic nerve stimulation were quite variable. To further investigate this reflex, Kohrman, Nolasco and Wiggers (63) performed bilateral phrenic nerve stimulation at different stimulation intensities and frequencies. At low stimulation frequencies the blood pressure fell, but when the stimulation intensity was sufficiently increased, reflex vasoconstriction led to a rise in blood pressure. Changes in heart rate were less pronounced than the alterations in blood pressure.

More recently, McCallister *et al.* (88) measured the compound action potentials produced by stimulating one phrenic nerve at different stimulation intensities. Blood pressure rose only when stimulation was sufficient to activate many group IV afferent fibers. Heart rate, however, remained unchanged at all stimulation intensities. Road, West and van Vliet (108) reported that high intensity stimulation of the left phrenic nerve caused mean arterial pressure to rise by 17%, in contrast to an increase of 23%

caused by gastrocnemius nerve stimulation. This response was eliminated by either section of the cervical dorsal roots or cooling of the phrenic nerve to 1-2 degrees C, indicating the participation of thin-fiber afferents in the reflex (108).

The activation of primarily group IV diaphragmatic afferents by capsaicin was investigated by Hussain *et al.* (42) using an *in situ*, vascularly isolated and innervated left hemi-diaphragm. Capsaicin injection into the left hemi-diaphragm increased mean arterial blood pressure and heart rate. In addition, there was a redistribution of cardiac ouput blood toward the brain and away from the left hemi-diaphragm, kidney and intestinal circulations. Neither cardiac output nor gastrocnemius (resting) muscle blood flow were altered. Furthermore, capsaicin injection into the circulation of a vascularly isolated gastrocnemius muscle evoked similar, but larger, cardiovascular changes.

In summary, activation of phrenic thin-fiber afferents elicits a pressor response and a redistribution of cardiac output. Similar to limb muscle afferent stimulation, these changes appear to be due to the activation of group IV phrenic afferents. The alteration of sympathetic motor drive probably occurs via both spinal and supraspinal reflexes.

1.3.b. Effect on the ventilatory system

A great deal of research has shown that afferent fibers originating in contracting muscle are intimately linked to the generation of exercise hyperpnea. Most studies have

shown that stimulation of large fiber afferents from skeletal muscle has a smaller effect on ventilatory drive than the activation of thin-fiber afferents. The next section of this thesis is comprised of a selection of studies supporting these findings.

Effects of limb muscle large fiber afferent activation

To investigate the effect of large fiber (groups Ia, Ib and II) afferents on breathing, studies have employed mechanical, chemical and electrical means to activate these fibers. The activation of muscle spindles by vibration or succinylcholine has either had no effect (38,89,139) or small effects (26,75) on ventilation. Electrical stimulation of large fibers can have a small but significant effect on ventilation. Senapati (121) found that low intensity electrical stimulation of a cut hindlimb nerve augmented ventilation by 6%. Similar findings were reported by Carcassi *et al.* (5), who stimulated motor nerves in the hindlimb of anesthetized cats, and simultaneously recorded compound action potentials from the dorsal root of the first sacral nerve. A fine gradation of stimulation of the nerve to the knee flexor muscles (posterior biceps and semitendinosus) caused either selective activation of predominantly Ia afferent fibers, or both Ia and Ib fibers. Ventilation increased by about 28% only when Ib fibers were activated, and this change was primarily due to an increase in tidal volume. In another study, stimulation of the

triceps surae nerve at intensities activating only large afferent fibers produced a 12% increase in minute ventilation (99). This response was eliminated by anodal blockade of the afferent fiber activity. In addition, since ventilatory stimulation occurred before the accompanying pressor response, the ventilatory response was not secondary to the cardiovascular response.

Larger changes in ventilation occurred when aortic and carotid sinus baroreceptor input was eliminated. Hussain *et al.* (44) maintained carotid sinus pressure constant during stimulation of the cut gastrocnemius nerve in vagotomized dogs. During stimulation at five times twitch threshold (activating primarily groups I and II afferents), minute ventilation increased by 65%, due to equal changes in breathing frequency and tidal volume.

Effects of phrenic large fiber afferent activation

Some studies have shown that activation of phrenic large-fiber afferents does not affect ventilatory drive. In 1965, Sant'Ambrogio and Widdicombe (117) suggested that the activation of mechanically sensitive receptors had no effect on eupneic breathing. However, Jammes *et al.*(49) reported that phrenic large fiber afferents may participate in the regulation of respiration during eupnea. These authors found that cold blockade of phrenic large fiber activity in anesthetized, vagotomized spinal cats (spinal cord severed at the eighth cervical level) led to a prolongation of phrenic

nerve firing and a slowing of respiratory frequency. A similar finding was that stimulation of phrenic large fiber afferents shortened the duration of phrenic nerve firing, indicating that large fiber afferents have a facilitatory effect on efferent phrenic discharge. However, ventilation did not change during unilateral phrenic nerve stimulation at 2.5-5 times twitch threshold in anesthetized dogs (108,140).

More recent studies have demonstrated that the activation of phrenic large fiber afferents can cause ventilatory inhibition. Cheeseman and Revelette (8) reported that, in low cervical spinal dogs, a small increase in diaphragm length (17% via abdominal compression) led to a 20% decrease in electromyographic activity of the crural diaphragm. A subsequent study indicated that this inhibitory response may be mediated by Golgi tendon organs, since the activity of these receptors increased by 30% during abdominal compression (105). In support of these findings, Speck and Revelette (124) have described a very short latency (5 milliseconds), ipsilateral phrenic-phrenic inhibitory reflex in cats, which involves the activation of large fiber afferents.

Mechanically sensitive afferents also appear to cause an overall inhibition of inspiratory motor drive. Paralysis of an innervated left hemi-diaphragm (which is both mechanically and vascularly isolated) led to an augmentation of electromyographic activity of the alae nasi, parasternal and

contralateral diaphragm muscles, as well as an increase in respiratory frequency (131). These findings may be caused by the elimination of sensory input from tendon organs or contraction-sensitive group III afferent fibers.

Effects of limb muscle thin-fiber afferent activation

A number of studies have indicated that limb muscle thin-fiber afferent activation significantly increases ventilation by augmentating and redistributing motor drive, and increasing respiratory frequency. Katz and Perryman (53) demonstrated that the ventilatory response to somatic nerve stimulation was modified by altering the intensity and frequency of stimulation. Stimulation of the distal end of a cut gastrocnemius-soleus nerve at 40 and 100 times motor threshold (intensities sufficient to activate thin-fiber afferents) increased minute ventilation by 18% and 24%, respectively (121). This augmentation of ventilation was significantly greater than that which occurred when only large fiber afferents were activated.

The fact that thin-fiber afferents are responsible for the increase in ventilation during ventral root stimulation was demonstrated by McCloskey and Mitchell (90), who showed that anesthetic blockade of groups III and IV fibers prevented the ventilatory response during isometric exercise. Tibes (135) subsequently confirmed that thin-fiber afferents were also responsible for the ventilatory changes caused by dynamic exercise. In addition, Kalia *et al.* (51)

demonstrated that the selective activation of unmyelinated (group IV) fibers led to an increase in ventilation.

The activation of thin-fiber afferents from skeletal muscles causes disproportionate changes in respiratory muscle activity. In a dog, stimulation of a cut sciatic nerve at 10 times threshold (for α-fiber activation) increased the electromyographic activity of the alae nasi, genioglossus and posterior cricoarytenoid muscles, while having no effect on the diaphragm. As stimulation intensity increased, electromyographic activity increased more in the upper airway dilator muscles than in the diaphragm (35). Slightly different results were reported by Hussain *et al.*(44) in a vagotomized dog when carotid sinus pressure was kept constant. Activities of the alae nasi, parasternal intercostal, transversus abdominis and diaphragm muscles increased similarly during gastrocnemius nerve stimulation at 10 times twitch threshold, while genioglossus activity increased to a greater degree. At a higher stimulation intensity, genioglossus and transversus abdominis activities increased disproportionately to the other muscles, and triangularis sterni activity increased slightly (but less than the increases occurring in diaphragmatic activity). The overall increase in ventilation was caused by an increase in respiratory frequency as well as tidal volume. Discrepant results from the Haxhiu and Hussain studies may be due to differences in anesthesia, or vagal (136) or baroreceptor

influences (116).

Road, West and van Vliet (108) demonstrated that anesthesia has an effect on the relative contributions of volume and frequency changes to increases in minute ventilation. In pentobarbitol anesthetized dogs, high intensity stimulation of the gastrocnemius nerve caused increases in respiratory frequency, whereas in animals anesthetized with alpha-chloralose, both tidal volume and breathing frequency increased during nerve stimulation. Chemical activation of gastrocnemius muscle thin-fiber afferents by capsaicin also caused increases in inspiratory muscle electromyographic activity and respiratory frequency in anesthetized dogs (43).

Eldridge *et al.* (21) investigated the influence of supraspinal activity on the response to limb muscle afferent stimulation in paralyzed cats. In intact cats, pinching or stretching a limb muscle caused a decrease in phrenic nerve activity for several seconds, followed by an increase in activity for the remaining minute of stimulation. However, after high cervical cordotomy, phrenic nerve activity decreased during muscle stimulation. This indicates that limb muscle afferent stimulation leads to spinal inhibitory and supraspinal facilitatory influences on phrenic motor drive. The spinal inhibition appears to be mediated by the neurotransmitter glycine (22).

In a similar study, Kumazawa and Tadaki (71) reported that electrical stimulation of the gastrocnemius nerve at 10-50 times twitch threshold caused an initial inhibition of phrenic nerve activity in the cat. (These authors noted that this response was never seen during similar experiments in dogs.) However, stimulation at 100 times twitch threshold caused 1) a facilitatory response during the stimulation, and 2) an inhibition of phrenic nerve activity to below baseline levels commencing one to two minutes after cessation of the stimulus. This post-stimulus inhibition of ventilation was also described in cats by Waldrop, Eldridge and Millhorn (137) following tibial nerve stimulation at intensities sufficient to activate all afferent fiber groups. Full development of this response required the presence of the cerebellum and suprapontine regions of the brain. Kumazawa *et al.* (72) confirmed the presence of this post-stimulus inhibition after high intensity stimulation in dogs. The neurotransmitters involved in this response are endogenous opiates (69,138) and gamma-aminobutyric acid (138).

Effects of phrenic thin-fiber afferent activation

Similar to what has been reported during limb muscle thin-fiber activation, most studies have shown that the activation of phrenic thin-fiber afferents stimulates ventilatory drive. Several studies, however, have reported inhibitory effects.

Excitatory effects on ventilatory motor drive

In 1947, Khorman (63) described that bilateral stimulation of cut phrenic nerves caused an increase in the depth and, usually, the frequency of respiratory movements. Although the level of stimulation was not quantitated, the large blood pressure response obtained indicates that the intensity was adequate to activate all afferent fiber groups. Similarly, Road, West and van Vliet (108) found that unilateral phrenic nerve stimulation at high intensities increased both tidal volume and frequency (under alpha-chloralose anesthesia). These responses disappeared when thin-fiber afferent activation was blocked by cooling the nerve. Selective activation of diaphragmatic thin-fiber (predominantly group IV) afferents by capsaicin has also been shown to increase tidal volume, breathing frequency, and diaphragmatic activity in anesthetized, vagotomized dogs (43,106).

Changes in the pattern of respiratory muscle activation in response to phrenic thin-fiber afferent activation were assessed by several studies. In anesthetized, vagotomized dogs, arterial injection of capsaicin into a vascularly isolated left hemi-diaphragm was associated with increased electromyographic activities of the left and right diaphragm, parasternal intercostal and mylohyoid muscles (43). To eliminate influences from aortic and carotid baroreceptors, carotid sinus pressure was maintained constant during

electrical stimulation of a cut phrenic nerve in vagotomized dogs (140). At high intensities of stimulation, electromyographic activities of the alae nasi, parasternal and contralateral diaphragm increased similarly, while the genioglossus and transversus abdominis activities increased to a greater extent. Triangularis sterni activity, however, did not change.

Capsaicin is a nonphysiologic substance which, when injected into the vasculature of skeletal muscle, causes large, sudden changes in thin-fiber afferent firing, local vasoconstriction and severe increases in systemic blood pressure (55). Use of a natural stimulant of afferent fibers should approximate more closely the consequences of fiber activation under physiologic conditions. **I injected bradykinin (a substance produced in muscle during exercise) into an vascularly isolated left hemi-diaphragm to assess the effect of diaphragmatic thin-fiber activation on inspiratory motor drive. These results are presented in Chapter 2.**

Ischemia has been shown to activate thin-fiber afferents and inhibit the firing of large fiber afferents. A vascularly isolated, innervated, left hemi-hemidiaphragm was made ischemic for twenty minutes, while changes in inspiratory motor drive were monitored (132). Alae nasi, parasternal, right and left hemi-diaphragmatic electromyographic activity increased from 30-70% above baseline, and respiratory frequency increased by 30%. These

changes were due to the activation of thin-fiber afferents by ischemia (133).

Inhibitory effects on ventilatory motor drive

Only one study has been reported which describes the effect of phrenic thin-fiber afferent activity on eupneic breathing. Jammes *et al.*(49) found that anesthetic blockade of thin-fiber phrenic activity did not alter phrenic efferent activity in anesthetized, vagotomized, low cervical spinalized cats. Since these afferents have supraspinal projections, further research using intact animals needs to be done to assess this issue. Electrical stimulation of thin-fiber phrenic afferents was associated with a decreased frequency (inhibition) of phrenic motoneuron firing. These authors hypothesized that group IV afferents were responsible for these results. Speck and Revelette (124) also demonstrated in cats the presence of an ipsilateral and contralateral inhibition of phrenic efferent activity. However, the response latencies underlying this reflex indicate that group III afferent fibers were involved. Both spinal and supraspinal reflexes are implicated in this response (85). The presence of a contralateral inhibitory reflex is further supported by the finding of Gill and Kuno (27) that stimulation of a phrenic nerve root caused hyperpolarization of many contralateral phrenic motoneurons.

Marlot, Macron and Duron (84) reported the effects of activating myelinated (groups I, II and III) afferents from

the fifth and sixth cervical branches of the phrenic nerve. Ventilation was initially inhibited for 2-3 respiratory cycles, as shown by decreases in tidal volume and inspiratory time. This brief inhibition was followed by a facilitation of ventilatory drive, characterized by an increase in tidal volume and respiratory frequency, and a decrease in inspiratory time. This biphasic response is similar to that reported during limb muscle afferent stimulation in cats (21,71). However, Marlot, Macron and Duron (84) found that gamma-aminobutyric acid (rather than glycine) was involved in the initial inhibitory response during phrenic nerve stimulation.

It is possible that thin-fiber afferent activity is an important regulator of respiratory muscle function during diaphragmatic fatigue. However, there is very little literature which addresses this question. When diaphragm fatigue was produced by thirty minutes of direct muscle stimulation, large fiber afferent activity decreased while thin-fiber afferent activity increased (48). During the fatigue trial, respiratory frequency and phrenic efferent firing rate decreased. Since these changes did not occur when fatigue was induced after phrenic nerve section, the changes in afferent fiber activity are implicated in the ventilatory inhibition.

In the preceding study, artificial stimulation to used fatigue the diaphragm, and fatigue was maintained for a short

period of time. Ischemia is a more physiologic stimulus which can be used to activate thin-fiber afferents for long periods of time. **As described in Chapter 5, I occluded circulation to the left hemi-diaphragm for three hours, to assess the effect of prolonged activation of thin-fiber afferents by ischemic fatigue on inspiratory motor drive.**

Lastly, Road, Osborne and Wakai (107) demonstrated the presence of post-stimulation inhibition following phrenic nerve stimulation. High intensity stimulation of a cut thoracic phrenic nerve was performed for one minute. Phrenic nerve activity fell to 19% of baseline approximately nine minutes after the cessation of stimulation, and returned to baseline values within thirty minutes. These changes were similar in magnitude and duration to the post-stimulus inhibition following gastrocnemius nerve stimulation.

1.3.c. Effect on lung resistance

In addition to increasing the motor drive to ventilatory muscles, exercise hyperpnea is usually associated with a significant reduction in lung resistance. Sensory information from working muscles (ventilatory and nonventilatory) may be involved in reflexes leading to these alterations in the control of ventilation.

The effect of skeletal muscle afferent activation on bronchomotor tone has been assessed by measuring total lung resistance or alterations in the tension or volume of an innervated *in situ* tracheal segment. Resistance measurements

in intubated animals indicates intrathoracic changes in the resistance to airflow. Monitoring of the tracheal segment reveals alterations in smooth muscle of a large, extrathoracic airway which contributes to total respiratory system resistance in the intact state. In these studies, afferent activity has been changed by either direct chemical or electrical stimulation, or by eliciting muscle contraction.

Trachealis muscle tension

The trachealis muscle is innervated by the superior laryngeal nerve and the recurrent laryngeal nerve (64,76). Activation of these nerves causes trachealis muscle contraction, and stimulation of sympathetic nerves will cause bronchodilation (4). Trachealis muscle relaxation can also be caused by a nonadrenergic pathway in cats and humans (45), but this pathway is not present in dogs (113).

Two *in situ* preparations have been used to assess changes occurring in the tracheal segment of the airway. In one method, a balloon is inserted into the trachea just superior to the site of a tracheostomy through which the anesthetized animal is being ventilated. Pressure changes within the balloon are indicative of trachealis muscle contraction or dilation. A second method was developed by Brown *et al.* (3). With this technique, several tracheal cartilages superior to a low tracheostomy are bisected in the midline. Sutures are placed in the ends of the cartilages;

one end of the tracheal segment is attached to a stable rod, and the other end is attached to a force displacement transducer. The effect of various interventions on trachealis muscle activation is determined by monitoring changes in tension of the tracheal segment. In both of these methods, the nerve and blood supply to the tracheal segment is maintained.

Using the method involving an open tracheal segment, Coleridge *et al.* (10) injected capsaicin (which selectively activates types III and IV afferent fibers) into several sites in anesthetized cats. Increases in trachealis muscle tension occurred when capsaicin was injected into the right atria or bronchial arteries, indicating that the activation of pulmonary thin-fiber afferents cause tracheal constriction. Conversely, injection into a femoral artery caused tracheal dilation, demonstrating that skeletal muscle thin-fiber afferent activation caused the trachealis muscle relaxation. Kaufman *et al.* (57) injected several doses of capsaicin and bradykinin (also a selective stimulant of thin-fiber afferents) into the arterial circulation of a skinned hindlimb in dogs. (The hindlimb was skinned to prevent the activation of cutaneous afferent fibers.) Both drugs caused a dose-dependent decrease in trachealis muscle tension, although the response following bradykinin injection had a longer latency.

Kaufman and Rybicki (58) assessed the effect of bilateral gracilis nerve stimulation at 5 and 40 Hertz on trachealis muscle tension in dogs. The lower frequency of stimulation produced an intermittent contraction, while the higher stimulation frequency caused a static (isometric) contraction. Both types of stimulation caused similiar decreases in trachealis tension. In addition, tracheal relaxation did not occur under two conditions: when the hindlimb muscles were paralyzed, and when muscle contraction was elicited by stimulation of the peripheral end of cut gracilis nerves. Therefore, the reflex causing tracheal relaxation during muscle contraction appears to originate from afferent fibers within the active muscle.

To determine if these findings are relevant to the regulation of ventilation during exercise, Longhurst (79) investigated the effect of static muscle contraction on trachealis muscle tension in cats. Contraction of hindlimb muscle was elicited by electrical stimulation of the seventh lumbar and first sacral ventral roots, exposed by a laminectomy. Trachealis muscle tension decreased during isometric muscle contraction, and the magnitude of the response was related to the amount of tension generated by the hindlimb muscle.

Rybicki and Kaufman (114) sought to determine which autonomic pathways participate in the trachealis muscle

dilation which occurs during afferent fiber activation. They administered atropine methylnitrate (a cholinergic antagonist), phentolamine mesylate (an alpha-adrenergic antagonist) or propranolol hydrochloride (a beta-adrenergic antagonist) before stimulating somatic or visceral afferents with capsaicin. Atropine was the only drug which eliminated trachealis muscle relaxation, indicating that this reflex is caused by an inhibition of tonic vagal input to the trachealis muscle (114).

No studies have been reported concerning the role of phrenic afferents on trachealis muscle tension. **Accordingly, I studied the simultaneous changes in trachealis tension and lung resistance caused by unilateral and bilateral activation of phrenic afferent fibers. This study is presented in Chapter 3.**

Total lung resistance

Two studies have been reported which investigate whether the reflex linking somatic thin-fiber afferent activity to trachealis muscle relaxation is part of a more generalized bronchodilation response throughout the lung. These reports measured breath-by-breath changes in total pulmonary resistance using a Buxco pulmonary mechanics analyzer (1). In the first study (115), electrical stimulation of gracilis nerves was performed in anesthetized, paralyzed dogs using different stimulation intensities and frequencies. The types of afferent fibers being activated were subsequently

determined by measuring compound action potentials generated by the stimulation parameters used. Total pulmonary resistance was unchanged when groups I and II afferents were activated (3 times motor threshold). However, resistance decreased when groups I-III afferents were activated, with even greater relaxation occurring once group IV afferents were activated.

A second study (60) investigated the effect of static and intermittent contraction of hindlimb muscles (elicited by unilateral stimulation of sixth and seventh lumbar ventral roots) on total pulmonary resistance. Both types of contraction caused significant decreases in total pulmonary resistance, although trachealis tension decreased more during static contraction than during intermittent contraction (approximately 83% and 93% of baseline values, respectively).

There are very few reports concerning the effect of phrenic afferents on lung resistance. In 1937 Thornton (134) observed that traction of the phrenic nerve caused bronchodilation in the cat. In addition, electrical stimulation of the phrenic nerve close to the diaphragm produced bronchodilation whether stimulation was performed at low intensity (when blood pressure remained unchanged) or at high intensity (causing a pressor response). Section of the cervical vagus nerves eliminated the bronchodilator response to phrenic nerve stimulation, indicating that the vagus was the efferent pathway for this reflex (134).

The next paper addressing the phrenic-bronchomotor reflex was published almost fifty years later. McCallister *et al.* (88) investigated the role of different types of phrenic afferents on total lung resistance in tracheostomized, anesthetized and paralyzed dogs. One phrenic nerve was stimulated in the thorax over a range of stimulation intensities (3-200 times motor twitch threshold). Total lung resistance was measured on a breath-by-breath basis. In a separate part of the study, compound action potentials were recorded from the cervical phrenic nerve to determine which fiber types were being activated at each stimulation intensity. Total lung resistance decreased by 9% when stimulation was sufficient to activate groups I-III afferents (20 times twitch threshold), and progressively decreased as stimulation intensity was increased (thereby activating group IV afferent fibers). The maximum decrease in resistance, 23% of baseline, occurred during stimulatiom at 200 times twitch threshold. Since mean arterial blood pressure increased only at the highest stimulation intensities (140 and 200 times twitch threshold), the decrease in total lung resistance was not secondary to a pressor response. Cholinergic and adrenergic pharmacologic blockade indicated that bronchodilation was caused by an inhibition of cholinergic input, in agreement with the studies investigating the effect of hindlimb muscle afferents on trachealis muscle tension (88).

In summary, limb muscle thin-fiber afferents are involved in the regulation of resistance by affecting both extrathoracic and intrathoracic airways. A few studies have indicated that phrenic nerve afferents may also participate in reflexes associated with the regulation of bronchomotor tone.

1.4 INFLUENCE OF VAGAL INPUT ON VENTILATORY DRIVE

The vagus nerve contains afferent fibers from the lung, heart, aorta, esophagus, stomach and intestines, and these fibers are activated by mechanical or chemical stimuli. Pulmonary afferent fibers are classified as pulmonary stretch receptors, irritant receptors, and J receptors. J receptors are also referred to as C fibers (102).

Pulmonary stretch receptors are slowly adapting receptors found in intrathoracic and extrathoracic airways, which exhibit either tonic or phasic firing. Phasic firing is caused by airway distension during lung inflation, and leads to the cessation of inspiratory muscle activity. Activation of these fibers is also associated with a dilation of airway smooth muscle (142) and an increase in heart rate (54).

Irritant receptors are rapidly adapting receptors located in large airways. They are activated when lung compliance is decreased, or during rapid inflation and deflation of the lung. Stimulation of these receptors leads to bronchoconstriction and cough and, at times, triggers a

gasp reflex (102). J receptors, named for their juxta-capillary location, are connected to slowly conducting, unmedullated nerve fibers. During lung congestion, these receptors are stimulated by the stretching of interstitial lung tissue (102). Stimulation of these receptors leads to shallow, rapid breathing, and increased diaphragmatic post-inspiratory activity (40). In addition, strong J receptor stimulation is associated with apnea, decreased heart rate and blood pressure (102).

Hollstein *et al.* (39) have shown that abdominal muscle activity is linked to the balance of excitatory pulmonary stretch receptor activity and inhibitory J receptor activity. In addition, tonic vagal activity has been shown to modulate the pattern of breathing, and may be involved in the response to hypercapnia (103).

One investigator has shown that vagal input may potentiate the excitatory effects of gastrocnemius nerve afferent stimulation on ventilatory drive (122). **It is not known what role vagal input may have on the effects of phrenic nerve afferent activation. Therefore, in Chapter 4, I have asssessed the effects of phrenic afferent stimulation on ventilatory drive before and after bilateral cervical vagotomy.**

1.5 REFERENCES

1. AMDUR, M.O. and J. MEAD. Mechanics of respiration in unanesthetized guinea pigs. *Am J Physiol* 192(2): 364-368, 1958.

2. BALZAMO, E., F. LAGIER-TESSONNIER, and Y. JAMMES. Fatigue-induced changes in diaphragmatic afferents and cortical activity in the cat. *Respir Physiol* 90: 213-226, 1992.

3. BROWN, J.K., A.R. LEFF, M.J. FREY, B.R. REED, and W.M. GOLD. Physiological and pharmacological properties of canine trachealis muscle in vivo. *J Appl Physiol:Respirat Environ Exercise Physiol* 49(1): 84-94, 1980.

4. CABEZAS, G.A., P.D. GRAF, and J.A. NADEL. Sympathetic versus parasympathetic nervous regulation of airways in dogs. *J Appl Physiol* 31(5): 651-655, 1971.

5. CARCASSI, A.M., A. CONCU, M. DECANDIA, M. ONNIS, G.P. ORANI, and M.B. PIRAS. Respiratory responses to stimulation of large fibers afferent from muscle receptors in cats. *Pflugers Arch* 399: 309-314, 1983.

6. CAREW, T.J. Spinal Cord I: Muscles and Muscle Receptors. In: *Principles of Neural Science*, edited by Kandel, E.R. and Schwartz, J.H. New York: Elsevier/North-Holland, 1981, pp. 284-292.

7. CARPENTER, M.B. *Core Text of Neuroanatomy*, Baltimore: Williams and Wilkins Co, 1972.

8. CHEESEMAN, M. and W.R. REVELETTE. Phrenic afferent contribution to reflexes elicited by changes in diaphragm length. *J Appl Physiol* 69: 640-647, 1990.

9. COGGESHALL, R.E. Law of separation of function of the spinal roots. *Physiol Rev* 60: 716-755, 1980.

10. COLERIDGE, J.C.G, H.M. COLERIDGE, A.M. ROBERTS, M.P. KAUFMAN, and D.G. BAKER. Tracheal contraction and relaxation initiated by lung and somatic afferents in dogs. *J Appl Physiol:Respirat Environ Exercise Physiol* 52(4): 984-990, 1982.

11. CORDA, M., C. VON EULER, and G. LENNERSTRAND. Proprioceptive innervation of the diaphragm. *J Physiol (Lond)* 178: 161-177, 1965.

12. CRAYTON, S.C., J.H. MITCHELL, and F.C. PAYNE. Reflex cardiovascular response during injection of capsaicin into skeletal muscle. *Am J Physiol* 240: H315-H319, 1981.

13. CUENOD, M. Reflexes proprioceptifs du diaphragma chez le lapin. *Helv Physiol Acta* 19: 360-372, 1961.

14. DAVENPORT, P.W., F.J. THOMPSON, R.L. REEP, ad A.N. FREED. Projection of phrenic nerve afferents to the cat sensorimotor cortex. *Brain Res* 328: 150-153, 1985.

15. DINGLE, J.T., G.T. KENT, L.L. WILLIAMS, and C.J. WIGGERS. A study of alleged quantitative criteria of vasomotor action. *Am J Physiol* 130: 63-68, 1940.

16. DURON, B. Postural and ventilatory functions of intercostal muscles. *Acta Neurobiol Exp* 33: 355-380, 1073.

17. DURON, B. Intercostal and diaphragmatic muscle endings and afferents. In: *Regulation of breathing, Part I*, edited by Hornbein, T.F. New York: Dekker, 1981, pp. 473-540.

18. DURON, B., M.C. JUNG-CAILLOL, and D. MARLOT. Myelinated nerve fiber supply and muscle spindles in the respiratory muscles of cat: quantitative study. *Anat Embryol* 152: 171-192, 1978.

19. DURON, B. and D. MARLOT. The non-myelinated fibers of the phrenic and the intercostal nerves in the cat. *Z mikrosk. Anat Forsch* 94: 257-269, 1980.

20. DURON, B., D. MARLOT, N. LARNICOL, M.C. JUNG-CAILLOL, and J.-M. MACRON. Somatotopy in the phrenic motor nucleus of the cat as revealed by retrograde transport of horseradish peroxidase. *Neurosci Lett* 14: 159-163, 1979.

21. ELDRIDGE, F.L., P. GILL-KUMAR, D.E. MILLHORN, and T.G. WALDROP. Spinal inhibition of phrenic motoneurones by stimulation of afferents from peripheral muscles. *J Physiol (Lond)* 311: 67-79, 1981.

22. ELDRIDGE, F.L., D.E. MILLHORN, and T. WALDROP. Spinal inhibition of phrenic motoneurones by stimulation of afferents from leg muscle in the cat: blockade by strychnine. *J Physiol (Lond)* 389: 137-146, 1987.

23. FELL, C. Changes in blood flow distributuion produced by central sciatic nerve stimulation. *Am J Physiol* 214:

561-565, 1968.

24. FERGUSON, J. The phrenic nerve. *Brain* 14: 282-283, 1891.

25. FRANZ, D.N. and A. IGGO. Conduction failure in myelinated and non-myelinated axons at low temperatures. *J Physiol (Lond)* 199: 319-345, 1968.

26. GAUTIER, H., A. LACAISSE, and P. DEJOURS. Ventilatory response to muscle spindle stimulation by succinylcholine in cats. *Resp Physiol* 7: 383-388, 1969.

27. GILL, P.K. and M. KUNO. Excitatory and inhibitory actions on phrenic motoneurones. *J Physiol (Lond)* 168: 274-289, 1963.

28. GLEBOVSKII, V.D. Stretch receptors of the diaphragm. *Fed Proc Transl Suppl* 22: T405-T410, 1963.

29. GORDON, G. The mechanism of the vasomotor reflexes produced by stimulating mammalian sensory nerves. *J Physiol (Lond)* 102: 95-107, 1943.

30. GOSHGARIAN, H.G. and P.J. ROUBAL. Origin and distribution of phrenic primary afferent nerve fibers in the spinal cord of the adult rat. *Exp Neuro* 92: 624-638, 1986.

31. GRAHAM, R., Y. JAMMES, S. DELPIERRE, C. GRIMAUD, and CH. ROUSSOS. The effects of ischemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. *Neurosci Lett* 67: 257-262, 1986.

32. GRANIT, R., S. SKOGLUND, and S. THESLEFF. Activation of muscle spindles by succinylcholine and decamethonium. The effects of curare. *Acta Physiol Scand* 28: 134-157, 1953.

33. GREENE, C.W. Control of the coronary blood flow by reflexes arising in widely distributed regions of the body. *Am J Physiol* 113: 399-415, 1935.

34. GUYTON A.C. *Textbook of Medical Physiology*, Philadelphia: W.B. Saunders Co., 1986. 7th Ed. pp. 578-579.

35. HAXHIU, M.A., E. VAN LUNTEREN, J. MITRA, N.S. CHERNIACK, and K.P. STROHL. Comparison of the responses of the diaphragm and upper airway muscles to central stimulation of the sciatic nerve. *Respir Physiol* 58: 65-76, 1984.

36. HERTEL, H.C., B. HOWALDT, and S. MENSE. Responses of group IV and group III muscle afferents to thermal

stimuli. *Brain Res* 113: 201-205, 1976.

37. HINSEY, J.C., K. HARE, and R.A. PHILLIPS. Sensory components of the phrenic nerve of the cat. *Proc Soc Exp Biol Med* 41: 411-414, 1939.

38. HODGSON, H.J.F. and P.B.C. MATTHEWS. The ineffectiveness of excitation of the primary endings of the muscle spindle by vibration as a respiratory stimulant in the decerebrate cat. *J Physiol (Lond)* 194: 555-563, 1968.

39. HOLLSTEIN, S.B., M.L. CARL, E.S. SCHELEGLE, AND J.F. GREEN. Role of vagal afferents in the control of abdominal expiratory muscle activity in the dog. *J Appl Physiol* 71(5): 1795-1800, 1991.

40. HOLMES, H.R. AND J.E. REMMERS. Stimulation of vagal C-fibers alters timing and distribution of respiratory motor output in cats. *J Appl Physiol* 67(6): 2249-2256, 1989.

41. HUNT, R. The fall of blood pressure resulting from the stimulation of afferent nerves. *J Physiol (Lond)* 18: 381-410, 1895.

42. HUSSAIN, S.N.A., A. CHATILLON, A. COMTOIS, C. ROUSSOS, and S. MAGDER. Chemical activation of thin-fiber phrenic afferents 2. Cardiovascular responses. *J Appl Physiol* 70(1): 77-86, 1991.

43. HUSSAIN, S.N.A., S. MAGDER, A. CHATILLON, and CH. ROUSSOS. Chemical activation of thin-fiber phrenic afferents: respiratory responses. *J Appl Physiol* 69(3): 1002-1011, 1990.

44. HUSSAIN, S.N.A., M.E. WARD, A.G. GATENSBY, CH. ROUSSOS, and A. DESCHAMPS. Respiratory muscle activation by limb muscle afferent stimulation in anesthetized dogs. *Respir Physiol* 84: 185-198, 1991.

45. IRVIN, C.G., R.R. MARTIN, and P.T. MACKLEM. Nonpurigenic nature and efficacy of non-adrenergic bronchodilation. *J Appl Physiol* 52: 562-569, 1982.

46. IWAMOTO, G.A. and B.R. BOTTERMAN. Peripheral factors influencing expression of pressor reflex evoked by muscular contraction. *J Appl Physiol* 58: 1676-1682, 1985.

47. JACK, J.J.B. Some methods for selective activation of muscle afferent fibres. In: *Studies in neurophysiology*, edited by Porter, R. London: Cambridge University Press, 1978, pp. 155-176.

48. JAMMES, Y. and E. BALZAMO. Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73(3): 894-902, 1992.

49. JAMMES, Y., B. BUCHLER, S. DELPIERRE, A. RASIDAKIS, C. GRIMAUD, and C. ROUSSOS. Phrenic afferents and their role in inspiratory control. *J Appl Physiol* 60(3): 854-860, 1986.

50. JOHANSSON, B. Circulatory responses to stimulation of somatic afferents. *Acta Physiol Scand* 198S: 5-91, 1962.

51. KALIA, M., J.M. SENAPATI, B. PARIDA, and A. PANDA. Reflex increase in ventilation by muscle receptors with nonmedullated fibers (C fibers). *J Appl Physiol* 32: 189-193, 1972.

52. KATO, M. and FUKUSHIMA, K. Selective activation of group II muscle afferents and its effects on cat spinal neurones. In: *Understanding the stretch reflex*, edited by Homma, S. Amsterdam: Elsevier, 1976, pp. 182-222.

53. KATZ, S. and J.H. PERRYMAN. Respiratory and blood pressure responses to stimulation of peripheral afferent nerves. *Am J Physiol* 108(5): 993-999, 1965.

54. KAUFMAN, M.P., IWAMOTO, G.A., ASHTON, J.H., AND S.S. CASSIDY. Responses to inflation of vagal afferents with endings in the lungs of dogs. *Circ Res* 51: 525-531, 1982.

55. KAUFMAN, M.P., G.A. IWAMOTO, J.C. LONGHURST, and J.H. MITCHELL. Effects of capsaicin and bradykinin on afferent fibers with endings in skeletal muscle. *Circ Res* 50: 133-139, 1982.

56. KAUFMAN, M.P., J.C. LONGHURST, K.J. RYBICKI, J.H. WALLACH, and J.H. MICHELL. Effects of static muscular contraction on impulse activity of groups III and IV afferents in cats. *J Appl Physiol: Respirat Environ Exer Physiol* 55: 105-112, 1983.

57. KAUFMAN, M.P., G.A. ORDWAY, J.C. LONGHURST, and J.H. MITCHELL. Reflex relaxation of tracheal smooth muscle by thin-fiber muscle afferents in dogs. *Am J Physiol* 243: R383-R388, 1982.

58. KAUFMAN, M.P. and K.J. RYBICKI. Muscular contraction reflexly relaxes tracheal smooth muscle in dogs. *Respir Physiol* 56: 61-72, 1984.

59. KAUFMAN, M.P. and K.J. RYBICKI. Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. *Circ Res* 61(S): I.60-I.65, 1987.

60. KAUFMAN, M.P., K.J. RYBICKI, and J.H. MITCHELL. Hindlimb muscular contraction reflexly decreases total pulmonary resistance in dogs. *J Appl Physiol* 59(5): 1521-1526, 1985.

61. KAUFMAN, M.P., K.J. RYBICKI, T.G. WALDROP, and G.A. ORDWAY. Effect of ischemia on responses of group III and IV afferents to contraction. *J Appl Physiol* 57: 644-650, 1984.

62. KNIFFKI, K.-D., S. MENSE, and R.F. SCHMIDT. Responses of group IV afferent units from skeletal msucle to stretch, contraction and chemical stimulation. *Exp Brain Res* 31: 511-522, 1978.

63. KOHRMAN, R.M., J.B. NOLASCO, and C.J. WIGGERS. Types of afferent fibers in the phrenic nerve. *Am J Physiol* 151: 547-557, 1947.

64. KONNO, A. Innervation of the smooth muscle in the cervical trachea. On the descending motor fibers to the cervical tracheal muscle in the inner branch of the superior laryngeal nerve. *J Otolaryn Jpn* 79: 725-732, 1976.

65. KOSTREVA, D.R. and S.P. PONTUS. Pericardial mechanoreceptors with phrenic afferents. *Am J Physiol* 264: H1836-H1846, 1993.

66. KOSTREVA, D.R. and S.P. PONTUS. Hepatic vein, hepatic parenchymal, and inferior vena caval mechanoreceptors with phrenic afferents. *Am J Physiol* 265: G15-G20, 1993.

67. KRNJEVIC, K. and R. MILEDI. Motor units in the rat diaphragm. *J Physiol (Lond)* 140: 427-439, 1958.

68. KUMADA, M., K. NOGAMI, and K. SAGAWA. Modulation of carotid sinus baroreceptor reflex by sciatic nerve stimulation. *Am J Physiol* 228: 1535-1541, 1975.

69. KUMAZAWA, T., K. EGUCHI, and E. TADAKI. Naloxone-reversible respiratory inhibition induced by muscular thin-fiber afferents in decerebrated cats. *Neurosci Lett* 53: 81-85, 1985.

70. KUMAZAWA, T. and K. MIZUMURA. Thin-fibre receptors responding to mechanical, chemical, and thermal stimulation in the skeletal muscle of the dog. *J Physiol (Lond)* 273: 179-194, 1977.

71. KUMAZAWA, T. and E. TADAKI. Two different inhibitory effects on respiration by thin-fiber muscular afferents in cats. *Brain Res* 272: 364-367, 1983.

72. KUMAZAWA, T., E. TADAKI, K. MIZUMURA, and K. KIM. Post-stimulus facilitatory and inhibitory effects on respiration induced by chemical and electrical stimulation of thin-fiber muscular afferents in dogs. *Neurosci Lett* 35: 283-287, 1983.

73. LANGFORD, L.A. and R.F. SCHMIDT. An electron microscopic analysis of the left phrenic nerve in the rat. *Anat Record* 205: 207-213, 1983.

74. LARNICOL, N., D. ROSE, and B. DURON. Identification of phrenic afferents to the external cuneate nucleus: a fluorescent double-labelling study in the cat. *Neurosci Lett* 62: 163-167, 1985.

75. LEITNER, L.-M. and P. DEJOURS. Reflex increase in ventilation induced by vibrations applied to the triceps surae muscles in the cat. *Resp Physiol* 12: 199-204, 1971.

76. LEMERE, F. Innervation of the larynx. *Anat Rec* 54: 389-407, 1932.

77. LIPSKI, J., R.E.W. FYFFE, and J. JODKOWSKI. Recurrent inhibition of cat phrenic motoneurons. *J Neurosci* 5: 1545-1555, 1985.

78. LITTLE, M.G.A. and B.A. MCSWINEY. Afferent fibres from the diaphragm. *J Physiol (Lond)* 94: 2P-3P, 1938.

79. LONGHURST, J.C. Static contraction of hindlimb muscles in cats reflexly relaxes tracheal smooth muscle. *J Appl Physiol:Respirat Environ Exercise Physiol* 57(2): 380-387, 1984.

80. MACRON, J.-M., D. MARLOT, and B. DURON. Phrenic afferent input to the lateral medullary reticular formation of the cat. *Respir Physiol* 59: 155-167, 1985.

81. MACRON, J.M. and D. MARLOT. Effects of stimulation of phrenic afferent fibers on medullary respiratory neurons in cat. *Neurosci Lett* 63: 231-236, 1986.

82. MARLOT, D., J.-M. MACRON, and B. DURON. Projections of phrenic afferences to the cat cerebellar cortex. *Neurosci Lett* 44: 95-98, 1984.

83. MARLOT, D., J.-M. MACRON, and B. DURON. Projection of phrenic afferents to the external cuneate nucleus in the cat. *Brain Res* 327: 328-330, 1985.

84. MARLOT, D., J.-M. MACRON, and B. DURON. Inhibitory and excitatory effects on respiration by phrenic nerve afferent stimulation in cats. *Respir Physiol* 69: 321-333, 1987.

85. MARLOT, D., J.-M. MACRON, and B. DURON. Effects of ipsilateral and contralateral cervical phrenic afferents stimulation on phrenic motor unit activity in the cat. *Brain Res* 450: 373-377, 1988.

86. MARTIN, J.H. Somatic sensory system I: Receptor physiology and submodality coding. In: *Principles of Neural Science*, edited by Kandel, E.R. and Schwartz, J.H. New York: Elsevier/North/Holland, 1981, pp. 163.

87. MARTIN, J.H., Somatic sensory system II: Anatomical substrates for somatic sensation. In: *Principles of Neural Science*, edited by Kandel, E.R. and Schwartz, J.H. New York: Elsevier/North-Holland, 1981, pp. 170-183.

88. MCCALLISTER, L.W., K.W. MCCOY, J.C. CONNELLY, and M.P. KAUFMAN. Stimulation of groups III and IV phrenic afferents reflexly decreases total lung resistance in dogs. *J Appl Physiol* 61(4): 1346-1351, 1986.

89. MCCLOSKEY, D.I., P.B.C. MATTHEWS, and J.H. MITCHELL. Absence of appreciable cardiovascular and respiratory responses to muscle vibration. *J Appl Physiol* 33: 623-626, 1972.

90. MCCLOSKEY, D.I. and J.H. MITCHELL. Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol (Lond)* 224: 173-186, 1972.

91. MENSE, S. Nervous outflow from skeletal muscle following chemical noxious stimulation. *J Physiol (Lond)* 267: 75-88, 1977.

92. MENSE, S. Effects of temperature on the discharges of muscle spindles and tendon organs. *Pflueg Arch* 374: 159-166, 1978.

93. MENSE, S. and A.D. CRAIG. Spinal and supraspinal terminations of primary afferent fibers from the gastrocnemius-soleus muscle in the cat. *Neurosci* 26: 1023-1035, 1988.

94. MENSE, S. and R.F. SCHMIDT. Activation of group IV afferent units from muscle by algesic agents. *Brain Res* 72: 305-310, 1974.

95. MENSE, S. and M. STAHNKE. Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the cat. *J Physiol (Lond)* 342: 383-397, 1983.

96. MITCHELL, J.H. and SCHMIDT, R.F. Cardiovascular reflex control by afferent fibers from skeletal muscle receptors. In: *The Cardiovascular System. Vol. III, Peripheral Circulation and Organ Blood Flow, Part 2,* edited by Shepherd, J.R. and Abboud, F.M. Bethesda: American Physiological Society, 1983, pp. 623-658.

97. MIZUMURA, K. and T. KUMAZAWA. Reflex respiratory response induced by chemical stimulation of muscle afferents. *Brain Res* 109: 402-406, 1976.

98. NATHAN, P.W. and T.A. SEARS. Some factors concerned in differential nerve block by local anesthetics. *J Physiol (Lond)* 157: 565-580, 1961.

99. ORANI, G.P. and M. DECANDIA. Group I afferent fibers: effects on cardiorespiratory system. *J Appl Physiol* 68: 932-937, 1990.

100. OSCARSSON, O. Functional organization of spinocerebellar pathways. In: *Handbook of sensory physiology, Vol. 2,* edited by Iggo, A. Berlin: Springer-Verlag, 1973,

101. PAINTAL, A.S. Functional analysis of group III afferent fibres of mammalian muscles. *J Physiol (Lond)* 152: 250-270, 1960.

102. PAINTAL, A.S. Vagal sensory receptors and their reflex effects. *Physiol Rev* 53(1): 159-227, 1973.

103. PHILLIPSON, E.A. Vagal control of breathing pattern independent of lung inflation in conscious dogs. *J Appl Physiol* 37(2): 183-189, 1974.

104. PICKAR, J.G., J.M. HILL, and M.P. KAUFMAN. Dynamic exercise stimulates group III muscle afferents. *J Neurophysiol* 71: 753-760, 1994.

105. REVELETTE, R., S. REYNOLDS, D. BROWN, and R. TAYLOR. Effect of abdominal compression on diaphragmatic tendon organ activity. *J Appl Physiol* 72: 288-292, 1992.

106. REVELETTE, W.R., L.A. JEWELL, and D.T. FRAZIER. Effect of diaphragm small-fiber afferent stimulation on ventilation in dogs. *J Appl Physiol* 65(5): 2097-2106, 1988.

107. ROAD, J.D., S. OSBORNE, and Y. WAKAI. Delayed poststimulus decrease of phrenic motoneuron output produced by phrenic nerve afferent stimulation. *J Appl Physiol* 74: 68-72, 1993.

108. ROAD, J.D., N.H. WEST, and B.N. VAN VLIET. Ventilatory effects of stimulation of phrenic afferents. *J Appl Physiol* 63(3): 1063-1069, 1987.

109. ROSE, D., N. LARNICOL, and B. DURON. The cat cervical dorsal root ganglia: general cell-size characteristics and comparative study of neck muscle, neck cutaneous and phrenic afferents. *Neurosci Res* 7: 341-357, 1990.

110. ROTTO, D.M. and M.P. KAUFMAN. Effect of metabolic products of muscular contraction of discharge of group III and IV afferents. *J Appl Physiol* 64: 2306-2313, 1988.

111. ROTTO, D.M., H.D. SCHULTZ, J.C. LONGHURST, and M.P. KAUFMAN. Sensitization of group III muscle afferents to static contraction by arachidonic acid. *J Appl Physiol* 68: 861-867, 1990.

112. RUCKEBUSCH, Y. Influx afferents d'origine pericardique dans les nerfs phreniques. *CR Soc Biol* 155: 524-527, 1961.

113. RUSSELL, J.A. Noradrenergic inhibitory innervation of canine airways. *J Appl Physiol:Respirat Environ Exercise Physiol* 48(1): 16-22, 1980.

114. RYBICKI, K.J. and M.P. KAUFMAN. Atropine prevents the reflex tracheal relaxation arising from the stimulation of intestinal and skeletal muscle afferents in dogs. *Brain Res* 270: 159-161, 1983.

115. RYBICKI, K.J. and M.P. KAUFMAN. Stimulation of group III and IV muscle afferents reflexly decreases total pulmonary resistance in dogs. *Respir Physiol* 59: 185-195, 1985.

116. SALAMONE, J.A., K.P. STROHL, D.M. WEINER, J. MITRA, and N.S. CHERNIACK. Cranial and phrenic nerve responses to changes in systemic blood pressure. *J Appl Physiol: Respirat Environ Exercise Physiol* 55(1): 61-68, 1983.

117. SANT'AMBROGIO, G. and J.G. WIDDICOMBE. Respiratory reflexes acting on the diaphragm and inspiratory intercostal muscles of the rabbit. *J Physiol (Lond)* 180: 766-779, 1965.

118. SATO, A., A. KAUFMAN, K. KOIZUMI, and C. MCBROOKS. Afferent nerve groups and sympathetic pathways. *Brain Res* 14: 575-587, 1969.

119. SATO, A. and R.F. SCHMIDT. Somatosympathetic reflexes: afferent fibers, central pathways, discharge characteristics. *Physiol Rev* 53: 916-947, 1973.

120. SCHLAEPFER, K. A further note on the motor innervation of the diaphragm. *Anat Record* 32: 143-150, 1926.

121. SENAPATI, J.M. Effect of stimulation of muscle afferents on ventilation of dogs. *J Appl Physiol* 21: 242-246, 1966.

122. SIMBULAN,D., TADAKI, E., KOZAKI, Y., EGUCHI, K., AND T. KUMAZAWA. Augmentation of muscle nociceptive respiratory reflex facilitation by vagal afferents. *Jap J Physiol* 40: 253-272, 1990.

123. SPECK, D.F. and W.R. REVELETTE. Excitation of dorsal and ventral respiratory group neurons by phrenic nerve afferents. *J Appl Physiol* 62: 946-951, 1987.

124. SPECK, D.F. and W.R. REVELETTE. Attenuation of phrenic motor discharge by phrenic nerve afferents. *J Appl Physiol* 62(3): 941-945, 1987.

125. SPECK, D.F., W.R. REVELETTE, and D.T. FRAZIER. Medullary projections of phrenic nerve afferents. *Federation Proc* 44: 1586, 1985.

126. STEBBINS, C.L., B. BROWN, D. LEVIN, and J.C. LONGHURST. Reflex effect of skeletal muscle mechanoreceptor stimulation on the cardiovascular system. *J Appl Physiol* 65: 1539-1547, 1988.

127. STEBBINS, C.L. and J.C. LONGHURST. Bradykinin-induced chemoreflexes from skeletal muscle: implications for the exercise reflex. *J Appl Physiol* 59(1): 56-63, 1985.

128. STEBBINS, C.L. and J.C. LONGHURST. Bradykinin in reflex cardiovascular responses to static muscle contraction. *J Appl Physiol* 61(1): 271-279, 1986.

129. STEBBINS, C.L. and J.C. LONGHURST. Potentiation of the exercise pressor reflex by muscle ischemia. *J Appl Physicl* 66(3): 1046-1053, 1989.

130. TALLARIDA, G., F. BALDONI, G. PERUZZI, F. BRINDISI, G. RAIMONDI, and M. SANGIORGI. Cardiovascular and respiratory chemoreflexes from the hindlimb sensory receptors evoked by intra-arterial injection of bradykinin and other chemical agents in the rabbit. *J Pharmacol Exp Ther* 208: 319-329, 1979.

131. TEITELBAUM, J., C.O. BOREL, S. MAGDER, R.J. TRAYSTMAN, and S.N.A. HUSSAIN. Effect of selective diaphragmatic paralysis on the inspiratory motor drive. *J Appl Physiol* 74: 2261-2268, 1993.

132. TEITELBAUM, J., S.A. MAGDER, CH. ROUSSOS, and S.N.A. HUSSAIN. Effects of diaphragmatic ischemia on the inspiratory motor drive. *J Appl Physiol* 72(2): 447-454, 1992.

133. TEITELBAUM, J., G. VANELLI, and S.N.A. HUSSAIN. Thin-fiber phrenic afferents mediate the ventilatory response to diaphragmatic ischemia. *Resp Physiol* 91: 195-206, 1993.

134. THORNTON, J.W. Bronchodilation by stimulation of the phrenic nerve. *J Physiol (Lond)* 90: 85P, 1937.

135. TIBES, U. Reflex inputs to the cardiovascular and respiratory centers from dynamically working canine muscles. *Circ Res* 41: 332-341, 1977.

136. VAN LUNTEREN, E., K.P. STROHL, D.M. PARKER, E.N. BRUCE, W.B. VAN DE GRAAFF, and N.S. CHERNIACK. Phasic volume-related feedback on upper airway muscle activity. *J Appl Physiol:Respirat Environ Exercise Physiol* 56(3): 730-736, 1984.

137. WALDROP, T.G., F.L. ELDRIDGE, and D.E. MILLHORN. Prolonged post-stimulus inhibition of breathing following stimulation of afferents from muscle. *Resp Physiol* 50: 239-254, 1982.

138. WALDROP, T.G., F.L. ELDRIDGE, and D.E. MILLHORN. Inhibition of breathing after stimulation of muscle is mediated by endogenous opiates and GABA. *Respir Physiol* 54: 211-222, 1983.

139. WALDROP, T.G., K.J. RYBICKI, and M.P. KAUFMAN. Chemical activation of group I and II muscle afferents has no cardiorespiratory effects. *J Appl Physiol:Respirat Environ Exercise Physiol* 56(5): 1223-1228, 1984.

140. WARD, M.E., A. DESCHAMPS, CH. ROUSSOS, and S.N.A. HUSSAIN. Effect of phrenic afferent stimulation on pattern of respiratory muscle activation. *J Appl Physiol* 73(2): 563-570, 1992.

141. WEBB-PELOE, M.M., D. BRENDER, and J.T. SHEPHERD. Vascular responses to stimulation of receptors in muscle by capsaicin. *Am J Physiol* 222: 189-195, 1972.

142. WIDDICOMBE, J.G. AND J.A. NADEL. Reflex effects of lung inflation on tracheal volume. *J Appl Physiol* 18(4): 681-686, 1963.

143. WILLIAMS, P.L. and R. WARWICK. *Gray's Anatomy*, Philadelphia: WB Saunders Co, 1980. 36th Ed. pp. 1093-1094.

144. WINCKLER, G. and B. DELALOYE. A propos de la presence de fuseaux neuro-musculaires dans le diaphragme humain. *Acta Anat* 29: 114-116, 1957.

145. WOODBURNE, R.T. *Essentials of Human Anatomy*, New York: Oxford University Press, 1973. 5th Ed. pp. 30.

146. YASARGIL, G.M. Proprioceptive Afferenzen im N phrenicus der Katze. *Helv Physiol Acta* 20: 39-58, 1962.

CHAPTER 2

PHRENIC AFFERENT STIMULATION BY BRADYKININ AND THE DISTRIBUTION OF THE INSPIRATORY MOTOR DRIVE

2.1 LINK TO CHAPTER 2

Most studies have used electrical stimulation to investigate the effect of phrenic nerve activation on ventilatory drive, and the findings have been equivocal. The activation of phrenic nerve afferents has been associated with an increase, decrease, or biphasic change in inspiratory motor drive. This lack of congruity may be due to the poor selectivity of fiber activation provided by electrical stimulation. Chemical stimulation of afferent fibers can be more selective with respect to fiber group activation and may, in addition, provide a better simulation of *in vivo* events. Selective activation of diaphragmatic thin-fiber afferents by capsaicin (a nonphysiological substance which activates thin-fiber, predominantly group IV, afferents) increases inspiratory motor drive. However, capsaicin injection also causes a severe limitation of diaphragmatic blood flow and a large increase in systemic blood pressure. Bradykinin is a substance which is produced within the muscle during contraction, and is a selective stimulant of groups III and IV afferent fibers. During exercise, it is probable that the local production of bradykinin may stimulate the activity of ventilatory muscle thin-fiber afferents. This afferent stimulation may, in turn, affect ventilatory drive. Therefore, the first project addresses the changes in inspiratory motor drive associated with the injection of bradykinin into a vascularly isolated, innervated, *in situ* left hemi-diaphragm preparation.

2.2 ABSTRACT

Activation of thin-fiber (groups III and IV) afferents from the diaphragm using capsaicin or ischemia increases the respiratory muscle activity. To assess whether bradykinin causes similar effects, we injected boluses of bradykinin into the phrenic artery of *in situ*, isolated and innervated left hemi-diaphragm preparations in 8 α-chloralose anesthetized, vagotomized, mechanically ventilated dogs. Inspiratory motor drive during spontaneous breathing attempts was assessed from the integrated EMG activity of several inspiratory muscles. Fifty μg of bradykinin increased peak integrated EMG activities of alae nasi to 110%, genioglossus to 189%, left diaphragm to 115% ($p<0.05$) and parasternal to 109% ($p<0.01$) of baseline activity 60 sec after the injection. Inspiratory time decreased by 10% ($p<0.01$). The mean arterial blood pressure increased by about 10 mmHg. Responses were similar with 10, 25 and 100 μg of bradykinin. After left phrenicotomy, bradykinin did not affect inspiratory muscle EMG or respiratory timing. In conclusion, thin-fiber phrenic afferent activation by bradykinin exerts an excitatory but disproportionate influence on the inspiratory motor drive.

2.3 INTRODUCTION

Most studies assessing the role of phrenic afferents in the regulation of ventilation have shown that inspiratory motor drive increases when group III and IV phrenic afferents are stimulated by electrical (14,22,29) or chemical (9,21) means. A biphasic ventilatory response (inhibition followed by excitation) has also been described (17).

Recently, Teitelbaum and colleagues (28) have shown that diaphragmatic ischemia elicited a progressive increase in the respiratory frequency as well as the inspiratory motor drive to several inspiratory muscles. Since ischemia is known to activate thin-fiber afferents (7), it was concluded that group III and IV phrenic afferents exert an excitatory effect on the inspiratory motor drive. A criticism of this method of activating thin-fiber afferents is that complete diaphragmatic ischemia rarely develops under physiological circumstances.

In the current study, we have used a physiological substance, bradykinin, to assess the effects of thin-fiber phrenic afferent activation on the inspiratory motor drive. This substance is known to activate only groups III and IV muscular afferents (18) in a more gradual fashion than capsaicin. The main advantage of using bradykinin is that it is produced during muscle contraction <u>in vivo</u> (26). This is the first study to assess the effect of bradykinin activation of phrenic afferents on the distribution of inspiratory motor

drive.

2.4 METHODS

Animal preparation

Mongrel dogs (25-32 kg) were anesthetized with thiopental sodium (20 mg/kg) followed by α-chloralose (60-80 mg/kg). Additional α-chloralose was provided as required to eliminate jaw tone yet maintain quadriceps reflexes. Animals were supine, intubated and mechanically ventilated with a constant volume ventilator (Harvard pump, initial setting of 12-15 ml/kg for tidal volume and 20 breaths/minute for ventilator frequency). These settings were later adjusted as necessary to maintain end-tidal CO_2 at 3-4% (Ametek analyzer). During the surgical procedure, supplemental O_2 was provided to maintain arterial P_{O2} > 100 mmHg. Both cervical vagi were cut though a midline neck incision to eliminate effects from vagal afferents. The left carotid artery was cannulated to measure arterial pressure (Part). The external jugular vein was cannulated to allow fluid administration.

Isolated hemi-diaphragm

The rectus abdominis, external oblique and latissimus dorsi muscles were dissected to expose the lower six ribs on the left side of the chest. Each intercostal space was bisected, and the incision was extended (5-10 cm) to the origin of the costal diaphragm. Intercostal vessels were

ligated and the ribs were removed. The left internal mammary artery was ligated through a midline incision in the xiphisternal cartilage. The lateral abdominal wall was incised just caudal to the inferior margin of the costal diaphragm. This incision extended from midline to the inferior margin of the last rib, which was cut at the left costophrenic angle. The two halves of the costal diaphragm were incised from the xiphisternal cartilage to the central tendon. The ribs and cartilages of the free costal margin were secured by silk threads to three metal bars (5 cm long). The bars were attached to rack and pinion adjustable clamps (Harvard Instruments Model 50-2633), which were fixed horizontally to a rigid metal frame surrounding the animal's thorax. The clamps were adjusted so that the costal diaphragm was at its in vivo, open thorax, end expiratory length. This was determined by measuring the distance between three pairs of sutures placed in the anterior, middle and posterior regions of the costal diaphragm. Body temperature was monitored using a rectal thermometer, and a heating lamp was used to maintain the temperature above 37°C. Plastic film (Saran Wrap) was applied to the chest of the animal to avoid drying of the exposed tissues.

Phrenic artery catheterization

The stomach, liver, spleen and left kidney were retracted to expose the left phrenic artery. This artery was dissected free from the surrounding tissues 4-6 cm above the

left adrenal gland. The proximal part of the artery was ligated, and a polyethylene catheter (PE-160, 1.14 mm inner diameter (ID), 1.57 mm outer diameter (OD), 3 cm long) was introduced into the distal portion. The catheter was connected to a 15 cm-long polyethylene tube (4 mm ID, 6 mm OD) that was joined to a Y-shaped connector. One arm of the Y was connected to a catheter in the left femoral artery so that arterial flow was diverted from that vessel into the left inferior phrenic artery. The other arm of the Y was used for the infusion of bradykinin. A saline/heparin mixture was infused regularly from a pressurized bag to prevent clotting.

Catheterization of the phrenic vein

A polyethylene catheter (PE-280, 2.15 mm ID, 3.25 mm OD) was introduced into the right femoral vein, and threaded through the inferior vena cava into the left inferior phrenic vein. The catheter was secured in place by a silk suture placed through the central tendon and around the vein. The distal tip of the catheter was placed 5-10 cm below the vena cava, so that diaphragmatic venous blood could be collected into a container and reinfused into the animal.

Electromyographic (EMG) activity

Pairs of hook electrodes (1 cm apart) were placed in the right and left diaphragmatic costal fascicles. Fine wire electrode pairs were placed in the left alae nasi, genioglossus and left 2nd or 3rd parasternal muscles. The

signals were amplified and filtered (20-1000 Hz, Disa 05A0L), and then filtered (50-500 Hz), rectified and integrated (0.1 sec time constant) by a resistance capacitance integrator. Breath-by-breath EMG activity for a given muscle was quantified in terms of peak values. Inspiratory (T_I) and expiratory (T_E) times were measured from the integrated EMG activity of the diaphragm. Breathing frequency (f_R) was calculated from T_I and T_E.

Bradykinin preparation

Bradykinin (Sigma Chemical, St. Louis, MO) was dissolved in a saline vehicle to obtain concentrations of 10, 25, 50 and 100 μg/ml. Solutions were placed in small vials (2.5 ml) and kept frozen at -5 °C. On the day of the experiment the number of vials required were thawed at room temperature just prior to usage.

Experimental Protocol

General procedure: After completion of the surgical procedure, a 30-min stabilization period was allowed. To obtain a stable spontaneous breathing pattern, the frequency of the ventilator was reduced to 15 breaths/min. Baseline measurements of arterial blood gases, EMG activities and Part were then taken.

Group 1 (n=8): A 1 ml bolus of bradykinin was injected into the phrenic artery catheter, followed by a flush of 2-3 ml of saline to ensure that the bradykinin reached the diaphragmatic vasculature. Phrenic arterial and venous

catheters were clamped immediately after the saline flush. The occlusion was maintained for one min to allow sufficient time for bradykinin to reach afferent nerve endings. Diaphragmatic venous blood was collected for two min after bradykinin injection and discarded. Since, during the first experiment, inspiratory motor drive was unaffected by bolus injections of .05-5 μg bradykinin, 10, 25, 50 and 100 μg bradykinin were administered in random order. Bradykinin injections were given when the peak integrated EMG activities and respiratory timing returned to pre-injection values, which usually required about four min. Saline was injected to control for the effect of the vehicle. (See Fig. 1.)

Group 2 (n=5): To determine if changes in inspiratory motor drive were mediated by phrenic afferent activation, one dose of bradykinin was injected before and after section of the left phrenic nerve in five animals.

Data Analysis

Baseline Part was determined as the mean value obtained over twenty sec immediately before each injection. Following bradykinin injection, mean Part was measured over 10 sec intervals for two min at each bradykinin dose. Baseline EMG activity and respiratory timing were obtained by averaging ten breaths immediately preceeding each injection. The response values were then obtained by averaging 3 to 5 breaths at intervals of 30, 60, 90 and 120 sec after bradykinin injection.

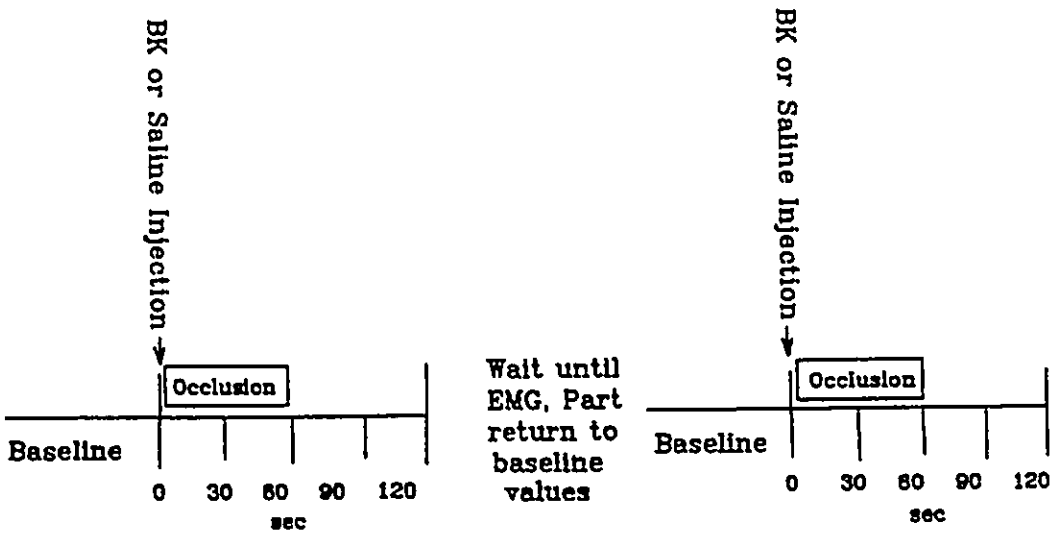

Fig. 2.1: A schema of the experimental protocol.

Statistical Analysis

Preliminary inspection of the data showed that the peak response to bradykinin was detected at 60 sec after injection, so this time interval was used in the statistical analysis. To compare the effect of bradykinin on inspiratory motor drive, three way analysis of variance was done using dosage and condition (baseline and response) as factors, blocked by animal. In addition, responses to saline and 50 μg bradykinin injections were compared by paired t-test. Group 2 responses to the 50 μg bradykinin injection were analyzed with a one sample t test. Significance was determined at $p<0.05$. Values are presented as means ± SE.

2.5 RESULTS

For all animals, baseline arterial pH, P_{o_2} and P_{co_2} after surgery averaged 7.28 ± 0.03, 87 ± 8 mmHg, and 41 ± 3 mmHg, respectively.

Group 1:

The experimental records from two animals are shown in Fig. 2. Baseline mean Part averaged 121 ± 10 mmHg. Mean Part increased to 131 ± 10 mmHg ($p<0.01$) by 60 sec after bradykinin injection, and then declined to control values by 2 min after the injection (Fig. 3). Genioglossus EMG activity was recorded in 7 animals at the 25 μg dosage, and in 8 animals at the 10, 50 and 100 μg dosages. Parasternal

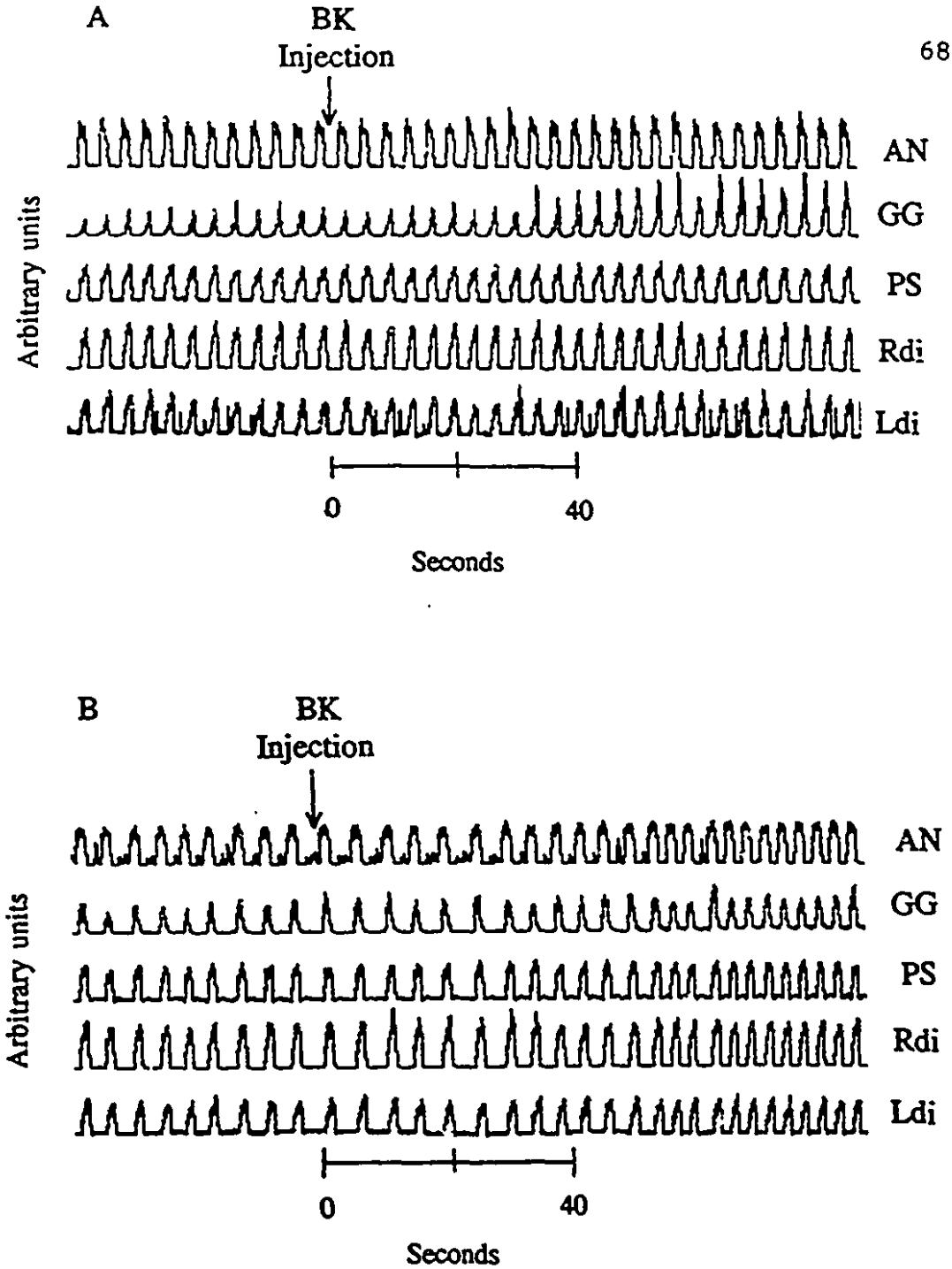

Fig. 2.2: Experimental records from two animals during bradykinin injections into the left phrenic artery. AN-alae nasi, GG=genioglossus, PS=parasternal, Rdi=right diaphragm, Ldi=left diaphragm. A. Note the marked change in genioglossus EMG activity after bradykinin injection. B. Note the marked change in respiratory frequency following bradykinin injection.

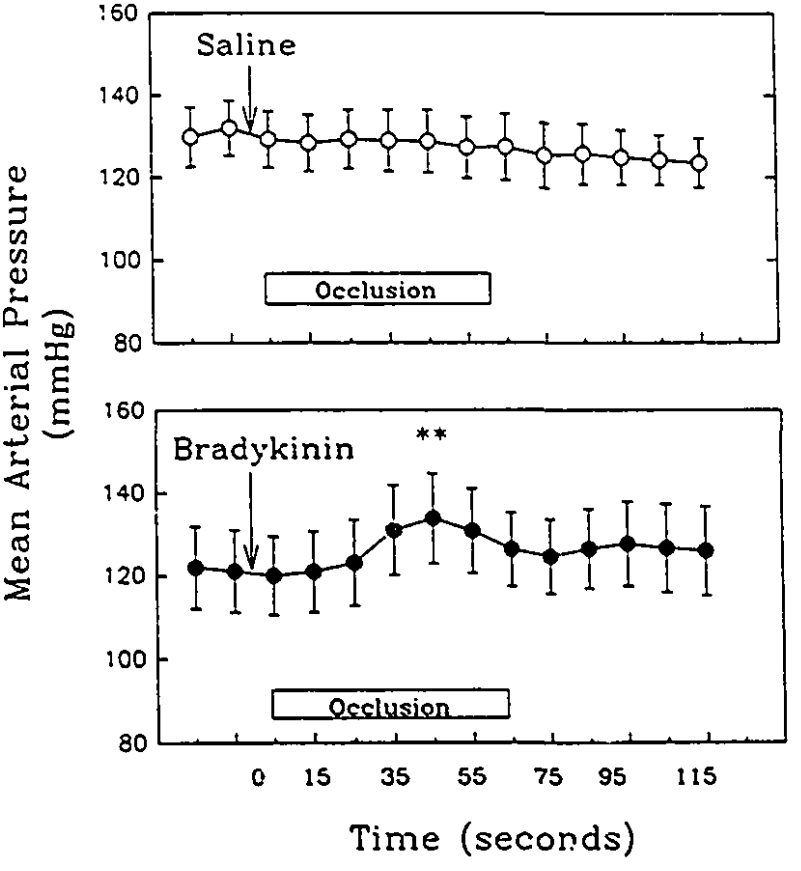

Fig 2.3: Systemic arterial blood pressure response to saline and 50 μg bradykinin injections into the left phrenic artery. Mean ± SE. ** $p < 0.01$ compared to baseline values. Note the increase in pressure caused by bradykinin injection.

EMG activity was recorded in 7 animals at the 25, 50 and 100 μg dosages, and 8 animals at the 10 μg dosage.

Effect of 50 μg injection

For most muscles, peak integrated EMG activity increased within 30 sec, and peaked by 60 sec after the injection. However, the magnitude of the response was dissimilar. There was a marked increase in genioglossus (GG) activity, whereas alae nasi (AN), parasternal (PS) and left diaphragmatic (Ldi) activity only increased slightly and right diaphragmatic (Rdi) remained unchanged (Fig. 4).

Baseline T_I, T_E and f_R averaged 1.59 ± 0.33 sec, 4.26 ± 1.35 sec, and 16.3 ± 3.0 breaths/min, respectively. Sixty sec after bradykinin injection, T_I declined significantly to 1.45 ± 0.31 sec ($p<0.01$ compared with control), whereas T_E (3.94 ± 1.41 sec) and f_R (18.0 ± 3.1 breaths/min) did not change significantly (Fig. 5). Figure 4 also illustrates the effect of saline injection on EMG activity of various muscles. Saline injections did not elicit any significant changes in respiratory timing or EMG activity of any of the muscles. When bradykinin response was compared to saline response, AN, GG, PS, Ldi and T_I responses remained significantly different ($p<0.05$).

Effect of different doses

Injections of 10, 25, 50 and 100 μg of bradykinin increased mean Part by a similar degree (5.6 ± 1.9, 8.5 ± 2.5, 9.4 ± 1.8 and 8.1 ± 2.4 mmHg, respectively).

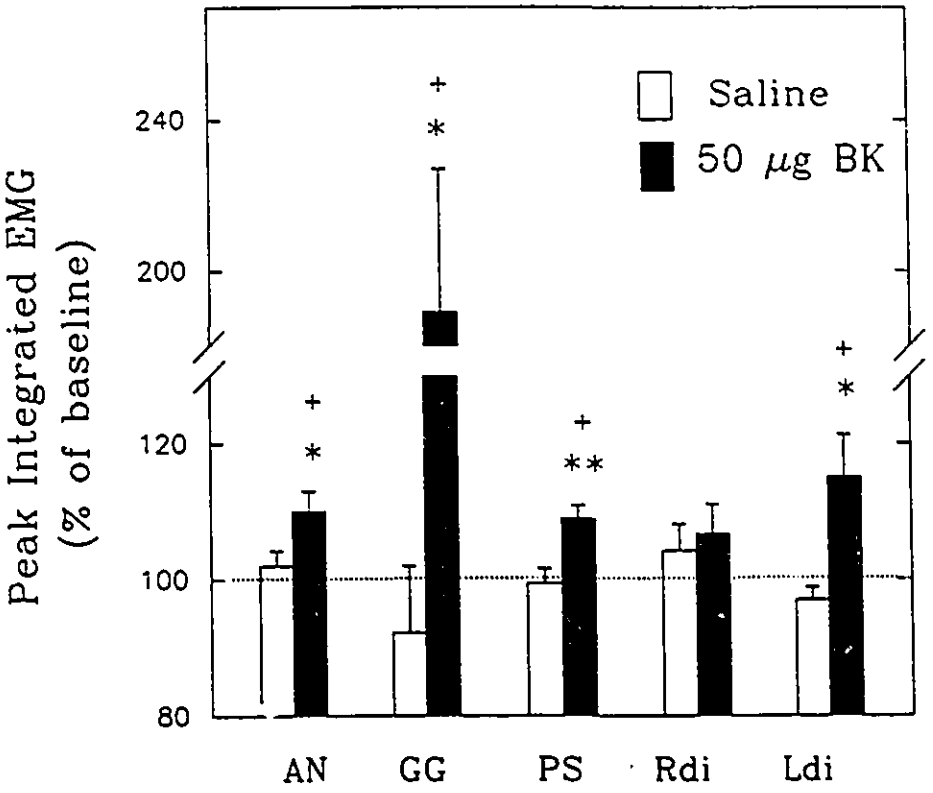

Fig. 2.4: EMG response of alae nasi (AN), genioglossus (GG), parasternal (PS), right (Rdi) and left (Ldi) diaphragms 60 sec after injection of saline or 50 μg bradykinin into the left phrenic artery. *,** $p < 0.05$ and 0.01, respectively, compared to baseline values. + $p < 0.05$ compared to saline values. Note the large change in genioglossus activity compared with the responses of the other muscles.

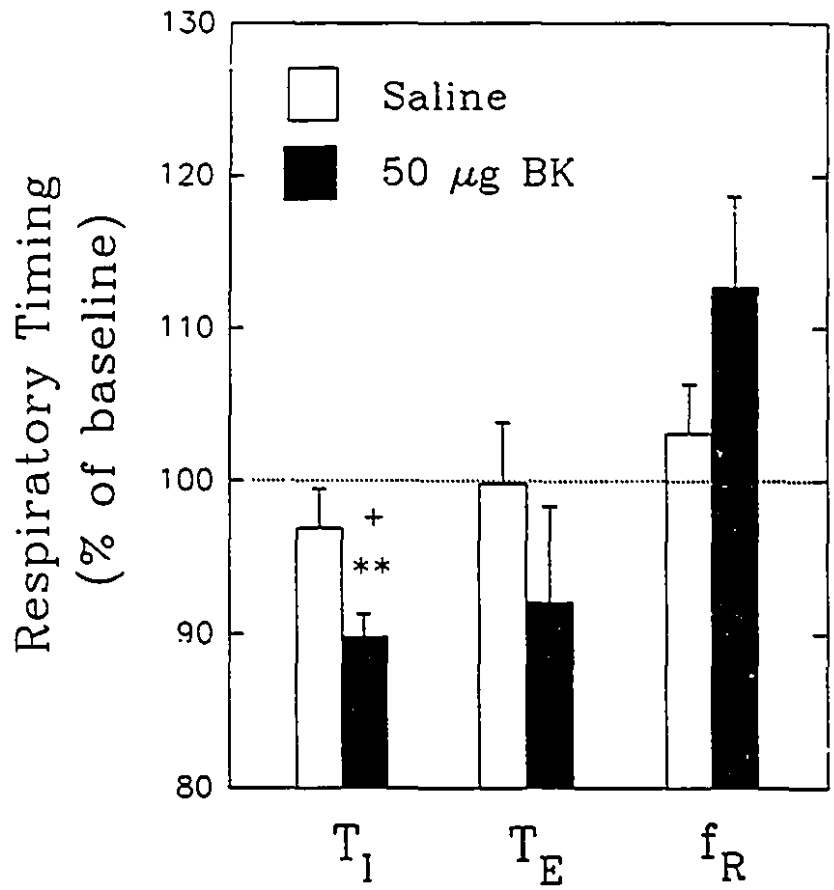

Fig 2.5: Changes in inspiratory time (T_I), expiratory time (T_E) and respiratory frequency (f_R) 60 seconds after injection of saline or 50 μg bradykinin into the left phrenic artery. * $p < 0.05$ compared to baseline values. + $p < 0.05$ compared to saline values.

Different doses of bradykinin elicited a similar increase in the peak integrated EMG activity of AN, GG, Rdi and Ldi, although PS was elevated more by the 50 μg injection (9% increase versus 4-6% increase with the other doses, $p<0.05$). T_I was significantly shortened after bradykinin injection, however, no differences were observed between different doses (97, 93, 90 and 96% of control in response to 10, 25, 50 and 100 μg, respectively). Bradykinin injection had no effect on T_E ($p>0.05$), although f_R increased slightly to 105, 113, 113 and 110% of control in response to 10, 25, 50 and 100 μg, respectively ($p<0.05$).

Group 2: After left phrenicotomy, baseline values of mean Part, T_I, T_E and f_R in this group averaged 116 $\pm$ 8.7 mmHg, 1.58 $\pm$ 0.36 sec, 3.02 $\pm$ 0.71 sec and 17.6 $\pm$ 4.6 breaths/min, respectively. With the injection of bradykinin, Part declined to 104.2 $\pm$ 11.1 mmHg ($p<0.05$). Peak EMG activity of AN (n=5) and Rdi (n=4) remained unchanged (101 and 90% of baseline, respectively). The effect of bradykinin injection on GG activity after phrenicotomy was assessed in three animals. Phrenicotomy resulted in the disappearance of GG activity in one animal. In the remaining two animals, bradykinin injection caused an average 61% increase in GG activity before phrenicotomy, and only 2% increase in GG activity after phrenicotomy. In addition, bradykinin did not appear to affect PS activity in the two animals with measurable PS EMG signals (91% of baseline levels).

Respiratory timing was also unaffected by bradykinin injection.

2.6 DISCUSSION

The main findings of this study are that:

1) Injection of bradykinin into the in-situ isolated and innervated left hemi-diaphragm vasculature resulted in a small increase in activity of alae nasi, parasternal and left diaphragm, a large increase in genioglossus activity, and no change in right diaphragmatic activity.

2) Responses were similar with 10, 25, 50 and 100 μg bradykinin injections.

3) The response could be eliminated by cutting the left phrenic nerve.

Critique

The main assumption of this study is that alterations in inspiratory motor drive were caused by bradykinin-induced activation of phrenic afferents. This requires that the left diaphragmatic vasculature be completely isolated from the systemic circulation (10). In our preliminary experiments, systemic contamination with bradykinin caused apnea and hypotension, in contrast to the augmentation of inspiratory muscle activity and arterial pressure that we report. Our assumption of phrenic afferent activation by bradykinin is also supported by the absence of any changes in respiratory timing or inspiratory muscle EMG activity when bradykinin was

injected after sectioning the left phrenic nerve in five animals. Finally, it is possible that the increase in the inspiratory motor drive we observed might have been modulated by afferent inputs from non-diaphragmatic sources such as arterial baroreceptors. Although we have eliminated inputs from aortic baroreceptors by sectioning the vagi, the increase in arterial pressure in response to bradykinin injection (Fig. 3) might have influenced carotid baroreceptor activity. An increase in arterial pressure is known to decrease ventilatory drive (2) and decrease hypoglossal nerve activity (23). It is possible, therefore, that the augmentation of the inspiratory motor drive, particularly that of the genioglossus, by phrenic afferent activation might have been larger if baroreceptor activity had been controlled.

One may also argue that the occlusion of phrenic arterial and venous flows following the injection of bradykinin might have elicited the increase in inspiratory motor drive by producing ischemia (28). We think this was unlikely, however, because saline injection followed by occlusion of diaphragmatic blood flow did not elicit ventilatory changes. Moreover, changes in ventilatory motor drive in response to diaphragmatic ischemia were evident only after 8 min of complete diaphragmatic ischemia in another study (28).

Physiology of Bradykinin

Bradykinin is a nonapeptide that is formed in tissue or plasma when kininogen is cleaved by the enzyme kallikrein, and is degraded by kininase II converting enzyme (5). Strong muscle contractions produce a significant increase in skeletal muscle bradykinin concentration, especially when ischemia is present (26). Although the mechanism of the increased production of bradykinin within muscle is not known, alterations in pH and lactate concentration have been shown to influence bradykinin metabolism (5,6).

In addition to its vasodilating effects, bradykinin is known to stimulate thin-fiber muscle afferents. Mense (18) recorded action potentials from fine filaments of group III and IV afferent fibers from the triceps surae muscles of anesthetized, paralyzed cats. Injection of bradykinin into the sural artery increased the frequency of action potential firing in 46% of the group III and 71% of group IV units. Similarly, Kaufman *et al.* (13) found that in anesthetized dogs injection of bradykinin elicited a four-fold increase in the rate of firing of 47% of group III and 52% of group IV afferent fibers in sciatic and gracilis nerves. There was no effect on the firing rate of group I and II afferent fibers. Based on these findings, we propose that the augmentation of inspiratory drive and arterial pressure observed in our study are mediated by the activation of thin-fiber (groups III and

IV) phrenic afferents. However, we do not know if our results can be attributed to a predominate activation of group III or group IV fibers, or to similar activation of both afferent groups.

Effects of groups III and IV phrenic afferents

Bradykinin elicited smaller increases in diaphragmatic activity and arterial pressure than those evoked by capsaicin injection (9). In addition, response latencies after bradykinin injection of 20 to 30 sec were longer than the 7 sec latencies observed after capsaicin injection (9), but similar to the latencies reported by Mizumura and Kumazawa (20) after bradykinin injection into the isolated medial gastrocnemius muscle of dogs. This might be attributed to differences in the pattern and intensity of afferent activation by these substances at the receptor level. Capsaicin elicits a sudden and strong increase in the firing rate of mainly group IV and few group III muscle afferents, whereas bradykinin causes afferent activity to rise more slowly and to a lesser extent than capsaicin (13). In addition, the response latency of capsaicin is 6 sec, while that of bradykinin is 16 sec (13).

We found that 10 μg of bradykinin was required to elicit changes in the ventilatory drive and arterial pressure. By comparison, Mizumura and Kumazawa (20) found a significant increase in minute ventilation in response to a 5 μg bradykinin injection into the medial gastrocnemius. This

difference could be attributed to the existence of fewer thin-fiber afferents in the diaphragm than in the gastrocnemius. Similar findings have been reported when thin-fiber afferents in these two muscles were activated electrically or chemically (9,22,29).

The absence of a dosage effect may be due to the steep dose response curve reported for activation of thin-fiber afferents by bradykinin (18). Another possibility may be the presence of tachyphylaxis. Kumazawa and Mizumura (15) reported a decrease in the firing rate of thin-fiber afferents when bradykinin injections were given every ten min. However, Mense (18) and Mense and Schmidt (19) demonstrated reproducibility of thin-fiber firing rates with injection intervals of 2-4 min. In addition, in a study on the effect of bradykinin on respiratory rate and cardiovascular parameters, Tallarida *et al.* (27) found no tachyphylaxis when injections were spaced 3-5 min apart. We speculate that the inability of higher doses of bradykinin to further augment inspiratory motor drive may be due to a maximal activation of afferents at the lowest bradykinin dose.

Differences in the inspiratory muscle response

Bradykinin elicited a relatively greater increase in genioglossus muscle activity compared with other inspiratory muscles (Fig. 4). This might have been due to the selective effects of anesthesia on central pathways. Barbiturate or

halothane anesthesia suppresses the activity of upper airway muscles more than the diaphragm through a selective action on the reticular formation (1,12). In contrast, α-chloralose, unlike other anesthetic agents, does not affect reticular formation activity (4), and should have an equal effect on the activity of the genioglossus and other inspiratory muscles. Indeed, the changes in phrenic and hypoglossal nerve activities in response to CO_2 rebreathing and hypoxia were influenced similarly by α-chloralose (see Fig. 8 in the Hwang report (12)). The preservation of reticular formation activity is particularly important in this protocol, since phrenic thin-fiber afferents seem to project predominately onto reticular nuclei rather than medullary respiratory group nuclei (16).

An alteration in baroreceptor activity as a result of the increase in arterial pressure from the bradykinin injection might have influenced the relative degree of genioglossus muscle activation. However, baroreceptor stimulation induces a greater depression of genioglossus activity than phrenic or recurrent laryngeal nerve activity (23). Thus, baroreceptor activity is an unlikely explanation for the disproportionate increase in genioglossus activity.

When compared to artificial electrical or chemical stimulation, bradykinin appears to be a weaker stimulant of inspiratory motor drive. For example, high intensity stimulation of the left phrenic nerve increased AN, GG, PS

and Rdi peak activities by 64%, 440%, 55% and 76% (29). Similarly, injection of 50 μg of capsaicin into the left hemi-diaphragm increased PS, Rdi and Ldi peak activities by 35%, 40%, and 67% (9). Neither type of stimulation changed respiratory timing. In our study, however, AN, PS and Ldi activities increased by 10-18%, GG activity increased by 80%, and inspiratory time decreased by 10%. In contrast, bradykinin did not significantly alter Rdi activity. It is possible that bradykinin selectively activates a greater proportion of group III phrenic afferents, which may be involved in a contralateral phrenic-phrenic inhibitory reflex (24). Activation of this reflex may have attenuated the augmentation of motor drive to the right diaphragm.

The differential activation of the inspiratory muscles observed in response to bradykinin injection is not unique to phrenic afferent activation. High intensity electrical stimulation of the sciatic (8) and gastrocnemius nerves (11) causes genioglossus EMG activity to increase more than other inspiratory muscles. In addition, tactile, auditory and visual stimuli have also been shown to cause a disproportionate increase in genioglossus muscle activity (3). The similarity of these effects may indicate a generalized response to afferent inputs on the reticular formation. Finally, the disproportionate increase in genioglossus activity induced by bradykinin under α-chloralose anesthesia supports the hypothesis that the

genioglossus muscle is predominantly controlled by reticular mechanisms, whereas the diaphragm is predominantly controlled by the medullary respiratory center (25).

2.7 REFERENCES

1. BENNETT, F.M. and W.M. ST. JOHN. Anesthesia selectively reduces hypoglossal nerve activity by actions upon the brain stem. *Pflugers Arch* 401: 421-423, 1984.

2. BISHOP, B. Carotid baroreceptor modulation of diaphragm and abdominal muscle activity in the cat. *J Appl Physiol* 36(1): 12-19, 1974.

3. BROUILLETTE, R.T. and B.T. THACH. Control of genioglossus muscle inspiratory activity. *J Appl Physiol* 49(5): 801-808, 1980.

4. DUDLEY, R.E., S.R. NELSON, and F. SAMSON. Influence of chloralose on brain regional glucose utilization. *Brain Res* 233: 173-180, 1982.

5. EDERY, H. and G.P. LEWIS. Inhibition of plasma kininase activity at slightly acid pH. *Brit J Pharmacol* 19: 299-305, 1962.

6. ERDOS, E.G. Hypotensive peptides: bradykinin, kallidin, and eledoisin. In: *Advances in Pharmacology*, edited by Garattini, S. and Shore, P.A. New York: Academic Press, 1966, p. 30-90.

7. GRAHAM, R., Y. JAMMES, S. DELPIERRE, C. GRIMAUD, and CH. ROUSSOS. The effects of ischemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. *Neurosci Lett* 67: 257-262, 1986.

8. HAXHIU, M.A., E. VAN LUNTEREN, J. MITRA, N.S. CHERNIACK, and K.P. STROHL. Comparison of the responses of the diaphragm and upper airway muscles to central stimulation of the sciatic nerve. *Respir Physiol* 58: 65-76, 1984.

9. HUSSAIN, S.N.A., S. MAGDER, A. CHATILLON, and CH. ROUSSOS. Chemical activation of thin-fiber phrenic afferents: respiratory responses. *J Appl Physiol* 69(3): 1002-1011, 1990.

10. HUSSAIN, S.N.A., CH. ROUSSOS, and S. MAGDER. In situ isolated perfused and innervated left hemidiaphragm preparation. *J Appl Physiol* 67(5): 2141-2146, 1989.

11. HUSSAIN, S.N.A., M.E. WARD, A.G. GATENSBY, CH. ROUSSOS, and A. DESCHAMPS. Respiratory muscle activation by limb muscle afferent stimulation in anesthetized dogs. *Respir Physiol* 84: 185-198, 1991.

12. HWANG, J., W. ST.JOHN, and D. BARTLETT, JR. Respiratory-related hypoglossal nerve activity: influence of anesthetics. *J Appl Physiol* 55(3): 785-792, 1983.

13. KAUFMAN, M.P., G.A. IWAMOTO, J.C. LONGHURST, and J.H. MITCHELL. Effects of capsaicin and bradykinin on afferent fibers with endings in skeletal muscle. *Circ Res* 50: 133-139, 1982.

14. KOHRMAN, R.M., J.B. NOLASCO, and C.J. WIGGERS. Types of afferent fibers in the phrenic nerve. *Am J Physiol* 151: 547-557, 1947.

15. KUMAZAWA, T. and K. MIZUMURA. Thin-fibre receptors responding to mechanical, chemical, and thermal stimulation in the skeletal muscle of the dog. *J Physiol (Lond)* 273: 179-194, 1977.

16. MACRON, J.-M., D. MARLOT, and B. DURON. Phrenic afferent input to the lateral medullary reticular formation of the cat. *Respir Physiol* 59: 155-167, 1985.

17. MARLOT, D., J-M. MACRON, and B. DURON. Inhibitory and excitatory effects on respiration by phrenic nerve afferent stimulation in cats. *Respir Physiol* 69: 321-333, 1987.

18. MENSE, S. Nervous outflow from skeletal muscle following chemical noxious stimulation. *J Physiol (Lond)* 267: 75-88, 1977.

19. MENSE, S. and R.F. SCHMIDT. Activation of group IV afferent units from muscle by algesic agents. *Brain Res* 72: 305-310, 1974.

20. MIZUMURA, K. and T. KUMAZAWA. Reflex respiratory response induced by chemical stimulation of muscle afferents. *Brain Res* 109: 402-406, 1976.

21. REVELETTE, W.R., L.A. JEWELL, and D.T. FRAZIER. Effect of diaphragm small-fiber afferent stimulation on ventilation in dogs. *J Appl Physiol* 65(5): 2097-2106, 1988.

22. ROAD, J.D., N.H. WEST, and B.N. VAN VLIET. Ventilatory effects of stimulation of phrenic afferents. *J Appl Physiol* 63(3): 1063-1069, 1987.

23. SALAMONE, J.A., K.P. STROHL, D.M. WEINER, J. MITRA, and N.S. CHERNIACK. Cranial and phrenic nerve responses to changes in systemic blood pressure. *J Appl Physiol: Respirat Environ Exercise Physiol* 55(1): 61-68, 1983.

24. SPECK, D.F. and W.R. REVELETTE. Excitation of dorsal and ventral respiratory group neurons by phrenic nerve afferents. *J Appl Physiol* 62: 946-951, 1987.

25. ST. JOHN, W. Influence of reticular mechanisms upon hypoglossal, trigeminal and phrenic activities. *Respir Physiol* 66: 27-40, 1986.

26. STEBBINS, C.L., O.A. CARRETERO, T. MINDROIU, and J.C. LONGHURST. Bradykinin release from contracting skeletal muscle of the cat. *J Appl Physiol* 69(4): 1225-1230, 1990.

27. TALLARIDA, G., F. BALDONI, G. PERUZZI, F. BRINDISI, G. RAIMONDI, and M. SANGIORGI. Cardiovascular and respiratory chemoreflexes from the hindlimb sensory receptors evoked by intra-arterial injection of bradykinin and other chemical agents in the rabbit. *J Pharmacol Exp Ther* 208: 319-329, 1979.

28. TEITELBAUM, J.S., S.A. MAGDER, CH. ROUSSOS, and S.N.A. HUSSAIN. Effects of diaphragmatic ischemia on the inspiratory motor drive. *J Appl Physiol* 72(2): 447-454, 1992.

29. WARD, M.E., G. VANELLI, M. HASHEFI, and S.N.A. HUSSAIN. Ventilatory effects of the interaction between phrenic and limb muscle afferents. *Respir Physiol* 88: 63-76, 1992.

CHAPTER 3

PHRENIC AFFERENT ACTIVATION REDUCES TRACHEALIS TENSION AND LUNG RESISTANCE

3.1 LINK TO CHAPTER 3

Previous studies have shown that the activation of thin-fiber afferents from limb muscles causes both hyperpnea and an increase in systemic blood pressure. In the first project I demonstrated that activation of diaphragmatic thin-fiber afferents by bradykinin (a physiological substance produced during muscle contraction) stimulates inspiratory motor drive and increases systemic blood pressure. This indicates that activation of afferents from ventilatory muscles play a role in the generation of exercise hyperpnea.

Another component of the exercise response is a decrease in air flow resistance. Previous studies have shown that the activation of limb muscle thin-fiber afferents decreases large airway tone (specifically tracheal tension) and lung resistance. Therefore, limb muscle afferent input may be involved in the control of airway muscle tone during exercise. Since ventilatory muscles are strongly recruited during exercise, afferent feedback from the diaphragm may also be involved in the regulation of airway smooth muscle tone. In the next project I investigated the relative effects of right, left and bilateral phrenic nerve stimulation on trachealis tension and lung resistance, and compared these effects to those found during tibial nerve stimulation.

3.2 ABSTRACT

Phrenic thin-fiber afferent activation stimulates inspiratory motor drive and breathing frequency. To assess the role of these fibers in airway muscle tone regulation, we monitored *in vivo* trachealis muscle tension (T_{tr}) and lung resistance (R_L) during phrenic and tibial nerve stimulation in 7 anesthetized, paralyzed, mechanically ventilated dogs. The proximal cut ends of the left and right phrenic (LP, RP) and the left tibial (T) nerves were stimulated separately and in combination (P+P) at high and low intensities (20-50 and 2-5 times twitch threshold, respectively) for 30-60 sec. High intensity stimulation of LP, RP, T, and P+P significantly decreased T_{tr} by 14, 18, 23 and 21%, respectively, whereas R_L significantly decreased by 8% and 11% during RP and P+P stimulation, respectively. Low intensity LP and RP stimulation did not affect T_{tr} or R_L. These findings indicate that phrenic afferent fiber activation decreases trachealis muscle tone and lung resistance. This reflex may alter airflow resistance when respiratory muscle activity is increased.

3.3 INTRODUCTION

Exercise induces a number of physiological changes in the cardiorespiratory system, many of which have been proposed to be due to the activation of sensory fibers of limb muscles. For example, activation of thin-fiber (groups III and IV) afferents of limb muscles has been shown to increase mean arterial blood pressure, tidal volume and respiratory frequency (20,28), and to redistribute ventilatory motor drive (28). In addition, the resistance to air flow is strongly influenced by these afferents as evidenced by the decline in trachealis muscle tone (7) and a decrease in lung resistance (22).

Like limb muscles, the respiratory muscles are strongly recruited during physical exercise. These muscles contain all four groups of afferent fibers which are present in other skeletal muscles. Therefore, it is possible that respiratory muscle afferents may also be involved in the cardiorespiratory response to exercise. Indeed, previous studies have illustrated that phrenic thin-fiber afferent activation leads to a rise in arterial blood pressure, redistribution of cardiac output (6), increased tidal volume and respiratory frequency (20), and redistribution of ventilatory motor drive (27,31). Unilateral stimulation of the phrenic nerve has been shown to decrease lung resistance (16). However, the influence of phrenic afferent activation on large airway smooth muscle tone, which provides the major

contribution to pulmonary resistance, remains unknown. The first aim of our study, therefore, was to evaluate simultaneous changes in trachealis muscle tension and lung resistance during electrical activation of thin-fiber phrenic afferents.

In most previous studies concerning the role of phrenic afferents, unilateral phrenic afferent activation has been used without consideration to anatomical differences in the number and origin of afferent fibers in the right and left phrenic nerves (5,11,12). For example, Kostreva and Pontus (12) have demonstrated that the right phrenic nerve contains mechanoreceptive afferent fibers from subdiaphragmatic structures. The physiological consequences of these anatomical differences have not yet been investigated. Accordingly, the second aim of our study was to evaluate the effect of activation of right versus left phrenic nerve afferents on trachealis muscle tone and lung resistance. In addition, bilateral phrenic nerve stimulation was performed.

Finally, it has been shown that chemical and electrical activation of limb muscle (e.g. gastrocnemius) afferents leads to stronger ventilatory and vascular responses than those elicited by phrenic afferent activation (6,19,28). Our third aim was to determine if the airway response is greater during tibial nerve stimulation than phrenic nerve stimulation.

3.4 METHODS

Animal preparation

Seven mongrel dogs (23-29 kg) were initially anesthetized with thiopental sodium (20 mg/kg) followed by α-chloralose (80-100 mg/kg). Supplemental boluses of α-chloralose were given as needed. The animals were intubated with a cuffed endotracheal tube (Sheridan, 9 mm ID), and ventilated with a constant volume ventilator (Harvard pump) with a tidal volume of 12-15 ml/kg and a frequency of 20 breaths/min. Ventilator settings were adjusted as necessary to maintain arterial P_{co_2} between 30-40 mmHg during the subsequent surgical preparation. Supplemental oxygen was provided to maintain arterial P_{o_2} above 100 mmHg. Air flow was measured using a pneumotachograph (Fleisch #2) and a differential pressure transducer (Validyne). Airway pressure was measured from a sideport located between the pneumotachograph and the endotracheal tube using a differential pressure transducer (Validyne). A catheter was placed in the right femoral artery to monitor systemic blood pressure (Trantec) and collect blood samples for arterial blood gas analysis (AVL 995). A second catheter was placed in the left femoral vein for fluid and drug administration.

Surgical procedure

The preparation developed by Brown *et al.* (1) was used to measure *in situ* changes in tension in the posterior tracheal membrane. The advantage of this preparation is that

the innervation to the trachealis muscle is left largely intact, enabling the investigation of reflexes under physiologic conditions. Briefly, a midline skin incision was made in the neck extending from the thyroid cartilage to just above the thoracic inlet. The endotracheal tube was removed, and the animal was reintubated through a low tracheostomy. The mid-cervical trachea above the tracheostomy site was exposed, and four contiguous cartilaginous rings (6-10) were bisected. Sutures were placed in the ends of each cartilage. One side of the tracheal segment was attached to a rod, and the other side was attached to a force displacement transducer (Grass FT03). Each side was attached to a rack and pinion system so that the trachealis muscle length could be adjusted.

A sternotomy was then performed, and the ribs were widely retracted. Both right and left phrenic nerves were isolated in the thorax, and bipolar stainless steel stimulating electrodes were placed close to the diaphragm. The twitch thresholds (TT) for a visible contraction were determined (Grass Stimulator Model S48, Grass Constant Current Unit CCU1), and the nerves were cut between the stimulating electrodes and the diaphragm. Similarly, the portion of the left tibial nerve which supplies the gastrocnemius muscle was isolated, the twitch threshold determined, and the nerve cut. The animals were then paralyzed with vecuronium (4-7 mg iv).

Experimental Protocol

The integrity of the trachealis preparation was tested using asphyxia produced by stopping the ventilator. This caused a marked increase in trachealis tension (T_{tr}) within approximately 30-60 sec. At the beginning of the experiment, the trachealis muscle length was adjusted to obtain a maximal decrease in tension in response to tibial nerve stimulation. The average trachealis muscle length utilized was 15 $\pm$ 1 mm. Baseline measurements of arterial blood gases, T_{tr} and arterial blood pressure (P_{art}) were recorded. During the experiment, end-tidal P_{CO_2} was monitored continuously (Amedec infrared analyzer), and oxygenation was monitored by periodic measurement of arterial blood gas tensions.

In each animal, phrenic and tibial nerves were individually stimulated in random order at high intensities (20-50 times twitch threshold, 40 Hz, 1 ms pulse duration) for 30-60 sec. In 5 animals, bilateral high intensity phrenic nerve stimulation was also performed. In addition, unilateral phrenic nerve stimulation was performed at low intensity (2-5 times twitch threshold, 100 Hz, 1 ms) in five animals. Stimulations were separated by 3-5 min, by which time monitored parameters had returned to their baseline levels. Air flow, airway pressure (P_{aw}), T_{tr} and P_{art} were recorded continuously for one min prior to and during each stimulation.

Data Analysis

Baseline T_{tr} and P_{art} were determined as the mean values obtained over twenty sec immediately before each stimulation. The peak change in T_{tr} and the length of time from the onset of stimulation was noted. Mean P_{art} was determined over 10 sec intervals throughout the stimulation, and the peak change in mean P_{art} was identified.

Breath-by-breath determination of lung resistance was done by applying multiple linear regression analysis to the equation of motion (P_{aw} = elastance * tidal volume + resistance * air flow + constant) as previously described (26). Baseline lung resistance (R_L) was determined as the mean of 3-5 breaths before each stimulation. Peak change in R_L during each stimulation was determined as the mean value of three consecutive breaths which deviated maximally from baseline. Resistance of the endotracheal tube was calculated as previously described (4) and subtracted from the resistance values provided by the regression analysis.

Analysis of variance for a randomized block design was used (30). Scheffé post hoc tests were used to compare baseline values to the results obtained during single nerve stimulation, and to assess differences in response to unilateral phrenic or tibial nerve stimulation. Due to different sample sizes, separate ANOVAS were performed for high and low intensity stimulations. Responses to bilateral phrenic nerve stimulation were compared with a one sample t

test. Significance was determined at $p < 0.05$. Values are presented as means ± SE.

3.5 RESULTS

After the surgical preparation was completed, arterial P_{O_2} and P_{CO_2} averaged 129 ± 17 mmHg and 39 ± 1 mmHg, respectively. Arterial pH was 7.33 ± 0.01. Figure 1 shows recordings of T_{tr} and P_{art} during separate high intensity stimulation of the left phrenic and tibial nerves in one animal. Stimulation of either the phrenic or tibial nerve produced a considerable reduction in T_{tr}. The maximum effect occurred within 10-20 sec, and T_{tr} then gradually returned toward baseline. In contrast, P_{art} increased and remained elevated throughout the period of stimulation of the phrenic or tibial nerve.

Phrenic Nerve Stimulation

High Intensity Stimulation

Group mean values for baseline T_{tr} are shown in Table 1. Baseline T_{tr} was comparable before left, right, and bilateral phrenic nerve stimulation (Table 1). As shown in Fig. 2, T_{tr} decreased significantly to 86%, 82% and 79% of baseline values during high intensity stimulation of the left phrenic, right phrenic and both phrenic nerves, respectively. Stimulation intensities were similar for all three conditions (left phrenic 28 ± 4 times TT; right phrenic 26 ± 3 times TT;

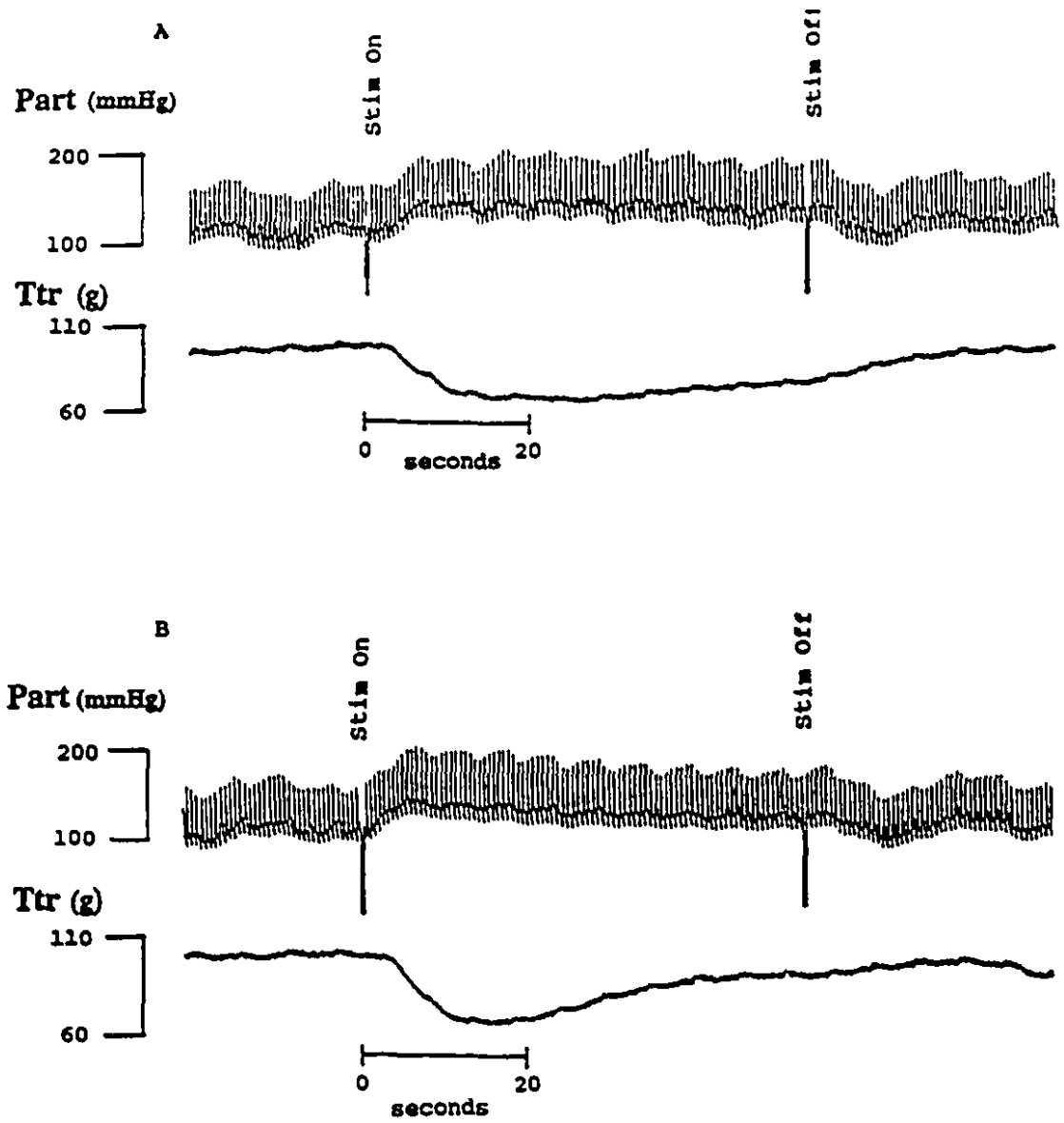

Fig. 3.1: Experimental recordings of tracheal tension (T_{tr}) and arterial blood pressure (P_{art}) from one animal during high intensity individual stimulation of the left phrenic (A) and left tibial (B) nerves.

Nerve	Intensity	Baseline (g)	Stimulation (g)
Left Phrenic	Low	136 (20)	128 (19)
	High	130 (15)	111* (12)
Right Phrenic	Low	134 (19)	126 (16)
	High	131 (14)	106** (11)
Bilat Phrenic	High	127 (19)	97* (10)
Left Tibial	High	130 (15)	99** (11)

Table 3.1: Trachealis muscle tension before and after nerve stimulation. Values are means. Values in parentheses are SE. *, ** $p < 0.05$, 0.01, respectively, compared to baseline values.

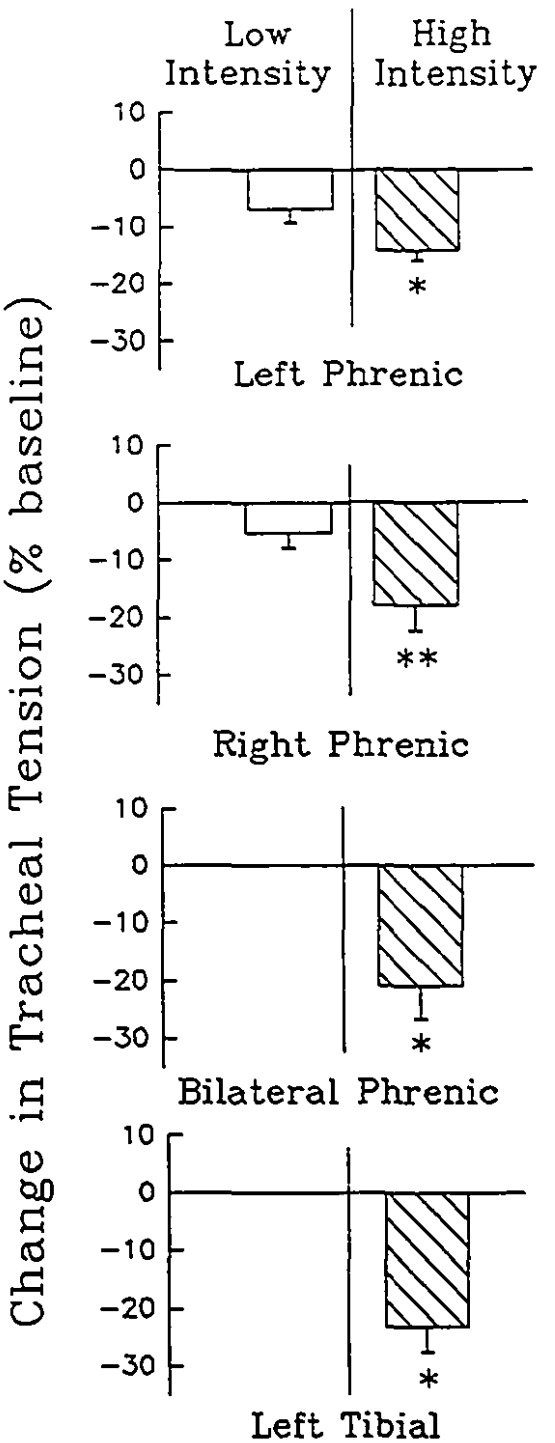

Fig. 3.2: Peak changes in tracheal tension during high and low intensity stimulation of the left phrenic (high: n=7, low: n=5), the right phrenic (high: n=7, low: n=5), both phrenic (high: n=5) and the left tibial (high: n=7) nerves. *,** $p < 0.05$, 0.01, respectively, compared to baseline values.

left phrenic nerve 34 ± 6 times TT and right phrenic nerve 23 ± 2 times TT). There were no significant differences between changes in T_{tr} during left and right phrenic nerve stimulation. The latency between the onset of stimulation and the peak change in T_{tr} averaged 17.3 ± 1 sec, and was no different between conditions.

After subtracting endotracheal tube resistance, baseline R_L was comparable before stimulation of the left, right and both phrenic nerves (Table 2). High intensity stimulation of the right phrenic nerve and both phrenic nerves significantly decreased R_L to 92% and 89% of baseline values, respectively (Fig. 3). However, the decrease in R_L caused by left phrenic nerve stimulation (to 94% of baseline) did not reach significance ($p = 0.10$) (See Fig. 3).

As shown in Table 3, there was a significant increase in mean P_{art} with both unilateral and bilateral phrenic nerve stimulations.

Low Intensity Stimulation

In the 5 animals tested, baseline T_{tr} and R_L were similar before left and right phrenic nerve stimulation (Tables 1 and 2). Unilateral phrenic nerve stimulation at low intensity (3 ± 1 times TT) had no significant effect on T_{tr} or R_L. A significant pressor response was found during right, but not left, phrenic nerve stimulation (Table 3).

Nerve	Intensity	Baseline ($cmH_2O/l/s$)	Stimulation ($cmH_2O/l/s$)
Left Phrenic	Low	2.78 (0.36)	2.63 (0.33)
	High	2.98 (0.35)	2.80 (0.33)
Right Phrenic	Low	2.74 (0.35)	2.62 (0.32)
	High	2.83 (0.31)	2.61* (0.29)
Bilat Phrenic	High	3.34 (0.43)	2.93* (0.31)
Left Tibial	High	2.95 (0.32)	2.75 (0.30)

Table 3.2: Lung resistance before and after nerve stimulation. Values are means. Values in parentheses are SE. * $p < 0.05$, compared to baseline values.

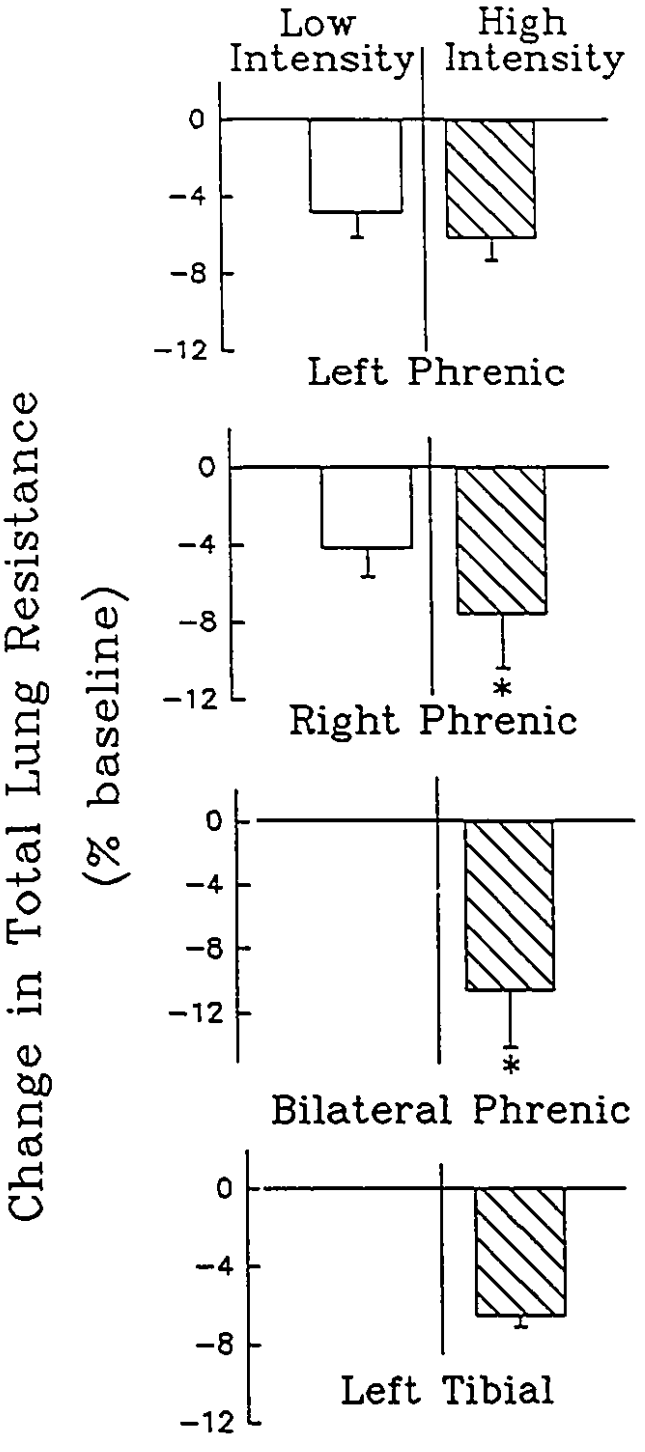

Fig. 3.3: Peak changes in lung resistance during high and low intensity stimulation of the left phrenic (high: n=7, low: n=5), the right phrenic (high: n=7, low: n=5), both phrenic (high: n=5) and the left tibial (high: n=7) nerves. * $p < 0.05$, compared to baseline values.

Nerve	Intensity	Baseline (mmHg)	Stimulation (mmHg)
Left Phrenic	Low	121 ± 7	124 ± 6
	High	125 ± 6	142 ± 7**
Right Phrenic	Low	119 ± 8	128 ± 8*
	High	126 ± 7	153 ± 8**
Bilat Phrenic	High	119 ± 6	149 ± 7*
Left Tibial	High	127 ± 6	144 ± 8**

Table 3.3: Mean systemic blood pressure before and after nerve stimulation. Values are means ± SE. *, ** $p < 0.05$, 0.01, respectively, compared to baseline values

Tibial Nerve Stimulation

Baseline T_{tr} before high intensity tibial nerve stimulation (22 ± 1 times TT) was similar to the baseline tension preceding high intensity phrenic nerve stimulation (Table 1). During tibial nerve stimulation, T_{tr} decreased to 77% of baseline (Fig. 2). There were no significant differences in the decreases in T_{tr} caused by stimulation of the tibial nerve when compared to phrenic nerve stimulation. Although R_L decreased to 93% of baseline (Fig. 3), this change was not statistically significant ($p = 0.08$). Finally, tibial nerve stimulation caused a significant rise in P_{art} (Table 3).

3.6 DISCUSSION

The main findings of this study are that:

1. High intensity electrical stimulation of the proximal end of one or both cut phrenic nerves elicited a significant decline in trachealis muscle tension, whereas low intensity stimulation of these nerves had no effect on trachealis muscle tension. These results were comparable to those obtained with tibial nerve stimulation.

2. Lung resistance decreased during high intensity stimulation of the right phrenic nerve and both phrenic nerves. However, lung resistance remained unchanged with high inte

nsity individual stimulation of the left phrenic and tibial

nerves, and low intensity stimulation of either phrenic nerve.

Critique

One could argue that the changes in trachealis muscle tension and lung resistance could have been caused by afferent inputs other than the phrenic afferents. Changes in chemoreceptor stimulation, such as hypoxia (1) and hypercapnia (18) are known to cause contraction of airway smooth muscle. Since experiments were performed during controlled mechanical ventilation, it is unlikely that chemoreceptor input varied during phrenic or tibial nerve stimulation.

Airway smooth muscle tone can also be affected by the activation of pulmonary mechanoreceptors. Lung inflation and deflation reflexly dilate (29) and constrict (9) airway smooth muscle, respectively. Considering the fact that nerve stimulation was performed in open chested animals with a fixed breathing pattern, changes in pulmonary mechanoreceptor activity should not have influenced the results obtained. However, in these animals, inspiratory muscle activity occurred during was not entrained to the ventilator rate. Therefore, our results could be affected by the phase differences between pulmonary stretch receptor activity and the central respiratory rhythm.

It might be argued that the decrease in trachealis tension during phrenic and tibial nerve stimulation was

produced by the concomitant pressor response. Nadel and Widdicombe (18) have shown that a large, sudden change in systemic blood pressure (from 20 mmHg to 200 mmHg) causes a modest increase (2.9%) in the volume of a tracheal segment, with no change in total lung resistance. In addition, Schultz *et al.* (23) reported a fall in trachealis tension when carotid sinus pressure was increased by 25 mmHg for one min. Although the mean peak changes in systemic blood pressure reported in our study (from 15-27 mmHg) lasted only ten sec, it is possible that these changes may have had a minimal effect on trachealis tension. However, others have shown that when animals are pretreated with phentolamine and/or propranolol, electrical or chemical thin-fiber afferent activation continues to elicit decreases in trachealis tension (7) and lung resistance (16) despite only minimal changes in mean arterial pressure. In further support of this point, we found that stimulation of phrenic or tibial afferents caused inhibition of trachealis tension in some animals when mean arterial pressure was either unchanged or even depressed. Similar findings have also been previously reported during activation of hindlimb muscle afferents (7,8,14).

In our protocol, we performed high and low intensity stimulations to activate different groups of afferent fibers. Although we did not perform nerve recordings, previous studies have shown that stimulation at less than 5 times the

stimulation threshold for motor (22) nerve fibers activates only group I and II afferent fibers, while stimulation at higher intensities activates groups III and IV (thin-fiber) afferents. We have therefore assumed that responses which occurred during high, but not low, intensity stimulation are due to the activation of thin-fiber afferents. This assumption is drawn from reports that cardiorespiratory responses to either muscle contraction (17) or nerve stimulation (20) do not occur when thin-fiber afferent activation is blocked by anesthetics or cooling.

Trachealis Tension and Lung Resistance

In our study, high intensity stimulation of one or both phrenic nerves had an inhibitory effect on trachealis tension, while low intensity stimulation had no significant effect. We also found that nonrespiratory (tibial) nerve stimulation caused similar decreases in trachealis tension. The activation of limb muscle afferents has been shown in previous studies to cause alterations in trachealis tension which are comparable to our findings. For instance, static contractions of one hindlimb (14) and both gracilis muscles (8) caused dilation of trachealis muscle to 89% and 73% of baseline levels, respectively. Similar results have been reported when muscular thin-fiber afferents have been activated by capsaicin (2,7).

Previous studies have demonstrated that lung resistance is affected by activation of respiratory and non-respiratory

skeletal muscle thin-fiber afferents. For example, McCallister *et al.*(16) reported that unilateral phrenic nerve stimulation at 20-200 times twitch threshold decreased resistance to 91-78% of baseline values. Similarly, Rybicki and Kaufman (22) found that stimulation of both gracilis nerves at 5-200 times twitch threshold decreased resistance to 96-82% of baseline values. We found that lung resistance decreased during high intensity stimulation of the right and both phrenic nerves, and that the changes in lung resistance during stimulation of the left phrenic and tibial nerves were not statistically significant. Some of the differences between our findings and those of McCallister *et al.* could be attributed to differences in the sample size, animal size, anesthetic level, endotracheal tube resistance, and stimulation protocol. In addition, in the McCallister study, resistance was determined by the Buxco Pulmonary Mechanics Analyzer (which uses the iso-volume method to determine resistance), while we used a multiple linear regression technique (26). Differences in flow rates in the two studies could affect the calculated resistance values and, in fact, the baseline resistance values (when uncorrected for endotracheal tube resistance) in our study were lower than those in the McCallister *et al.* and Rybicki and Kaufman studies.

It is interesting that the changes in trachealis tension were, in general, larger than the changes in lung resistance.

This is consistent with a previous finding that trachealis tension is a more sensitive indicator of airway contraction than lung resistance, perhaps due to differences in cholinergic receptor concentration (13) and inherent variability in trachealis tension versus lung resistance measurements. Leff *et al.* (13) have shown that lung resistance does not change until the alterations in trachealis tension exceed 11 g/cm. In our study, the decreases in trachealis tension ranged from 8-14 g/cm. This may explain why our changes in lung resistance were small.

Comparison of Responses to Nerve Stimulation

We have observed that high intensity stimulation of the right phrenic nerve tends to cause slightly larger (although not significantly different) changes in trachealis tension, lung resistance, and mean arterial pressure than stimulation of the left phrenic nerve. Very few studies discuss anatomical differences between the right and left phrenic nerves. Hinsey *et al.* (5) state that, in the cat, the number of nerve fibers in the right and left phrenic nerves in the portion of the nerve located between the heart and the diaphragm are not identical. In addition, a recent study of the right phrenic nerve in the dog indicates that it contains fibers which are responsive to mechanical stimulation of the hepatic parenchyma, hepatic veins and inferior vena cava (12). The phrenic nerves in our preparation were cut as close as possible to the diaphragm, so it is probable that

few, if any, fibers from the pericardium and posterior mediastinum were stimulated (11). However, any fibers arising from subdiaphragmatic structures (e.g. the inferior vena cava, liver or hepatic veins) would have been activated during right phrenic nerve stimulation. It may be that anatomical variations in the fibers of the two phrenic nerves are responsible for the slight differences in trachealis tension and lung resistance during single nerve stimulation.

Bilateral phrenic nerve stimulation produced a significant decrease in trachealis tension (21%) which was less that the sum of the responses to unilateral phrenic nerve stimulation (32%). Similarly, stimulation of both phrenic nerves produced a significant decrease in lung resistance (11%) which was slightly less than the sum of the responses to unilateral phrenic nerve stimulation (14%). Stimulation of both gracilis nerves has also been reported to produce an effect on total pulmonary resistance which was less than additive (22). However, combined stimulation of phrenic and gastrocnemius nerves has been shown to increase minute ventilation by 150%, an amount similar to that predicted by summating the effects of individual nerve stimulation (28).

We found that changes in trachealis tension in response to unilateral tibial nerve stimulation tended to be greater than the changes caused by unilateral phrenic nerve stimulation, and similar to the decreases caused by bilateral

phrenic nerve stimulation. Differences in muscle mass and the number of afferent fibers activated may affect these responses. In fact, the gastrocnemius muscle weighs about 35% more than the left hemi-diaphragm (6,20), and the entire diaphragm muscle weighs about 27% more than the gastrocnemius muscle (20). However, the ratio of thin-fiber afferents per unit mass of muscle may not be constant. This was indicated by an earlier finding that, even when the amount of capsaicin delivered was adjusted by muscle weight, gastrocnemius stimulation by capsaicin produced larger responses than hemi-diaphragm stimulation (6).

Reflex Pathways involved in the Regulation of Airway Smooth Muscle Tone

The precise anatomical pathway through which phrenic afferent activation alters airway smooth muscle tone is not known. However, the latency observed between onset of phrenic afferent stimulation and peak response suggest that a long loop reflex is involved. This is not surprising since phrenic afferents project upon several supraspinal structures, including the medullary respiratory groups (24), cerebellum (15), and sensorimotor cerebral cortex (3). The canine trachealis muscle is innervated by the superior and the recurrent largyngeal nerves (10), and the lower airways are innervated by nerve filaments from the anterior and posterior pulmonary plexuses. The caudal ventrolateral medulla (19) and ventral medullary surface (25) have been

implicated as regions associated with the regulation of airway caliber. One study has indicated that trachealis dilation during limb muscle afferent stimulation is due to an inhibition of cholinergic input (21). This indicates that the mechanism whereby phrenic afferent activation alters trachealis tension and lung resistance may also involve inhibition of cholinergic input to the large and small airways.

In summary, we have shown that phrenic thin-fiber afferent activation inhibits trachealis muscle tension and affects lung resistance. This reflex provides a mechanism to modulate large and small airway resistance in accord with changes in diaphragmatic activity. The ability to decrease airway resistance when respiratory muscle activity is increased may be an important component of the respiratory controller response to exercise or pathologic conditions which require an augmented ventilatory drive.

3.7 REFERENCES

1. BROWN, J.K., A.R. LEFF, M.J. FREY, B.R. REED, and W.M. GOLD. Physiological and pharmacological properties of canine trachealis muscle in vivo. *J Appl Physiol:Respirat Environ Exercise Physiol* 49(1): 84-94, 1980.

2. COLERIDGE, J.C.G, H.M. COLERIDGE, A.M. ROBERTS, M.P. KAUFMAN, and D.G. BAKER. Tracheal contraction and relaxation initiated by lung and somatic afferents in dogs. *J Appl Physiol:Respirat Environ Exercise Physiol* 52(4): 984-990, 1982.

3. DAVENPORT, P.W., F.J. THOMPSON, R.L. REEP, and A.N. FREED. Projection of phrenic nerve afferents to the cat sensorimotor cortex. *Brain Res* 328: 150-153, 1985.

4. GOTTFRIED, S.B., A. ROSSI, and B.D. HIGGS. Noninvasive determination of respiratory system mechanics during mechanical ventilation for acute respiratory failure. *Am Rev Respir Dis* 131: 414-420, 1985.

5. HINSEY, J.C., K. HARE, and R.A. PHILLIPS. Sensory components of the phrenic nerve of the cat. *Proc Soc Exp Biol Med* 41: 411-414, 1939.

6. HUSSAIN, S.N.A., S. MAGDER, A. CHATILLON, and CH. ROUSSOS. Chemical activation of thin-fiber phrenic afferents: respiratory responses. *J Appl Physiol* 69(3): 1002-1011, 1990.

7. KAUFMAN, M.P., G.A. ORDWAY, J.C. LONGHURST, and J.H. MITCHELL. Reflex relaxation of tracheal smooth muscle by thin-fiber muscle afferents in dogs. *Am J Physiol* 243: R383-R388, 1982.

8. KAUFMAN, M.P. and K.J. RYBICKI. Muscular contraction reflexly relaxes tracheal smooth muscle in dogs. *Respir Physiol* 56: 61-72, 1984.

9. KONDO, T., K. TAMURA, K. ONOE, H. TAKAHIRA, Y. OHTA, and H. YAMABAYASHI. In vivo recording of electrical activity of canine tracheal smooth muscle. *J Appl Physiol* 72(1): 135-142, 1992.

10. KONNO, A. Innervation of the smooth muscle in the cervical trachea. On the descending motor fibers to the cervical tracheal muscle in the inner branch of the superior laryngeal nerve. *J Otolaryn Jpn* 79:

725-732, 1976.

11. KOSTREVA, D.R. and S.P. PONTUS. Pericardial mechanoreceptors with phrenic afferents. *Am J Physiol* 264: H1836-H1846, 1993.

12. KOSTREVA, D.R. and S.P. PONTUS. Hepatic vein, hepatic parenchymal, and inferior vena caval mechanoreceptors with phrenic afferents. *Am J Physiol* 265: G15-G20, 1993.

13. LEFF, A.R., N.M. MUNOZ, and B. ALDERMAN. Measurement of airway response by isometric and nonisometric techniques in situ. *J Appl Physiol:Respirat Environ Exercise Physiol* 52(5): 1363-1367, 1982.

14. LONGHURST, J.C. Static contraction of hindlimb muscles in cats reflexly relaxes tracheal smooth muscle. *J Appl Physiol:Respirat Environ Exercise Physiol* 57(2): 380-387, 1984.

15. MARLOT, D., J.-M. MACRON, and B. DURON. Projections of phrenic afferences to the cat cerebellar cortex. *Neurosci Lett* 44: 95-98, 1984.

16. MCCALLISTER, L.W., K.W. MCCOY, J.C. CONNELLY, and M.P. KAUFMAN. Stimulation of groups III and IV phrenic afferents reflexly decreases total lung resistance in dogs. *J Appl Physiol* 61(4): 1346-1351, 1986.

17. MCCLOSKEY, D.I. and J.H. MITCHELL. Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol (Lond)* 224: 173-186, 1972.

18. NADEL, J.A. and J.G. WIDDICOMBE. Effect of changes in blood gas tensions and carotid sinus pressure on tracheal volume and total lung resistance to airflow. *J Physiol (Lond)* 163: 13-33, 1962.

19. PADRID, P.A., J.R. HASELTON, AND M.P. KAUFMAN. Role of caudal ventrolateral medulla in reflex and central control of airway caliber. *J Appl Physiol* 71(6): 2274-2282, 1991.

20. ROAD, J.D., N.H. WEST, and B.N. VAN VLIET. Ventilatory effects of stimulation of phrenic afferents. *J Appl Physiol* 63(3): 1063-1069, 1987.

21. RYBICKI, K.J. and M.P. KAUFMAN. Atropine prevents the reflex tracheal relaxation arising from the stimulation of intestinal and skeletal muscle

afferents in dogs. *Brain Res* 270: 159-161, 1983.

22. RYBICKI, K.J. and M.P. KAUFMAN. Stimulation of group III and IV muscle afferents reflexly decreases total pulmonary resistance in dogs. *Respir Physiol* 59: 185-195, 1985.

23. SCHULTZ, H.D., T.E. PISARRI, H.M. COLERIDGE, and J.C.G. COLERIDGE. Carotid sinus baroreceptors modulate tracheal smooth muscle tension in dogs. *Circ Res* 60: 337-345, 1987.

24. SPECK, D.F. and W.R. REVELETTE. Excitation of dorsal and ventral respiratory group neurons by phrenic nerve afferents. *J Appl Physicl* 62: 946-951, 1987.

25. STROHL, K.P., M.P. NORCIA, A.D. WOLIN, M.A. HAXHIU, E. VAN LUNTEREN, and E.C. DEAL, JR. Nasal and tracheal responses to chemical and somatic afferent stimulation in anesthetized cats. *J Appl Physiol* 65(2): 870-877, 1988.

26. WALD, A., D. JASON, T.W. MURPHY, and V.D.B. MAZZIA. A computers system for respiratory parameters. *Comp Biomed Res* 2: 411-429, 1969.

27. WARD, M.E., A. DESCHAMPS, CH. ROUSSOS, and S.N.A. HUSSAIN. Effect of phrenic afferent stimulation on pattern of respiratory muscle activation. *J Appl Physiol* 73(2): 563-570, 1992.

28. WARD, M.E., G. VANELLI, M. HASHEFI, and S.N.A. HUSSAIN. Ventilatory effects of the interaction between phrenic and limb muscle afferents. *Respir Physiol* 88: 63-76, 1992.

29. WIDDICOMBE, J.G. and J.A. NADEL. Reflex effects of lung inflation on tracheal volume. *J Appl Physiol* 18(4): 681-686, 1963.

30. WILKINSON, L. SYSTAT: The system for statistics. Evanston IL: SYSTAT, Inc., 1990.

31. WILSON, C.R., G. VANELLI, S. MAGDER, and S.N.A. HUSSAIN. Phrenic afferent stimulation by bradykinin and the distribution of the inspiratory motor drive. *Respir Physiol* 96:1-12, 1994.

CHAPTER 4

THE EFFECTS OF VAGAL AFFERENTS AND PHASIC ACTIVATION OF PHRENIC AFFERENTS ON THE INSPIRATORY MOTOR DRIVE

4.1 LINK TO CHAPTER 4

Most studies which have shown that phrenic thin-fiber afferent activation increases ventilatory drive have used continuous stimulation (40-60 sec) in vagotomized preparations. However, thin-fiber afferents are activated by mechanical and chemical stimuli, and mechanosensitive fibers are usually phasically active. Indeed, it has recently been demonstrated that group III hindlimb afferents fire phasically in response to slow locomotion in decerebrate cats. To mimic *in vivo* activation of mechanoreceptive thin-fiber afferents, electrical stimulation during either neural inspiration or expiration must be investigated. One study has indicated that activation of limb muscle thin-fiber afferents during inspiration causes more profound excitation of phrenic drive than expiratory stimulation. The effect of phasic activation of phrenic afferents is not known. In the next project, I determined the effect of phasic activation of large and thin-fiber phrenic nerve afferents on inspiratory motor drive, and compared these findings to changes evoked by tibial nerve stimulation.

Furthermore, it is known that vagal input affects ventilatory drive. However, it is not known whether vagal input modulates the inspiratory motor response to phrenic thin-fiber activation. Therefore, we also investigated the effect of phasic phrenic nerve stimulation before and after vagotomy. We repeated the protocol while performing tibial nerve stimulation, to compare vagal influences on respiratory and non-respiratory muscle afferent input.

4.2 ABSTRACT

We assessed the interaction between phrenic and vagal afferents in the control of inspiratory motor drive. In chloralose anesthetized, mechanically ventilated dogs, the central end of cut phrenic or tibial nerves were stimulated for 8 breaths during neural inspiration or expiration, at high and low intensity, and before and after bilateral vagotomy. Inspiratory motor drive was assessed using the integrated EMG activity of the right diaphragm (Rdi), parasternal (PS) and alae nasi (AN) muscles. Inspiratory and expiratory high intensity phrenic or tibial nerve stimulation produced similar augmentation of inspiratory motor drive. Enhanced inspiratory motor drive in response to inspiratory phrenic afferent stimulation was attenuated by vagotomy. Vagotomy, however, did not influence the excitatory effects of phasic tibial nerve stimulation. These results indicate that the presence of the vagi during inspiration potentiates the stimulating effect of phrenic thin-fiber afferent activation, and that vagal afferents exert differential influences on phrenic and tibial afferent inputs.

4.3 INTRODUCTION

Stimulation of phrenic afferents by chemical or electrical means leads to a rise in arterial blood pressure, a redistribution of cardiac output (9), an increase in tidal volume and respiratory frequency (20), and a redistribution of ventilatory motor drive (25,27). In addition, activation of phrenic thin-fiber (groups III and IV) afferents has been shown to cause a decrease in lung resistance (15). However, most studies have employed stimulation for relatively long periods of time (30-60 seconds), and the effect of brief stimulation of phrenic afferents on the distribution of inspiratory motor drive has not yet been described. In addition, thin-fiber afferents (predominately type III afferents) can be activated by mechanical stimuli (16). Hence, *in vivo*, some thin-fiber phrenic afferents may be activated during either inspiration or expiration. Activation of these afferents during different portions of the respiratory cycle may evoke distinct changes in ventilatory drive. Therefore, the first aim of this study was to determine if the inspiratory motor drive response to phrenic nerve stimulation is phase dependent.

To eliminate vagal influences, vagotomized preparations have been used frequently to investigate the effect of phrenic afferent stimulation on ventilatory drive. It is possible, however, that the responses to phrenic nerve stimulation may be modified by vagal input. In limb muscles, Simbulan *et al.* (23) found that activation of tibial nerve afferents caused greater increases in phrenic nerve activity in vagi-intact preparations

than in vagotomized preparations. These authors attributed their findings to differences in vagal activity (e.g. volume-related feedback) during the inspiratory and expiratory phases of the respiratory cycle. Therefore, the second aim of this study was to determine if the inspiratory drive response to phasic phrenic nerve stimulation is different in vagi-intact versus vagotomized animals.

4.4 METHODS

Surgical preparation

Eight mongrel dogs (21-33 kg) were initially anesthetized with thiopental sodium (20 mg/kg) followed by α-chloralose (80 mg/kg). Supplemental boluses of α-chloralose were given as needed. The animals were intubated with a cuffed endotracheal tube and ventilated with a constant volume ventilator (Harvard pump) with a tidal volume of 12-15 ml/kg and a frequency of 20 breaths/min. Ventilator settings were adjusted as necessary to maintain arterial PCO_2 between 30-40 mmHg during the subsequent surgical preparation. Supplemental oxygen was provided to maintain arterial PO_2 above 100 mmHg.

A median sternotomy was performed, and the chest was widely retracted. The left phrenic nerve was isolated and bipolar stimulating electrodes were placed on the nerve. A twitch threshold (TT) was determined by stimulating the nerve until a contraction of the left diaphragm was visible. The nerve was cut distal to the stimulating electrode, which was then secured and wrapped in gauze soaked in liquid paraffin. The left tibial

nerve was similarly prepared. Both vagus nerves were isolated in the cervical region, and surrounded with gauze soaked in liquid paraffin. One catheter was placed in the right carotid artery to monitor arterial blood pressure, and a second catheter was placed in the left jugular vein for fluid administration. End-tidal carbon dioxide ($ETCO_2$, Amedec infrared analyzer) was monitored during the stimulation protocol and maintained between 3-4%.

To monitor electromyographic activity, fine wire electrodes were placed in the right alae nasi, parasternal (2nd or 3rd intercostal space) and right costal diaphragm. The signals were amplified and filtered (20-1000 Hz, Disa 05A01), and then filtered (50-500 Hz), rectified and integrated (0.1 sec time constant) by a resistance capacitance integrator. Breath-by-breath integrated EMG activity for a given muscle was quantified in terms of peak values. Inspiratory (Ti) and expiratory (Te) times were measured from the integrated EMG activity of the diaphragm. Breathing frequency (fR) was calculated from Ti and Te.

Experimental Protocol

After completion of the surgical procedure, a 30 minute stabilization period was allowed. To obtain a stable spontaneous breathing pattern, the frequency of the ventilator was reduced to 15 breaths/min. Baseline measurements of arterial blood gases, EMG activity and arterial pressure were taken.

The left phrenic and tibial nerves were then individually stimulated in random order for 8 consecutive breaths during

neural inspiration or expiration (as determined from the integrated signal of the right costal diaphragm). Stimulations were either done at low intensity (2 times twitch threshold, 1 ms pulse duration, 100 Hz), or high intensity (35-40 times twitch threshold, 1 ms pulse duration, 12 Hz). A bilateral cervical vagotomy was then performed and, after a 30 min recovery period, the stimulation protocol was repeated.

Data Analysis

Baseline arterial pressure was determined as the mean value obtained over twenty sec immediately before each stimulation. Baseline EMG activity and respiratory timing were obtained by averaging eight breaths immediately preceeding each stimulation. The response values were determined by averaging the values of all eight stimulated breaths.

Some difficulties arise when comparing changes in inspiratory drive occurring during vagi-intact and vagotomized conditions. In the open thorax conditions used in our experiment, vagotomy increased baseline respiratory frequency and inspiratory duration, and decreased expiratory time (Tables 1-4). When inspiratory time changes, peak integrated EMG activity does not provide sufficient information to interpret changes in inspiratory drive (e.g. peak integrated EMG activity could be increased simply because inspiratory time is prolonged). To obtain an index of ventilatory drive, we measured the rate of rise of parasternal and right diaphragm integrated EMG during the first 200 msec of each breath.

Analysis of variance (26) was done to determine 1) the significance of the response, 2) the effect of stimulation

phase, and 3) the importance of vagal input on the changes occuring in inspiratory drive during afferent stimulation. Significance was determined at $p < 0.05$. Values are presented as means ± SE.

4.5 RESULTS

Baseline arterial pH, PO_2 and PCO_2 after surgery averaged 7.27 ± 0.02, 135 ± 8 mmHg and 46 ± 4 mmHg, respectively.

High Intensity Phrenic Stimulation

VAGI INTACT

Inspiratory and expiratory phrenic nerve stimulation increased inspiratory muscle activity to similar levels (Fig. 1 and 2). In addition, phasic stimulation elicited similar increases in breathing frequency, due to a shortening of both inspiratory and expiratory times (Fig 3 and Table 1). Mean arterial pressure increased similarly when stimulation was performed during inspiration or expiration (baseline, 135 ± 2 and 135 ± 2 mmHg; during stimulation, 160 ± 2 and 163 ± 3 mmHg, respectively, $p < 0.05$).

VAGI CUT

After vagotomy, phasic phrenic nerve stimulation increased parasternal and right diaphragm peak EMG activity to the same degree as in the vagi-intact condition (Fig. 1). However, the increase in AN activity was attenuated (Fig. 1). Furthermore, expiratory, but not inspiratory, stimulation significantly

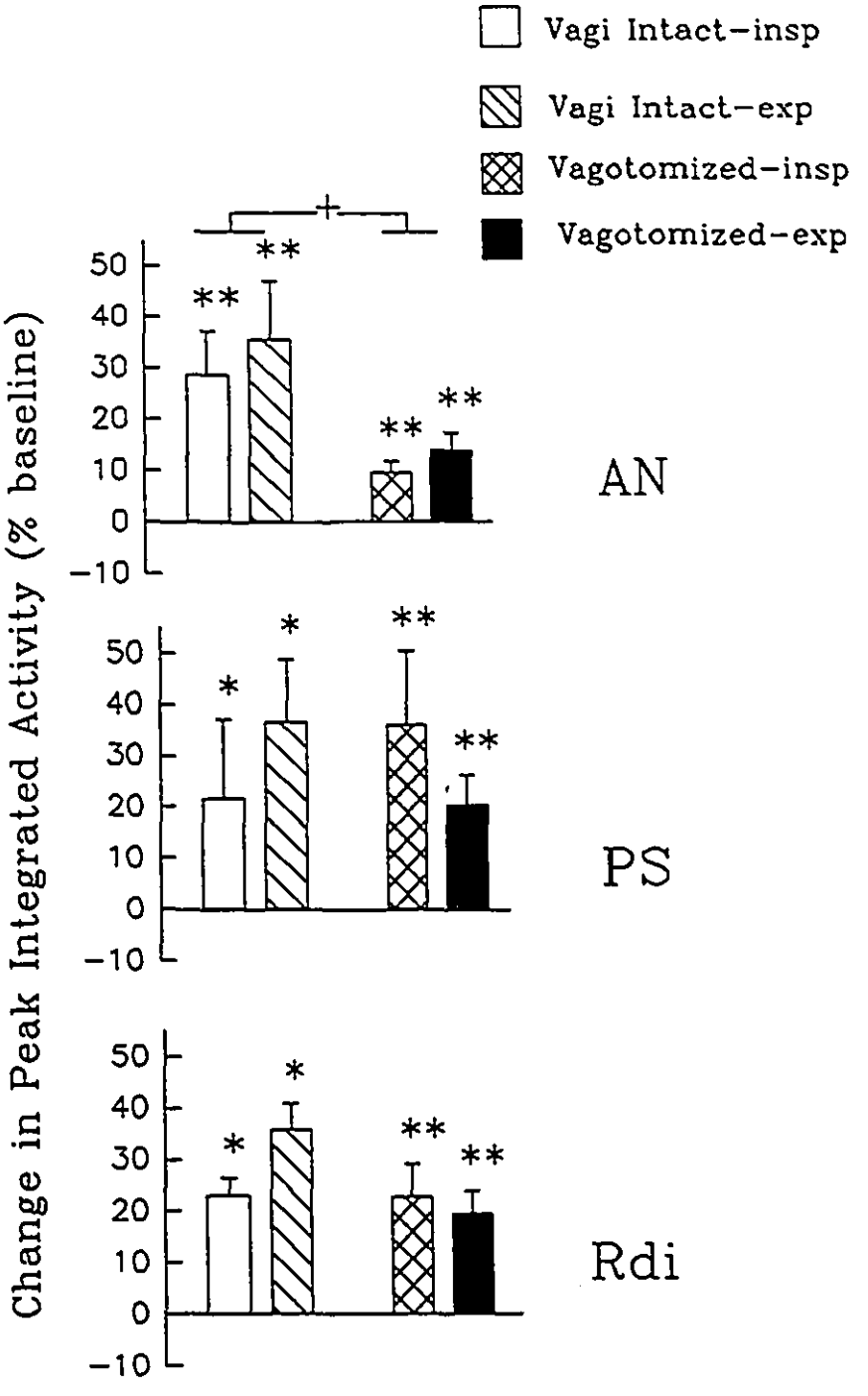

Fig. 4.1: Changes in peak integrated EMG of alae nasi (AN), parasternal (PS) and right diaphragm (Rdi) with high intensity phrenic nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. *,** $p < 0.05$, 0.01, respectively, compared to baseline values; + $p < 0.05$ compared to vagotomized condition.

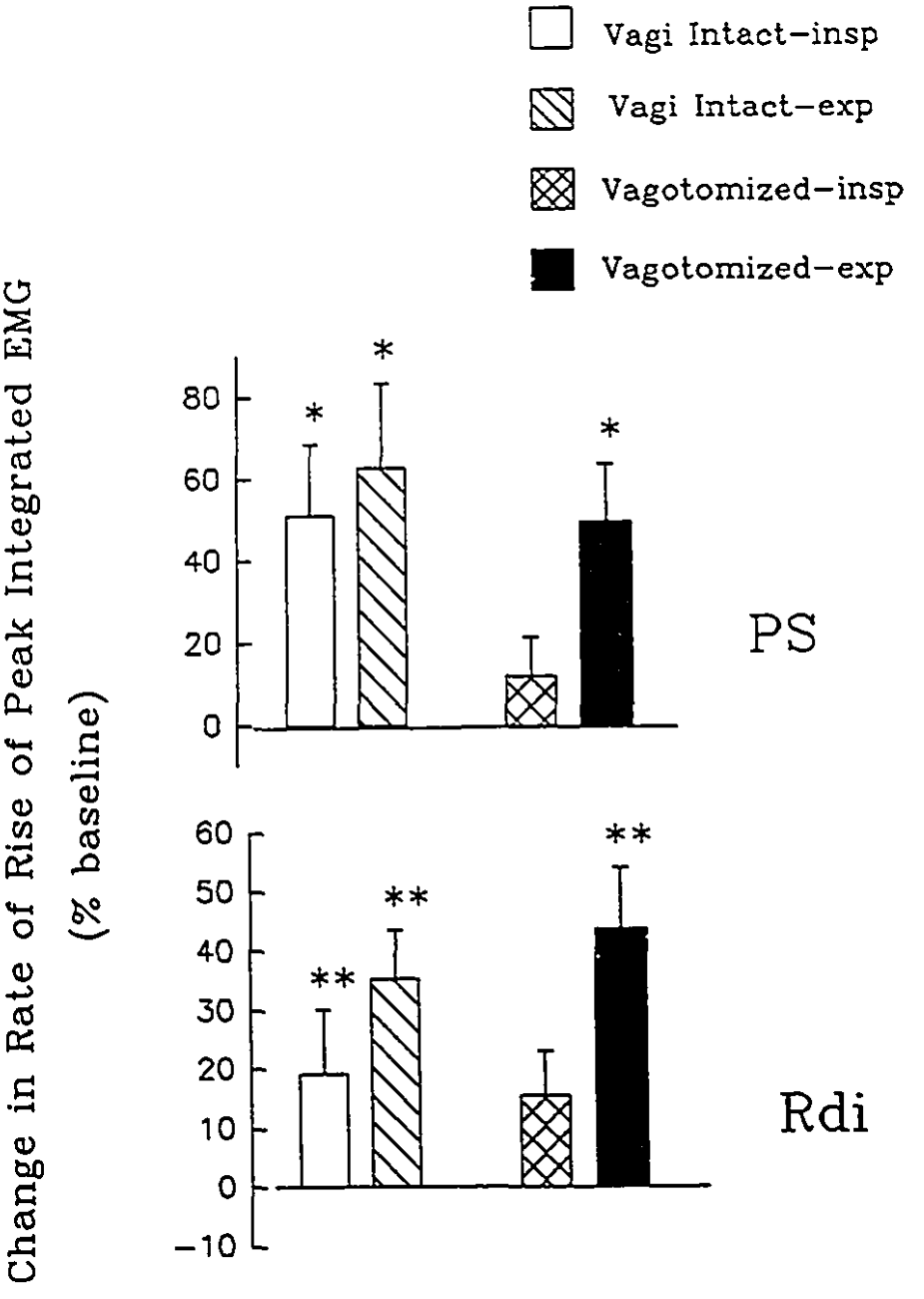

Fig. 4.2: Changes in rate of rise of parasternal (PS) and right diaphragm (Rdi) EMG with high intensity phrenic nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. *,** $p < 0.05$, 0.01, respectively, compared to baseline values.

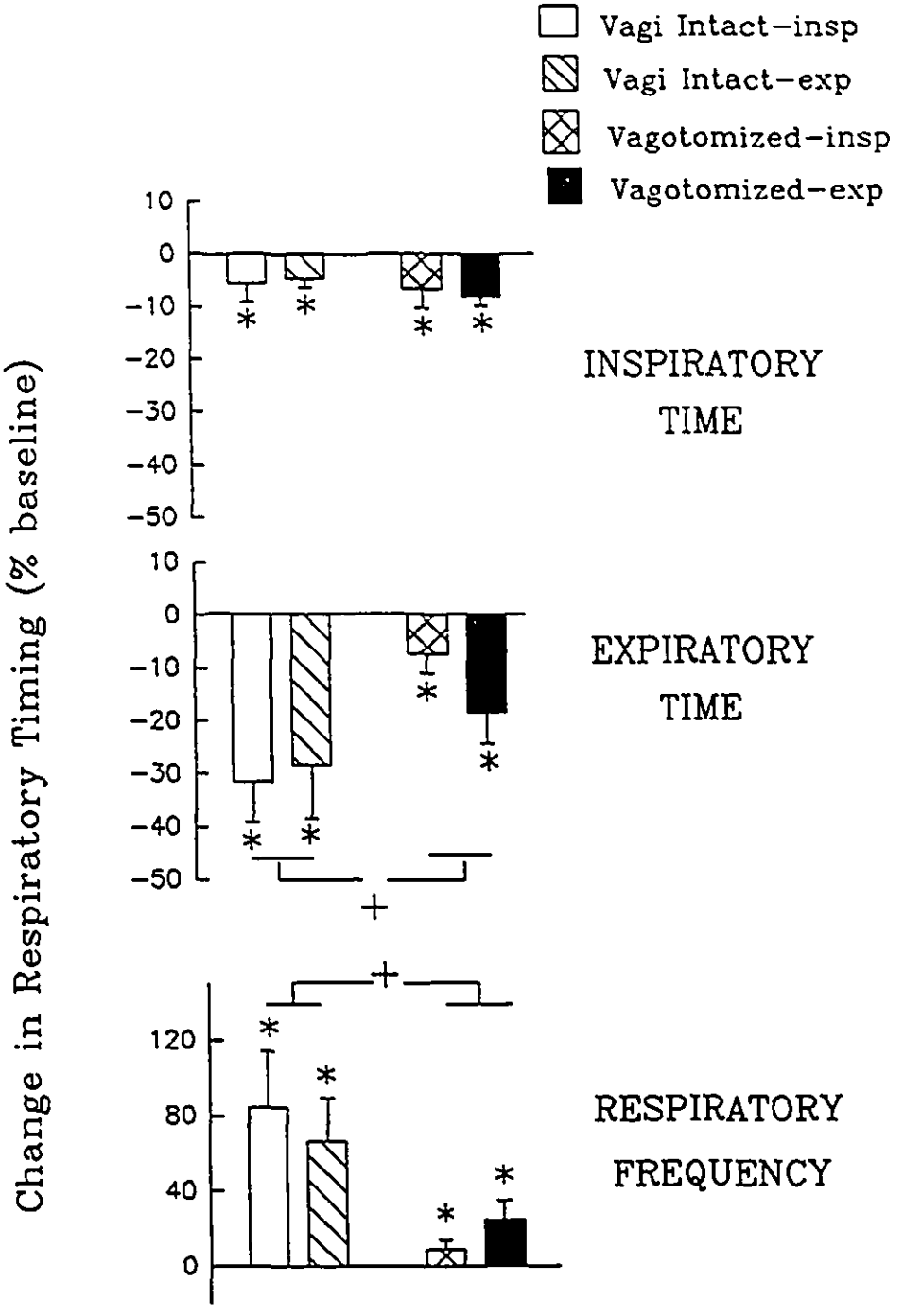

Fig. 4.3: Changes in respiratory timing with high intensity phrenic nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. * $p < 0.05$ compared to baseline values. + $p < 0.05$ compared to vagotomized condition.

	Ti(s)	Te(s)	f_R(br/min)
VAGI INTACT			
Stimulation during Inspiration			
Baseline	0.96 (0.12)	5.24 (0.62)	11.5 (1.9)
Response	0.88*+ (0.09)	3.45*+ (0.45)	19.5**+ (2.9)
Stimulation during Expiration			
Baseline	0.97 (0.13)	5.22 (0.52)	10.7 (1.1)
Response	0.92* (0.12)	3.73*+ (0.61)	17.6**+ (2.7)
VAGI CUT			
Stimulation during Inspiration			
Baseline	1.41 (0.16)	2.45 (0.42)	18.6 (2.8)
Response	1.30* (0.14)	2.20* (0.32)	19.8* (2.8)
Stimulation during Expiration			
Baseline	1.55 (0.20)	2.95 (0.5)	16.8 (3.2)
Response	1.40* (0.16)	2.21* (0.28)	19.6* (2.9)

Table 4.1: Changes in respiratory timing during high intensity stimulation of the central end of cut left phrenic nerve in animals with intact vagi and after bilateral vagotomy. Values are means. Values in parentheses are SE. *,** $p < 0.05$ and 0.01, respectively, compared to baseline. + $p < 0.05$ compared to vagi cut. Ti: inspiratory time, Te: expiratory time, f_R: respiratory frequency

increased the rate of rise of PS and Rdi (Fig. 2).

Phasic stimulation caused similar increases in breathing frequency. These increases were less than before vagotomy, due to an attenuated decrease in expiratory time (Fig. 3, Table 1). Inspiratory and expiratory stimulation caused similar increases in mean arterial pressure (baseline, 137 ± 2 and 137 ± 2 mmHg; during stimulation, 164 ± 3 and 163 ± 3 mmHg, respectively, $p < 0.05$), which were like those observed in the vagi-intact condition.

Low Intensity Phrenic Stimulation

VAGI INTACT

Phrenic nerve stimulation during either inspiration or expiration had no significant effect on inspiratory muscle activity or respiratory timing (Table 2). Mean arterial pressure (137 ± 2 and 138 ± 2 mmHg before inspiratory and expiratory stimulation, respectively) was unchanged by these stimulations.

VAGI CUT

Similarly, phasic phrenic nerve stimulation in vagotomized animals had no significant effect on inspiratory muscle activity or respiratory timing (Table 2). Mean arterial pressure (137 ± 2 and 138 ± 2 mmHg before inspiratory and expiratory stimulations, respectively) did not change during stimulation.

High Intensity Tibial Stimulation

VAGI INTACT

In vagi-intact animals, phasic tibial nerve stimulation caused similar increases in the inspiratory motor drive (Fig.

	Ti(s)	Te(s)	f_R(br/min)
VAGI INTACT			
Stimulation during Inspiration			
Baseline	1.03	4.48	13.0
	(0.13)	(0.45)	(1.7)
Response	1.11	4.45	13.5
	(0.16)	(0.52)	(2.0)
Stimulation during Expiration			
Baseline	1.06	4.91	11.3
	(0.14)	(0.47)	(1.2)
Response	1.03	4.80	12.6
	(0.13)	(0.63)	(2.0)
VAGI CUT			
Stimulation during Inspiration			
Baseline	1.49	2.80	16.9
	(0.19)	(0.44)	(2.8)
Response	1.45	2.65	17.3
	(0.18)	(0.40)	(2.7)
Stimulation during Expiration			
Baseline	1.50	2.74	16.9
	(0.18)	(0.40)	(2.8)
Response	1.45	2.70	17.2
	(0.17)	(0.39)	(2.8)

Table 4.2: Changes in respiratory timing during low intensity stimulation of the central end of cut left phrenic nerve in animals with intact vagi and after bilateral vagotomy. Values are means. Values in parentheses are SE. Ti: inspiratory time, Te: expiratory time, f_R: respiratory frequency

4 and 5), which were greater than those observed during phrenic nerve stimulation. Phasic stimulation also elicited similar increases in breathing frequency and decreases in expiratory time (Fig. 6, Table 3). However, inspiratory time decreased during expiratory, but not inspiratory, stimulation (Fig. 6, Table 3). In addition, mean arterial blood pressure increased during inspiratory and expiratory stimulation (baseline, 135 ± 6 and 137 ± 6 mmHg; during stimulation, 149 ± 7 and 161 ± 8 mmHg, respectively, $p < 0.05$).

VAGI CUT

After vagotomy, tibial nerve stimulation increased inspiratory motor drive to a lesser extent than before vagotomy (Fig. 4 and 5). Surprisingly, inspiratory time decreased more after vagotomy than before vagotomy (Fig. 6 and Table 3). In addition, inspiratory and expiratory stimulation increased mean arterial blood pressure (baseline, 138 ± 7 and 136 ± 8 mmHg; during stimulation, 155 ± 9 and 155 ± 12 mmHg, respectively, $p < 0.05$).

Low Intensity Tibial Stimulation

VAGI INTACT

In contrast to phrenic nerve stimulation, low intensity tibial nerve stimulation significantly increased alae nasi, parasternal and right diaphragm peak integrated EMG during inspiratory (142 ± 35%, 135 ± 16% and 126 ± 14% of baseline, respectively, $p < 0.05$) and expiratory (113 ± 4%, 127 ± 16% and 110 ± 4% of baseline, respectively, $p < 0.05$) stimulation. The rate of rise of PS increased significantly during inspiratory and expiratory stimulation (148 ± 27% and

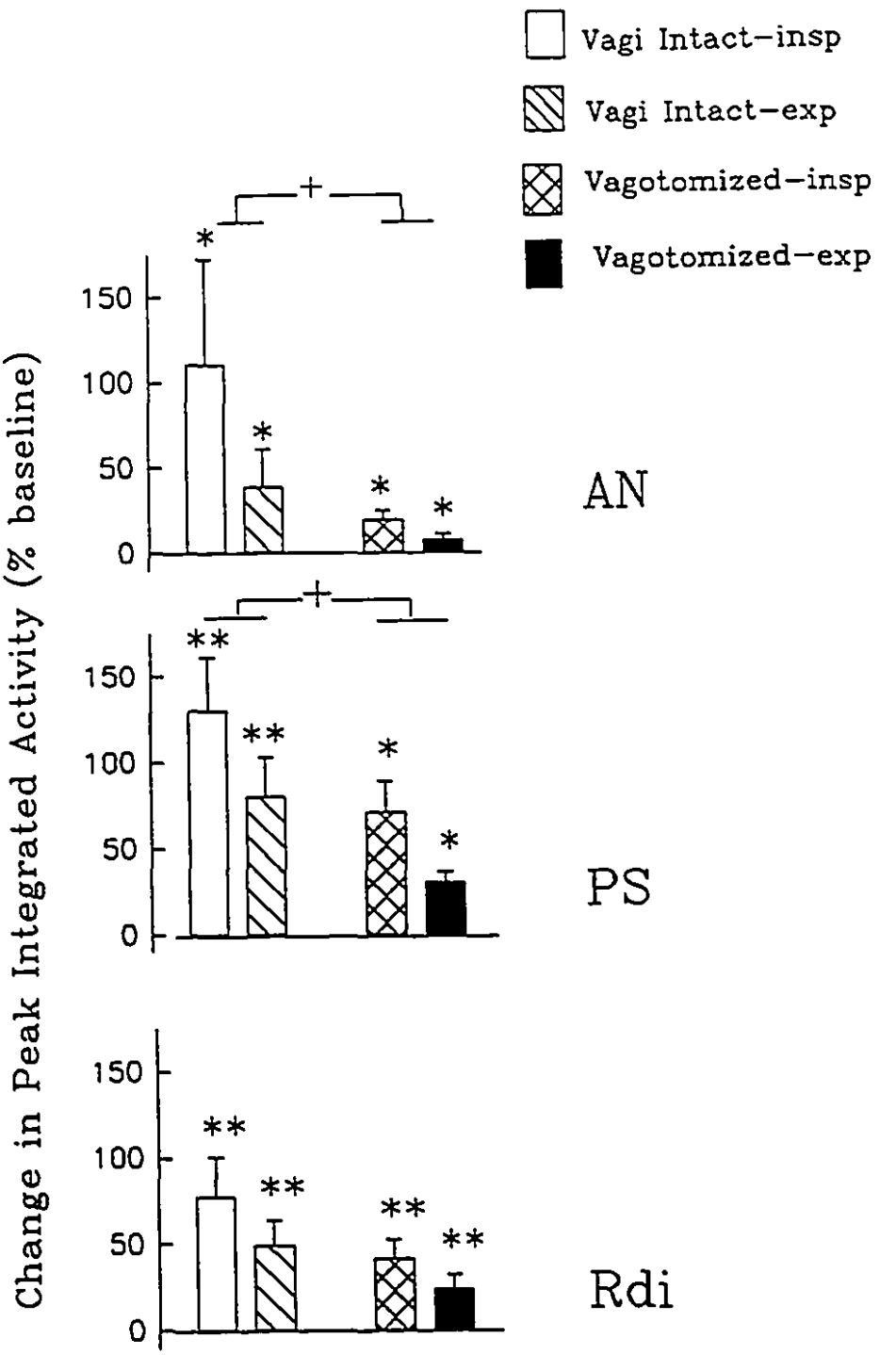

Fig. 4.4: Changes in peak integrated EMG of alae nasi (AN), parasternal (PS) and right diaphragm (Rdi) with high intensity tibial nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. *,** $p < 0.05$, 0.01, respectively, compared to baseline values; + $p < 0.05$ compared to vagotomized condition.

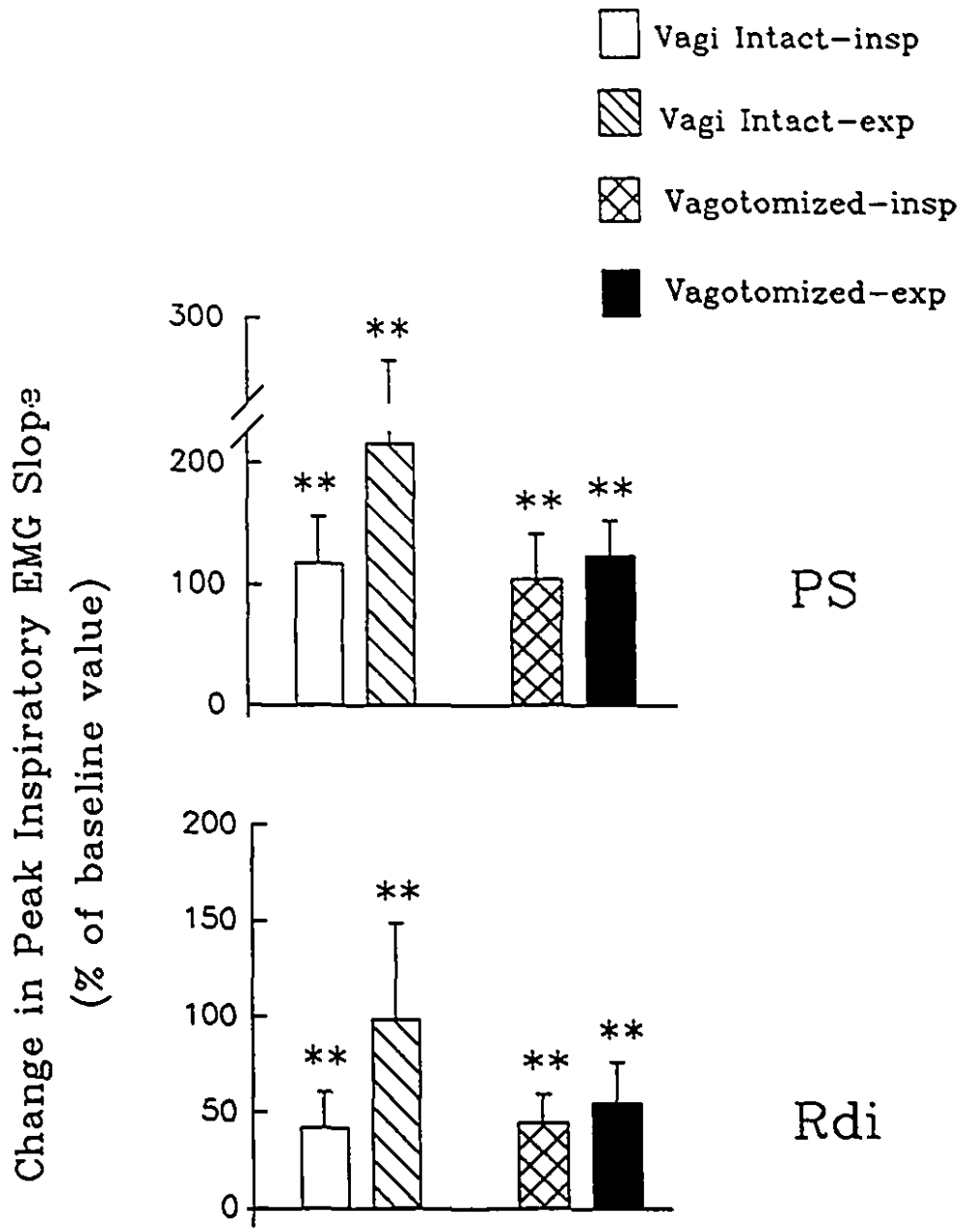

Fig. 4.5: Changes in rate of rise of parasternal (PS) and right diaphragm (Rdi) EMG with high intensity tibial nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. ** $p < 0.01$ compared to baseline values.

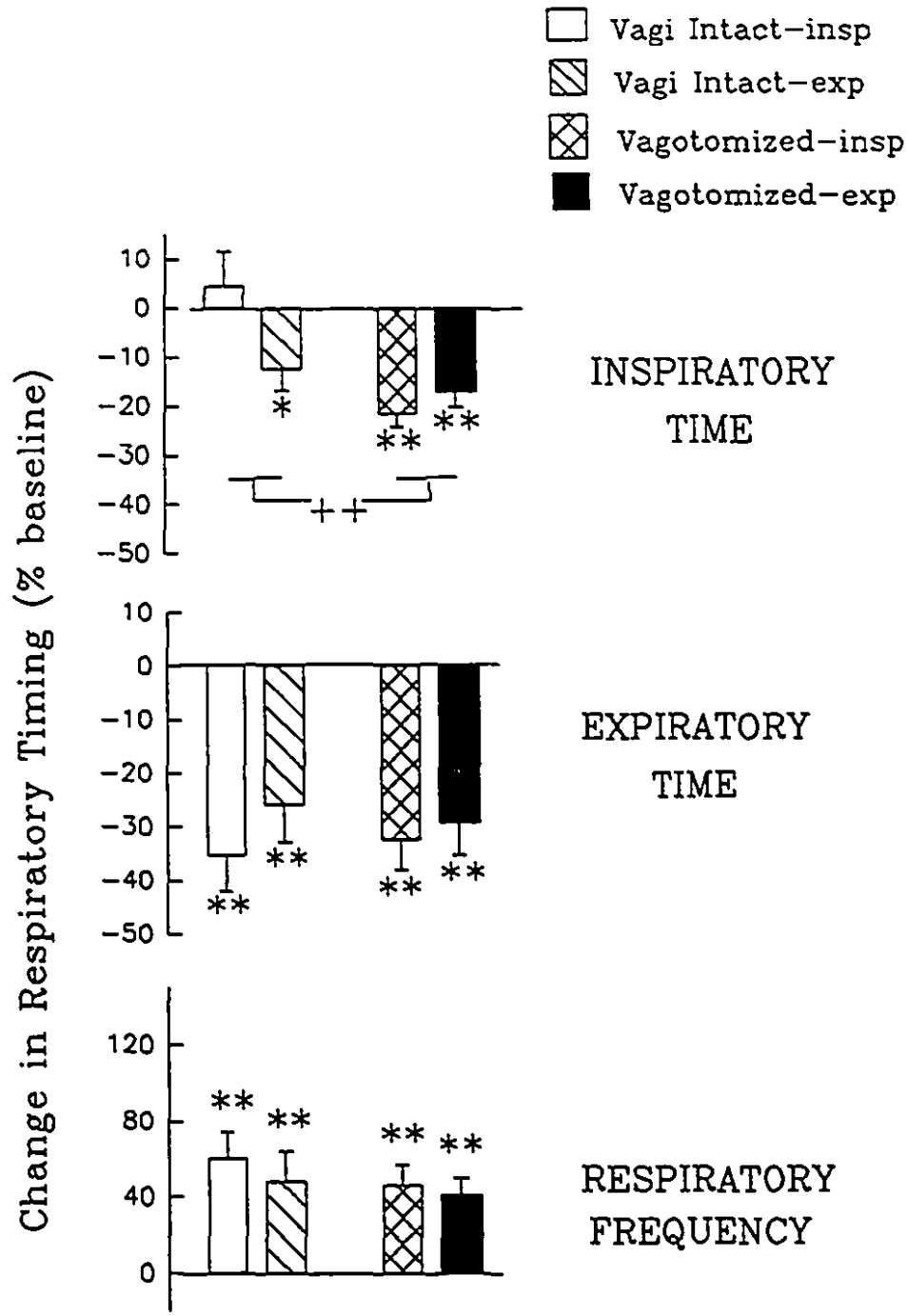

Fig. 4.6: Changes in respiratory timing with high intensity tibial nerve stimulation during inspiration or expiration, before and after bilateral vagotomy. Mean ± SE. *, $p < 0.05, 0.01$, respectively, compared to baseline values; ++ $p < 0.01$ compared to vagotomized condition.**

	Ti(s)	Te(s)	f_R(br/min)
VAGI INTACT			
Stimulation during Inspiration			
Baseline	0.98 (2.4)	4.17 (0.52)	14.7 (2.4)
Response	0.97 (0.10)	3.75** (0.50)	23.3** (3.8)
Stimulation during Expiration			
Baseline	1.04 (0.15)	4.38 (0.60)	14.0 (2.2)
Response	0.91* (0.13)	3.11** (0.37)	19.7** (3.2)
VAGI CUT			
Stimulation during Inspiration			
Baseline	1.51 (0.18)	2.71 (0.40)	16.7 (2.4)
Response	1.18** (0.14)	1.72* (0.23)	23.6** (2.8)
Stimulation during Expiration			
Baseline	1.45 (0.20)	2.51 (0.47)	18.6 (2.8)
Response	1.20** (0.17)	1.62** (0.23)	25.1** (3.2)

Table 4.3: Changes in respiratory timing during high intensity stimulation of the central end of cut left tibial nerve in animals with intact vagi and after bilateral vagotomy. Values are means. Values in parentheses are SE. *,** $p < 0.05$ and 0.01, respectively, compared to baseline. Ti: inspiratory time, Te: expiratory time, f_R: respiratory frequency

140 ± 26% of baseline, respectively, $p < 0.05$), whereas the rate of rise of Rdi remained unchanged. Breathing frequency increased and expiratory time decreased during inspiratory stimulation (Table 4). However, inspiratory and expiratory times showed small but significant decreases during expiratory stimulation only. Mean arterial pressure increased during inspiratory, but not expiratory, stimulation (baseline, 135 ± 5 mmHg; during stimulation, 143 ± 5 mmHg, $p < 0.05$).

VAGI CUT

After vagotomy, phasic tibial nerve stimulation caused no significant changes in inspiratory muscle activity or respiratory timing (Table 4). In addition, mean arterial blood pressure remained unchanged during both inspiratory and expiratory phase stimulation (baseline values of 139 ± 7 and 136 ± 8 mmHg).

4.6 DISCUSSION

The main findings of this study are that:

1) High intensity phrenic stimulation during either inspiration or expiration produced similar increases in inspiratory motor drive. Similar observations were seen during tibial nerve stimulation.

2) Vagotomy attenuated the increases in the rate of rise of parasternal and right diaphragm EMG during inspiratory, but not expiratory, phrenic nerve stimulation. These findings suggest that the presence of the vagus nerves

	Ti(s)	Te(s)	f_R(br/min)
VAGI INTACT			
Stimulation during Inspiration			
Baseline	1.04 (0.14)	4.12 (0.49)	14.2 (1.8)
Response	1.01 (0.12)	3.25* (0.52)	19.4* (2.9)
Stimulation during Expiration			
Baseline	1.01 (0.13)	4.04 (0.53)	15.0 (2.0)
Response	0.92* (0.15)	3.69** (0.50)	16.2 (2.1)
VAGI CUT			
Stimulation during Inspiration			
Baseline	1.47 (0.21)	2.58 (0.50)	18.2 (2.7)
Response	1.36 (0.18)	2.04 (0.31)	20.7 (2.8)
Stimulation during Expiration			
Baseline	1.46 (0.17)	2.44 (0.37)	17.9 (2.5)
Response	1.38 (0.17)	2.16 (0.29)	19.3 (2.4)

Table 4.4: Changes in respiratory timing during low intensity stimulation of the central end of cut left tibial nerve in animals with intact vagi and after bilateral vagotomy. Values are means. Values in parentheses are SE. *,** $p < 0.05$ and 0.01, respectively, compared to baseline. Ti: inspiratory time, Te: expiratory time, f_R: respiratory frequency

during inspiration potentiates the stimulatory effect of phrenic thin-fiber afferent activation.

3) Tibial nerve stimulation caused similar increases in the rate of rise of parasternal and right diaphragm EMG before and after vagotomy.

Critique

This study has focussed on changes in inspiratory motor drive caused by phrenic nerve stimulation. We speculate that these changes result from the activation of phrenic afferent, rather than efferent, fibers. Retrograde activation of phrenic motoneurons inhibits phrenic efferent drive, due to the activation of Renshaw cells by efferent axon collaterals (11). We, therefore, propose that the stimulatory effects observed in our study were due to the activation of phrenic afferent fibers.

Other potentially confounding influences on ventilatory drive include chemoreceptor and baroreceptor afferent inputs. To avoid alterations in chemoreceptor input, end-tidal CO_2 was maintained at 3-4% before each stimulation, and arterial PO_2 was maintained above 100 mmHg. However, increases in mean arterial blood pressure during high intensity stimulation must have changed baroreceptor input. Since rising arterial pressure is associated with an inhibition of ventilatory drive (3), the augmentation of ventilatory drive observed in our study may have been even larger if blood pressure did not change. In addition, since mean arterial blood pressure rose to similar levels during all high intensity stimulations, differences in inspiratory motor

drive and timing in the vagi-intact and vagotomized conditions are probably not due to alterations in baroreceptor input. However, it must be noted that aortic baroreptor input is not present in the vagotomized animals.

The stimulation intensities used in our protocol are within the ranges of intensities shown previously to activate large and thin-fiber afferents (21). Therefore, we presume that thin-fiber afferent activation is responsible for changes which occurred during high intensity nerve stimulation, but were not present during low intensity stimulation.

Phrenic nerve afferents and inspiratory muscle activation

Previous studies have shown that electrical activation of phrenic afferents increases minute ventilation by 45-70% (20,25) and respiratory frequency by about 30% (25). Ward *et al.* (25) found that, in vagotomized dogs, left phrenic nerve stimulation for one minute increased the alae nasi, parasternal and right diaphragm activities by 50%, 75% and 60%, respectively. These increases are larger than the changes we currently report during phasic stimulation for only 8 breaths in both vagi-intact and vagotomized preparations. The shorter duration of stimulation in our protocol could explain the smaller rise in inspiratory motor drive in our study.

Phasic activation of muscle afferents

We used phasic stimulation because it is possible that rhythmic activation of mechanosensitive afferents has differential effects on inspiratory motor drive. It is well

known that muscle spindles and tendon organs (innervated by groups I and II afferents) are activated phasically. In addition, it has recently been shown that type III afferent fibers in limb muscle are phasically active during dynamic exercise (18).

Recordings of spontaneously active phrenic afferent fibers has demonstrated both phasic (5,19) and tonic (6,10) firing patterns. Phasic firing has been attributed to large fiber afferents, and tonic firing to thin-fiber afferents. To date, there is no literature on the effects of phasic activation of phrenic afferents on ventilatory drive. We observed that inspiratory and expiratory phrenic nerve stimulation produced similar changes in the peak and rate of rise of EMG and respiratory timing. These changes were also similar during phasic tibial nerve stimulation. This finding is surprising since, although the ratio of stimulus duration to phase duration was similar during inspiration and expiration (26-52% of phase duration), fewer stimuli were delivered during inspiration. One interpretation of these results is that afferent fiber activation during inspiration appears to have a more potent effect on ventilatory drive when the vagi are intact.

The only published reports on the effects of phasic limb muscle afferent stimulation are those of Simbulan *et al.* (22,23). These authors reported that, in paralyzed cats, high intensity gastrocnemius nerve stimulation during inspiration produced a greater augmentation of phrenic nerve activity than expiratory stimulation (77% versus 21% increase

above baseline). The differences between our results and those of Simbulan *et al.* may be due to differences in species or methodology. Simbulan *et al.* used paralyzed cats, thereby eliminating dynamic afferent input from contracting inspiratory muscles. In addition, the greater stimulation intensity used in that study (400-600 times twitch threshold) may have caused activation of a greater number of group IV afferent fibers.

In our study, low intensity stimulation of the phrenic nerve had no effect on inspiratory motor drive, whereas similar stimulation of the tibial nerve increased respiratory drive. These results may be explained by the anatomical finding that limb muscles contain more muscle spindles and tendon organs than the diaphragm and, hence, a greater number of large afferent fibers.

Effect of Vagotomy

It has been well established that the presence of the vagus nerve has a significant effect on the pattern of respiratory muscle activation as well as the timing of respiration. Ainsworth *et al.* (1) reported that vagal blockade in awake dogs during eupneic breathing increased activity of the parasternal, diaphragm and triangularis sterni muscles but inhibited transversus abdominus activity. When the vagi were cooled during exercise, similar changes were found in all muscles except the transversus abdominus (2). Hollstein *et al.* (7) reported that the vagal influence on abdominal muscle activity was caused by a balance between excitatory activity (from pulmonary stretch receptors) and

inhibitory activity (from lung C fibers).

Vagal blockade also affects respiratory timing. Phillipson (17) reported that vagal blockade and occlusion in awake dogs slowed breathing frequency and lengthened inspiratory time. These results were probably due to the influence of tonic vagal inputs, since lung stretch remained constant during occlusion. In contrast, activation of vagal C fibers has been reported to increase respiratory frequency and tidal volume, and increase expiratory flow deceleration (8). It is apparent that there are both phasic and tonic vagal influences on respiratory motor activity.

We found that vagotomy attenuated the increased rate of rise of EMG of the parasternal and right diaphragm during inspiratory, but not expiratory, phrenic nerve stimulation. However, vagotomy did not affect the rate of rise of EMG during tibial nerve stimulation. These results indicate that the presence of the vagus during inspiration augments the excitatory effect of thin-fiber phrenic afferents. Our results with tibial nerve stimulation contrast with the findings of Simbulan *et al.* (23), who reported that vagotomy markedly attenuated the increase in phrenic nerve activity during both inspiratory and expiratory gastrocnemius nerve stimulation.

The anatomical pathways involved in the facilitatory action of the vagus nerve on phrenic nerve afferent inputs are not known. Integration of vagal and phrenic reflexes could occur in brain regions which receive afferent fiber projections from both nerves. Brainstem phrenic afferent

fiber projections include the dorsal and ventral respiratory groups of the medulla (12,13,24), lateral reticular formation (12), and external cuneate nucleus (14). Vagal afferent fibers have been found to project to many nuclei within the solitary tract, including the ventrolateral nucleus. This nucleus appears to be the relay station for sensory input from the respiratory system. Fibers coming from the ventrolateral nucleus project to the nucleus ambiguus and nucleus retroambiguus of the ventral respiratory group (4). The site of integration of vagal and phrenic afferent inputs might therefore be within the ventral respiratory group. Another region which contains both vagal (4) and phrenic afferent fibers is the external cuneate nucleus.

In summary, we have shown that high intensity stimulation of the phrenic or tibial nerves during inspiration or expiration produces similar changes in inspiratory motor drive when the vagi are intact. The presence of the vagi appears to potentiate the stimulatory effect of inspiratory phrenic thin-fiber afferent activation, but has no similar effect during the activation of tibial thin-fiber afferents.

4.7 REFERENCES

1. AINSWORTH, D.M., C.A. SMITH, B.D. JOHNSON, S.W. EICKER, K.S. HENDERSON, and J.A. DEMPSEY. Vagal contributions to respiratory muscle activity during eupnea in the awake dog. *J Appl Physiol* 72(4): 1355-1361, 1992.

2. AINSWORTH, D.M., C.A. SMITH, B.D. JOHNSON, S.W. EICKER, K.S. HENDERSON, and J.A. DEMPSEY. Vagal modulation of respiratory muscle activity in awake dogs during exercise and hypercapnia. *J Appl Physiol* 72(4): 1362-1367, 1992.

3. BISHOP, B. Carotid baroreceptor modulation of diaphragm and abdominal muscle activity in the cat. *J Appl Physiol* 36(1): 12-19, 1974.

4. CIRIELLO, J., A.W. HRYCYSHYN, and F.R. CALARESU. Glossopharyngeal and vagal afferent projections to the brain stem of the cat: a horseradish peroxidase study. *J Auton Nerv Sys* 4: 63-79, 1981.

5. DURON, B. Intercostal and diaphragmatic muscle endings and afferents. In: *Regulation of breathing, Part I*, edited by Hornbein, T.F. New York: Dekker, 1981, p. 473-540.

6. GRAHAM, R., Y. JAMMES, S. DELPIERRE, C. GRIMAUD, and CH. ROUSSOS. The effects of ischemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. *Neurosci Lett* 67: 257-262, 1986.

7. HOLLSTIEN, S.B., M.L. CARL, E.S. SCHELEGLE, and J.F. GREEN. Role of vagal afferents in the control of abdominal expiratory muscle activity in the dog. *J Appl Physiol* 71: 1795-1800, 1991.

8. HOLMES, H.R. and J.E. REMMERS. Stimulation of vagal C-fibers alters timing and distribution of respiratory motor output in cats. *J Appl Physiol* 67(6): 2249-2256, 1989.

9. HUSSAIN, S.N.A., S. MAGDER, A. CHATILLON, and CH. ROUSSOS. Chemical activation of thin-fiber phrenic afferents: respiratory responses. *J Appl Physiol* 69(3): 1002-1011, 1990.

10. JAMMES, Y. and E. BALZAMO. Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73(3): 894-902, 1992.

11. LIPSKI, J., R.E.W. FYFFE, and J. JODKOWSKI. Recurrent inhibition of cat phrenic motoneurons. *J Neurosci* 5: 1545-1555, 1985.

12. MACRON, J.-M., D. MARLOT, and B. DURON. Phrenic afferent input to the lateral medullary reticular formation of the cat. *Respir Physiol* 59: 155-167, 1985.

13. MACRON, J.M. and D. MARLOT. Effects of stimulation of phrenic afferent fibers on medullary respiratory neurons in cat. *Neurosci Lett* 63: 231-236, 1986.

14. MARLOT, D., J.-M. MACRON, and B. DURON. Projection of phrenic afferents to the external cuneate nucleus in the cat. *Brain Res* 327: 328-330, 1985.

15. MCCALLISTER, L.W., K.W. MCCOY, J.C. CONNELLY, and M.P. KAUFMAN. Stimulation of groups III and IV phrenic afferents reflexly decreases total lung resistance in dogs. *J Appl Physiol* 61(4): 1346-1351, 1986.

16. MITCHELL, J.H. and SCHMIDT, R.F. Cardiovascular reflex control by afferent fibers from skeletal muscle receptors. In: *The Cardiovascular System. Vol. III, Peripheral Circulation and Organ Blood Flow, Part 2,* edited by Shepherd, J.R. and Abboud, F.M. Bethesda: American Physiological Society, 1983, pp. 623-658.

17. PHILLIPSON, E.A. Vagal control of breathing patterns independent of lung inflation in conscious dogs. *J Appl Physiol* 37(2): 183-189, 1974.

18. PICKAR, J.G., J.M. HILL, and M.P. KAUFMAN. Dynamic exercise stimulates group III muscle afferents. *J Neurophysiol* 71: 753-760, 1994.

19. REVELETTE, R., S. REYNOLDS, D. BROWN, and R. TAYLOR. Effect of abdominal compression on diaphragmatic tendon organ activity. *J Appl Physiol* 72: 288-292, 1992.

20. ROAD, J.D., N.H. WEST, and B.N. VAN VLIET. Ventilatory effects of stimulation of phrenic afferents. *J Appl Physiol* 63(3): 1063-1069, 1987.

21. RYBICKI, K.J. and M.P. KAUFMAN. Stimulation of group III and IV muscle afferents reflexly decreases total pulmonary resistance in dogs. *Respir Physiol* 59: 185-195, 1985.

22. SIMBULAN, D., E. TADAKI, Y. KOZAKI, K. EGUCHI, and T. KUMAZAWA. Phrenic responses to muscle afferent nerve stimulation applied to different respiratory phases. *Environ Med* 31: 61-67, 1987.

23. SIMBULAN, D.,JR., E. TADAKI, Y. KOZAKI, K. EGUCHI, and T. KUMAZAWA. Augmentation of muscle nociceptive respiratory reflex facilitation by vagal afferents. *Jap J Physiol* 40: 253-272, 1990.

24. SPECK, D.F. and W.R. REVELETTE. Excitation of dorsal and ventral respiratory group neurons by phrenic nerve afferents. *J Appl Physiol* 62: 946-951, 1987.

25. WARD, M.E., A. DESCHAMPS, CH. ROUSSOS, and S.N.A. HUSSAIN. Effect of phrenic afferent stimulation on pattern of respiratory muscle activation. *J Appl Physiol* 73(2): 563-570, 1992.

26. WILKINSON, L. SYSTAT: The system for statistics. Evanston IL: SYSTAT, Inc., 1990.

27. WILSON, C.R., G. VANELLI, S. MAGDER, and S.N.A. HUSSAIN. Phrenic afferent stimulation by bradykinin and the distribution of the inspiratory motor drive. *Respir Physiol* 96:1-12, 1994.

CHAPTER 5

THE EFFECT OF PROLONGED ISCHEMIA OF THE LEFT HEMI-DIAPHRAGM ON THE INSPIRATORY MOTOR DRIVE

5.1 LINK TO CHAPTER 5

In the first two projects I assessed the effects of diaphragmatic thin-fiber afferent activation by providing short term (one minute) chemical or electrical stimulation. In the third project, the phrenic nerve was electrically stimulated during either inspiration or expiration to mimic the action of mechanoreceptive thin-fiber afferents. Therefore, all of these studies used protocols involving stimulation for short periods of time. However, during clinical conditions causing diaphragmatic fatigue, thin-fiber afferents can be activated for long periods of time (e.g. hours).

Therefore, in the fourth project I assessed the effects of prolonged activation of phrenic thin-fiber afferents on inspiratory motor drive. To approximate physiological conditions, diaphragmatic fatigue was caused by selective ischemia of the left-hemidiaphragm for three hours. A previous study has shown that short term (30 minutes) ischemia of the left hemi-diaphragm in vagotomized animals stimulates ventilatory drive. We have found that prolonged ischemic fatigue of the diaphragm alters this response.

5.2 ABSTRACT

We tested the effect of prolonged diaphragmatic ischemia on the inspiratory motor drive. In 6 chloralose anesthetized, mechanically ventilated dogs, the left hemi-diaphragm was made ischemic for 3 hours and reperfused for 1 hour. Peak integrated EMG of the alae nasi (AN), parasternal (PS), right (Rdi) and left (Ldi) hemi-diaphragm muscles, spontaneous Ldi tension (SpT), and peak Ldi M wave amplitude (LdiM) and Ldi tension (TT) (elicited by supramaximal twitch stimulation) were measured. Ldi, SpT, LdiM and TT decreased throughout ischemia (18%, 32%, 39%, and 23% of baseline, respectively, at 180 min). Rdi rose (183% of baseline, 130 min) and then fell (137% of baseline, 180 min) during ischemia. A similar trend was found in the AN and PS. Inspiratory time increased during the last 30 min of ischemia (122% of baseline at 180 min). There were no further changes in inspiratory motor drive during reperfusion. Mean systemic blood pressure decreased thoughout the experiment (from 129 to 107 mmHg). These results indicate that prolonged ischemic fatigue of the diaphragm causes an initial stimulation of inspiratory motor drive, with subsequent failure to maintain this increase in drive.

5.3 INTRODUCTION

During attempts at weaning from mechanical ventilation, Cohen *et al.* (4) observed that minute ventilation sometimes increased initially, and then fell to baseline values, despite increasing carbon dioxide levels. The development of diaphragmatic fatigue is these patients, indicated that sensory input from the diaphragm may be involved in this response. Similar changes in ventilatory drive have been described during diaphragm fatigue caused by cardiogenic shock (1) or septic shock (7).

More recently, Jammes and Balzamo (8) induced diaphragmatic fatigue using intermittent direct muscle stimulation for thirty minutes. When fatigue was produced in this non-physiologic manner, respiratory frequency and phrenic efferent firing rates decreased. In contrast, under hypercapnic conditions, ischemic fatigue of an *in situ*, isolated diaphragmatic muscle strip stimulated contralateral diaphragm EMG and respiratory frequency (15). Similarly, Teitelbaum *et al.* (18) reported that twenty minutes of left hemi-diaphragmatic ischemia increased inspiratory motor drive and respiratory frequency. In all of these studies, diaphragmatic fatigue was present for a short period of time (< 30 minutes).

The goal of our study was to investigate changes in ventilatory drive during both the early and late phases of

diaphragmatic fatigue. Ischemia was chosen as the stimulus in an effort to approximate physiological conditions.

5.4 METHODS

Animal preparation

Six mongrel dogs (24-34 kg) were anesthetized with thiopental sodium (20 mg/kg) followed by alpha-chloralose (60-80 mg/kg). Supplemental doses of alpha-chloralose were given as needed. Animals were supine, intubated and mechanically ventilated with a constant volume ventilator (Harvard pump, initial setting of 12-15 ml/kg for tidal volume and 20 breaths/min for ventilator frequency, 5 cm H_2O PEEP). These settings were adjusted as necessary to maintain end-tidal CO_2 at 3-4% (Ametek analyzer). During the surgical procedure and protocol, supplemental oxygen was provided to maintain arterial PO_2 > 100 mmHg. A catheter was placed in the left femoral artery to monitor systemic blood pressure (Trantec) and collect blood samples for arterial blood gas analysis (AVL995). A second catheter was placed in the right external jugular vein for fluid and drug administration.

Surgical procedure

The *in situ*, vascularly isolated and innervated left hemi-diaphragm preparation was used for this experiment (6). In this protocol, however, the vagus nerves were left intact. Briefly, the muscles and ribs of the left hemi-thorax were excised to expose the left hemi-diaphragm. The rib

attachments of the left hemi-diaphragm were left attached to the muscle. The two halves of the costal diaphragm were surgically divided, and the left hemi-diaphragm was suspended from a frame above the animal. The frame was adjusted so that the diaphragm was at its *in vivo*, open thorax, end expiratory length, as determined by measuring the distance between three pairs of sutures placed in the anterior, middle and posterior regions of the costal diaphragm. The rib segments of these three regions were surgically divided. The middle costal region of the diaphragm was attached to a force displacement transducer (Grass model FT03) to measure the force generated by the contracting diaphragm.

A catheter system joining the left inferior phrenic artery and left femoral artery provided autoperfusion of the left hemidiaphragm. An electromagnetic flow probe (Carolina Medical Electronics) was contained within the system, and provided information about left phrenic arterial flow throughout the experiment. A second catheter was threaded into the left inferior phrenic vein, to monitor the integrity of the vascular isolation. Body temperature was monitored using a rectal thermometer, and a heating pad was used to maintain the temperature above 37 degrees C. Plastic film was applied to the chest of the animal to avoid drying of the exposed tissues.

Electromyographic (EMG) activity

Fine wire bipolar electrode pairs were placed in the

left alae nasi, right 2nd or 3rd parasternal muscles and left costal hemi-diaphragm. A pair of hook electrodes (1 cm apart) was placed in the right costal diaphragm. The signals were amplified and filtered (20-1000 Hz, Disa 05A0L) and then filtered (50-500Hz), rectified and integrated (0.1 sec time constant) by a resistance-capacitance integrator.

Stimulation of the left phrenic nerve

A bipolar stimulating electrode was placed on the left phrenic nerve as close as possible to the diaphragm. The twitch threshold was determined by delivering single stimuli (0.1 ms pulse duration) to the phrenic nerve (Grass Stimulator Model S48, Grass Constant Current Unit CCU1) until a contraction of the left costal diaphragm was visible (range 4-9 volts). The electrode was then secured in place and covered with paraffin-soaked gauze.

Experimental Protocol

After completion of the surgical procedure, a 30 minute stabilization period was allowed. To obtain a stable spontaneous breathing pattern, the frequency of the ventilator was reduced to about 15 breaths/min. Baseline measurements of arterial blood gases, EMG activities, systemic blood pressure, end-tidal CO_2, spontaneous and twitch tensions were then taken. During expiration, supramaximal stimulation (2.7-3.5 times twitch threshold) of the left phrenic nerve was performed three times, and the twitch tension and EMG activities of the left costal

diaphragm were recorded.

Throughout the protocol, arterial blood gases were controlled in several ways. Ventilator frequency was adjusted to maintain CO_2, which was monitored continuously (end-tidal CO_2) in addition to the blood gas sampling done at ten minute intervals. Boluses of bicarbonate were administered to maintain pH. Oxygen saturation was maintained by delivering supplemental oxygen, and using positive end-expiratory pressure in this open-chested preparation. Lastly, dextran was administered when necessary to maintain adequate systemic blood pressure.

After taking baseline measurements, the phrenic artery was occluded. Ischemia was maintained for three hours. Every ten minutes, EMG activities, spontaneous left hemi-diaphragm tension, respiratory frequency, and systemic blood pressure were recorded, and arterial blood gas sampled. Three supramaximal twitch stimulations of the left phrenic nerve were given every 20-30 minutes, and twitch tension and Ldi EMG activities were recorded.

After three hours of ischemia, the left phrenic artery catheter was unclamped, and reperfusion of the left hemi-diaphragm commenced. Recordings of variables continued at ten minute intervals, and supramaximal twitches were done at 20-30 minute intervals. At the end of the protocol, the animal was euthanized.

Data analysis

Measurements of integrated EMG activities and spontaneous left hemi-diaphragm tension were made for ten breaths before the onset of ischemia (baseline values) and for 5 breaths every ten minutes during ischemia and reperfusion. Breathing frequency, inspiratory and expiratory times were measured from the right costal diaphragm EMG signal. Mean systemic blood pressure (P_{art}) was calculated by averaging over twenty seconds. Peak Ldi M wave amplitude and Ldi twitch tension measurements were calculated as the average of three twitches performed before ischemia and at 20-30 minute intervals during ischemia and reperfusion. These values were then grouped into 6 time intervals during ischemia (20-30 min, 50-60 min, 80-90 min, 110-120 min, 140-150 min and 170-180 min) and two time intervals during reperfusion (20-30 min and 50-60 min).

Repeated measures analysis of variance was done separately for the ischemic and reperfusion periods (20). Dunnetts post-hoc analysis was used to compare changes during ischemia and reperfusion to pre-ischemic baseline values. Significance was determined at $p < 0.05$. Values are reported as means $\pm$ SE.

5.5 RESULTS

Baseline arterial pH, pCO_2, HCO_3 and pO_2 averaged 7.30 $\pm$ 0.01, 47.8 $\pm$ 1.5 mmHg, 22.1 $\pm$ 0.4 mmoles/L and 156.0 $\pm$ 10.1

mmHg, respectively (Fig. 1). Mean systemic blood pressure was 129.1 ± 4.7 mmHg. Respiratory frequency, inspiratory and expiratory times were 11.9 ± 0.8 breath/min, 0.9 ± 0.09 s and 4.2 ± 0.3 s, respectively. Measurements of left diaphragm EMG activity, spontaneous tension and peak Ldi M wave amplitude are presented for 5 dogs.

Ischemia

Arterial blood gas values did not change significantly from pre-ischemic values (Fig. 1), with the exception of a momentary fall in pH at 130 min of ischemia ($p < 0.05$). Mean systemic blood pressure fell during the ischemic period, reaching 118 ± 2 mmHg at 180 min ($p < 0.02$).

During ischemia, spontaneous Ldi EMG activity decreased significantly at 40 min, and reached 18 ± 15% of baseline at 180 min ($p < 0.01$, Fig. 2). Spontaneous tension of the left diaphragm also significantly decreased at 40 min, attaining 32 ± 14% of baseline ($p < 0.01$) by the end of the ischemic period (Fig. 2). After 60 min of ischemia, supramaximal twitch stimulation yielded Ldi tension and EMG activation which was significantly less than before ischemia (Fig. 3). These variables continued to decrease until the end of the ischemic period, when twitch Ldi tension was 39 ± 12% of baseline, and peak Ldi M wave amplitude was 23 ± 9% of baseline (Fig. 3).

Right diaphragm EMG activity increased significantly by 100 min (Fig. 2). This stimulation was not maintained: Rdi

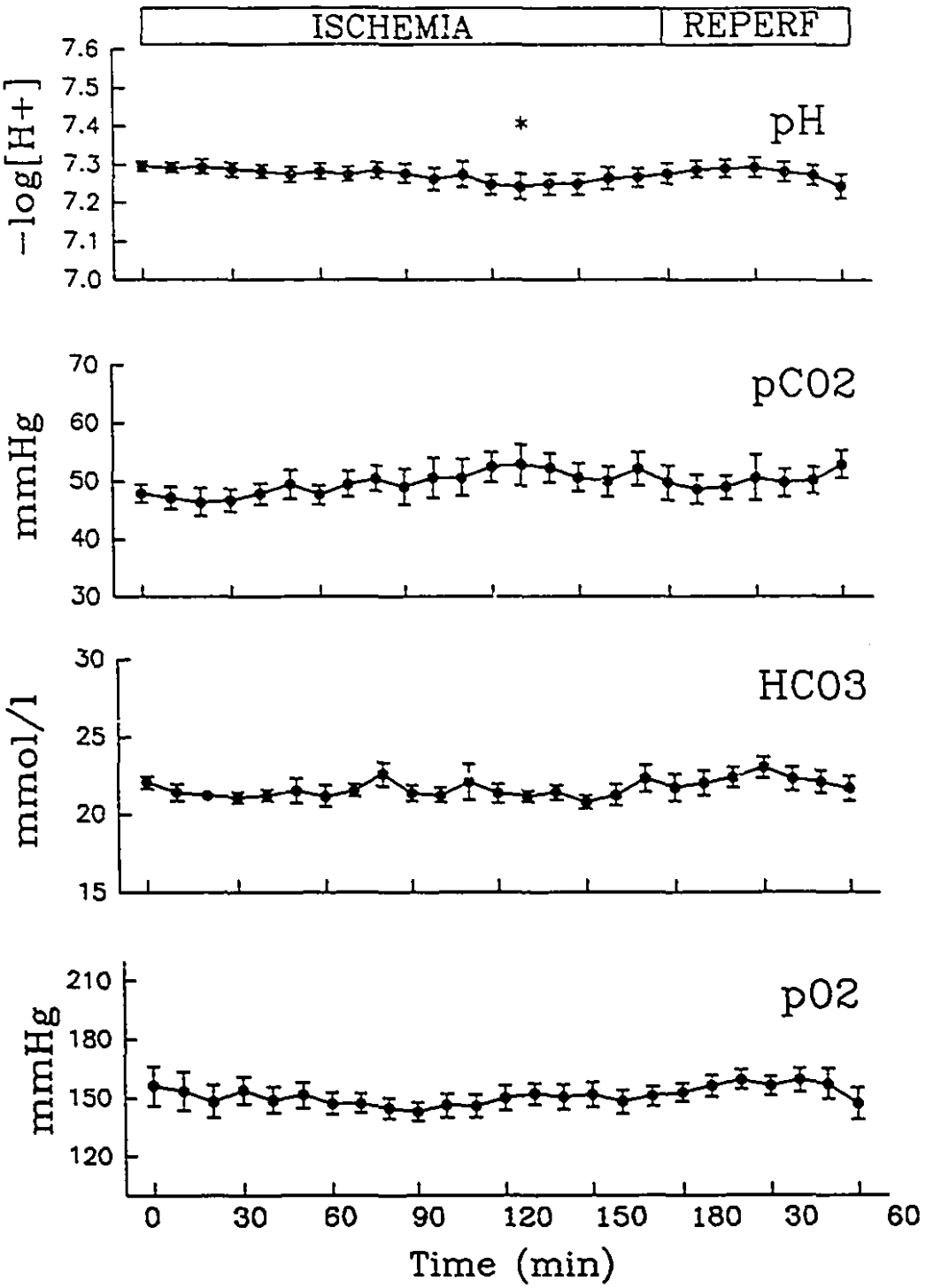

Fig. 5.1: Arterial blood gases were maintained relatively constant throughout the ischemia-reperfusion trials. Mean ± SE. * $p < 0.05$, compared to pre-ischemic baseline values.

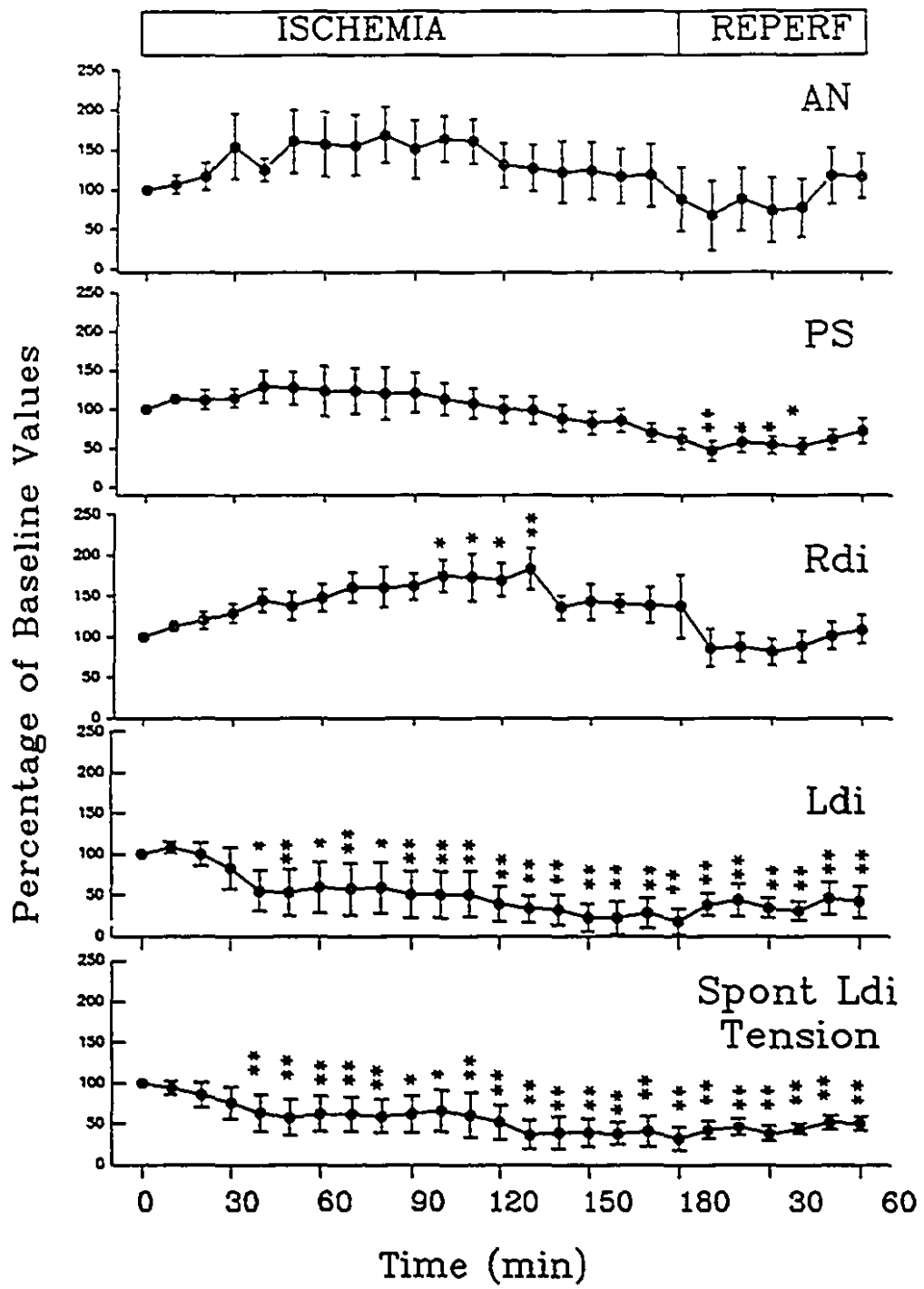

Fig. 5.2: Changes in peak integrated EMG activities of the alae nasi (AN), parasternal (PS), right hemi-diaphragm (Rdi) and left hemi-diaphragm (Ldi) and spontaneous Ldi tension (Spont Ldi Tension) during ischemia-reperfusion of the left hemi-diaphragm. Mean ± SE. *,** $p < 0.05$, 0.01, respectively, compared to pre-ischemic baseline values.

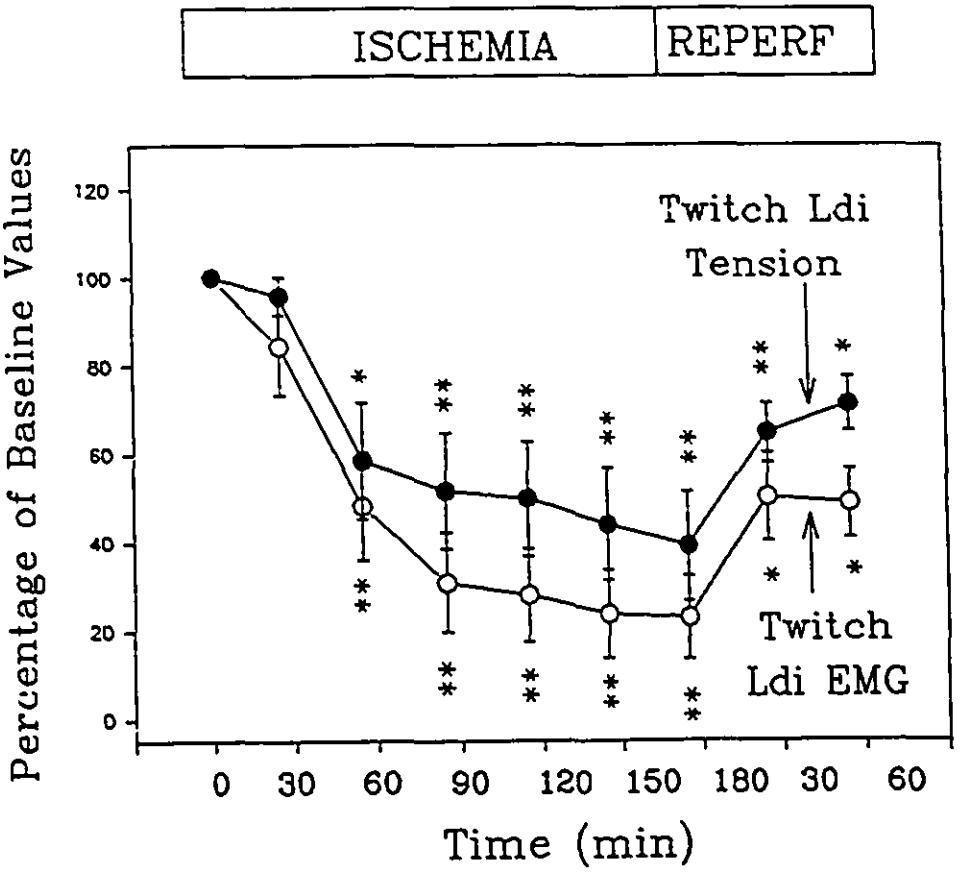

Fig. 5.3: Twitch stimulation of the left phrenic nerve demonstrates decreases in the maximal EMG activity (Twitch Ldi EMG) and tension generating capability (Twitch Ldi Tension) of the left hemi-diaphragm during ischemia, and some recovery during reperfusion. Mean ± SE. *, $p < 0.05$, 0.01, respectively, compared to pre-ischemic baseline values.**

activity fell at 140 min, and reached 137 ± 39% at the end of the ischemic period. EMG activities of the alae nasi and parasternal muscles also showed a biphasic response to ischemia, although these changes were not significantly differently from baseline values (Fig. 2).

Respiratory timing was variable during the ischemic period, yielding no significant changes in respiratory frequency or expiratory time. Inspiratory time significantly increased at 150 min ($p < 0.01$, Fig. 4), and was 122 ± 10% of pre-ischemia baseline at the end of the ischemic period.

Reperfusion

Arterial blood gas values during reperfusion were similar to those obtained preceding ischemia (Fig. 1). Mean systemic pressure fell significantly at 10 min, reaching 107 ± 4 mmHg after one hour of reperfusion ($p < 0.01$, compared to baseline).

Reperfusion of the left diaphragm caused Ldi EMG activity to increase slightly at 10 min, and remain constant throughout the remainder of the reperfusion period (41 ± 18% of baseline at 60 min, $p < 0.01$). Spontaneous tension of the left diaphragm recovered slightly during reperfusion, but remained significantly less than pre-ischemic values at 60 min (51 ± 8 %, $p < 0.01$, Fig. 2). Although some recovery occurred, peak Ldi M wave amplitude and twitch tension at 60 min of reperfusion remained below pre-ischemia values (49 ± 8% and 71 ± 6 %, respectively).

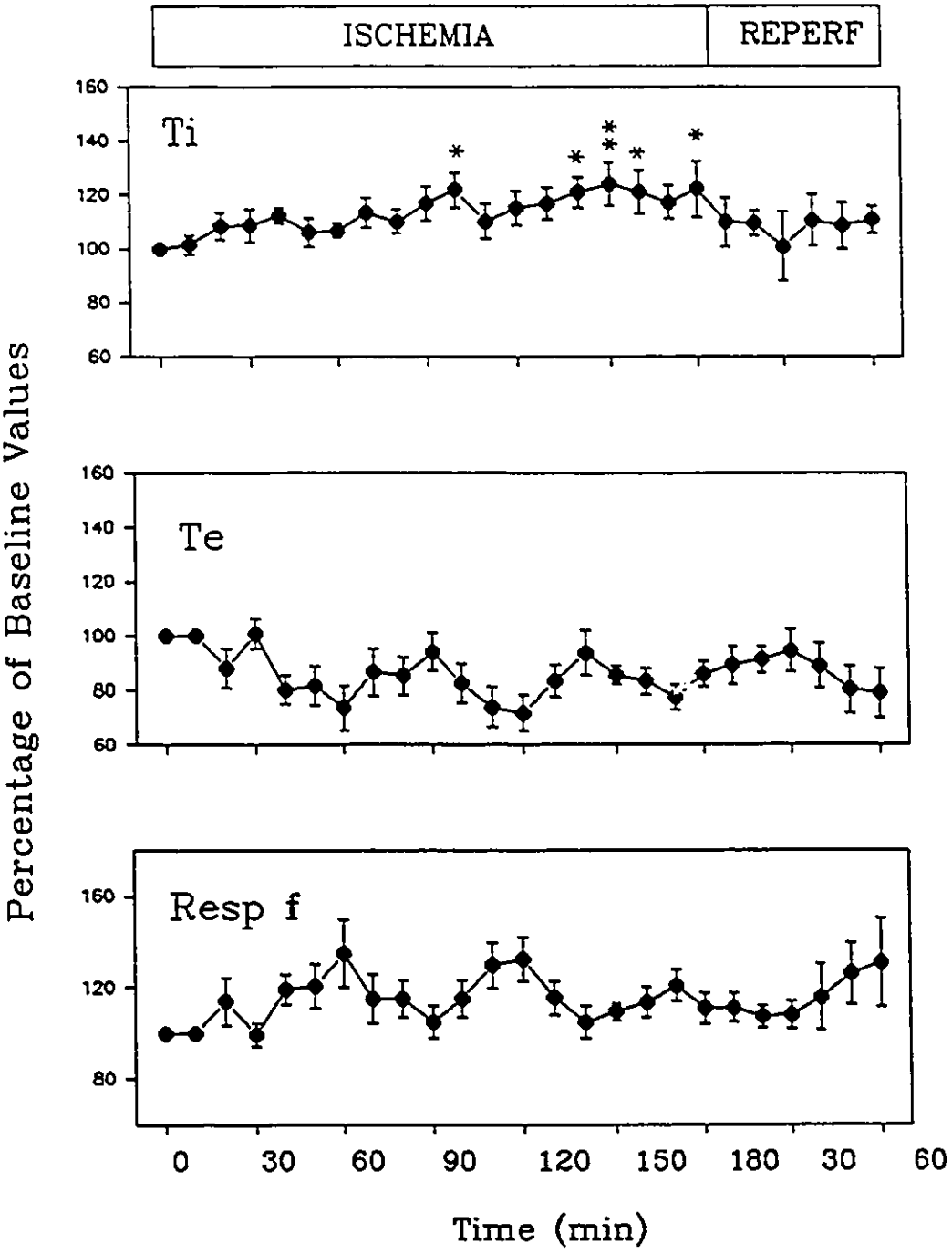

Fig. 5.4 Inspiratory time (Ti), expiratory time (Te), and respiratory frequency (Resp f) during ischemia-reperfusion of the left hemi-diaphragm. Mean ± SE. *,** $p < 0.05$, 0.01, respectively, compared to pre-ischemic baseline values.

Parasternal activity significantly decreased at 10 min of reperfusion, but was not significantly different from pre-ischemia baseline values at the end of the reperfusion period (72 ± 16% of baseline). No significant changes were found in either right diaphragm or alae nasi EMG activity during reperfusion (109 ± 18% and 117 ± 28% of baseline, respectively, at 60 min of reperfusion, Fig. 2).

Respiratory frequency, inspiratory and expiratory times did not change throughout the period of reperfusion (Fig. 4).

5.6 DISCUSSION

The main findings of this study are that 1) prolonged ischemia of the left hemi-diaphragm causes an initial rise in inspiratory motor drive followed by a return to baseline levels; and 2) there were no further changes in inspiratory motor drive during one hour of reperfusion of the left hemi-diaphragm.

Critique

This study was designed to test the effects of prolonged stimulation of phrenic afferents during ischemic fatigue on inspiratory motor drive. However, it is possible that the responses observed could be due to non-diaphragmatic afferent inputs. Arterial blood gases were maintained relatively constant throughout the protocol, so it is doubtful that alterations in chemoreceptor stimulation are responsible for the results. However, because the vagus nerves were left

intact, sensory information coming from vagally innervated receptors (e.g. pulmonary or vascular baroreceptors) could be involved in the responses obtained. Positive end-expiratory pressure and ventilator tidal volume was kept constant throughout the protocol, and the changes in respiratory frequency which were required to maintain end-tidal CO_2 constant were minimal. Therefore, pulmonary stretch receptor information remained relatively stable throughout the baseline ischemia and reperfusion periods.

Baroreceptor information, however, was not constant during the protocol. Mean systemic blood pressure (baseline = 129 mmHg) fell 11 mmHg during the three hours of ischemia, and continued to fall during the last hour of reperfusion (107 mmHg at end of reperfusion period). Brunner *et al.* (3) have shown that a fall in carotid sinus pressure from 125 mmHg to 100 mmHg in vagotomized, anesthethetized dogs caused a decrease in tidal volume (to 87% of baseline values), an increase in respiratory frequency, and decreases in both inspiratory and expiratory times. In addition, in anesthetized vagi intact cats, Bishop (2) found that a decrease in carotid sinus pressure by common carotid occlusion was associated with increased diaphragmatic and abdominal muscle activation, and increased respiratory frequency. In our protocol, inspiratory motor drive increased, decreased, and stabilized while mean arterial pressure continued to fall. In addition, respiratory

frequency did not change significantly, although inspiratory time lengthened during the last hour of ischemia. Therefore, although baroreceptor input was certainly changing during the protocol, it cannot explain the biphasic nature of the changes seen during ischemia and reperfusion.

Local effects of ischemia

Some of the initial changes which occur during muscle ischemia include a decrease in the membrane potential of the muscle cell, disruption of the plasma membrane, loss of energy stores, commencement of anaerobic metabolism, hypoxia, acidosis, and leakage of lactate, hydrogen and potassium ions constituents into the interstitial space (9). These changes lead to problems with action potential propagation and the generation of post-synaptic events leading to muscle contraction. The decreases in Ldi EMG and tension observed during spontaneous breathing and twitch stimulation are presumably due to these local effects of ischemia.

Ischemia also affects afferent fiber activity. In a contracting diaphragm, ischemia increases the activity of tonically active (5) thin-fiber afferents (11), while decreasing the activity of phasic afferents (presumably large-fiber afferents (5). Thin-fiber afferent activity is further augmented during skeletal muscle ischemia by bradykinin (10,12). In addition, it is possible that the free oxygen radical compounds produced in the diaphragm

during ischemia-reperfusion (14) may activate thin-fiber afferents. Lastly, Teitelbaum *et al.* (17) have shown that thin-fiber afferent activity mediates the changes in inspiratory motor drive which occur during diaphragmatic ischemia. Based on these findings, changes which occurred in this protocol during ischemia may be largely due to diaphragmatic thin-fiber afferent stimulation.

Changes in inspiratory motor drive

We found that three hours of left hemi-diaphragm ischemia caused an initial increase and subsequent decrease in right hemi-diaphragm, alae nasi and parasternal EMG activity (although the increased activity of the latter two muscles was not significant). A previous study from our laboratory reported that twenty minutes of ischemia caused significant increases in drive to these same inspiratory muscles (Teitelbaum et al. 1992).

Despite fairly large changes in alae nasi and parasternal EMG activity, statistical significance was not reached in our study due to inter-animal variability. We have found that EMG activities in vagi-intact preparations exhibit more breath-to-breath variability than occurs in vagotomized preparations. In addition, peak increases in alae nasi and parasternal EMG occurred at different times during ischemia. When the peak values for each animal were averaged and compared by paired t-test, statistically significant increases were found for both the alae nasi (172%

of baseline, $p < 0.001$) and parasternal muscles (146% of baseline, $p < 0.016$).

Our finding that left hemi-diaphragm EMG activity and spontaneous tension decreased during ischemia is in agreement with the response to fatigue of a diaphragmatic muscle strip reported by Supinski *et al.*(15). However, Teitelbaum *et al.* (16) reported that Ldi EMG activity increased and spontaneous tension either increased or decreased during 20 min of ischemia. The greater degree of hypercapnia present in our animals and during the study reported by Supinski *et al.* may be implicated in these different responses.

As indicated by the decrease in peak Ldi M wave amplitude and twitch tension during ischemia, fatigue occurred after 60 min of ischemia. We also found that inspiratory muscle EMGs, spontaneous tension, peak Ldi M wave amplitude and twitch Ldi tension stabilized and began to recover toward baseline during reperfusion.

Potential mechanisms underlying the changes in inspiratory motor drive

When left hemi-diaphragmatic ischemia was preceded by capsaicin-induced depletion of neurotransmitter substance from thin-fiber afferent terminals, no changes in inspiratory motor drive occurred (17). This indicates that diaphragmatic thin-fiber afferents are responsible for the initial increase in inspiratory motor drive during hemi-diaphragmatic ischemia. The subsequent decrease in EMG activity could be

caused by 1) the depletion of neurotransmitter substances from the terminals of afferent or other nerve fibers in the reflex pathway, or 2) the development of central inhibition (e.g. the production of endogenous opioids) of ventilatory drive (13,19). Our data support the former interpretation, since ventilatory drive returned toward baseline values during the latter part of ischemia. However, the development of central inhibition of ventilatory drive cannot be completely ruled out.

In conclusion, we have shown that prolonged activation of diaphragmatic thin-fiber afferents by ischemia causes an initial increase in ventilatory drive, with subsequent failure to maintain this early rise. Reperfusion of the ischemic diaphragm causes no additional changes in the inspiratory motor drive. While the initial increase in drive has been previously shown to be due to the activation of diaphragmatic thin-fiber afferents, we postulate that the subsequent return to baseline activity is due to the depletion of neurotransmitter substance in afferent pathways.

5.7 REFERENCES

1. AUBIER, M., T. TRIPPENBACH, and C. ROUSSOS. Respiratory muscle fatigue during cardiogenic shock. *J Appl Physiol* 51: 499-508, 1981.

2. BISHOP, B. Carotid baroreceptor modulation of diaphragm and abdominal muscle activity in the cat. *J Appl Physiol* 36(1): 12-19, 1974.

3. BRUNNER, M., M. SUSSMAN, A. GREENE, C. KALLMAN, and A. SHOUKAS. Carotid sinus baroreceptor reflex control of respiration. *Circ Res* 51: 624-636, 1982.

4. COHEN, C.A., G. ZAGELBAUM, D. GROSS, CH. ROUSSOS, and P.T. MACKLEM. Clinical manifestations of inspiratory muscle fatigue. *Am J Med* 73: 308-316, 1982.

5. GRAHAM, R., Y. JAMMES, S. DELPIERRE, C. GRIMAUD, and CH. ROUSSOS. The effects of ischemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. *Neurosci Lett* 67: 257-262, 1986.

6. HUSSAIN, S.N.A., CH. ROUSSOS, and S. MAGDER. In situ isolated perfused and innervated left hemidiaphragm preparation. *J Appl Physiol* 67(5): 2141-2146, 1989.

7. HUSSAIN, S.N.A., G. SIMKUS, and C. ROUSSOS. Respiratory muscle fatigue: a cause of ventilatory failure in septic shock. *J Appl Physiol* 58: 2033-2040, 1985.

8. JAMMES, Y. and E. BALZAMO. Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73(3): 894-902, 1992.

9. JENNINGS, R.B., C.E. GANOTE, and K.A. REIMER. Ischemic tissue injury. *Am J Pathol* 81: 179-198, 1975.

10. KAUFMAN, M.P., G.A. IWAMOTO, J.C. LONGHURST, and J.H. MITCHELL. Effects of capsaicin and bradykinin on afferent fibers with endings in skeletal muscle. *Circ Res* 50: 133-139, 1982.

11. KAUFMAN, M.P., K.J. RYBICKI, T.G. WALDROP, and G.A. ORDWAY. Effect of ischemia on responses of group III and IV afferents to contraction. *J Appl Physiol* 57: 644-650, 1984.

12. MENSE, S. Nervous outflow from skeletal muscle following chemical noxious stimulation. *J Physiol (Lond)* 267: 75-88, 1977.

13. ROAD, J.D., S. OSBORNE, and Y. WAKAI. Delayed poststimulus decrease of phrenic motoneuron output produced by phrenic nerve afferent stimulation. *J Appl Physiol* 74: 68-72, 1993.

14. SUPINSKI, G., D. STOFAN, and A. DIMARCO. Effect of ischemia-reperfusion on diaphragm strength and fatigability. *J Appl Physiol* 75: 2180-2187, 1993.

15. SUPINSKI, G.S., A.F. DIMARCO, F. HUSSEIN, and M.D. ALTOSE. Alterations in respiratory muscle activation in the ischemic fatigued canine diaphragm. *J Appl Physiol* 67(2): 720-729, 1989.

16. TEITELBAUM, J., S.A. MAGDER, CH. ROUSSOS, and S.N.A. HUSSAIN. Effects of diaphragmatic ischemia on the inspiratory motor drive. *J Appl Physiol* 72(2): 447-454, 1992.

17. TEITELBAUM, J., G. VANELLI, and S.N.A. HUSSAIN. Thin-fiber phrenic afferents mediate the ventilatory response to diaphragmatic ischemia. *Resp Physiol* 91: 195-206, 1993.

18. TEITELBAUM, J.S., S.A. MAGDER, CH. ROUSSOS, and S.N.A. HUSSAIN. Effects of diaphragmatic ischemia on the inspiratory motor drive. *J Appl Physiol* 72(2): 447-454, 1992.

19. WALDROP, T.G., F.L. ELDRIDGE, and D.E. MILLHORN. Inhibition of breathing after stimulation of muscle is mediated by endogenous opiates and GABA. *Respir Physiol* 54: 211-222, 1983.

20. WILKINSON, L. SYSTAT: The system for statistics. Evanston IL: SYSTAT, Inc., 1990.

CHAPTER 6

GENERAL CONCLUSIONS

6.1 CONCLUSIONS

This thesis addresses the influence of diaphragmatic afferents, particularly thin-fiber afferents, on the control of breathing. The majority of previous studies concerned with effect of phrenic nerve afferents on ventilatory drive have used electrical stimulation. These studies showed that stimulation caused ventilatory drive to either increase (4,8,11) or decrease (3,9). In addition, a biphasic response has been reported (5).

Differences in these results are most probably because of different methodologies and modes of stimulation. Chemical stimulation can be more selective in the types of fibers activated, but requires vascular isolation of the diaphragm. Capsaicin activation of diaphragmatic thin-fiber afferents increased inspiratory motor drive (1,7). Capsaicin is a selective stimulant of group III and, primarily, group IV afferents. However, it is a non-physiologic substance which causes large and sudden changes in afferent nerve activity, which may not be representative of activation under more natural conditions. In the first thesis project, I used bradykinin, a physiologic substance produced in the muscle during exercise (10), to stimulate left hemi-diaphragmatic thin-fiber afferents. Small increases in electromyographic activity occurred in the alae nasi, parasternal and left diaphragm muscles, while genioglossus EMG activity increased

greatly, and right diaphragm EMG activity was unchanged. In addition, inspiratory time decreased significantly. These changes did not occur when bradykinin was injected after left phrenicotomy, indicating that phrenic afferents were responsible for the changes we observed. We conclude that selective activation of diaphragmatic thin-fiber afferents causes mild stimulation of inspiratory motor drive, decreases inspiratory time, and redistributes inspiratory motor drive.

The increase in ventilation which occurs during exercise is associated with increases in ventilatory muscle activity and respiratory frequency, and a decrease in airway muscle tone. Limb muscle thin-fiber afferents may be involved in this response, since activation of these fibers causes increases in inspiratory motor drive and decreases in trachealis muscle tone and lung resistance. We demonstrated in the first project that diaphragmatic thin-fiber afferents also participate in reflexes leading to an augmentation of inspiratory motor drive. Unilateral stimulation of the phrenic nerve has been shown to decrease lung resistance (6). In the second project we assessed the effects of unilateral and bilateral phrenic afferent activation on large airway smooth muscle tone and lung resistance. We found that unilateral and bilateral phrenic nerve stimulation at high intensities decreased trachealis tension. In addition, significant decreases in lung resistance occurred during high intensity stimulation of the right or both phrenic nerves.

Since tracheal tension and lung resistance are unchanged by low intensity unilateral nerve stimulation, we presume that the responses observed during high intensity stimulation are caused by the activation of thin-fiber afferents. We have thus shown that phrenic thin-fiber afferent activation is associated with changes in autonomic control of airway muscle tone as well as inspiratory motor drive.

Many thin-fiber afferents are mechanically sensitive and may, therefore, be activated in phase with the ventilatory cycle. In the third thesis project, I studied whether the response to phrenic nerve stimulation is phase dependent, i.e. if inspiratory drive responses are similar when stimulation is done during either inspiration or expiration. In addition, I have investigated the role of vagal input in modulating this response. I found that, in vagi-intact animals, high intensity inspiratory and expiratory phrenic nerve stimulation caused similar changes in inspiratory motor drive and respiratory timing. Vagotomy attenuated the rate of rise of inspiratory muscle EMG caused by inspiratory phrenic, but not tibial, nerve stimulation. Therefore, vagal input potentiates the stimulatory effect of inspiratory phrenic thin-fiber afferent activation.

As previously mentioned, studies concerning the ventilatory effects of phrenic thin-fiber afferent activation have used protocols where chemical or electrical stimulation was done for short periods of time. Thin-fiber afferents may

provide important input during the development of muscle fatigue. Jammes and Balzamo (2) found that tonic afferent fiber activity increased progressively over 30 minutes when diaphragmatic fatigue was elicited by direct muscle stimulation. When diaphragmatic fatigue was caused by weaning from mechanical ventilation, septic shock or cardiogenic shock, ventilatory drive has been shown to increase and then decrease over a time period ranging from 20 min to several hours. Phrenic afferents may play a role in this response.

In the fourth project, we used ischemia to assess the effect of prolonged activation of phrenic thin-fiber afferents on ventilatory drive. We showed that, unlike short term ischemia, three hours of left hemi-diaphragmic ischemia caused an initial increase in nonischemic inspiratory motor activity, followed by a return to baseline levels. In addition, as ischemic fatigue progressed, spontaneous EMG activity and tension generated by the left hemi-diaphragm fell. After one hour of reperfusion, non-ischemic inspiratory motor drive remained at pre-ischemic baseline levels. However, spontaneous motor drive to the left hemi-diaphragm remained low and, despite some recovery, fatigue was still present after reperfusion. Previous studies have shown that the initial increase in ventilatory drive is due to the activation of phrenic thin-fiber afferents. We propose that the subsequent decrease in drive may be due to

the depletion of neurotransmitter substances within afferent pathways.

In summary, I have demonstrated that chemical and electrical phrenic thin-fiber afferent activation leads to an increase in inspiratory motor drive in vagotomized dogs. When I performed phasic stimulation in vagi-intact animals, the increases in inspiratory motor drive were similar whether stimulation is performed during inspiration or expiration. Vagal input during inspiration appears to modulate the stimulatory effect of phrenic, but not tibial, thin-fiber afferent activation. Finally, I have demonstrated that prolonged activation of diaphragmatic thin-fiber afferents by ischemia causes biphasic changes in ventilatory drive to non-ischemic respiratory muscles, as well as fatigue of the ischemic hemi-diaphragm. Fatigue of the left-hemidiaphragm was not eliminated by one hour of reperfusion. Finally, I postulate that the fall in activity of non-ischemic muscles to baseline levels during prolonged ischemia may indicate depletion of neurotransmitter substances in afferent pathways.

In conclusion, phrenic nerve thin-fiber afferents participate in a number of ventilatory reflexes, and appear to play an important role in the regulation of breathing during physiological and pathophysiological states.

6.2 REFERENCES

1. HUSSAIN, S.N.A., S. MAGDER, A. CHATILLON, and CH. ROUSSOS. Chemical activation of thin-fiber phrenic afferents: respiratory responses. *J Appl Physiol* 69(3): 1002-1011, 1990.

2. JAMMES, Y. and E. BALZAMO. Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *J Appl Physiol* 73(3): 894-902, 1992.

3. JAMMES, Y., B. BUCHLER, S. DELPIERRE, A. RASIDAKIS, C. GRIMAUD, and C. ROUSSOS. Phrenic afferents and their role in inspiratory control. *J Appl Physiol* 60(3): 854-860, 1986.

4. KOHRMAN, R.M., J.B. NOLASCO, and C.J. WIGGERS. Types of afferent fibers in the phrenic nerve. *Am J Physiol* 151: 547-557, 1947.

5. MARLOT, D., J.-M. MACRON, and B. DURON. Inhibitory and excitatory effects on respiration by phrenic nerve afferent stimulation in cats. *Respir Physiol* 69: 321-333, 1987.

6. MCCALLISTER, L.W., K.W. MCCOY, J.C. CONNELLY, and M.P. KAUFMAN. Stimulation of groups III and IV phrenic afferents reflexly decreases total lung resistance in dogs. *J Appl Physiol* 61(4): 1346-1351, 1986.

7. REVELETTE, W.R., L.A. JEWELL, and D.T. FRAZIER. Effect of diaphragm small-fiber afferent stimulation on ventilation in dogs. *J Appl Physiol* 65(5): 2097-2106, 1988.

8. ROAD, J.D., N.H. WEST, and B.N. VAN VLIET. Ventilatory effects of stimulation of phrenic afferents. *J Appl Physiol* 63(3): 1063-1069, 1987.

9. SPECK, D.F. and W.R. REVELETTE. Attenuation of phrenic motor discharge by phrenic nerve afferents. *J Appl Physiol* 62(3): 941-945, 1987.

10. STEBBINS, C.L., O.A. CARRETERO, T. MINDROIU, and J.C. LONGHURST. Bradykinin release from contracting skeletal muscle of the cat. *J Appl Physiol* 69(4): 1225-1230, 1990.

11. WARD, M.E., G. VANELLI, M. HASHEFI, and S.N.A. HUSSAIN. Ventilatory effects of the interaction between phrenic and limb muscle afferents. *Respir Physiol* 88: 63-76, 1992.

CLAIMS TO ORIGINALITY

I have made the following original contributions toward understanding the role of phrenic afferents in the control of ventilation:

1. The activation of diaphragmatic afferents by bradykinin (a substance which activates only group III and IV afferent fibers) elicits an increase in inspiratory muscle activity and a decrease in inspiratory time.

2. The increase in inspiratory muscle activity caused by bradykinin stimulation of diaphragmatic afferents is inhomogeneous (i.e. the increase in genioglossus EMG activity was ten fold greater than the increases in alae nasi, parasternal, or diaphragm EMG activity).

3. Ventilatory drive stimulation caused by bradykinin activation of diaphragmatic afferents is not dose dependent in the range of 10-100 micrograms. This finding suggests that, in the isolated hemi-diaphragm preparation, diaphragmatic afferents are maximally activated at bradykinin dosages $\geq$ 10 micrograms.

4. High intensity electrical stimulation of the proximal end of one or both cut phrenic nerves causes a decline in trachealis muscle tension. Trachealis muscle tension is unaffected by low intensity unilateral nerve stimulation, indicating that group III and IV afferents exert an inhibitory effect on large airway motor tone.

5. The effect of phrenic nerve stimulation on trachealis tension is qualitatively similar to, but less than, the inhibition elicited by tibial nerve stimulation. Bilateral phrenic nerve stimulation causes decreases in trachealis tension which are comparable to those which occur during unilateral tibial nerve stimulation, but less than the sum of the responses of unilateral stimulation of the right and left phrenic nerves.

6. Phasic high intensity phrenic nerve stimulation during either inspiration or expiration produces similar increases in inspiratory motor drive.

7. Vagotomy eliminates the increased rate of rise of inspiratory muscle integrated EMG activities caused by inspiratory phrenic, but not tibial, nerve stimulation. This indicates that vagal input during inspiration potentiates the stimulatory effect of phrenic thin-fiber afferent activation.

8. Unlike short periods of ischemia, prolonged (3 hours) left hemi-diaphragmatic ischemia causes an initial increase in motor drive to non-ischemic inspiratory muscles (e.g. alae nasi, parasternal and right hemi-diaphragm muscles), which is not sustained during the last hour of ischemia.

9. During three hours of left hemi-diaphragmatic ischemia, spontaneous EMG activity and tension of the ischemic muscle decline. These changes are probably due to the development of ischemic fatigue, indicated by a fall in the tension generated by the left hemi-diaphragm during supramaximal twitch stimulation.

10. After one hour of left hemi-diaphragmatic reperfusion, inspiratory drive to non-ischemic muscles remains at baseline levels, in contrast to the sustained decrease in spontaneous EMG activity and tension generated by the left hemi-diaphragm. Supramaximal twitch stimulation reveals that, despite some recovery, ischemic fatigue of the left hemi-diaphragm is still present after one hour of reperfusion.

APPENDIX 1

SI unit Equivalents

1 Pascal (Pa) = 0.075 mmHg

= 0.01 cmH_2O

APPENDIX 2

AN	alae nasi
ANOVA	analysis of variance
br	breaths
BK	bradykinin
C	Centigrade
cmH_2O	centimeters of water
CO_2	carbon dioxide
Fig.	figure
EMG	electromyogram
exp	expiration
f_R	respiratory frequency
g/cm	grams per centimeter
GG	genioglossus
HCO_3	bicarbonate
Hz	Hertz
ID	internal diameter
insp	inspiration
kg	kilogram
l	liter
-log[H+]	negative logarithm of the hydrogen ion concentration
Ldi	left hemi-diaphragm
LdiM	peak amplitude of M wave of Ldi
LP	left phrenic nerve
µg	microgram
mg/kg	milligrams per kilogram
min	minute
ml/kg	milliliters per kilogram
mmHg	millimeters of mercury
mmol/l	millimoles per liter
ms	milliseconds
O_2	oxygen
OD	outer diameter
Part	systemic arterial pressure
Paw	airway pressure
pCO_2	partial pressure of carbon dioxide
PEEP	positive end-expiratory pressure
pH	negative logarithm of the hydrogen ion concentration
pO_2	partial pressure of oxygen
P+P	bilateral phrenic
PS	parasternal muscle
Reperf	reperfusion (of the left hemi-diaphragm)
Resp f	respiratory frequency
Rdi	right hemi-diaphragm
RL	lung resistance
RP	right phrenic

APPENDIX 2 (cont'd)

s, sec	second
SE	standard error of the mean
SI units	standard international units of measurement
SpT, SpLdiTension	spontaneous tension generated by the left hemi-diaphragm
T	tibial
Te, T_E	expiratory time
Ti, T_I	inspiratory time
TT	twitch threshold
Ttr	trachealis muscle tension
%	percentage
<	less than