Doctor of Philosophy

Neural and humoral factors related to diaphragm fatigue.

By

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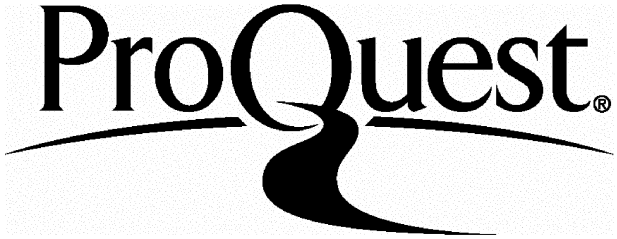

To Brenda for having faith in me and Generator of Diversity
for making life so fascinating.

WAS IT ALL WORTH IT

What is there left for me to do in this life
Did I achieve all I set in my sights
Am I a happy man on a natural high
Was it all worth it, was it all worth it

Here's my story, Let me tell you about it
I was given a science kit, I blew some circuits
I played a scientist, it was so perfect
Was it all worth it
Yeah, living, breathing Roussos and all
Staying up all nights
Was it all worth it
Yeah, giving all my heart and soul
This never ending fight
Was it all worth it, was it all worth it
Yes, it was a worthwhile experience

Inspired by Queen

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Finally, special thanks go to the loves of my life, my wife, my mother and my daughter for their understanding and support, and the late Freddie, Aretha, and Anita for keeping me sane.

Formal Declaration

I declare that I have written this thesis presented to University of London for the degree of Doctor of Philosophy. All the studies reported in the thesis are predominantly my own with exception of the following:

The construction of the Alec stimulator to my specifications and some of the testing of it was done by Mr A. Birkett.

ABSTRACT

The aim of project was to gain an insight into the neural control of breathing during the development of diaphragm fatigue.

In phase one, the role of vagal feedback in the control of breathing during the development of diaphragm fatigue was examined by comparing the ventilatory responses to inspiratory resistive loading (IRL) in vagally intact and vagally denervated rabbits. The results indicate that vagal inputs probably have no significant role to play in the control of breathing during the development of diaphragm fatigue.

In phase two, the effects of IRL on arterial blood chemistry were examined to identify noxious chemicals generated during fatiguing IRL. This was necessary to identify potential chemical stimuli of small-phrenic afferent fibres. Potassium was identified as one such stimulus. The increase in arterial potassium concentration ($[K_a^+]$) during IRL was associated with a combined metabolic and respiratory acidosis. On the basis of theoretical considerations, the increase in $[K^+]$ could have precipitated diaphragm fatigue. The effects of metabolic and respiratory acidoses on $[K_a^+]$ were independently assessed. Both produced a rise in $[K_a^+]$, but the sum was less than the rise in $[K_a^+]$ produced by IRL.

In the final phase, it was established that activation of small-phrenic afferents either by electrical stimulation or by K^+ applied to the abdominal surface of the diaphragm caused an increase in minute ventilation and a transient decrease in mean arterial blood pressure. In addition, K^+ was shown to excite phrenic afferents. Two patterns of discharge were observed; one was rapidly adapting characteristic of group III fibres, the other was slowly adapting characteristic of group IV fibres.

CONTENTS

A LIST OF FIGURES, PHOTOGRAPHS AND TABLES

Figures

Fig. 8 5. Average changes ± SE in expiratory time (T_E), inspiratory time (T_I), duty cycle (T_I/T_T) and mean inspiratory flow (V_T/T_I), expressed as percentages of their control values, during phrenic nerve stimulation.

Fig. 8.6. Average changes ± SE in end-tidal CO_2, expressed as percentages of their control values, during phrenic nerve stimulation.

Fig. 8.7. Effect of electrical stimulation of the central end of the right phrenic nerve on tidal volume, mean arterial blood pressure, and end-tidal CO_2 in spontaneously breathing rabbits.

Fig. 9.1. Comparison of the effect of 1.5 M KCl with the effects of normal saline on ventilation and arterial blood pressure in the same rabbit.

Fig. 10.1. Trace showing the effect of firm pressure on the activity of phrenic afferents

Fig. 10.2. Stimulation of phrenic afferents by topical application of potassium to the diaphragm. (A) Histogram showing rapid response to potassium by an individual fibre. (B) Recording of action potential impulses of a rapidly adapting afferent fibre to potassium.

Fig. 10.3. Stimulation of phrenic afferents by topical application of potassium to the diaphragm. (A) Histogram showing a prolong response to potassium by an individual fibre. (B) Recording of action potential impulses of a slow adapting afferent fibre to potassium.

Fig. A.1. Relationship between potassium concentrations measured using ABL 4 and concentrations measured using a flame photometer.

Fig. A.2. Relationship between potassium concentrations measured using ABL 4 and concentrations measured using a flame photometer.

Fig. B.1. Diagram of the set-up used to calibrate the Gaeltec pressure transducer.

Fig. B.2. Gaeltec pressure measurements over a range of pressures.

Fig. C.1. Diagram of the experimental set-up for determining the resistance of the resistor.

Fig. C.2. Relationship between airflow (f) and pressure gradient (ΔP) across the resistor.

Fig. D.1. Schematic diagram of Alec stimulator. Explanation of the diagram is given in the text below.

Fig. D.2. Determination of pulse amplitude and width.

Photographs

Tables

Table. B.1. Gaeltec Pressure transducer calibration at various pressures

Table D.1. Showing the range of frequencies, pulse width, and voltage generated by the Alec electrical stimulator.

CHAPTER 1

INTRODUCTION

The inspiratory muscles, of which the diaphragm is the major component, are essential for life and must contract rhythmically throughout life. It is important that these muscles should be able to maintain alveolar ventilation in face of changing demands imposed by such varied activities as sleep, speech, singing, playing a musical instrument, muscular exercise and disease. In such a well-controlled system, afferent inputs from inspiratory muscles should have a significant role to play in the control of breathing in response to a fatiguing workload.

The respiratory system could respond to a fatiguing load in two possible ways: one is for the inspiratory muscles to be driven to exhaustion in an attempt to maintain arterial blood gas tensions within normal limits; the other is for the respiratory controller to respond by reducing neural drive to protect inspiratory muscles from failure with a rise in arterial partial pressure of carbon dioxide as well as a fall in arterial partial pressure of oxygen. There is evidence to show that the latter response does occur (Cohen et al., 1982; Roussos, 1984 & 1990) but there is also evidence available to indicate that the diaphragm might be driven to exhaustion.

Stimulation of group III and IV phrenic afferents, which are sensory fibres arising from the diaphragm, has been shown to produce an increase in ventilation that could drive the diaphragm to exhaustion (Road et al., 1987; Revelette et al., 1988). However, stimulation of these afferents has also been shown to decrease total lung resistance (McCallister et al., 1988). These responses combined may ensure adequate oxygen supply to the loaded diaphragm and reduce its workload, thereby optimizing the overall endurance of the diaphragm. Experiments of this type provide evidence to

suggest that before the diaphragm is overtly fatigued, important interactions between it and the respiratory controller take place. The nature of the stimulus arising from the diaphragm remains to be discovered, and the ultimate aim of this thesis is identify the nature of this stimulus.

PHYSIOLOGY OF INSPIRATORY MUSCLE FATIGUE

Exercise-induced muscle fatigue has been defined as the transient loss of force-generating capacity resulting from preceding physical activity (Simonsen & Weiser 1976, Asmussen 1979). Alternatively, fatigue has been defined as a failure to maintain a required force or exercise intensity (Edward 1983). The latter could be considered as a definition of exhaustion, which is the final stage of fatigue. The former definition is consistent with the idea that the muscle is gradually fatiguing from the onset of exercise, since fatigue is an integral part of exercise (Bigland-Ritchie et al 1986). In the studying of inspiratory muscle fatigue, fatigue has been defined as the inability to develop sufficient pressure for an adequate alveolar ventilation. In this thesis, inspiratory muscle fatigue is defined as the inability of the inspiratory muscles to sustain the required transdiaphragmatic pressure (Pdi) for adequate alveolar ventilation when repeatedly contracting near or at maximal Pdi.

The site and mechanism of fatigue have been controversial for over a century and inspiratory muscle fatigue over the last two decades. It is, however, interesting that the consensus view emerging about the nature of diaphragm fatigue was outlined elegantly in the first experiments studying respiratory muscle fatigue performed by Davies, Haldane, and Priestly in 1919. They studied the ventilatory response to respiratory resistance in man. They found the following:

(1) Respiratory resistance produced either rapid shallow breathing or slow deep breathing.

(2) It, also, produced respiratory muscle fatigue, which was indicated by a substantial decrease in ventilation, and was usually associated with anoxemia.

(3) Anoxemia hastened greatly the onset of fatigue and the ease with which it was produced.

However, they attributed respiratory muscle fatigue to failure of the respiratory centre. This is not surprising as the prevailing view then was that muscular fatigue was central in origin and because they had no direct means of assessing the contractility of the diaphragm.

Some 60 years later, Roussos and Macklem (1977) confirmed much of Davies and colleagues' findings. In addition, Roussos and Macklem found that when subjects breathing through inspiratory resistors generated transdiaphragmatic pressures (Pdi) at predetermined fractions of their maximal transdiaphragmatic pressure (Pdi_{max}) the relationship between endurance of the diaphragm and Pdi/Pdi_{max} was curvilinear; above a Pdi/Pdi_{max} of 40%, the diaphragm fatigued rapidly with increasing force of contraction. They proposed that above a Pdi/Pdi_{max} of 40%, the energy demand of the diaphragm exceeds its energy supply, resulting in contractile fatigue.

The view that muscular fatigue is caused by central inhibition remained unchallenged until Merton showed in 1954 that fatigue in the adductor pollicis muscle was a consequence of failure in the muscle. The current view is that muscular fatigue can occur as a result of impairment in one or more steps of the command chain for muscle contraction; the site of failure could reside in (1) the central nervous system, (2) the final motor neuron, (3) the neuromuscular junction, or (4) the muscle (Fig 1.1). This

will depend upon the type of exercise being performed and the muscles used.

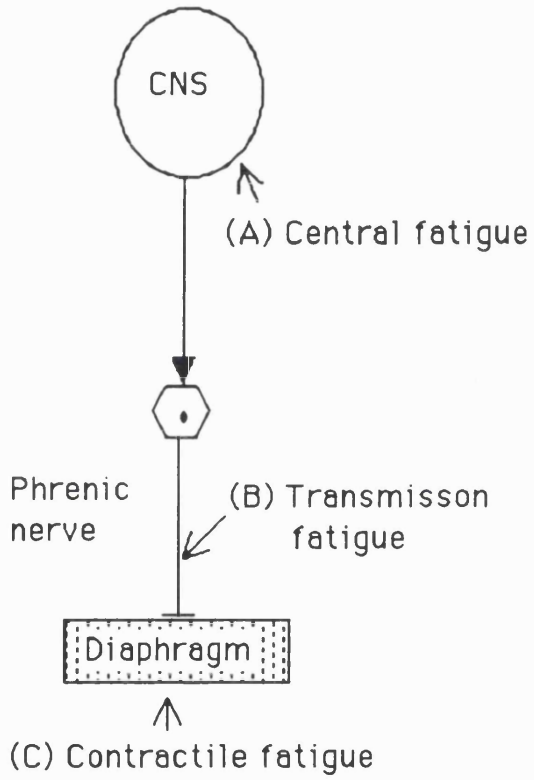


Fig. 1.1. Types of diaphragmatic fatigue. A schematic diagram showing the potential sites of fatigue. The letters A, B, and C denote the site where electrical stimulation can be applied to assess the function of the chain.

During voluntary effort, Pdi is reduced in all three types of fatigue. Central fatigue is the only type in which superimposed phrenic nerve stimulation augments the maximal voluntary response. Both contractile and transmission fatigue exhibit a decrease in Pdi response to stimulation at end-expiration. Only in contractile fatigue is Pdi in response to direct stimulation of the muscle is reduced. A summary of the methods used to diagnose diaphragm fatigue is presented in Table 1.1 below.

For the experiments presented in this thesis, the second and fourth methods were used to diagnose diaphragmatic fatigue as they are in combination the best methods to diagnose peripheral fatigue without requiring too much surgical intervention.

MECHANICAL RESPONSES	CENTRAL FATIGUE	TRANSMISSION FATIGUE	CONTRACTILE FATIGUE
(1) Voluntary Pdi_{max}	Reduced	Reduced	Reduced
(2) Voluntary tidal Pdi	Reduced	Reduced or Normal	Reduced or Normal
(3) Pdi response to phrenic nerve stimulation superimposed on Pdi_{max}	Present	Absent	Absent
(4) Pdi response to phrenic nerve stimulation applied at end-expiration	Normal	Reduced	Reduced
(5) Pdi response to direct stimulation of the diaphragm	Normal	Normal	Reduced

Table 1.1. Summary of some of the methods that can be used to diagnose each of the three types of fatigue.

Central fatigue

Central fatigue is the reversible decrease in central neural drive caused by over use of muscle. Edwards (1981) has described two types of central fatigue: motivational and non-motivational. In motivational fatigue of inspiratory muscles, any decrease in respiratory effort can be restored by voluntary supra-effort; whereas in non-motivational fatigue, the muscle can be maximally activated by supra-maximal electrical stimulation but no amount of exhortation can increase the level of respiratory effort. The mechanisms responsible for central fatigue are not known.

Both types of central fatigue have been demonstrated in humans. Bellemare and Bigland-Ritchie (1987) found that inspiratory resistive loading lead to a reduction in maximal voluntary Pdi generation. Although a small part of this reduction in Pdi could be accounted for by contractile failure, the major contribution to fatigue was apparently due to inability to voluntarily fully activate the diaphragm in the fatigue state, as diagnosed by superimposition of phrenic nerve stimuli on maximal voluntary effort. This finding suggests that the decrease in Pdi was due to the development of non-motivation fatigue. Gandevia and Mckenzie (1985) also showed that central inhibition was a major contributor to human diaphragm fatigue, but they found that voluntary efforts were capable of maximally activating the diaphragm at least once in all subjects, even when the diaphragm was fatigued. Their study showed that motivational fatigue occurs in humans. Both these studies provide evidence to suggest that central fatigue is a major contributor to diaphragm fatigue, at least in healthy humans.

Central fatigue has also been shown to be a contributor to diaphragm failure in the rabbit, the animal model used in this thesis. Aldrich (1988) showed that diaphragmatic effort decreased in the rabbit despite increased chemical drive and

despite the diaphragm being able to maximally contract in response to phrenic nerve stimulation.

A biochemical explanation for the cause of central fatigue has been provided for by Scardella and others (1986). They observed an increase in ß-endorphin level in cerebrospinal fluid in the goat, which was associated with a progressive decrease in tidal volume, during inspiratory resistive loading (IRL). They also found that administration of naloxone during IRL increased minute ventilation. Their results suggest that endogenous opioid may precipitate central fatigue during IRL. However, peripheral fatigue cannot be ruled out as Pdi_{max} was not measured. Similar results were found in a study on humans. In 1981, Santiago and others documented that some chronic obstructive airway disease patients have an abnormal compensatory response to resistive loading and normalize their compensatory response after receiving naloxone. In view of these reports, it seems that endogenous opioid can reduce central neural drive to the inspiratory muscles, and thereby contribute to central fatigue.

Transmission fatigue

Transmission fatigue is a reversible impairment in transmission of neural impulses through nerves or across neuromuscular junction as consequence of over use of the muscles. The idea that the neuromuscular junction fails during fatiguing contractions is attractive as exhausting the stores of neurotransmitter provides an obvious explanation.

Studies on isolated rat phrenic nerve-hemi-diaphragm preparations have demonstrated that transmission failure can occur at three sites, resulting in diaphragmatic fatigue (Krnjevic et al., 1958; Kurihara et al., 1975). These sites were axonal branch points, where impulses may fail to

conduct down them; the neuromuscular junction, where there may be insufficient release or re-uptake of neurotransmitter; or the muscle membrane which may become relatively inexcitable. Because the isolated diaphragm preparation is different from an intact diaphragm in terms of blood supply, metabolism and pattern of neural activation to produce fatigue, these findings cannot be said to have established the sites and causes of transmission fatigue in vivo.

Transmission fatigue, however, has been studied in vivo. In one such study, Aldrich (1987) showed that inspiratory resistive loaded breathing (IRLB) produced diaphragmatic transmission fatigue in the rabbit. He found that the diaphragmatic compound action potential produced by phrenic nerve stimulation was reduced during IRLB.

Teleologically, transmission fatigue like the other types of fatigue could be beneficial in some instances. This has been suggested by Nasser-Gentina and colleagues (1975) and Kugelberg and Lindergen (1969) who argued that the muscle is protected from excessive depletion of its adenosine triphosphate (ATP) store that would ultimately lead to rigor mortis.

Contractile fatigue

Contractile fatigue is the reversible impairment in muscle contractility to neural impulses as a result of over-use of the muscle. It has been subgrouped into two types: (1) high-frequency fatigue is characterized by a reduction in the force generated by a muscle to high frequency stimulation which is recovered within minutes; (2) low-frequency fatigue is characterized by a persistent reduction in force generated by a muscle to low frequency stimulation, which may not recover for at least 24 hours (Edwards, 1979). These two types of fatigue have been demonstrated to occur

in the human diaphragm (Aubier et al., 1981a) as well as in rabbit diaphragm (Aldrich & Appel, 1985). Both types of contractile fatigue occur together, but their different rates of recovery imply that at least two pathophysiological processes are taking placing.

The causes of the two types of contractile fatigue remain to be discovered. However, there are several hypotheses available. High-frequency fatigue is thought to be caused by accumulation of metabolic products of contraction, by altered Ca^{2+} or other electrolyte concentrations such as K^{+} (Sjøgaard, 1986 and 1990), or by a decrease in ATP concentration at critical sites in the muscle. Low-frequency fatigue is believed to be caused by minor muscle damage that must be repaired before full function is restored.

Pathophysiology of high frequency fatigue

Studies with non-destructive nuclear magnetic resonance technique by Dawson and colleagues (1978) provided evidence to show that tension decreases in proportion to an increase in the concentrations of hydrogen ions and free ADP and not to a decrease in the phosphocreatine concentration. Although a major increase in diaphragmatic lactate concentration does not necessarily lead to fatigue (Aldrich & Appel, 1987), an increase in intramuscular lactate concentration (Mertzger & Fitts, 1986) and a decrease in intramuscular pH have been correlated with diaphragm fatigue in vitro in rats (Mertzger & Fitts, 1987). At pH 6.5, about the lowest value found in normally fatigued muscle, the reduction in maximal contractile force is reported to be around 10% to 30%, with fast fibres more affected than slow ones (Donaldson & Hermansen, 1978; Fabiato & Fabiato, 1978). Moreover, a number of studies have shown that increased hydrogen ion concentrations interfere with excitation-contraction coupling (Fuchs et al., 1970; Nakamura et al., 1972; Inesi et al., 1983). Fuchs and colleagues found that an increase

in H^+ concentration reduces the affinity of troponin c for Ca^{2+}. In another study, Nakamura and colleagues showed that a decrease in pH increased the Ca^{2+}-binding capacity of sarcoplasmic reticulum. Both mechanisms would, probably, lead to a reduction in the number of Ca^{2+} able to bind to troponin c during excitation-coupling.

Although ATP depletion does not occur in a fatigued diaphragm, ATP levels may be insufficient to run the Ca^{2+}-pumps or K^+-Na^+-pumps efficiently in some parts of the muscle. Acceleration in the rate of onset of fatigue that occurs in hypoxia (Jardim et al., 1981) and circulatory failure (Aubier et al., 1981b), provides evidence to support this hypothesis. Since in vivo each of these mechanisms may result in increased production of lactic acid, the significance of ATP depletion in the development of contractile fatigue may be obscured. Results, presented by Dawson and colleagues in 1978, showed that changes in ATP during fatiguing contractions did not correlate with change in force. However, it indicated that the changes in ATP were related to the slowing of the rate of contraction.

It is probable that changes in the concentration of ions in muscle produced by muscular contraction could result in loss of membrane excitability leading to a reduced force. During muscular contraction, there are large fluxes of potassium from the muscle that can double the extracellular concentration of K^+ to 8 or 9 mmol/l (Hnik et al, 1976; Sjøgaard, 1986 and 1990; Friedland & Paterson, 1988). Friedland and Paterson (1988) reported that in renal patients and healthy volunteers that the rise in arterial K^+ during strenuous exercise peaked at the point where the subjects were unable to continue the exercise. This study indicates that increased arterial K^+ concentration may have a role to play in the development of fatigue. Doubling of extracellular K^+ during exercise is believed to be sufficient to impair membrane excitability and hence decrease muscle contractility (Sjøgaard, 1986 and 1990;

Friedland & Paterson, 1988). In addition, they argued that such a rise in extracellular K^+ would excite group III and IV afferents with their free endings in the muscle (Friedland & Paterson, 1988; Sjøgaard, 1990).

Pathophysiology of low frequency fatigue

Prolonged contractile failure has been attributed to minor muscle injury. Injury may occur at the T-tubule membrane slowing or stopping the propagation of action potentials in some parts of the muscle (Jones, 1981). A toxic by-product of muscle contraction could cause such an injury, for instance free radicals released from the mitochondria. The free radicals could be responsible for peroxidation of nearby membranes. Superoxide and other free radicals are by-products of electron transfer reactions in the mitochondria (Chance et al., 1978). It could be hypothesized that in a severely loaded muscle, mitochondrial activity increases, producing enough free radicals to overcome the intrinsic antioxidant defences. Evidence in support of this hypothesis has been provided by Davies and colleagues (1982). They used electron spin resonance spectroscopy to find higher than normal levels of free radicals in leg muscle of exhausted rats.

Effect of diaphragm fatigue on arterial blood gases

During the development of diaphragm fatigue induced by inspiratory resistive loading in animals (Aldrich & Appel, 1985 and 1987; Watchko et al., 1988) and as consequence of respiratory disease in humans (Cohen et al., 1982) blood gas tension and acid-base status are disturbed; that is decreased arterial pH and P_{O_2} and increased P_{CO_2}. However, none of these studies reported a change in arterial K^+ level. Changes in acid-base status, blood gas tensions or muscular exercise can produce a change in extracellular K^+

level. The evidence in support of this statement is reviewed in Chapters 5 to 7. An increase in extracellular K^+ level, irrespective of the source or mode of release from muscle, could alter ventilation.

Ventilatory response to increased extracellular potassium

The first experiments that showed that increased extracellular K^+ can alter ventilation were carried out by Comroe and Schmidt (1943). They observed an increase in ventilation after K^+ was injected into a femoral artery of the dog, and concluded that depolarization of pain fibres in or near the arterial wall is involved. Twenty-five years later, Wildenthal and colleagues (1968) showed that the potassium-induced hyperventilation could be abolished when femoral and sciatic nerves were cut in vascularly isolated dog hindleg. It was concluded that potassium activated pain afferents in the muscle, and the reflexes initiated may be functional during exercise.

Jarisch and colleagues (1952) were the first to show that high $[K^+]$ of 500 mmol/l injected intra-arterially close to the carotid bodies increased the discharge of the carotid sinus nerve. Subsequently, Linton and Band (1985) showed in the cat that more modest increases in arterial K^+, as seen during exercise, also increased carotid chemoreceptor discharge. Recently, Nye and Paterson (1987) found that slowly adapting pulmonary stretch receptors in the anaesthetized cat were excited also by physiological levels of extracellular K^+, but the sensitivity of the stretch receptors was four times less than that of the carotid body.

The above studies demonstrate that a number of receptors spread throughout the body can be excited by K^+. However, as far as is known, no previous study has shown that diaphragmatic receptors can be excited by K^+. One of the principal aims of this thesis was to investigate whether K^+

can excite diaphragmatic receptors and whether activation of diaphragmatic receptors initiates a change in ventilation.

Types of diaphragmatic receptors

The diaphragm is composed of two distinct skeletal muscles, crural and costal muscles that contain relatively few proprioceptors. Muscle spindles have been found in the diaphragm of man (Winkler & Delaloye, 1957), the cat (Duron et al., 1978; Duron, 1981), dog, guinea pig (Dogiel, 1902), rabbit (Cuenod, 1961), and rat (Hudson, 1966). In the cat, only seven to nine muscle spindles were located in crural portions but none was found in costal portions (Duron et al., 1978)

Nerve recordings from the cervical dorsal roots indicate that the majority of proprioceptor afferents arise from golgi tendon organs (GTO) (Corda et al., 1965). The GTO, have been found close to the tendinous portion of muscle and respond to changes in muscle tension. Spindles, on the other hand, respond to changes in muscle length. In the diaphragm, GTO are found mainly in the crural portion, few are found ventrally, and none in the sternal region (Corda et al., 1965). Rapidly adapting receptors, probably pacinian receptors, are distributed throughout the diaphragm (Corda et al., 1965). These receptors are not spontaneously active and respond to prodding of the diaphragm with rapid adaptation.

Based on deafferentation studies, 20%-35% of the myelinated fibres in the phrenic nerve were estimated to be afferents (Duron et al., 1978; Landau et al., 1962). Duron and colleagues (1978) have estimated that 12% of the myelinated afferent fibres innervate spindles (group I and IIa) with a slightly greater percentage arising from GTO (group IIb), and according to Hinsey and colleagues (1939), the bulk of myelinated afferent fibres in the cat is small diameter

group III afferents. Yasargil (1967) also found in the rabbit that the majority of myelinated afferent fibres in the phrenic nerve were group III. Unmyelinated fibres in phrenic nerve have been reported to be more numerous than myelinated ones by a ratio of 3:1 in the cat (Duron & Marlot, 1980; Hinsey et al., 1939) and 2:1 in the rat (Langford & Schmidt, 1983). Unmyelinated afferent fibres (group IV) like the small myelinated afferent fibres (group III) terminate as free nerve endings in muscle.

Free nerve endings are polymodal receptors and respond to temperature, pressure, and changes in local chemical environment. Stacey (1969) estimated that 75% of sensory fibres in the hindlimb of the cat terminate as free nerve endings. Considering the relative paucity of myelinated afferents in the diaphragm, the percentage of fibres with free nerve endings may be higher in the phrenic nerve. Free nerve endings are known to be excited by local pressure, hypoxia, isotonic potassium chloride, hypertonic sodium chloride, ischaemia, and prolong contraction. The essential role of both group III and IV afferents is the transmission of pain signals, and their free nerve endings are usually referred to as nociceptors.

Classification of muscle afferents

The classification of afferent fibres arising from receptors in skeletal muscles has been based on size, conduction velocity and whether they have a myelin sheath or not. Five main groups of afferent fibres that have been identified within the phrenic are given in Table 1.2.

Table 1.2. Types of afferents in the phrenic nerve.

Fibre type	Fibre diameter (μm)	Conduction velocity (m/s)	Number in the phrenic nerve
Ia (myelinated)	12-20	80-120	Rare (6-9) located in crura
Ib (myelinated)	12-18	71-110	Few but > Ias Ib: Ia ~ 47:38
II (myelinated)	5-11.5	31-70	Rare
III (myelinated)	1-5	2.6-30	Abundant
IV (unmyelinated)	0.5-2	0.5-2.5	Abundant

(Road, 1990)

Review of Phrenic nociceptors

The phrenic nerve contains both motor and sensory fibres. The significance of the sensory component of the phrenic nerve has been appreciated since the 1840s. Bourgery (1845) and Luschka (1853 and 1863) described the phenomenon by which stimulation or irritation of the central part of the diaphragm in man evokes pain referred to the shoulder region. It was later shown that electrical stimulation of the central end of a cut phrenic nerve in the dog induced an increase in blood pressure (Schreider, 1883), dilation of the coronary vessels (Greene, 1935) and bronchodilation (Thorton, 1937). Thorton (1937) also found that bronchodilation produced in response to phrenic nerve stimulation was abolished when the vagi were cut, indicating the efferent pathway for this reflex was the vagus. Because in these early experiments the stimulus parameters were not reported, it is not known which groups of phrenic afferents mediated the responses to electrical stimulation. In view of the evidence available today, the cardiovascular and bronchodilatory responses were probably initiated by activation group of III and IV phrenic afferents.

The first experiments that attempt to show that phrenic afferents can be excited by noxious stimuli were performed by Little and McSwiney (1938). They observed pupillo-dilation on application of acid, alkali, heat, or pinching of the diaphragm. In addition, they established that the afferent pathway of the pupillo-dilatory reflex enters the spinal cord by the dorsal roots of the fifth and sixth cervical segments. Some years later, Gernandt (1946) showed by application of noxious stimuli to the diaphragm such as pinching, heat above 40° C, 6% sodium chloride solution and acetic acid produced an increase in discharge of the small-phrenic afferents in the cat and the dog. He concluded that pain was transmitted in group III and IV phrenic afferents.

After a hundred years of investigation into the physiology of phrenic sensory fibres, it was established that the phrenic afferents can be activated by noxious stimuli, transmit pain signals, and the noxious responses are manifest by changes in cardiovascular status, bronchodilation, and referral pain. Surprisingly, perhaps the role that phrenic afferent inputs on ventilatory control were not investigated over this period. Over the next fifty years to address this gap in knowledge, research has concentrated upon the effect of phrenic afferent activation on ventilation. In 1940, Hinsey and Philips reported hypernea in the cat in response to electrical stimulation of the diaphragm, and demonstrated that afferent pathway of reflex was the phrenic nerve by sectioning combination of afferent pathways. Seven years later, Kohram and others (1947) found that electrical stimulation of the central cut end of the phrenic nerve of the anaesthetized cat and dog increased minute ventilation. These studies in 1940s demonstrate that phrenic afferent activity can influence breathing.

After a 40 year-gap, the ventilatory response to activation of small-phrenic afferents was again studied intensively. In 1986, Jammes and colleagues showed in vagotomized, C_8 spinalized cats that electrical stimulation of the central end of cut phrenic nerve produced a reduction in discharge frequency of the contralateral phrenic efferent fibres and a reduction in inspiratory duration. These responses to stimulation of the phrenic afferents occurred both when the conduction of action potentials in the large fibres was blocked by nerve cooling (circa 6°C) or when procaine was applied to the nerve to interrupt conduction in the small afferent fibres. They concluded that activation of phrenic afferents in general depressed ventilation.

In contrast, Road and colleagues (1987) reported that in the anaesthetized dog diaphragmatic activity, tidal volume, and breathing frequency increased in response to electrical

stimulation of the central end of a cut phrenic nerve with current intensity of 20-60 times twitch threshold. These authors suggested that this reflex was mediated by phrenic afferents III and/or IV and it was supra-spinal in nature.

The disagreement between Road's and Jammes's findings could be partly explained by the difference in preparation. For example, the spinalized vagotomized cat compared with the vagi intact dog. In the spinalized vagotomized animal, important reflexes involved in the control of inspiratory drive may have been interrupted. Therefore, Jammes and Road may have studied different afferent pathways. Moreover, cold block used by Jammes and colleagues may have been ineffective in blocking conduction in all large afferent fibres (Franz & Iggo, 1968). In other words, Jammes may have studied the effect of activation of large-phrenic afferents on ventilation, while Roads investigated the effect of activation of small-phrenic afferents. Finally, the stimulus parameters required to activate group III and IV afferents also recruit group I and II afferents making it difficult sometimes to separate the response initiated by group III and IV afferents from those initiated by group I and II afferents.

Because of the difficulties of selectively electrical stimulating small-phrenic afferent fibres, Revelette and colleagues (1988) selectively excited the small-phrenic afferents by infusing capsaicin into the phrenic artery of the anaesthetized vagotomized dog. They observed a short period of apneusis while tonic activity of the diaphragm and hypoglossal muscle increased significantly. This was followed by increased phasic activity in these muscles which was associated with a reduction in both T_I and T_E. When capsaicin was re-infused after spinalization at C_7, only an increase in tonic activity was observed. In view of these observations, they concluded that small-phrenic afferent fibres have an excitatory effect upon inspiratory drive. These results have been confirmed recently by Hussain and

colleagues (1990). They also infused capsaicin to activate small-phrenic afferents but their maximum dose was half that used by Revelette and colleagues and the arterial supply to and innervation to the left hemi-diaphragm of the dog was isolated. The studies using capsaicin as well as electrical stimulation to activate small-phrenic afferents have shown that indeed these afferents have a significant effect on ventilation.

Over the last decade, an interest into the potential role of the activity of group III and IV phrenic afferent in the control of breathing during diaphragm fatigue has grown with many researchers speculating about the role of these small afferents. Some researchers have speculated that changes in diaphragm metabolites during fatiguing contractions might induce ventilatory changes mediated by increased group III and IV phrenic afferents activities (Hussain et al., 1985; Jammes et al., 1986; Road et al., 1987 Lockhart et al., 1988; Roussos, 1990). Two research groups have provided evidence to give credence to this speculation (Graham at al., 1985; Jammes et al, 1986). These studies showed that activity of group III and IV increased when hypertonic sodium chloride, or when lactic acid was infused into the phrenic artery. These studies demonstrate that group III and IV phrenic afferents can be excited by some metabolites associated with muscle contraction without indicating whether effect of ventilation would have been excitatory or inhibitory.

A principal aim of this thesis is to identify a potential chemical stimulus for phrenic afferents that is generated during diaphragm fatigue and then investigate whether it can excite phrenic afferents producing a change in ventilation. This study was carried out on anaesthetized rabbits. The rabbit was chosen as the animal model because the fibre type composition of its diaphragm closely resembles that of man (Faulkner et al., 1979) and the ventilatory response of the rabbit to diaphragm fatigue induced by severe inspiratory

load, is similar to that of a patient being weaned off mechanical ventilation (Roussos, 1984; Aldrich & Appel, 1985). How muscular fatiguing affects the pattern of breathing is studied in non-vagotomized and vagotomized rabbits. In addition, the effect of IRLB on arterial blood chemistry is examined to identify a noxious chemical stimulus that might initiate ventilatory changes. Finally, the effect of the noxious stimulus on phrenic afferent activity is examined. It is anticipated that the findings of these studies will further the understanding of the pathophysiology associated with respiratory failure, and hence may have important implications for the management of patients with this condition.

BASIC PREPARATIONS AND METHODS

CHAPTER 2

CHAPTER 2

MATERIALS AND PREPARATION

Rabbit anaesthesia protocol

Premedication

Before the administration of premedication, each animal was weighed. The following drugs were injected intramuscularly (i.m) into a hindlimb:

(1) Atropine 0.6 mg
(2) Ketamine 60 mg/kg
(3) Diazepam 5 mg/kg

Premedication was given to some but not all rabbits.
The marginal vein in an ear was then cannulated for intravenous administration of drugs.

Initial anaesthesia

(1) Boluses of 0.5 or 1.0 ml of pentobarbitone (12 mg/ml) were given intravenously.

Usually 7-15 ml of pentobarbitone administered over 30-40 minutes was required to obtain surgical anaesthesia. The desired level of anaesthesia was achieved when the hindlimb withdrawal reflex to pinching was absent. As soon as anaesthesia was evident or once all major surgery was completed, a continuous infusion of anaesthetic was administered via the marginal vein.

Maintenance anaesthesia

To maintain anaesthesia one of two regimes was used:

(1) A continuous infusion of ketamine in isotonic saline (5 mg/ml) was set at 2 ml/kg/hr. An infusion rate of

6 to 8 ml/hr was effective in maintaining surgical anaesthesia for the duration of the experiment.

(2) A continuous infusion of 6 mg/ml of pentobarbitone in isotonic saline was started at a rate of 4-6 ml/hr and adjusted as necessary.

Most of the experiments were performed using anaesthetic regime number (1); only the experiments in Chapter 8 were carried out using regime (2).

Throughout an experiment, anaesthesia was verified by the absence of the hindlimb withdrawal reflex, presence of the corneal reflex, and by the depth and rate of breathing and end-tidal CO_2 between 4 and 5%.

NECK SURGERY

The rabbit was laid supine with neck extended. A mid-line incision, extending from the cricoid cartilage to the sternal notch, was made by diathermy. The trachea, both vagi and both phrenic nerves were exposed with minimal bleeding by teasing connective tissue and muscles apart using a collection of mosquito forceps. Any bleeding was arrested by diathermy or ties. The exposed trachea was sliced about 1 cm below the larynx with a carbon steel surgical size 11 blade (Swann-Morton), after which a plastic cannula was inserted into the trachea. The isolated nerves were each identified by a short piece of black cotton thread until cutting. Before the vagi were cut, they were tied off at two points with black cotton thread to prevent bleeding, and then they were cut between the ties. The phrenic cervical nerves 5 (C_5) to 7 (C_7) were isolated from surrounding tissue. The relative position of these nerves in the neck is illustrated in Figure 2.1. The middle root containing fibres from cervical nerves 5 and 6 (C_6) was cut. Immediately afterwards, either the rostral end of the cut phrenic was mounted on a pair of stimulating electrodes or the caudal

end was placed on a pair of recording electrodes. Desiccation of the phrenic nerve was prevented by either immersing it in a pool of mineral oil or by covering it with a piece cotton wool soaked in the same oil. The pool was formed between the cervical skin flap and vertebrae by retracting the skin flaps.

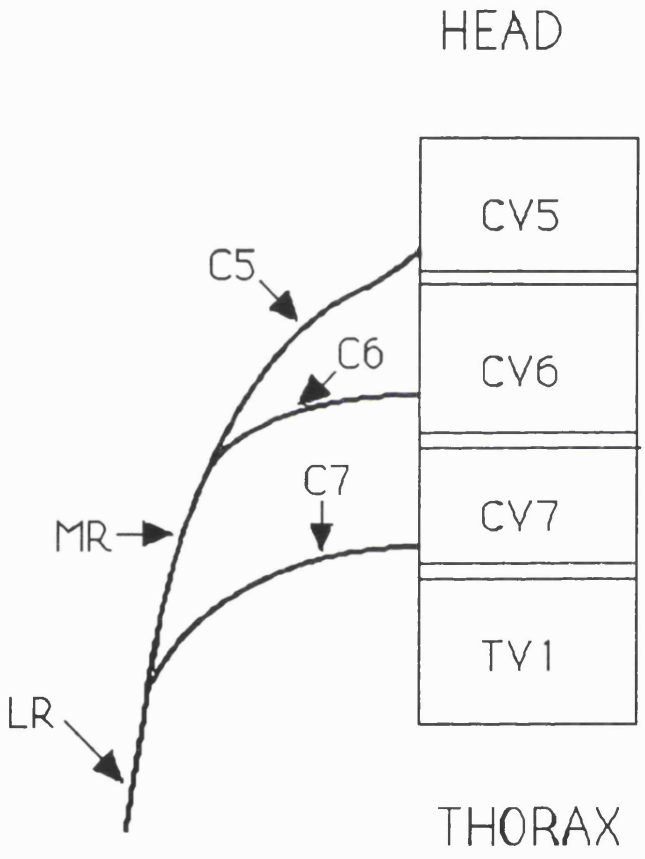


Fig. 2.1. Schematic diagram of the cervical phrenic nerve roots. The letter C refers to cervical nerve root. MR and LR denote middle root and lower root respectively. The abbreviations CV and TV refer to cervical vertebra and thoracic vertebra respectively.

CANNULATION OF VESSELS AND LUMENS

General cannulation for all experiments

The external marginal vein in both the left and right ears were cannulated with Venisystem 20 G abbocath cannulae (32 mm long, external diameter 1.1 mm, internal diameter 0.7 mm) to access veins for the administration of intravenous drugs. A tracheostomy was performed and the trachea cannulated with a 2 mm internal diameter tube. Either a femoral or carotid artery was catheterized with a Portex polyethylene catheter so that blood pressure could be monitored and arterial blood could be obtained for the determination of blood gas tension, acid-base, and potassium analysis. The cannula used had an internal diameter (ID) of 0.58 mm and an external diameter (ED) of 0.98 mm or occasionally ID of 0.76 and ED of 1.22, depending upon the size of arterial vessel.

Specific cannulations for Chapters 3 to 6

Gastric and oesophageal pressures were measured by balloon catheters coupled to Gaeltec 2b pressure transducers (Gaeltec, Isle of Skye, Scotland). The pressure transducers were calibrated over the appropriate pressure ranges. An oesophagostomy was performed for the insertion of two balloon-tipped catheters. Each thin-walled latex balloon was 3 cm in length mounted on a 50 cm polyvinyl catheter (see Photo. 2.1). The catheters were inserted so that the deflated balloon of one was within the stomach and that of the other was in the lower half of the oesophagus. Half a millilitre of air was added to the gastric balloon while 0.4 ml was added to the oesophageal balloon. The balloon volumes were checked frequently during the experiments. The position of the catheters was secured with ties placed around the oesophagus and catheters.

In a few experiments, tracheal pressure from the side port of the tracheal tube was measured instead of oesophageal pressure. The two pressure signals were electronically summed to give transdiaphragmatic pressure (Pdi = Pg - Po or Pt) using a differential amplifier.

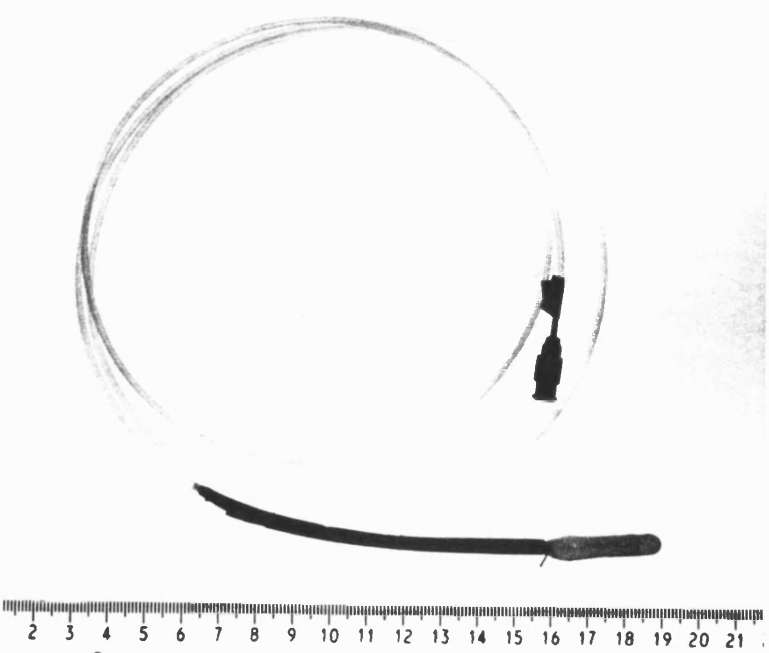


Photo. 2.1. Thin-walled latex balloon catheter (PK Morgan, Rainham Kent) used to monitor gastric and oesophageal pressures.

For transvenous stimulation of phrenic nerves

In the rabbit, the right and left phrenic nerves are attached to the right and left anterior vena cavae respectively for about two thirds of the length of the vena cavae. Because of their close proximity, the phrenic nerves can be electrically stimulated by the electrodes inserted into the anterior vena cavae (Wanner et al., 1973).

A 90 cm long Swan-Ganz size 5F bipolar pacing catheter (see Photo. 2.2) was inserted into either the internal or external branch of both jugular veins. They were advanced into the upper chest while monitoring the gastric or transdiaphragmatic pressure response to 1 Hz stimulation at 20 V. They were positioned to achieve maximal stimulation of the phrenic nerves. At autopsy, the tips of the stimulating catheters were found within the anterior vena cavae 2-4 cm below the lower border of the ipsilateral clavicles.

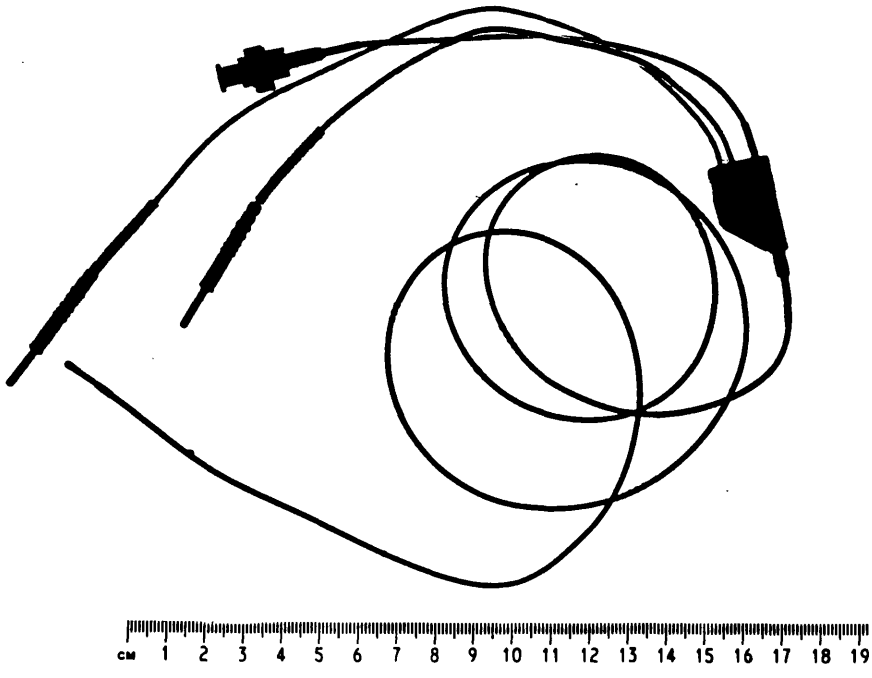


Photo. 2.2. A Swan-Ganz bipolar pacing catheter used for transvenous stimulation of the phrenic nerve.

SURGICAL PRECEDURE TO EXPOSE THE DIAPHRAGM

With the rabbit supine, a horizontal incision through three layers of tissue was made below the costal margin to open the abdominal cavity. The diaphragm was exposed by tearing the connective tissue between it and the liver. The liver and the gut were covered with gauze soaked in warm saline (37-40 °C) and then with a plastic sheet. This ensured that the abdominal content was kept moist and isolated from the diaphragm.

The diaphragm of the rabbit is divided clearly into left and right muscular digits (see Fig. 2.2). Each side has one sternal digit, six costal digits, and a crural digit from the central tendon (CT) to the lumbar vertebrae. The muscular digits were numbered anteroposteriorly: sternal digit (s) 1, costal digits (co) 2-7, and crural digit (cr) 8.

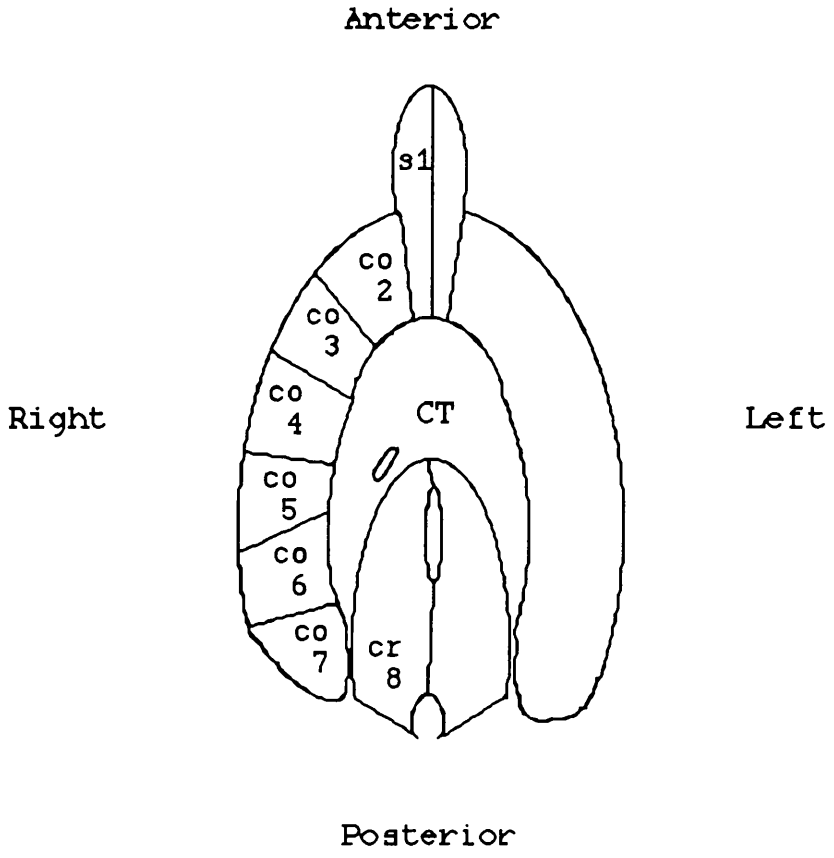


Fig. 2.2. A schematic diagram of the abdominal surface of the diaphragm. See text above for detail explanation.

TIME ALLOWED FOR STABILIZATION

To allow each preparation to stabilize, no measurement was made within 30 minutes following the completion of surgery.

MONITORING AIRFLOW AND PRESSURES

Inspiratory airflow was measured from the tracheal tube using a Gould pneumotachograph (Fleisch size 0). In Chapters 3 to 6, the other end of the Fleisch head was attached to a miniature unidirectional value (Hans Rudolf no. 2384). This valve had a dead space of 1.6 ml. The pneumotachograph integrates the airflow signal to derive tidal volume and respiratory rate. Tracheal gases were continuously sampled and analyzed by an Airspec MGA 2200 mass-spectrometer for CO_2 and O_2. Oesophageal or tracheal pressure from a side port of tracheal cannula and gastric pressure were monitored continuously using Gaeltec 2b pressure transducers. Transdiaphragmatic pressure was derived using a differential amplifier (constructed by University College London interdepartmental electronic workshop) from gastric and oesophageal or tracheal pressure signals. Arterial blood pressure was monitored using a Gould pressure transducer. All signals were recorded on either a Gould ES 1000 or ES 2000 electrostatic recorder.

Before each experiment, a two-point volume calibration was performed with a 100 ml syringe. In a study carried out by a G. Smith, a research technician at UCL, expired volume was simultaneously measured with a spirometer and found to be within 10% of the integrated pneumotachograph value. To ensure that the volume did not change with the attachment of the inspiratory resistor (IR), the pneumotachograph was calibrated on three separate occasions with or without the IR attached to the Fleisch via the Hans Rudolf valve. The volumes measured with IR were within 10% of that without IR. All pressures were measured relative to atmospheric

pressure. Both tidal and inspired minute volumes were corrected to body temperature, pressures and saturated with water vapour (BTPS).

METHOD OF ARTERIAL BLOOD SAMPLING

0.1 ml of warm isotonic heparinized saline (circa 38° C) was drawn up into a 2 ml sampling syringe. Any visible air bubbles in the syringe were expelled. Immediately before each blood sample, 0.5 ml of fluid was withdrawn from the arterial catheter into a flush syringe containing heparinized normal saline to remove the dead space containing heparinized saline from the catheter. Each 0.5 ml blood sample was collected anaerobically and immediately analyzed using a Radiometer ABL4 to determine the acid-base and blood gas status.

ABDOMINAL BINDER

The abdomen of the rabbit needed to be bound with an inelastic binder to minimize alteration of abdominal volume, ensuring that the resting diaphragmatic length remained constant. If the resting diaphragm fibre length is altered, the pressure generated by the diaphragm would vary and any loss of diaphragmatic tension could not then be attributed with confidence to inspiratory resistive loading. Orthoplast (Johnson and Johnson), an inelastic material at room temperature, is usually used to immobilize broken bones and joints. A sheet of this material was supplied by the Middlesex Hospital Occupational Therapy Department.

The abdominal binder was constructed from an Orthoplast sheet of 30 cm by 50 cm. To make the sheet pliable, it was immersed in a bucket of hot water above 65 °C. Once pliable, it was removed from hot water and allowed to cool. The sheet cooled rapidly and within 2 minutes could be handled. It was

then moulded around the abdomen and lower third of the thorax of a 3.0 kg rabbit and cut to size. Once cooled to room temperature, the Orthoplast set hard, forming an inelastic abdominal binder (Photo 2.3). If required, the cold sheet could have been remodelled by immersing in hot water.

The binder constructed from Orthoplast was placed snugly around the abdomen and lower third of the thorax throughout each experiment. There was no clear evidence that breathing was obstructed by the binder as there was no obvious change in respiratory rate or tidal volume on application of the binder. However, tidal Pg increased by as much as 200% on application of the binder.

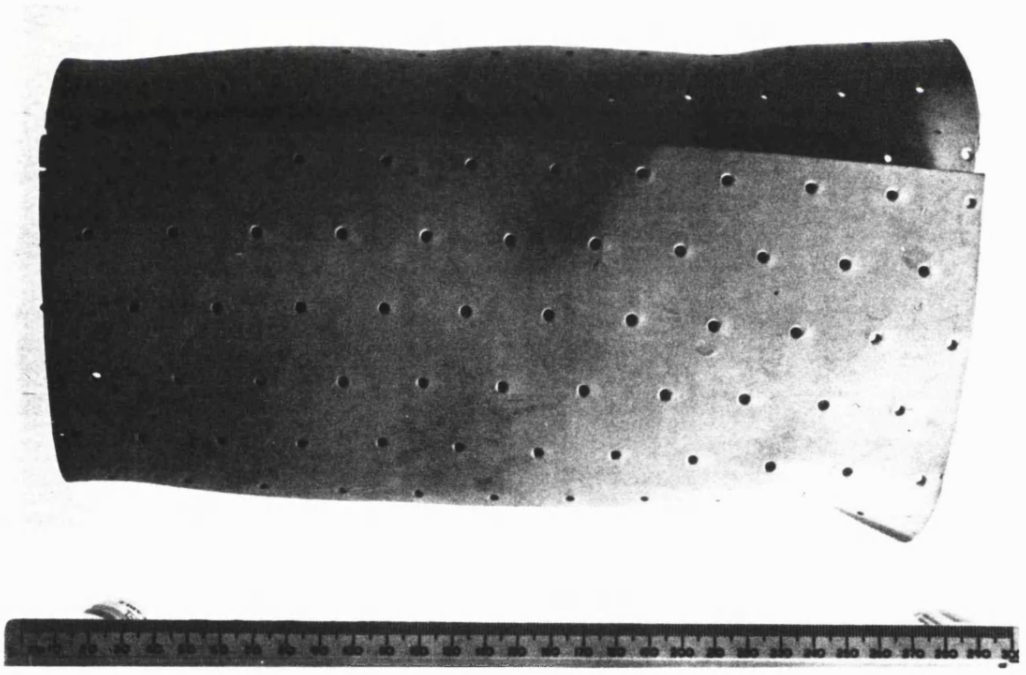


Photo. 2.3. Abdominal binder constructed from Orthoplast. The abdominal binder had an external circumference of 45.5 cm and internal one of 36.5 cm. Its length was 26.8 cm. This binder was used in all inspiratory resistive loading experiment.

ADDITIONAL PROCEDURES AND TECHNIQUES

Additional techniques and methods will be given at the start of the relevant chapter.

EXPERIMENTAL RESULTS

CHAPTERS 3 TO 10

CHAPTER 3

DIAPHRAGMATIC FATIGUE IN THE RABBIT

Introduction

When skeletal muscles contract above a certain threshold of tension and duration their force declines because their energy demand exceeds their energy supply. This process of muscle fatigue can be defined as the loss of muscle strength or force exerted by the muscle that is recoverable with rest. It can be distinguished from muscle weakness which is the loss of muscle strength that cannot be recovered with rest.

The muscle strength can be assessed independently from the effort of the subject or animal by electrical stimulation of the muscle. The force-frequency relationship can be established by stimulating with a supramaximal voltage at various frequencies, usually from 10 to 100 Hz. Immediately after fatiguing contractions, the force-frequency curve is shifted downward with the loss of force at all frequencies. The force generated at high frequencies (30-100 Hz) recovers within minutes, whereas the force generated at low frequencies (10-20 Hz) takes hours or even days to recover. This highlights the existence of two types of peripheral fatigue, high frequency and low frequency fatigue.

Before some of the neural and humoral factors related to diaphragm fatigue are investigated, three things must be established:

(i) that the loss of diaphragmatic force during inspiratory resistive loading (IRL) is due to fatigue rather than muscle weakness;

(ii) the nature of diaphragm fatigue induced by IRL;

(iii) whether pressure changes are due to a fatiguing or deteriorating preparation.

Methods

The methods to be outlined apply to the studies in this chapter and Chapters 4 and 5. Any additional procedures relevant to a particular chapter is given in the protocol of that chapter. The basic preparation for these studies is shown in Figure 3.1.

In these studies, 10 New Zealand white rabbits of both sexes, weighing 3.0-3.5 kg, were used. A plastic splint was placed snugly around the abdomen and lower half of the thorax to minimize changes in abdominal volume. Gastric pressure and oesophageal or tracheal pressure were measured as outlined in Chapter 2. To assess contractility of the diaphragm, the phrenic nerves were stimulated using bipolar pacing catheters while monitoring gastric pressure and oesophageal or tracheal pressure.

Induction of diaphragm fatigue

To induce diaphragm fatigue, an inspiratory resistor was applied to the inspiratory port of the Hans Rudolf valve as shown in Photo. 3.1 while the rabbit was spontaneously breathing. The inspiratory resistor (IR) was a three-way tap. Preliminary studies showed that the three-way tap could be adjusted to give four resistances. The lower two resistances had no significant effect on ventilation. The highest resistance caused asphyxia. The middle resistance affected ventilation without causing asphyxia. Therefore, the middle resistance was used in the studies presented in this thesis. The value of the middle resistance was calculated to be 23.4 $cmH_2O/l/min$ (Appendix C).

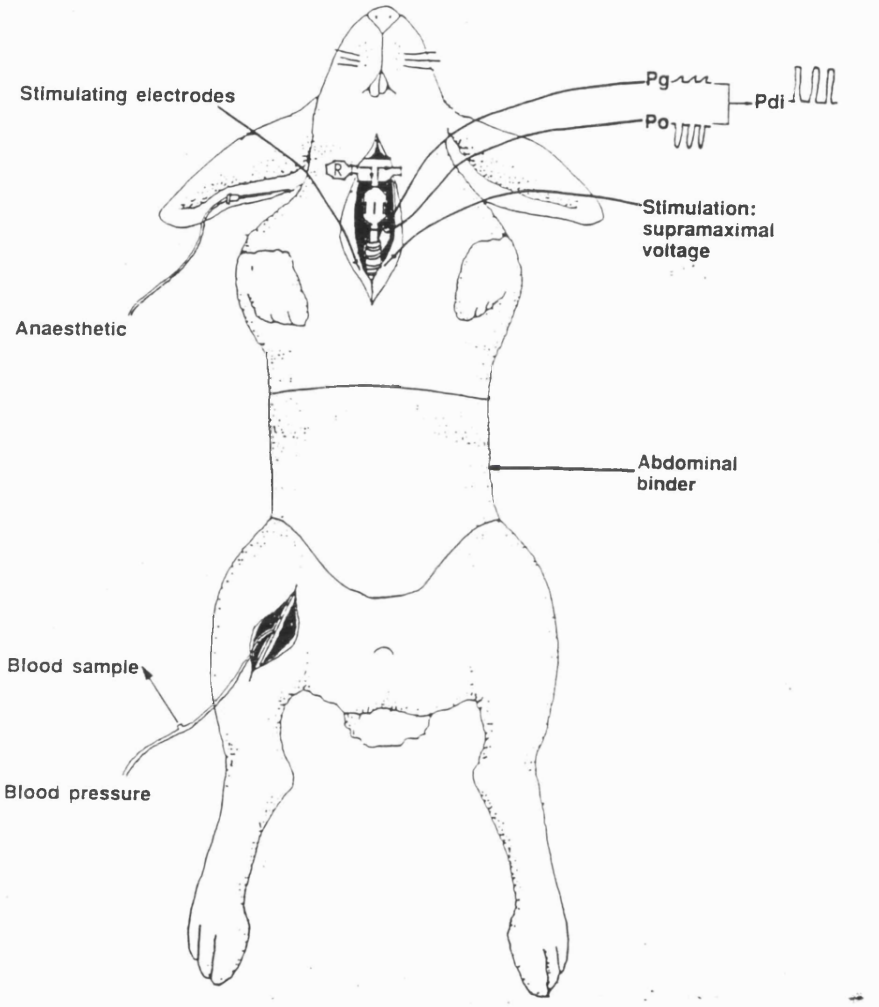


Fig. 3.1. Diagram of the experimental preparation.

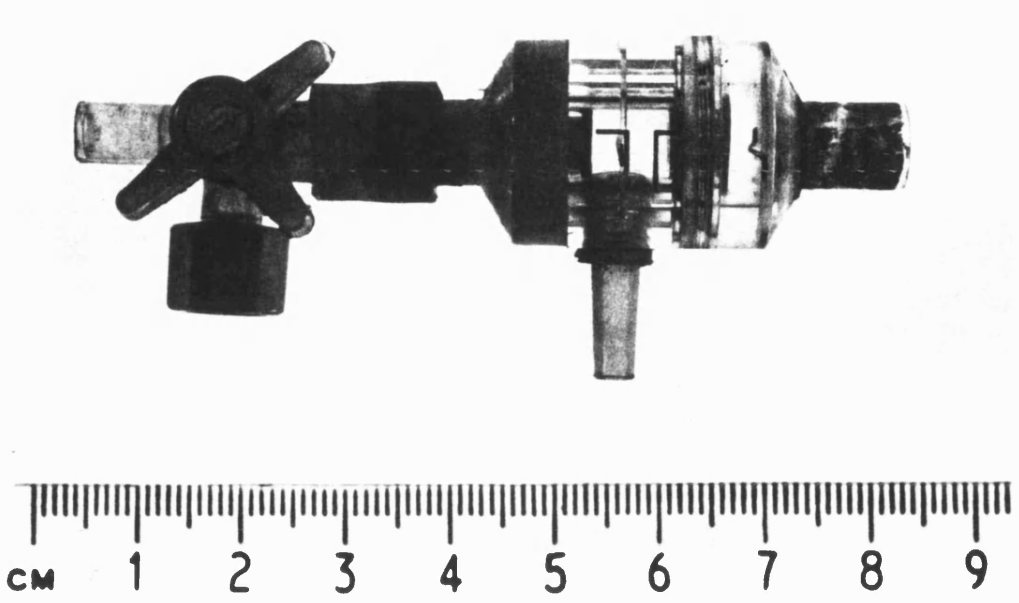


Photo. 3.1. Inspiratory resistor attached to Hans Rudolf valve.

Assessment of diaphragmatic function

Before determining the pressure-frequency relationship, the lowest voltage required to produce maximal transdiaphragmatic (Pdi) or gastric pressure (Pg) was determined. To do this, both phrenic nerves were stimulated for 1 or 2 seconds with trains of 0.2 ms impulses at 50 Hz and from 10 to 40 V while monitoring Pdi or Pg. A voltage of 1.2 times the lowest voltage required to produce maximal transdiaphragmatic or gastric pressure was considered supramaximal voltage. This voltage (25-40 V) was used for all further stimulation. Both nerves were stimulated in sequence at 10, 20, 50 and 100 Hz. Each stimulation train was 2 ms and each stimulation was started at the end of expiration during momentary occlusion of the inspiratory valve. Occlusion at the end of expiration maximized the opportunity of activating the diaphragm at its optimal resting fibre length. The stimulations were performed at intervals of two seconds or longer. The data were collected in triplicates. Baseline pressure-frequency curve for diaphragmatic contraction was determined from these data.

After the baseline pressure-frequency curve was measured, the resistor was applied to the inspiratory limb of the Hans Rudolf valve. After 5 minutes, 10 minutes, and then at 10 minute intervals thereafter, both phrenic nerves were stimulated at 100 Hz during inspiratory valve occlusion at the end of expiration. When the response of gastric or transdiaphragmatic pressure to 100 Hz-stimulation was less than 75% of that obtained before loading, the resistor was removed and pressure-frequency curve measurements were repeated.

Protocol

Recovery pattern and the nature of fatigue

To find out whether the loss in diaphragmatic strength was reversible, four animals were monitored during recovery from fatigue. The pressure-frequency relationship of the diaphragm was determined before IRL, at diaphragm fatigue, and then at recovery.

Recovery from diaphragm fatigue was deemed to have occurred when the Pg induced by supramaximal stimulation of the phrenic nerves at 100 Hz was within 5% of that induced before loading. Stimulation was performed at 10 minute intervals for the first three rabbits, and at 30 minute intervals for the last rabbit.

Control experiment

Time-control experiments were conducted in two of the 10 rabbits to verify the stability of a preparation over a comparable time-course to that employed during severe inspiratory resistive loading. Before IRL in rabbit 160 and following recovery from diaphragm fatigue in rabbit 76, tidal Pdi and ventilatory variables were monitored continuously for 90 minutes while the rabbits breathed spontaneously without added resistance. Supramaximal electrical stimulation at 100 Hz was performed at intervals of 30 minutes while recording Pg to assess contractility of the diaphragm.

Data analysis

Data for the pressure measurements at each frequency were collected in triplicate. The mean of each triplicate was expressed as a percentage of the preload value obtained at 100 Hz-stimulation. Pressure measurements obtained preload, during loaded breathing, and at recovery are all presented

as means ± standard error (SE). A simple linear regression analysis was performed to determine whether there was a significant correlation between Pdi_{max} and Pg_{max}. A correlation was deemed significant when $P < 0.05$.

Results

Relationship between Pdi and Pg

Sometimes, transvenous stimulation caused contraction of the oesophagus as well as diaphragmatic contraction, which made it impossible to determine transdiaphragmatic pressure from oesophageal and gastric pressure. For this reason, tracheal pressure was monitored instead of oesophageal pressure in four of the ten rabbits. In addition, gastric pressure in response to supramaximal stimulation was used to assess diaphragmatic contractility.

To determine whether Pg_{max} in response to supramaximal stimulation at 100 Hz was as good an index of diaphragmatic contractility as Pdi_{max}, the relationship between Pg_{max} and Pdi_{max} was examined in three rabbits, in which no oesophageal contractions were induced. In these rabbits, Pg_{max} and Pdi_{max} were simultaneously recorded before and during loading. A simple linear regression analysis revealed a highly significant correlation between Pdi_{max} and Pg_{max} for the pooled data from the three rabbits (Fig 3.2).

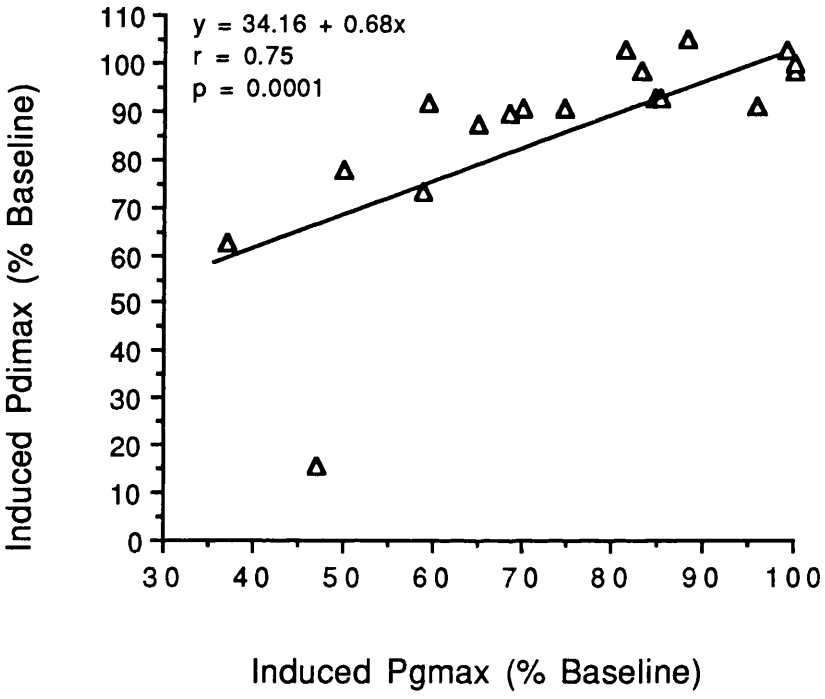


Fig. 3.2. Relationship between transdiaphragmatic pressure (Pdi_{max}) and gastric pressure (Pg_{max}) produced by stimulation of the phrenic nerves with a supramaximal voltage at 100 Hz. Each point is an average of three observations from one of the three rabbits.

Rate of recovery from diaphragm fatigue

An example of chart recordings from one rabbit is shown in Figure 3.3. This figure shows that gastric pressure induced by supramaximal stimulation at 100 Hz decreased during and recovered after inspiratory resistive loading. Figures 3.4 and 3.5 show the time course of the development of and recovery from diaphragmatic fatigue in the four rabbits. The gastric pressure generated with high frequency stimulation, an index of diaphragmatic contractility, was steadily lost during IRL. Once the load was removed, gastric pressure induced by 100 Hz-stimulation returned to its preload value after 20 to 50 minutes (Table 3.1). There was no correlation between the time taken for the diaphragm to be fatigued and time taken for the diaphragm to recover from fatigue (Table 3.1).

Table 3.1. The time taken for IRL to induce high frequency fatigue and to recover from fatigue.

Rabbit	Time taken to induce fatigue (min)	Recovery time (min.)
245	45	40
56	40	50
76	5	20
1110	60	30
Mean (± SE)	37.5 (11.6)	35.0 (6.4)

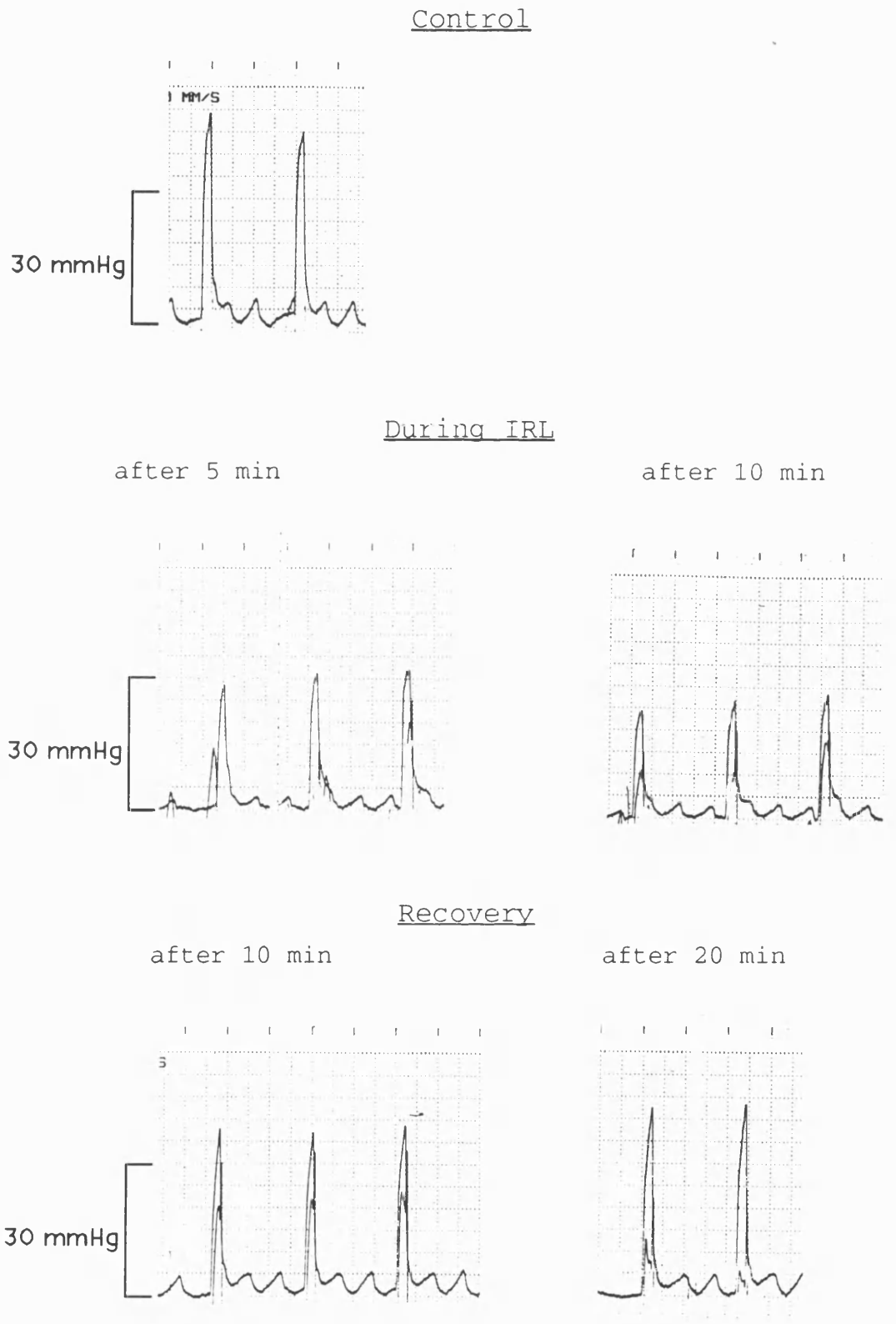


Fig. 3.3. Gastric pressures induced by supramaximal voltage at 100 Hz before, during, and after inspiratory resistive loading (IRL) for rabbit 76.

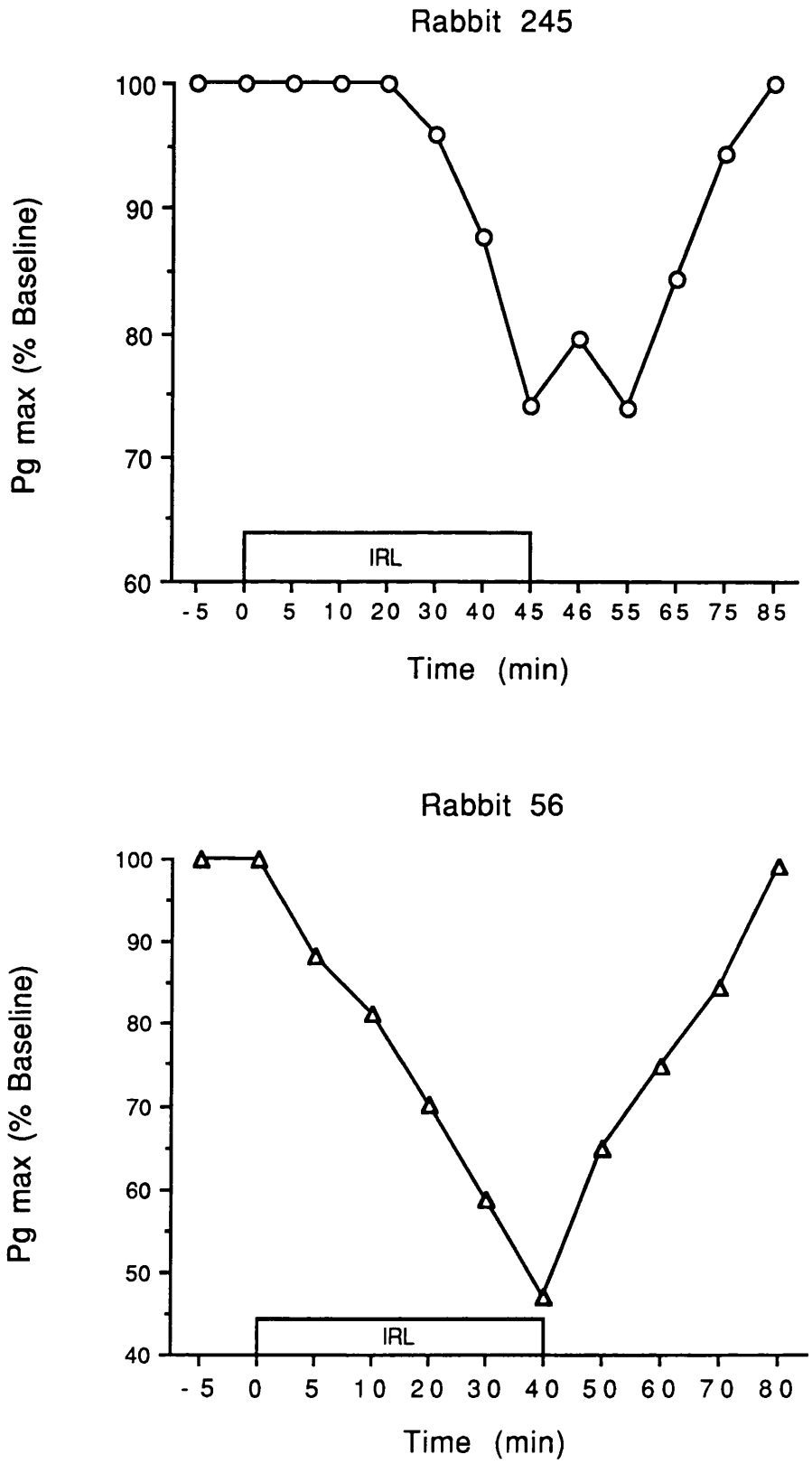


Fig. 3.4. The time course of the development of diaphragm fatigue and recovery in two rabbits. Pg_{max} is gastric pressure expressed as a percentage of pressure induced by 100 Hz stimulation made before inspiratory resistive loading (IRL).

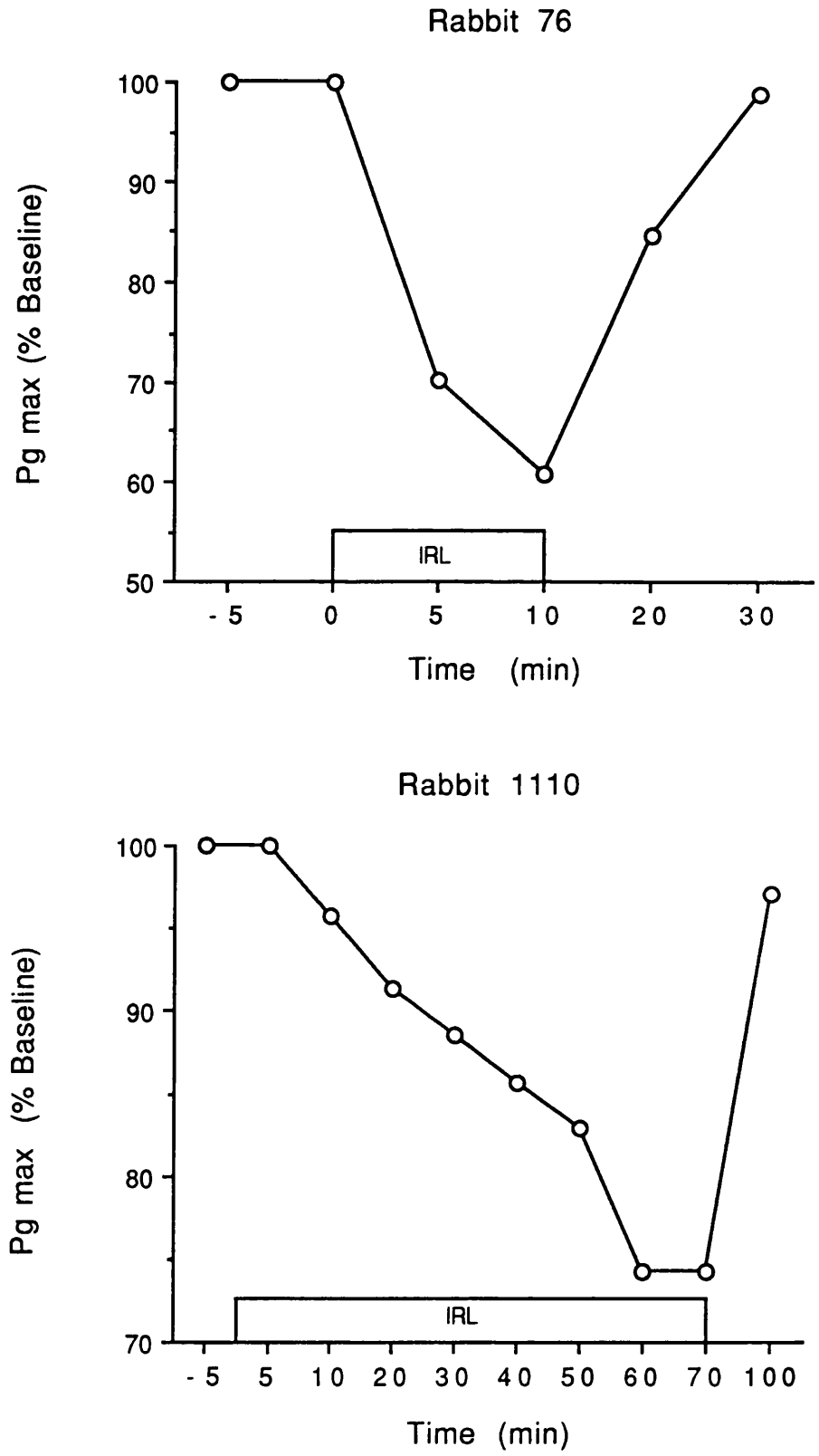


Fig. 3.5. The time course of the development of diaphragm fatigue and recovery in two rabbits. Pg_{max} is gastric pressure expressed as a percentage of pressure induced by 100 Hz stimulation made before inspiratory resistive loading (IRL).

Effect of IRL on frequency-pressure curves

The force developed with low frequency stimulation at control, end of loading and recovery is considerably smaller than that induced by high frequency stimulation (Fig. 3.6). The gastric pressure induced by both high and low frequency stimulations decreased in response to inspiratory resistive loading. The force induced by a 100 Hz-stimulation decreased by an average of 45%. The reductions in force at 50 Hz and the lowest frequencies were 33% and 30% respectively. The force at all frequencies recovered within 50 minutes of removing the load (Fig 3.7).

Effect of the abdominal binder on ventilation before IRL

During the application of the abdominal binder, ventilation was altered; once the binder was in position, ventilation returned to normal. However the presence of the binder did increase tidal Pg and tidal Pdi in most rabbits, indicating that the work of the diaphragm increased. Whether the presence of the abdominal binder influenced the ventilatory response to IRL was not determined.

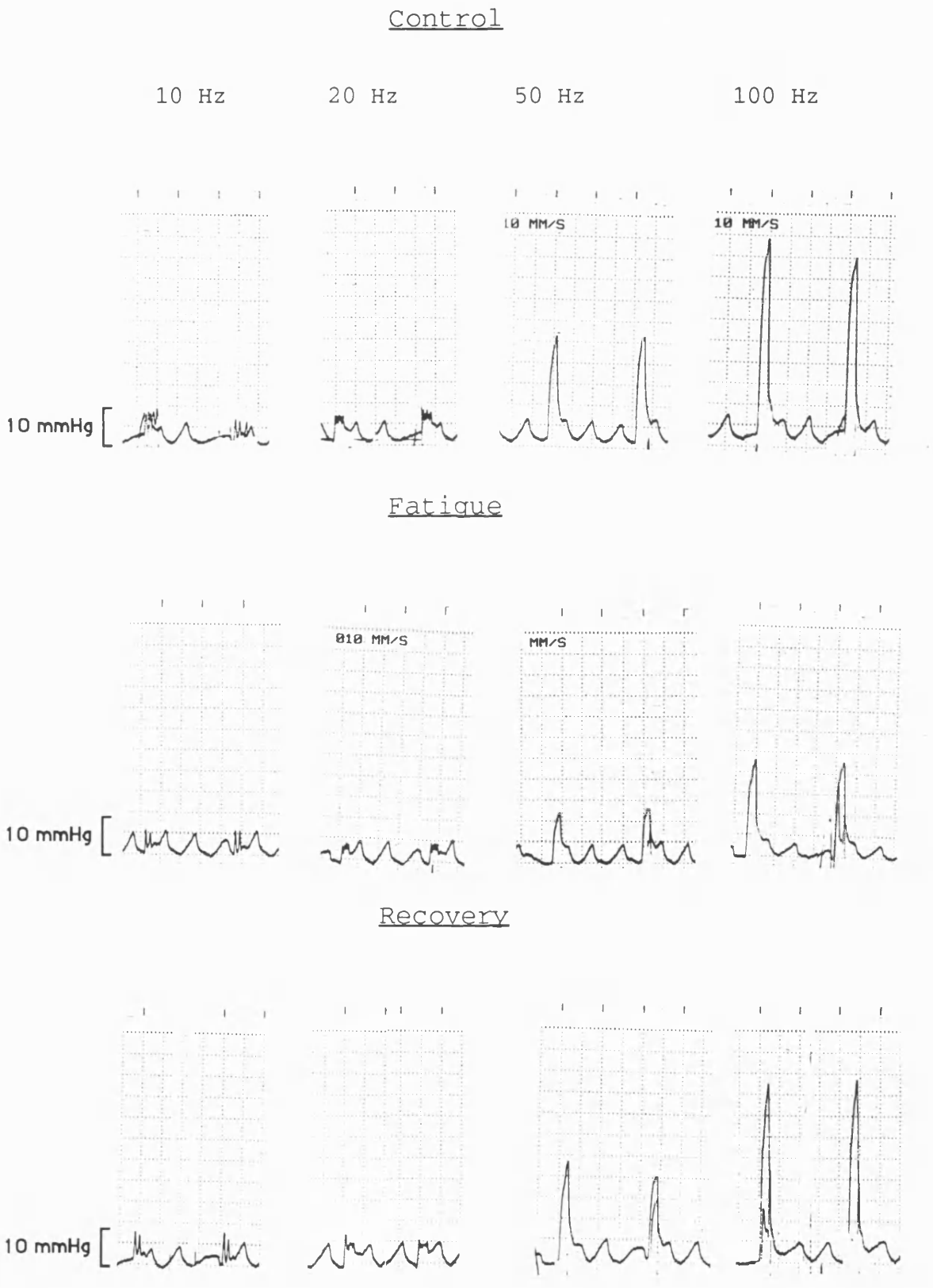


Fig. 3.6. Gastric pressure generated by supramaximal voltage at high and low frequencies before, during, and after inspiratory resistive loading (IRL) for rabbit 76.

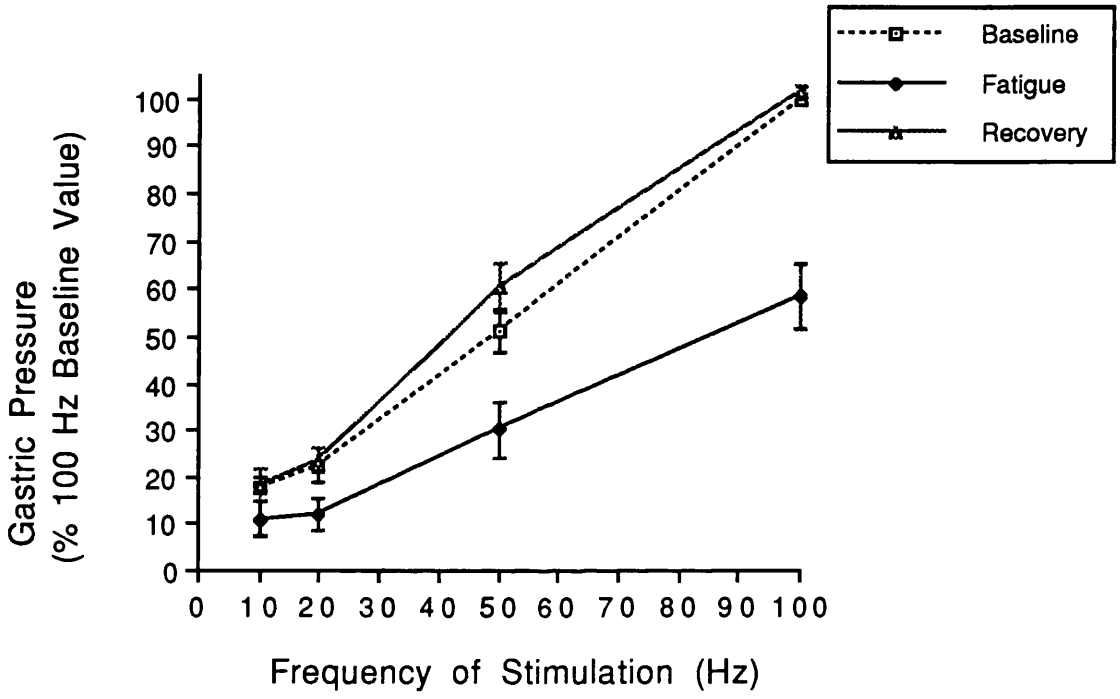


Fig. 3.7. Pressure-frequency curve of the diaphragm based on gastric pressure response to 100 Hz-stimulations before inspiratory resistive loading (IRL), at fatigue produced by IRL, and at recovery. The results are averaged data from four rabbits. Each point represents a mean of 12 observations.

Time-control studies

In the two rabbits in which control studies were performed, tidal Pdi remained constant while Pg_{max} increased insignificantly over the 90 minutes of unloaded breathing (Table 3.2). This result indicates that the reductions in tidal Pdi and Pg induced by supramaximal electrical stimulation observed during IRL was associated with a fatiguing diaphragm and not due to a time-dependent deterioration in the preparation.

Time (min)	Pg_{max} (mmHg)
0	18 0 ± 3.0
30	21.2 ± 4.4
60	21.5 ± 5.0
90	23.2 ± 7.2

Table. 3.2. Changes in Pg_{max} induced by supramaximal stimulation at 100 Hz during the control study. The values are mean maximal gastric pressure ± SE for the two rabbits.

Discussion

The time-control study indicated that the decrease in induced Pg during IRL resulted from diaphragm failure. This loss in diaphragmatic force occurred at both high and low frequency stimulations and was recoverable with rest. These observations indicate that IRL produced peripheral diaphragmatic fatigue.

Time-control study

Neither changes in chest wall geometry nor changes in diaphragmatic resting fibre length were assessed. However,

since the changes in Pg_{max} during the time-control experiments were insignificant, it was likely that any changes in chest wall geometry and diaphragmatic fibre length were minimal. Therefore, the decrease in pressures that occurred during inspiratory resistive loading was a consequence of diaphragmatic failure.

Relationship between Pg_{max} and Pdi_{max}

In this thesis, Pg induced by supramaximal stimulation at 100 Hz was used instead of Pdi to assess diaphragmatic strength. Transvenous stimulation of the phrenic nerves sometimes caused the oesophagus to contract. Contraction of the oesophagus was detected by the oesophageal balloon. Consequently, the recorded Pdi did not reflect strength of diaphragmatic contraction. Since Po generated is affected by the activity of both rib cage muscles and the diaphragm, any change in Pdi, a sum of Po and Pg, could have been the consequence of changes in the activity of rib cage muscles and not diaphragmatic activity. In contrast, Pg generated is determined by the actions of the diaphragm and abdominal muscles. As the abdomen was bound with an inelastic binder keeping the abdominal volume relatively constant, the effect of abdominal muscle contraction and relaxation on Pg was negated.

The results from the rabbits demonstrated that Pg_{max} was on the whole representative of Pdi_{max}. The outlier in Fig 3.2 was caused by a substantial decrease in oesophageal pressure, which was greater than would be predicted from the slope. The decrease in Po could be attributed to rib cage muscle fatigue. Evidently, in view of this observation, Pdi_{max} could be influenced not only by changes in contractility of the diaphragm but also by changes in contractility of the rib cage muscles. Therefore, it could be argued that Pg is a better index of the strength of diaphragmatic contraction than Pdi or at least as good an index of diaphragmatic

strength. Aldrich (1985), studying diaphragm fatigue in the rabbit, has provided evidence to support this argument.

Recovery from high and low frequency stimulation

The effects of fatigue and recovery on diaphragmatic force-frequency relationship in the rabbit were not totally consistent with the prevailing view. Diaphragm fatigue was indicated by a marked decrease in force generated at high and low frequency stimulation; this result is consistent with the prevailing view. During recovery the force generated at high frequency stimulation returned to non-fatigued levels within an hour, as would be expected, but unexpectedly the force generated at low frequency stimulation also recovered within the hour.

Previous studies have also shown that severe inspiratory resistive loaded breathing can induce diaphragm fatigue characterized by a decrease in Pdi generated at both high and low frequency electrical stimulations. However, they have found that loss in force at high frequencies recovers within an hour whereas the force generated by loss at low frequency stimulation may remain depressed for hours or days (Edwards, 1979; Aubier et al., 1981a; Aldrich, 1987). These findings would suggest the development of two pathophysiologically distinct forms of fatigue. Since the muscle recovers slowly from low frequency fatigue, this type of fatigue is believed to occur as a result of damage to contractile proteins, requiring a long time for repairs to be completed. In contrast, because muscles recover from high frequency fatigue rapidly, some researchers believe that it results from alteration in intracellular and extracellular ion concentrations (Fuch et al, 1970; Mertzger & Fitts, 1986) Changes in the distribution of K^+ across cell membranes are a cause of muscle fatigue (Friedland & Paterson, 1988; Sjøgaard, 1986 & 1990). In Chapters 8 to 10 changes in extracellular K^+ and their effects on diaphragm will be examined.

The most probable explanation for the apparent recovery in the loss of force at low frequency stimulation presented here relates to the difficulty in discriminating small changes in Pg in response to low frequency stimulation. The induced Pg could be measured to an accuracy of 0.5 mm. This level of discrimination was not important during high frequency stimulation, where it constituted less than 10% of the measurement of Pg. However, with low frequency stimulation, 0.5 mm could account for more than 10% of Pg measurement. It is conceivable then that what appeared to be a complete recovery may have been incomplete recovery. In retrospect, the gain of Pg should have been increased to facilitate more accurate determination of Pg induced by low frequency stimulation.

Conclusion

The conclusion that can be drawn from the data in Table 2 is that the loss of diaphragmatic strength induced by IRL is reversible in the rabbit, thereby showing that the loss of strength was due to fatigue. These results also showed that diaphragm fatigue was peripheral in nature, since force was lost at both high and low frequency stimulations during inspiratory resistive loaded breathing. The precis nature of the fatigue was not determined. However, a combination of neuromuscular transmission failure and contractile failure is believed to have contributed to diaphragmatic fatigue in these rabbits (Aldrich & Appel, 1985; Aldrich, 1987).

CHAPTER 4

EFFECT OF VAGAL FEEDBACK ON VENTILATION DURING INSPIRATORY RESISTIVE LOADING AND DIAPHRAGM FATIGUE

Introduction

The effect of diaphragm fatigue on the pattern of breathing has been studied extensively. The development of diaphragm fatigue is characterized by rapid shallow breathing and overt diaphragmatic fatigue is exhibited by bradypnea[1]. There are probably neural and humoral factors responsible for producing these patterns of breathing. The respiratory drive to the inspiratory muscles is believed to be predominantly controlled by central and peripheral chemoreceptors and vagal afferents (Sant' Ambrogio et al., 1985). Changes in vagal afferent activity, and alteration in arterial blood gas composition during diaphragm fatigue might be responsible for both tachypnea and bradypnea. A third mechanism for these patterns of breathing may arise from the diaphragm itself. The purpose of this study was to assess whether vagal inputs have any major influence on ventilation during the development of diaphragm fatigue in the rabbit.

(Cohen et al., 1982; Roussos, 1990)[1]

Protocol

This study was carried out in six non-vagotomized (Non-VGX) and four vagotomized (VGX) rabbits. Diaphragm fatigue was induced by severe inspiratory resistive loading (IRL) and diaphragmatic contractility was assessed as described in Chapter 3. Ventilation and Pdi were monitored continuously before and during IRL. For Non-VGX rabbits, arterial blood samples for blood gas and acid-base analysis were taken before IRL and at fatigue. For VGX rabbits, arterial blood samples for analysis were in addition taken at intervals of 20 minutes during IRL.

Data analysis

Respiratory variables were analyzed in the following manner. The control value of each respiratory variable was an average of 10 consecutive breaths measured before IRL. The period of inspiratory resistive loading was divided into four bins: 0 minutes, 5 minutes, 10+ minutes, and fatigue. The first ten breaths immediately following the imposition of the IRL were averaged and represented by 0 min. After five minutes of IRL, ten consecutive breaths were analyzed and averaged. Subsequently, ten consecutive breaths were analyzed every fifth minute until fatigue. Because the diaphragm fatigued at different rates for each rabbit, all data obtained after the first 5 minutes of IRL and before fatigue were averaged. This average is denoted by 10+ min. The ten consecutive breaths analyzed and averaged at fatigue constituted the final bin. All results are expressed as mean ± SE. The difference in a respiratory variable between Non-VGX and VGX rabbits for each bin was assessed using a one factor analysis of variance (ANOVA). To determine whether a variable changed significantly from control (unloaded) value during inspiratory resistive loaded breathing, ANOVA test was also performed. A difference was deemed significant in all cases when $P < 0.05$.

Results

Changes in respiratory variables during IRL and at diaphragm fatigue

Diaphragm fatigue was induced in all six Non-VGX rabbits after an average of 27.5 ± 5.9 minutes of IRL. In contrast, diaphragmatic fatigue was induced in only two of the four vagotomized rabbits, with an average endurance of the diaphragm (Tlim) of 50 ± 10 minutes. The non-fatiguable rabbits breathed through an inspiratory load for one and a half hour before IRL was aborted. Subsequent analysis was performed only on the data from fatigued rabbits.

Cutting the vagus nerves produced the predictable slow deep breathing. As a result, control measurements of tidal volume (V_T) and respiratory frequency (f_R) were significantly different between VGX and Non-VGX (see Table 4.1 and 4.2). A slower f_R for VGX was achieved by a prolonged inspiratory (T_I) and expiratory time (T_E) (see Table 4.1 and 4.2); but only T_I was significantly different from the Non-VGX control value. Inspired minute ventilation (V_I) was on average higher for VGX rabbits than for Non-VGX rabbits. This higher V_I in VGX rabbits was reflected in their significantly lower end-tidal CO_2. Averages of the duty cycle (T_I/T_T) and mean inspiratory flow (V_T/T_I) were not significantly different between the VGX and Non-VGX rabbits during the control period (Table 4.1 and 4.2).

On imposition of the inspiratory resistive load, V_T decreased in both groups of rabbits. f_R decreased for Non-VGX rabbits and remained unchanged for VGX (Fig. 4.2; Table 4.1 and 4.2). As a consequence of the changes in V_T and f_R, V_I decreased below its control value on imposition of IRL (Fig. 4.3). In both groups, tidal volume stabilized after the first ten breaths until fatigue in the Non-VGX group whereas it progressively decreased after 5 minutes of IRL in

the VGX group (Fig. 4.2). Both T_I and T_E increased during inspiratory resistive loading for Non-VGX rabbits. In contrast, T_I increased for part of IRL and T_E decreased for VGX rabbits (Fig. 4.4). The increase in f_R at five minutes in Non-VGX rabbits had the effect of increasing V_I slightly above the value for the first ten breaths (Figs 4.2 and 4.3). Subsequently V_I progressively decreased in these rabbits during IRL. Over the same period, V_I for VGX rabbits remained relatively constant (Fig. 4.3). End-tidal CO_2 rose progressively throughout IRL in both groups, although the rise was steepest for the Non-VGX group (Fig. 4.3). During the early phase of IRL (0 and 5 min), T_I/T_T increased above control values while V_T/T_I decreased in both (Tables 4.1 and 4.2).

At diaphragmatic fatigue, average f_R and V_T decreased below the previous measurement for both groups (Fig. 4.2). The reduction in f_R was achieved by both T_I and T_E being prolonged substantially. The reduction in V_I was greatest for the VGX rabbits (Fig. 4.3). End-tidal CO_2 peaked at fatigue in both Non-VGX and VGX rabbits (Fig. 4.3). The average duty cycle decreased in response to fatigue in both groups. Mean inspiratory flow remained unchanged at fatigue in Non-VGX rabbits. In contrast the VGX rabbits decreased their mean inspiratory flow at fatigue (Table 4.1 and 4.2).

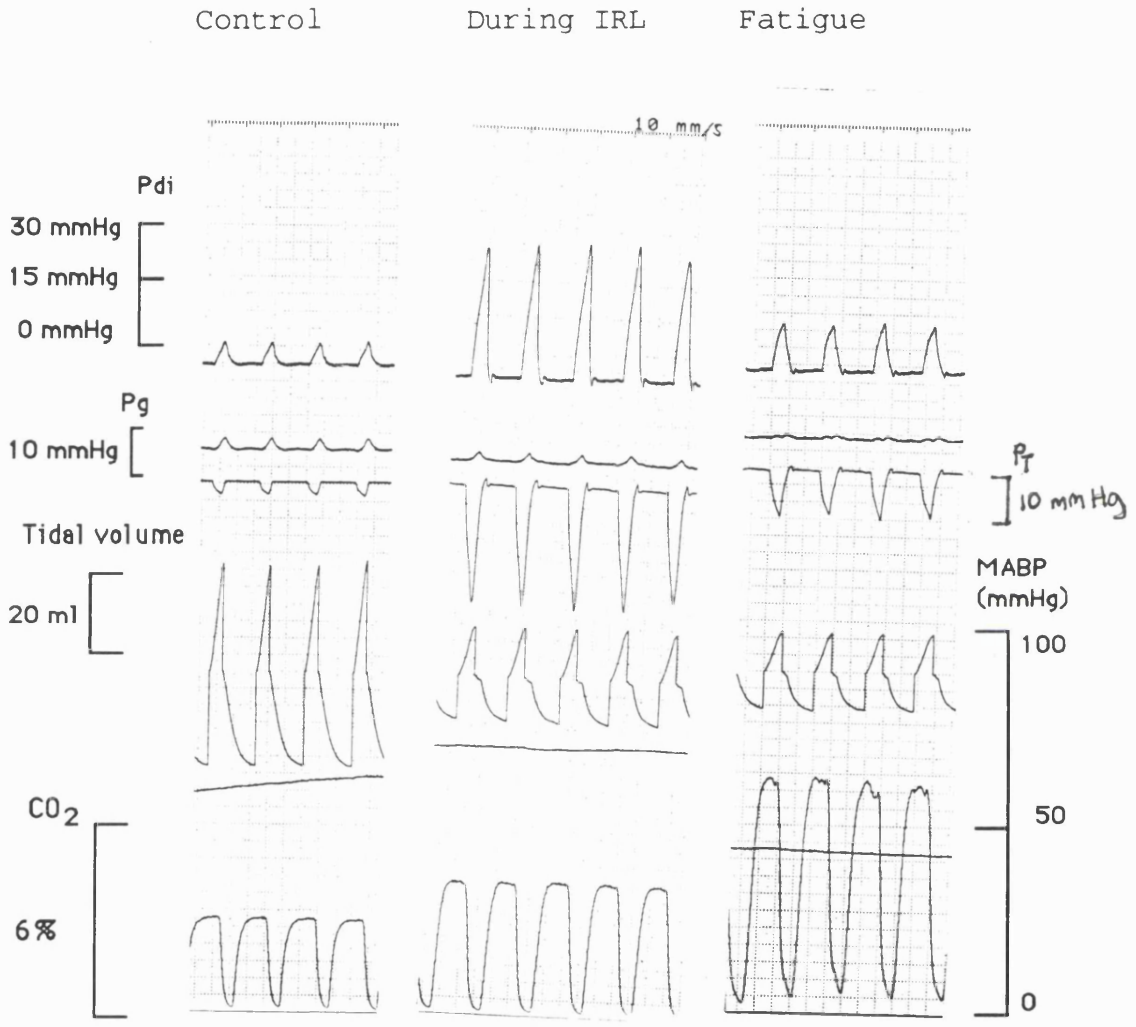


Fig 4.1. The effect of fatiguing inspiratory resistive loading on ventilation and mean arterial blood pressure (MABP).

Table 4.1. Changes in respiratory variables during severe inspiratory resistive loaded breathing in non-vagotomized rabbits. Values are means ± SE for six rabbits at control and fatigue but only five rabbits at 5 and 10+ minutes as one animal's diaphragm became fatigued after 5 minutes.
* Value is statistically different from control.

	V_T (ml)	f_R (breaths /min)	V_T/T_I (ml/sec)	T_I/T_T	V_I (ml/min)	T_I (sec)	T_E (sec)	E_TCO_2 (%)
Control	23.6	61	60.3	0.43	1625	0.40	0.57	3.87
	± 2.7	± 5	± 6.9	± 0.03	± 174	± 0.02	± 0.10	± 0.22
0 min	13.6*	56	28.5*	0.47	743*	0.48	0.60	4.54*
	± 0.9	± 7	± 2.8	± 0.05	± 89	± 0.03	± 0.15	± 0.21
5 min	15.1*	60	35.1*	0.45	907*	0.44	0.59	5.93*
	± 1.4	± 7	± 3.1	± 0.04	± 108	± 0.03	± 0.12	± 0.34
10+ min	14.0*	59	32.1*	0.45	789*	0.44	0.62	6.61*
	± 1.9	± 8	± 3.7	± 0.04	± 100	± 0.03	± 0.13	± 0.45
Fatigue	14.1*	56	33.3*	0.46	729*	0.49	0.67	7.48*
	± 2.1	± 4	± 4.7	± 0.04	± 146	± 0.06	± 0.10	± 0.77

Table 4.2. Changes in respiratory variables during severe inspiratory resistive loaded breathing in two vagotomized rabbits.

Rabbit 1110

	V_T (ml)	f_R (breaths /min)	V_T/T_I (ml/sec)	T_I/T_T	V_I (ml/min)	T_I (sec)	T_E (sec)	ET^{CO_2} (%)
Control	40.8	39	60.0	0.44	1591	0.68	0.86	3.0
0 min	25.9	38	34.0	0.49	984	0.76	0.80	3.4
5 min	24.7	44	36.9	0.49	1086	0.67	0.71	3.5
10+ min	20.7	46	33.8	0.47	952	0.61	0.70	5.8
Fatigue	17.5	32	24.9	0.38	582	0.70	1.16	8.3

Rabbit 249

	V_T (ml)	f_R (breaths /min)	V_T/T_I (ml/sec)	T_I/T_T	V_I (ml/min)	T_I (sec)	T_E (sec)	ET^{CO_2} (%)
Control	40.0	37	51.9	0.48	1480	0.77	0.85	2.7
0 min	30.5	37	32.4	0.58	1128	0.94	0.69	3.8
5 min	28.7	37	30.9	0.57	1062	0.93	0.71	3.8
10+ min	23.7	43	33.7	0.49	1019	0.69	0.70	4.1
Fatigue	23.0	31	28.7	0.41	713	0.80	1.13	4.6

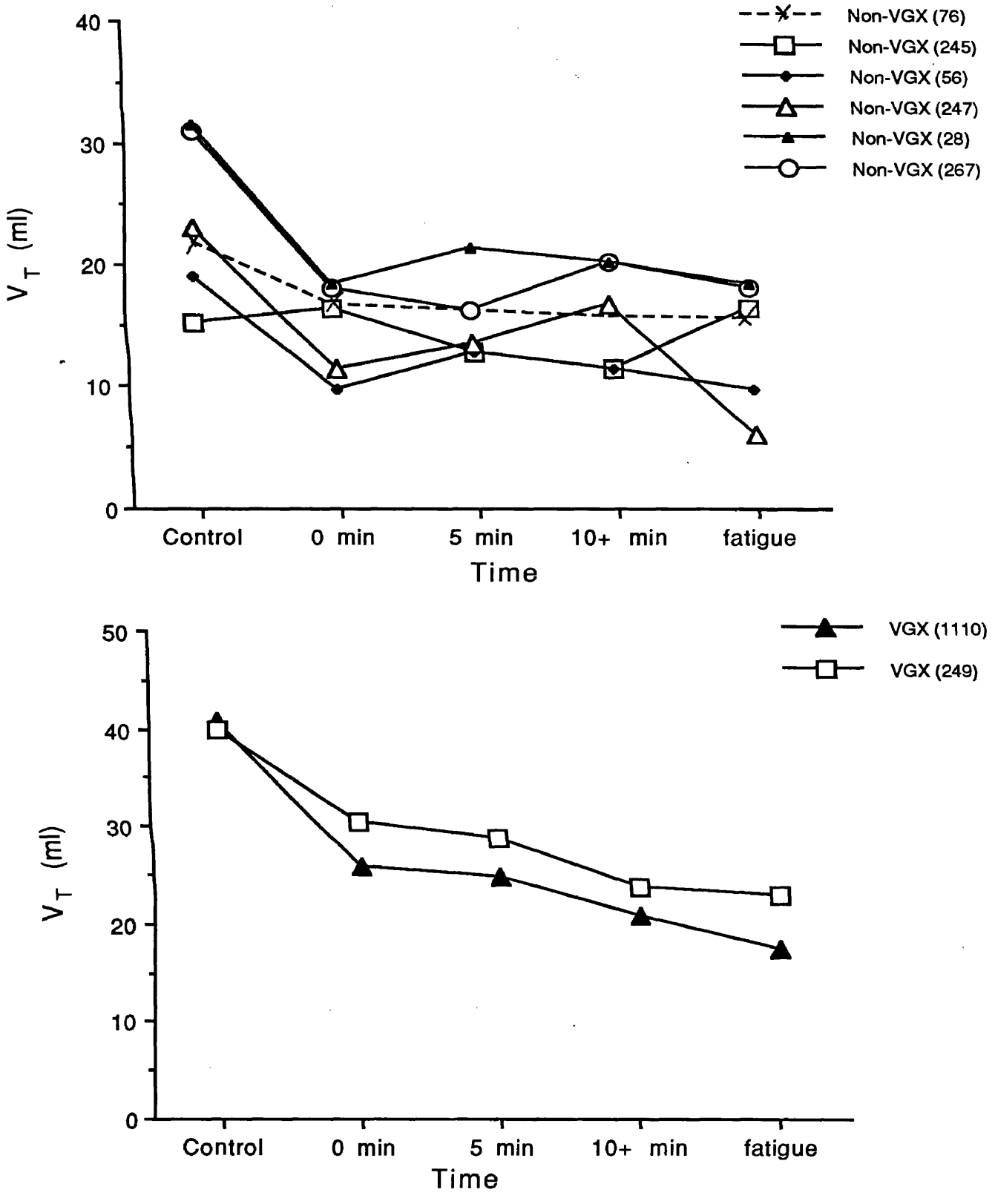


Fig. 4.2. Changes in tidal volume (V_T) during severe IRL in non-vagotomized (Non-VGX) and vagotomized (VGX) rabbits. Control is the period before IRL. 0 and 5 min denote respiratory changes during first ten breaths and after 5 minutes of IRL respectively. 10+ min denotes changes in respiratory variables from 10 minutes of IRL to immediately before fatigue. Fatigue denotes changes in respiratory variables at diaphragm fatigue. Dashed line for rabbit 76 is to signify that values for 5 min and 10 + min are not available.

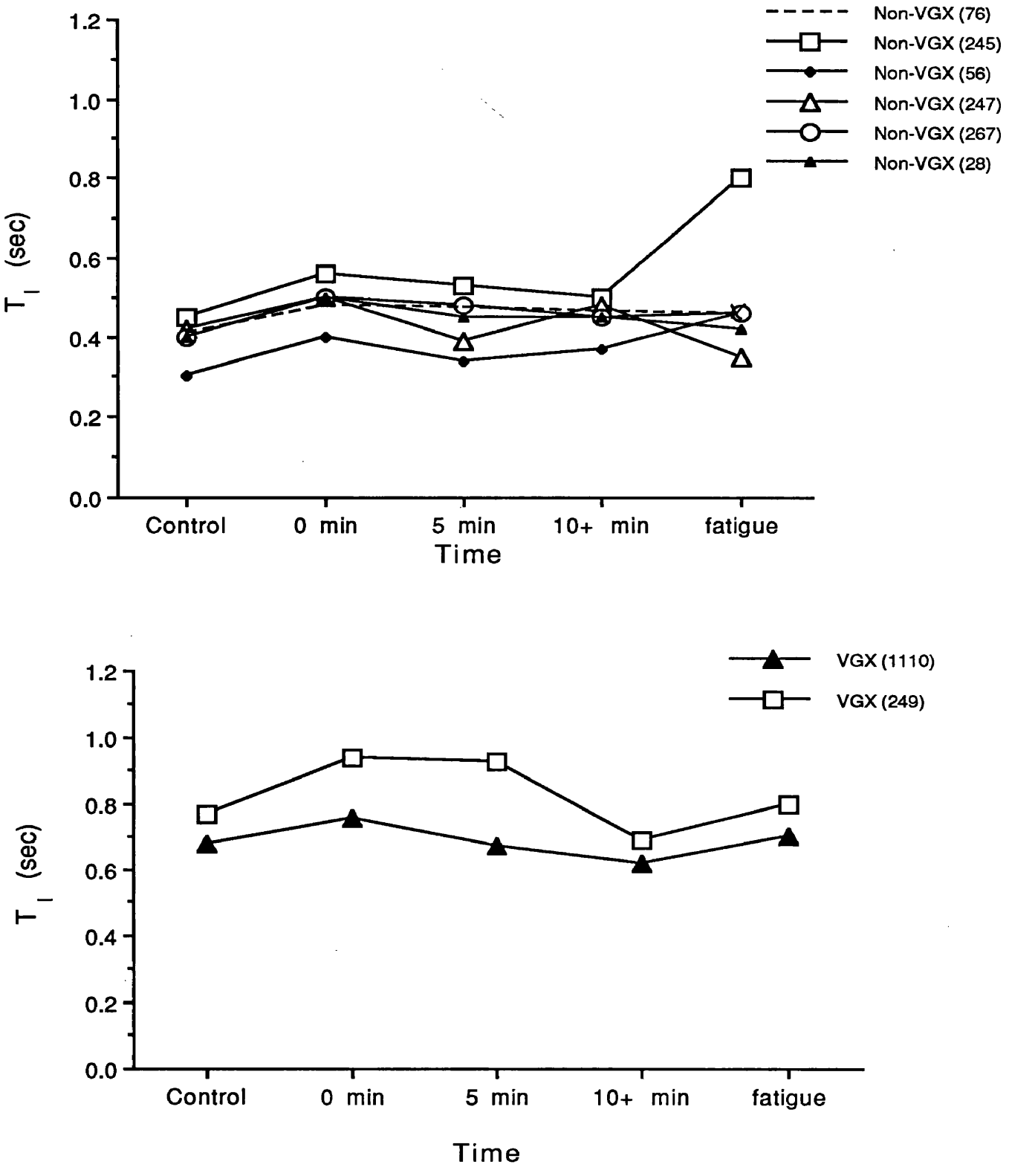
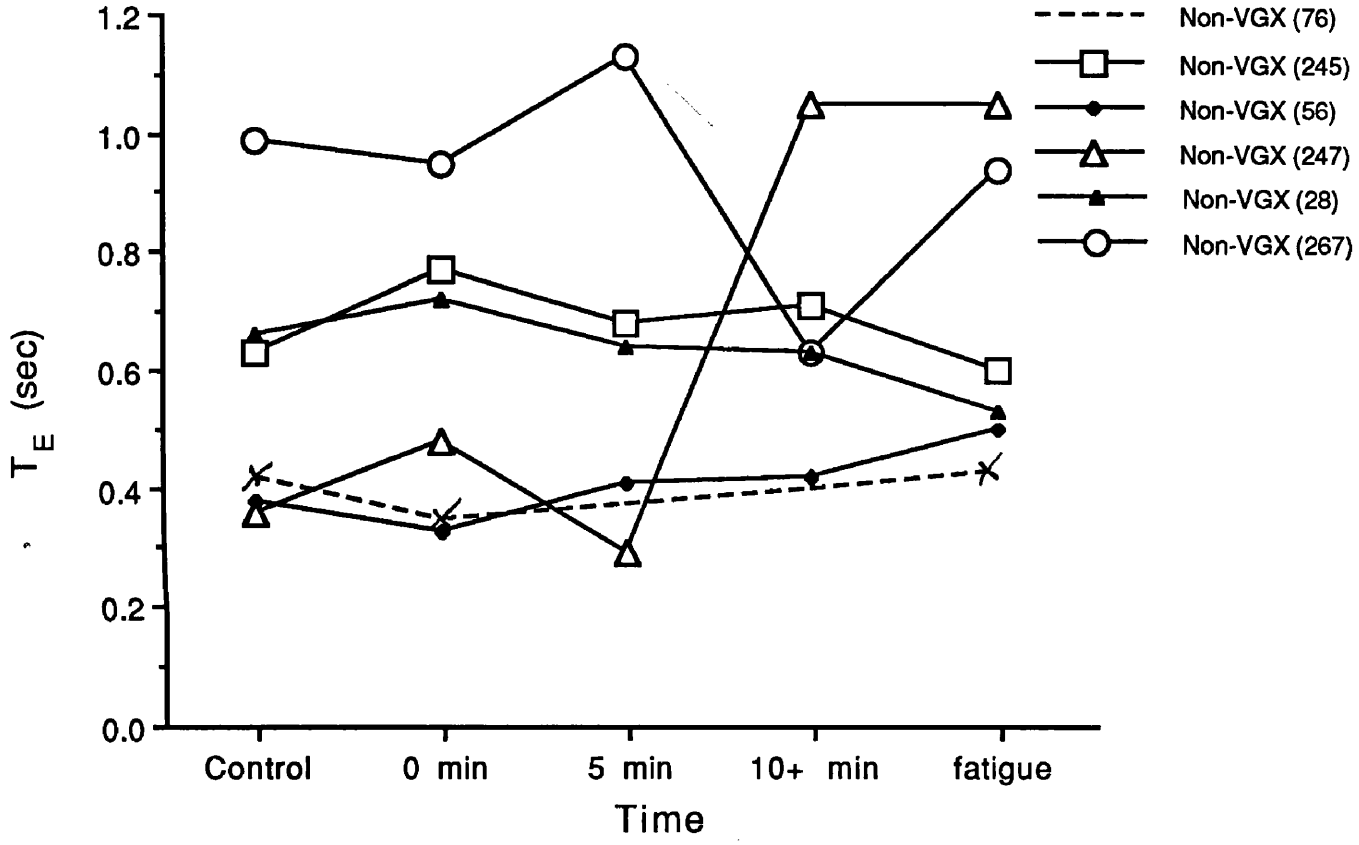


Fig. 4.3. Changes in inspiratory time (T_I) during severe IRL in non-vagotomized (Non-VGX) and vagotomized (VGX) rabbits. Control is the period before IRL. 0 and 5 min denote respiratory changes during first ten breaths and after 5 minutes of IRL respectively. 10+ min denotes changes in respiratory variables from 10 minutes of IRL to immediately before fatigue. Fatigue denotes changes in respiratory variables at diaphragm fatigue. Dashed line for rabbit 76 is to signify that values for 5 min and 10 + min are not available.



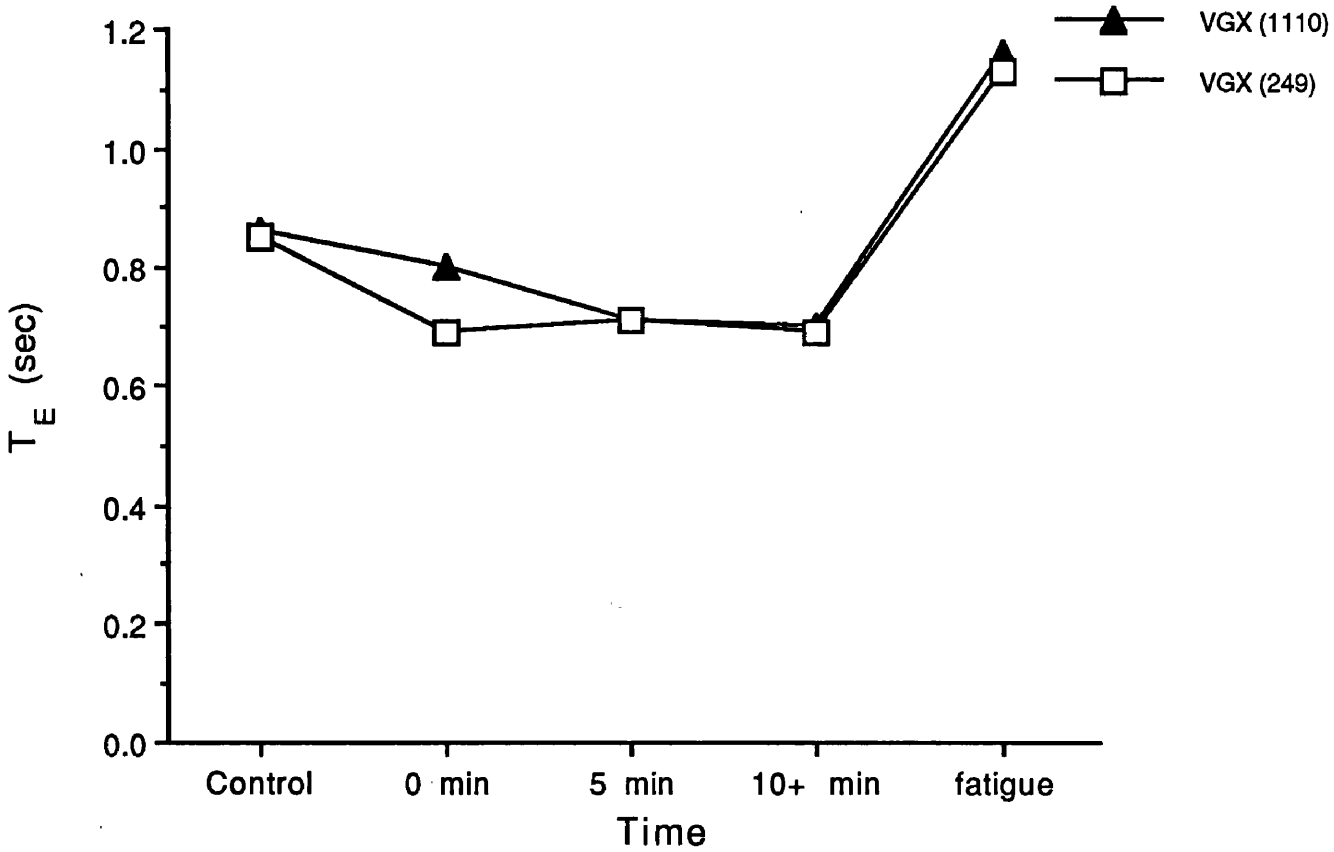


Fig. 4.4. Changes in expiratory time (T_E) during severe IRL in non-vagotomized (Non-VGX) and vagotomized (VGX) rabbits. Control is the period before IRL. 0 and 5 min denote respiratory changes during first ten breaths and after 5 minutes of IRL respectively. 10+ min denotes changes in respiratory variables from 10 minutes of IRL to immediately before fatigue. Fatigue denotes changes in respiratory variables at diaphragm fatigue. Dashed line for rabbit 76 is to signify that values for 5 min and 10 + min are not available.

Arterial blood gas composition before, during IRL, and at diaphragm fatigue

The results of arterial blood analysis for both Non-VGX and VGX rabbits are summarized in Table 4.3 and 4.4 respectively. Both groups of rabbits had normal arterial pH and P_{O_2} values before IRL. Control values of arterial pH and P_{O_2} for Non-VGX and VGX rabbits were not significantly different. Before IRL, Pa_{CO_2} for VGX rabbits was significantly lower than Pa_{CO_2} for Non-VGX rabbits. The VGX rabbits were hypocapnic resulting from an elevated alveolar ventilation.

During IRL of VGX rabbits, arterial pH and P_{O_2} decreased progressively, and P_{CO_2} stabilized after an initial fall. At fatigue, both Non-VGX and VGX rabbits were severely acidotic and hypoxic. Non-VGX rabbits were more acidotic and hypoxic than VGX rabbits. However, arterial pH and P_{O_2} for Non-VGX were not significantly different from arterial pH and P_{O_2} for VGX rabbits. Arterial P_{CO_2} rose sharply at fatigue for VGX animals. Both groups of animals were hypercapnic with Pa_{CO_2} being insignificantly higher in Non-VGX rabbits.

Table 4.3. Arterial blood gases and pH for five Non-VGX rabbits before IRL and at fatigue. Values are means ± SE.
* Value is significantly different from control.

	Control	Fatigue
pH	7.45 ± 0.05	7.04 ± 0.05 *
Pa_{CO_2}	29.6 ± 1.7	77.4 ± 9.0 *
Pa_{O_2}	114.5 ± 9.8	48.0 ± 10.8 *

Table 4.4. Arterial blood gases and pH for two VGX rabbits before, during IRL, and at fatigue. Values are means ± SE.
* Value is significantly different from control.

	Control	20 min	40 min	Fatigue
pH	7.49 ± 0.02	7.34 ± 0.07	7.29 ± 0.09	7.22 ± 0.16
Pa_{CO_2}	21.4 ± 1.7	35.9 ± 5.0	35.2 ± 2.1 *	52.8 ± 16.4
Pa_{O_2}	99.0 ± 8.5	63.9 ± 22.8	58.2 ± 19.1	54.8 ± 19.5

Discussion

The immediate response to inspiratory resistive loading in Non-VGX and VGX rabbits was a reduction in V_T. Non-VGX rabbits also decreased their f_R in response to the load whereas VGX maintained their f_R. The net effect in both groups of rabbits was a substantial reduction in V_I. After five minutes of inspiratory resistive loading, there was some recovery in V_I in Non-VGX and VGX rabbits but V_I remaining well below control levels. This partial recovery in V_I was solely due to an increase in f_R. In VGX rabbits, f_R rose above control values. At fatigue, breathing slowed in both Non-VGX and VGX rabbits.

In two of the four VGX rabbits, inspiratory resistive loading did not induce diaphragm fatigue whereas it did in all Non-VGX rabbits. It is tempting to conclude that in the absence of vagal activity, the onset of diaphragm fatigue may be postponed or even prevented. However, evidence obtained from this study indicates that the failure of IRL to induce diaphragm fatigue in two VGX rabbits was fortuitous. Although diaphragm fatigue was induced in only two of the four VGX rabbits, there was no obvious difference in the pattern of breathing adopted by the four during IRL. Moreover, although the average Tlim was longer for VGX rabbits, two of the Non-VGX rabbits had a Tlim that was either equal to or longer than the Tlim of one of the VGX rabbits.

Mechanisms of ventilatory response to load

Early phase (0 min). Over the first ten breaths of inspiratory resistive loading, the two principal changes were a reduction in V_T and an increase in T_I for both Non-VGX and VGX rabbits.

An increase in T_I in response to loading occurred in both the absence and presence of vagal feedback, indicating that the response was not vagally mediated. The increase in T_I may have been mediated by afferent feedback from the chest wall and the loaded inspiratory muscles. Evidence is available to indicate that afferent information from mechanoreceptors arising from the chest wall and diaphragm can alter respiratory timing. It has been reported that T_I prolongation in quadriplegic subjects was about half the magnitude observed in normal subjects (Hof et al., 1986). Since these quadriplegic subjects were devoid of afferent feedback from intercostal muscles and, with cervical lesions, feedback from a portion of the diaphragm, this result suggests that activity of receptors in the intercostal muscles and/or diaphragm may have participated in this response. Furthermore, medullary inspiratory-neuronal activity has been shown to increase in response to tracheal occlusion in anaesthetized vagotomized cats. This response was abolished following cervical (C_3-C_7) and thoracic (T_1-T_9) dorsal rhizotomies (Shannon et al., 1985). The nature and location of receptors influencing brainstem activity were not determined by Shannon and colleagues.

In the present study, it is difficult to attribute the T_I response to inspiratory muscle spindle activity; the activity from these receptors would increase during loading and increased activity from these receptors would be expected to decrease T_I instead of increasing it (Remmers & Marttila, 1975). There are three other types of muscle receptors, tendon organs, free nerve endings and pacinian corpuscles whose role in the control of respiratory timing is unclear (Duron, 1981).

Because there was averaging of the first ten breaths, any recovery in respiratory timing and tidal volume initiated by increased chemical drive to breathe, occurring within the first ten breaths, could have been obscured. In the rabbit, information is not available about how soon changes in

chemoreceptor activity occur after ventilation is decreased in response to loading. However, studies in the cat have shown that significant changes in chemoreceptor activity occur as soon as 12-15 seconds after initial decrease in ventilation (Bruce et al., 1974) or after changing the inspired gas from air to air plus 4-5% CO_2 (Biscoe & Purves, 1967). Assuming a similar time-course for a significant increase in chemoreceptor activity in the rabbit, chemoreceptor activity would have started to have an affect on ventilation on the 11th-14th loaded breath in Non-VGX rabbits, and on the 8th-10th loaded breath in VGX rabbits. Therefore, for the first ten breaths in Non-VGX rabbits at least, chemoreceptive feedback probably had minimal if any effect on ventilation.

Mid phase (5 and 10+ min). During the mid phase, f_R increased towards control in Non-VGX rabbits and above control in VGX rabbits. The increase in f_R was produced by a reduction in both T_I and T_E in both groups of rabbits (Figs. 4.2 and 4.4). Although the chest wall and diaphragm afferents may still be active during this phase, the increase in f_R in both groups of rabbits probably occurred as a result of increased chemical drive.

Inspiratory resistive loading in Non-VGX rabbits and VGX rabbits produced both hypoxia and hypercapnia, thus increasing the chemical drive to breathe. There is evidence to suggest that the hypercapnia and not the hypoxia might have been responsible for the increased chemical drive during IRL. Adams and colleagues (1988) have shown that non-fatiguing IRL can produce tachypnea in vagal intact anaesthetized dogs. This change in pattern of breathing was not abolished if hypoxia was prevented. However, it was abolished if hypercapnia was prevented.

Hypercapnia has been shown to reduce T_E in both Non-VGX and VGX cats (Gautier et al., 1973) and to a lesser extent T_I in Non-VGX goats and dogs (Isaza et al., 1976; Phillipson,

1974). In both groups of rabbits, T_E decreased during the mid-phase in respect to the initial phase of loading. However, VGX rabbits decreased their T_E below control value whereas the Non-VGX did not. This difference in response cannot be explained by the absence of vagal activity in the former and the presence in the latter. This is because increased vagal activity would have resulted in a decrease in T_E rather than an increase in it.

Fatigue. When the diaphragm became fatigued, both hypoxemia and hypercapnemia became more pronounced and breathing became slower in both Non-VGX and VGX rabbits. Tidal volume decreased and both T_I and T_E increased in the absence of vagal feedback (Figures 4.2 and 4.4). It is, therefore, reasonable to argue that the resulting slowing of breathing at fatigue is not mediated by activity of the vagal afferents.

The modest reduction in V_T probably occurred because the diaphragm failed to generate sufficient pressure to maintain it (see Chapter 3). The increase in T_E and T_I at fatigue could have resulted from a decrease in central neural drive. The resultant hypoxemia and hypercapnemia at fatigue may have produced such a reduction in central neural drive and accelerated diaphragm fatigue (See Chapter 5). Because these changes in arterial blood gas tensions may have been precipitated by bradypnea, hypoxic and hypercapnic central depression may not be responsible for bradypnea. Indeed, bradypnea may have been produced by non-vagal reflexes.

One such reflex might involve sensory information arising from the fatigued inspiratory muscles. Hussain and colleagues (1985) have reported the effect of sepsis on the development of diaphragmatic fatigue in the anaesthetized vagotomized dog. In their study, the dogs became bradypnoic in the absence of hypercapnia and hypoxia. Hussain and colleagues (1985) proposed that the response occurred as a

result of reflexes initiated by activation of type III and IV phrenic afferent fibres

Conclusion

The results in this chapter indicate that tachypnea is not always produced in response to IRL but when contractile diaphragm fatigue occurs slowing of breathing invariably ensues. Because these changes in breathing pattern during IRL cannot be attributed to vagal activity, the simplest explanation is that they were brought about by changes in arterial blood chemistry. Although there is no direct evidence to implicate non-vagal afferents, the role of these afferents in the control breathing during IRL in this study cannot be ruled out. Future neural investigations in this thesis will concentrate on the possible role played by phrenic afferents in the control of breathing during the development of diaphragm fatigue.

HUMORAL FACTORS RELATED TO DIAPHRAGM FATIGUE

CHAPTERS 5 TO 7

CHAPTER 5

EFFECT OF SEVERE INSPIRATORY RESISTIVE LOADING ON ARTERIAL BLOOD GASES, ACID-BASE, AND POTASSIUM

Introduction

Severe inspiratory resistive loading (IRL) produces alterations in arterial blood gases, and acid-base (Aldrich & Appel, 1985; Watchko et al., 1988). However, no study has reported the effect of IRL on arterial potassium. Plasma potassium (K^+) has been shown to increase from 4 mmol/l to 9 mmol/l during limb muscle exercise (Hnik et al, 1976; Linton at al., 1984; Sjøgaard, 1986; Medbø & Sejersted, 1990). An increase in plasma [K^+] during exercise results from a net efflux of K^+ from the exercising muscles (Hirche et al., 1980; Linton at al., 1984; Sjøgaard, 1986). Exercise-induced hyperkalemia is believed to contribute to the development of limb muscle fatigue (Friedland & Paterson, 1988; Sjøgaard, 1986 and 1990). Therefore, it is reasonable to assume that an increase in extracellular K^+ may also contribute to inspiratory muscle fatigue. The aim of this chapter is twofold: to investigate whether IRL can induce hyperkalemia; and to assess whether any rise in arterial [K^+] would be sufficient to precipitate diaphragm fatigue based on theoretical considerations.

Protocol

Details of the methods and preparations are given in Chapters 3 and 4. All rabbits except one breathed room air throughout the study. This one rabbit breathed 40% O_2 before inspiratory resistive loading and thereafter breathed room air. Arterial blood samples were taken before IRL and when diaphragm fatigue was induced by IRL. These samples were analyzed by Radiometer ABL4 for blood acid-base status, gas tensions, and $[K^+]$. The ABL4 potassium potentiometer measured arterial $[K^+]$ to an accuracy of 0.1 mmol/l. Its accuracy in measuring potassium was assessed as outlined in Appendix A. The results presented here are from six rabbits. Statistical comparisons between blood variables measured before IRL and those at fatigue were made using a paired t-test unless otherwise stated. Differences between means were considered significant when $P < 0.05$.

Calculations

(i) Determination of arterial hydrogen ion concentration.

The pH of the blood is the negative of the logarithm to the base 10 of its hydrogen ion concentration ($[H^+]$).

$$pH = -\log [H^+]$$

Therefore, $[H^+]$ can be expressed as nmol/l by using

$$[H^+] = \text{antilog } (9-pH) \qquad (1)$$

(ii) Estimation of diaphragmatic Resting Membrane Potential (RMP).

Changes in $[K^+]$ within intracellular and extracellular fluids can contribute to changes in RMP. Because the excitability of muscle membrane is essential for contraction, alteration of RMP may precipitate muscular fatigue. To assess whether the magnitude of the changes in arterial $[K^+]$ was sufficient to induce diaphragm fatigue by altering RMP, diaphragmatic RMP before IRL and at fatigue were estimated. It was calculated using the Hodgkin and Horowicz equation (1959):

$$RMP = 61.5 \times \log \frac{[K^+_e] + 0.01\,[Na^+_e]}{[K^+_i] + 0.01\,[Na^+_i]} \qquad (2)$$

For equation 2, interstitial potassium concentration is denoted by $[K^+_e]$ and intracellular potassium concentration by $[K^+_i]$. Interstitial sodium and intracellular sodium concentration are signified by $[Na^+_e]$ and $[Na^+_i]$ respectively.

In order to calculate diaphragmatic RMP, three assumptions were made:

(1) Arterial $[K^+]$ is representative of $[K^+_e]$

(2) $[K^+_i]$ remained constant at 150 mmol/l before and at fatigue.

(3) The effect of $[Na^+_e]$ and $[Na^+_i]$ on the RMP was negligible.

Results

Effect of inspiratory resistive loading on arterial blood gases, acid-base and potassium.

Arterial blood gases, acid-base status, and K^+ are shown in Table 5.1. At fatigue, all the measured blood chemistry variables except bicarbonate changed significantly. Arterial $[K^+]$ increased on average by 81%, and Pa_{CO2} by an average of 171%. The reduction in Pa_{O2} was 58%. Both pH and base-excess decreased. Bicarbonate increased insignificantly by 5.9%. With a rise in Pa_{CO2} and a fall in both pH and base-excess, the overall picture presented by this data at fatigue was a combined respiratory and metabolic acidosis.

Table 5.1

Mean changes in blood chemistry produced by IRL. Results are expressed as mean values ± SE for the six rabbits.

	Control	Fatigue	Change(Δ)	P
K^+ (mmol/l)	2.9 ± 0.1	5.2 ± 0.8	2.3	<0.05
pH (units)	7.45 ± 0.04	7.04 ± 0.04	-0.41	<0.05
Pa_{O2} (mmHg)	110.5 ± 8.9	46.1 ± 9.1	-64.4	<0.01
Pa_{CO2} (mmHg)	28.0 ± 2.2	76.0 ± 7.5	48.0	<0.01
BE (mmol/l)	-2.8 ± 1.8	-11.8 ± 2.3	-9.0	<0.05
HCO_3^+ (mmol/l)	20.4 ± 1.4	20.6 ± 1.38	1.2	NS

Figure 5.1 shows arterial [K^+] data in each rabbit during the control and at fatigue. Arterial [K^+] measured before inspiratory resistive loading in each rabbit ranged from 2.4 to 3.2 mmol/l. In contrast, arterial [K^+] measured at fatigue ranged from 2.7 to 8.3 mmol/l. In two rabbits [K^+] increased by less than 1 mmol/l, and by approximately 2.5 mmol/l in three others. In a sixth animal, [K^+] increased by 5 mmol/l.

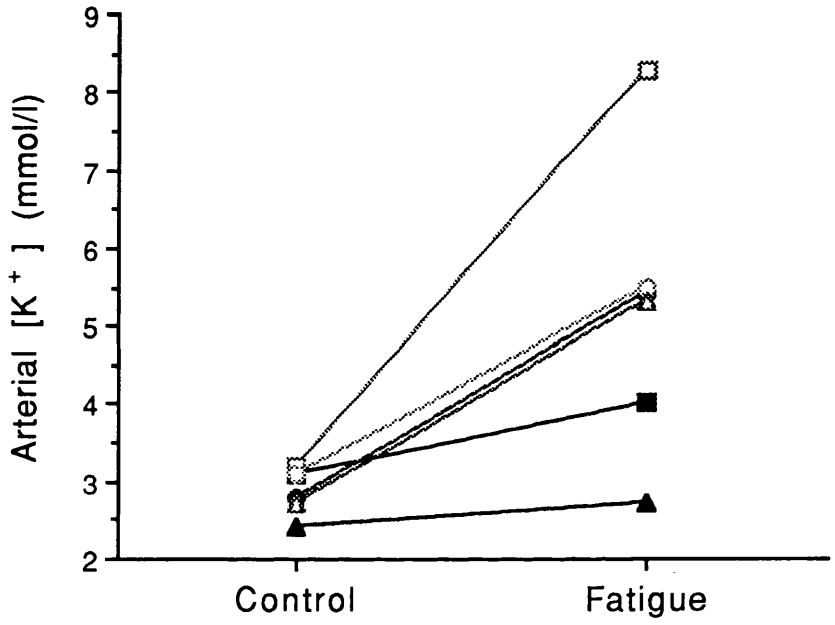


Fig. 5.1. Effect of IRL on arterial potassium in each rabbit. Each pair of points represents potassium measurements in one animal before and at the end of IRL. Inspiratory resistive loading ended when diaphragm fatigue was indicated.

Examining the relationship between the increased arterial potassium and acid-base disturbances

The rise in $[K^+]$ at fatigue was associated with a combined metabolic and respiratory acidosis. The increase in arterial potassium induced, however, did not correlate with either the magnitude of the changes in blood gas tensions or to the duration of loading (see Fig. 5.2). The rabbit with greatest rise in $[K^+]$ 5.1 mmol/l at fatigue did not have the greatest change in either Pa_{CO_2} or Pa_{O_2}. In two rabbits, the rise in $[K^+]$ at fatigue was of the same magnitude (2.6 mmol/l). However, the magnitude of the changes in their Pa_{CO_2} and Pa_{O_2}, and Tlim were dissimilar. Moreover, the rise in $[K^+]$ appears not to be time dependent. Although the animal with the shortest Tlim had also the smallest rise in $[K^+]$, the animal with longest Tlim had the second smallest rise in $[K^+]$ and one with greatest rise in K^+ had a short Tlim.

A multiple linear regression analysis between ΔK^+ and the changes in the other blood variables (ΔpH, ΔBE, ΔPa_{CO_2}, and ΔPa_{O_2}) was performed to quantify the relationship between ΔK^+ and the changes in other blood chemistry variables. The results of this analysis showed a strong correlation between the changes in K^+ and acid-base (see Figures 5.3 and 5.4) and a poor correlation between K^+ and blood gases (Pa_{CO_2} $r = 0.01$; Pa_{O_2} $r = 0.09$).

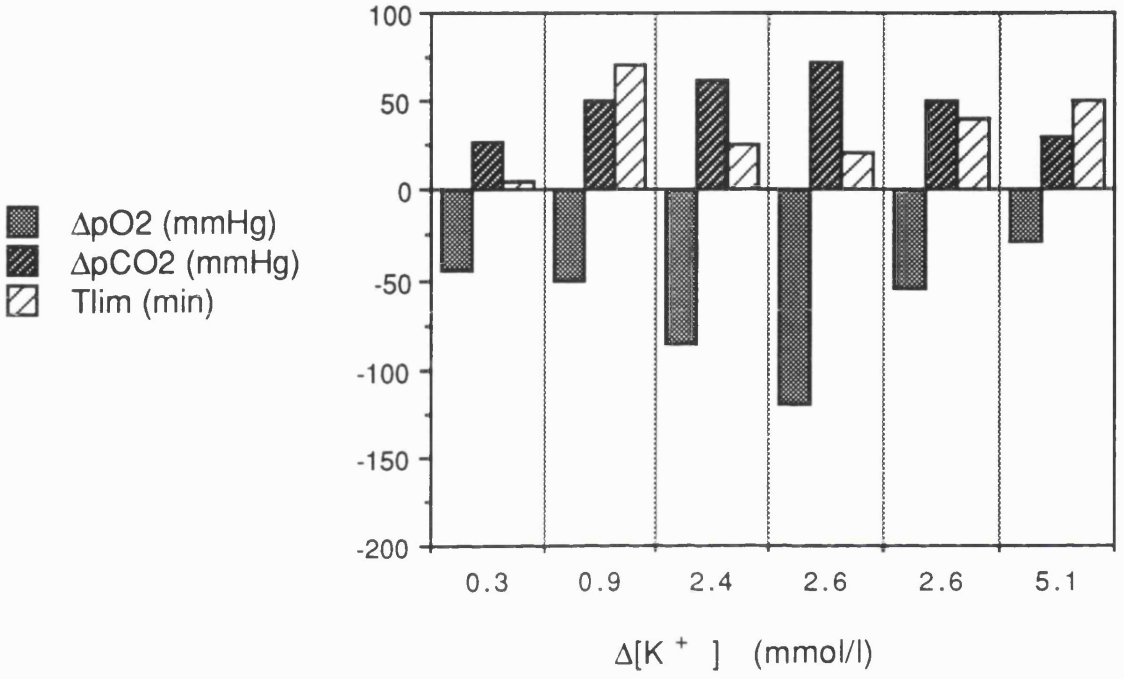


Fig 5.2. Histogram showing how changes in arterial [K^+] relate to changes in Pa_{CO_2}, Pa_{O_2}, and Tlim in each rabbit.

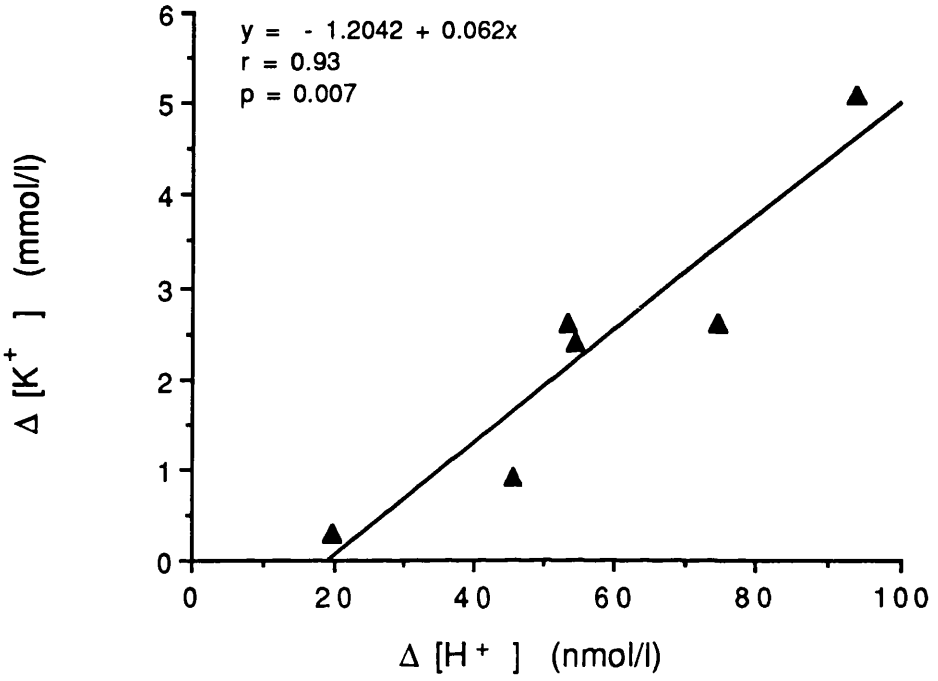


Fig. 5.3. Relationship between changes in arterial potassium ion concentration ($[K^+]$) and hydrogen ion concentration ($[H^+]$) resulting from severe inspiratory resistive loading. Each point represents values measured in one animal.

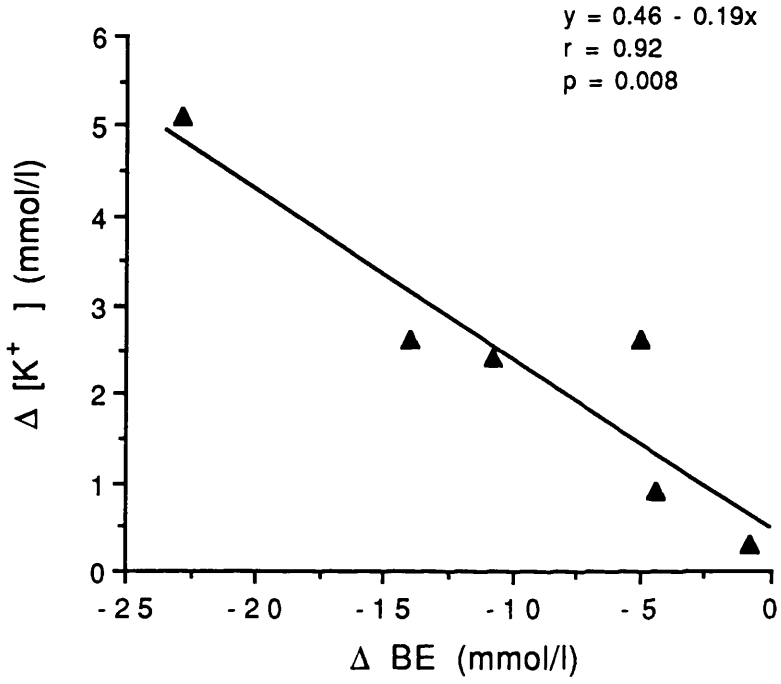


Fig. 5.4. Relationship between changes in arterial potassium concentration ([K^+]) and base-excess ([BE]) resulting from severe inspiratory resistive loading. Each point represents values measured in one animal.

Effect of increased plasma potassium concentration on resting membrane potential (RMP).

The RMP was calculated for each rabbit. The results are shown in Table 5.3.

RABBIT no.	REST [K] (mmol/l)	FATIGUE [K] (mmol/l)	REST RMP (mV)	FATIGUE RMP (mV)	ΔRMP (mV)
76	2,4	2.7	-110.4	-107.3	3.1
245	3.1	4.0	-103.6	-96.8	6.8
247	2.8	5.4	-106.3	-88.8	17.5
267	3.2	8.3	-102.8	-77.3	25.5
28	3.1	5.5	-103.6	-88.3	15.3
1110	2.7	5.3	-107.3	-89.3	18.0
		mean ± SE	-105.7 ± 1.2	-91.2 ± 4.1	14.4 ± 3.3

Table 5.3. A summary of the calculations of resting membrane potential of the diaphragm at rest and overt fatigue. Arterial potassium concentrations ($[K^+]$) were assumed to be equivalent to interstitial $[K^+]$. The intracellular $[K^+]$ was assumed to be 150 mmol/l at rest and fatigue. The effects of $[Na^+]$ on the RMP were ignored.

Discussion

The present study has shown that a fatiguing inspiratory resistive loading can induce a rise in arterial $[K^+]$. This rise in arterial $[K^+]$ was associated with a combined metabolic and respiratory acidosis. The increase in arterial K^+ correlated well with changes in arterial pH and BE. In contrast, changes in blood gas tensions appeared to be unrelated to arterial $[K^+]$.

Effect of hypercapnia and changes in serum bicarbonate levels on diaphragmatic function

Studies have shown that hypercapnia alone can produce a significant reduction in the force of diaphragmatic contraction in isolated rat diaphragm preparations and in

intact dogs (Fitzgerald et al., 1984; Schnader et al., 1985 and 1988). Moreover, Fitzgerald and colleagues showed that even when pH was kept constant, an increase in P_{CO_2} still precipitated a decrease in diaphragmatic force. The level of P_{CO_2} that impaired diaphragm contractility in the above studies ranged from 46 to above 102 mmHg. Arterial P_{CO_2} measured at fatigue in the present study ranged from 55 to 100 mmHg. The severity of hypercapnia induced by IRL in the present study falls within the range of P_{CO_2} known to reduce diaphragmatic contractility. Presumably, therefore, it is possible that increased arterial P_{CO_2} reported in this chapter could have contributed to a decrease in force of diaphragmatic contraction. How hypercapnia induces diaphragmatic contractile depression is not known, but one possible mechanism has been proposed by Lade and colleagues (1963) and Fitzgerald and colleagues (1983). Both groups of researchers showed that hypercapnia could produce a decrease in intracellular $[K^+]$ in limb muscles of man (Lade et al. 1963) and the rat diaphragm (Fitzgerald et al. 1983). Since most of the intracellular K is ionized, a decrease in its concentration would reduce the resting membrane potential. Such a change in RMP is known to reduce the force of contraction of a muscle.

Changes in intracellular $[H^+]$ could also explain the loss of diaphragmatic force of contraction induced by hypercapnia. Fitzgerald and colleagues (1986), studying the rat diaphragm, demonstrated a fall in intracellular pH secondary to hypercapnia using ^{31}P nuclear magnetic resonance technique. The effect of intracellular acidosis on muscle contractility will be outlined later in this discussion

In the present study, no relationship was found between changes in arterial bicarbonate concentration and arterial potassium concentration induced by diaphragmatic fatigue. Plasma bicarbonate rose on average by 1 mmol/l. A possible explanation for the increase in plasma bicarbonate is that increased Pa_{CO_2} yielded an increase in plasma carbonic acid,

which would dissociate into H^+ and HCO_3^-. There is evidence to show that extracellular bicarbonate must be lowered rather than raised to precipitate diaphragm fatigue.

An in vitro study on the rat diaphragm has shown that extracellular bicarbonate needs to be lowered by 17 mmol/l to produce a reduction in diaphragmatic force of contraction (Fitzgerald et al., 1984). Thus the minor changes in bicarbonate levels observed in the rabbit could not have induced diaphragmatic contractile failure.

Effect of hypoxia on diaphragmatic function

In four rabbits, Pa_{O2} ranged from 30 to 38 mmHg. These P_{O2} values have been reported to have reduced diaphragmatic tension in spontaneously breathing pigs (Watchko et al., 1986) and isolated rat diaphragm (Shee & Cameron, 1990). At present, how hypoxia induces contractile failure is not understood. However, it has been demonstrated that contractile failure in isolated rat diaphragm cannot be attributed to hypoxia-induced intracellular acidosis (Shee & Cameron, 1990).

The current view is that the reduction in diaphragmatic force of contraction during severe hypoxia results from a reduction in ATP production. Clearly if hypoxemia occurred during intense diaphragmatic activity, the decreased oxygen supply to the diaphragm and increased diaphragmatic energy demand would precipitate peripheral diaphragmatic fatigue. In support of this hypothesis, it has been shown in humans that the diaphragm, working against a severe IRL, fatigued sooner during hypoxic gas breathing (Jardim et al., 1981).

The reduction in Pa_{O2} observed in this study is severe enough to produce changes in $[H^+]$ (Shee & Cameron, 1990), and perhaps secondary changes in $[K^+]$ in the extracellular compartment. A low Pa_{O2} indicates the present of tissue hypoxia. Tissue hypoxia, induced by ventilatory or

circulatory dysfunction or increased oxygen consumption, stimulates lactic acid production. Plasma lactate concentration and $[H^+]$ are increased as they are released from the active muscles. An increase in extracellular $[K^+]$ may result as extracellular hydrogen ions are exchanged for intracellular potassium ions. Alternatively and probably more likely, an increase extracellular $[K^+]$ could have been achieved by a reduction in Na^+-K^+-adenosine triphosphatase (Na^+-K^+-ATPase) activity, resulting in a net loss of K^+ from active muscles. The activity of Na^+-K^+-ATPase is reduced when insufficient ATP is produced by anaerobic metabolism.

Hypoxia like hypercapnia can stimulate the release of catecholamines from the adrenal glands (Cunningham et al., 1965; Claustre & Peyrin, 1982). Increased levels of catecholamines will increase the oxygen consumption of tissues further exacerbating the severity of tissue hypoxia. Studies have shown that within the first 10 minutes after the injection of noradrenaline, plasma K^+ increases rapidly, followed by a much longer hypokalemic response (Brewer et al., 1939; Todd & Vick, 1971). The initial hyperkalemia results from an α-adrenoreceptor-mediated release of K^+ release from the liver (Craig & Honig, 1963; D'Silva, 1936). In contrast, hypokalemia results from β-adrenoreceptor-mediated net uptake of K^+ primarily by skeletal muscles but probably also by the heart and liver (Craig & Honig, 1963; Clausen, 1986). A reduction in ATP synthesis induced by hypoxia could have prevented an increase in Na^+-K^+-ATPase required for the net uptake of K^+ in muscles and liver, and thus hyperkalemia would be maintained. This explanation for the observed hyperkalemia in the six rabbits, although attractive, must be viewed with caution as catecholamine levels were not measured in this study and therefore it is known not whether they increased during IRL.

Effect of pH on diaphragmatic function

Substantial evidence has accumulated to indicate that intracellular acidosis can produce muscular fatigue (Hermansen & Osnes, 1972; Metzger & Fitts, 1987). The extracellular acidosis in the present study was both metabolic and respiratory in nature. This means that the changes in both Pa_{CO_2} and Pa_{O_2} reported here could have contributed to the changes in plasma pH. In the present study, arterial pH decreased to values of 6.91 to 7.19 at fatigue. For the intracellular pH to have fallen, extracellular pH must have decreased below 7.15 (Heisler, 1975). In five rabbits pH measured at fatigue was lower than 7.15. In these rabbits at least, the $[H^+]$ in the diaphragm should have increased. An increase in intracellular $[H^+]$ may have contributed to the development of diaphragm fatigue in these rabbits.

Possible mechanisms, by which increased intracellular $[H^+]$ may interfere with diaphragmatic force of contraction, will be outlined. Intracellular acidosis inhibits phosphofructokinase activity, resulting in a reduction in ATP synthesis (Trivedi et al., 1966). An increase in intracellular $[H^+]$ can prevent the interaction between contractile proteins. Hydrogen ions have been shown to increase Ca^{2+} binding to the sarcoplasmic reticulum (SR) (Nakamaru & Schwartz, 1972) and inhibit SR adenosine triphosphatase (Inesi & Hill, 1983). Both effects would reduce the availability of Ca^{2+} on excitation. Moreover, H^+ has been shown to compete with Ca^{2+} for the binding site on troponin C (Fuch et al., 1970). Intracellular acidosis may induce a net efflux of K^+ from myocytes. An efflux of K^+ from the diaphragm may result in alteration in its RMP which would reduce its excitability.

Effect of K^+ on RMP and diaphragm contractility

Inspiratory resistive loading in the six rabbits induced a rise in plasma [K^+]. There are several mechanisms that could explain this rise in plasma [K^+] during IRL. These include the following: (1) a shift in water from extracellular to intracellular compartment; (2) hypercapnia and increased lactate production by exercising inspiratory muscles; (3) α-adrenergic stimulation resulting from raised catecholamine levels; (4) an increased number of action potentials generated by exercising inspiratory muscles with incomplete re-uptake of K^+; (5) an inhibition of Na^+-K^+ pump caused by a reduction in available ATP. All or some of these factors may have contributed to the rise in plasma [K^+] observed at fatigue.

To assess whether the changes in arterial K^+ reported here could have altered diaphragmatic RMP, RMP was calculated. The calculation of RMP was based on three assumptions.

(1) Diaphragmatic blood flow can increase substantially during severe IRL (Rochester & Bettini 1976). With a high perfusion rate and rapid diffusion of K^+ from interstitial fluid, venous [K^+] should be close to [K+] in interstitial fluid. Sahlin and Broberg (1989) reported that a femoral venous-artery arterial [K^+] was within 0.2 mmol/l at fatigue. These findings indicate that arterial [K^+] at end of exhaustive exercise is at best representative or at worst an underestimate of interstitial fluid K^+ concentration.

(2) Intracellular [K^+] was assumed to have remained constant at 150 mmol/l. This value for diaphragmatic intracellular [K^+] may have been inappropriate for the preparation used in this study. During IRL intracellular [K^+] would have decreased as K^+ leaves the cells. Therefore, it is likely that intracellular [K^+] at fatigue may have been overestimated. A more accurate picture of the changes in intracellular K^+ concentrations as a consequence of

loading could have been achieved if biopsies from diaphragm were taken before and at fatigue.

(3) The effect of distribution of Na^+ across the membrane on the RMP was ignored. Sjøgaard (1986) has shown during exhaustive exercise of leg muscles in humans that extracellular Na^+ concentration does not change and only increases modestly during recovery. Besides the effect of $[Na^+]$ on diaphragmatic RMP would be negligible as indicated by the Hodgkin & Horowicz equation (see equation 2 in this chapter).

The predicated RMP at rest ranged from -102.8 to -110.4 mV. These values are higher than those measured in the isolated diaphragm of the rat by Creese and colleagues (1958). Their diaphragmatic RMP ranged from -60 mV at the periphery to -80 mV in the centre of the muscle. Esau (1991) measured diaphragmatic RMP in the hamster; her control RMP was -74 mV. In the limb muscles of the rat, RMP was -63 or -64 mV (Sjøgaard, 1986). The probable explanation for disagreement between the predicated RMP and measured RMP reported by others is that intracellular $[K^+]$ was probably overestimated for the calculation of RMP both at rest and at fatigue. Sjøgaard (1986) measured intracellular K^+ of rat extensor digitorium longus (EDL) and soleus (SOL) using microelectrodes. In the fresh muscles, intracellular $[K^+]$ was 131 mmol/l for SOL and 142 mmol/l for EDL. Moreover, he found that intracellular $[K^+]$ decreased by more than 35 mmol/l at fatigue in both EDL and SOL.

The predicted RMP of the diaphragm at fatigue ranged from -77.3 to -91.3 mV. In contrast, RMP measured in fatigued limb and respiratory muscles were around -43 mV (Sjøgaard, 1986; Esau, 1991). Although the predicated values for diaphragm RMP may not be accurate, the estimated changes in RMP induced by hyperkalemia are of a magnitude reported by other researchers. It has been reported that doubling of extracellular $[K^+]$ would depolarize the membrane of a cell

by 18 mV (Medbø & Sejersted, 1990). In Table 5.2, it can be seen that a near doubling of $[K^+]$ at fatigue in experiment 247 increased RMP by an estimated 17.5 mV.

Based on calculations of RMP, the RMP of diaphragm in each rabbit decreased at fatigue. The average decrease in RMP was 14.3 mV. A decrease in RMP of such a magnitude is thought to be able to precipitate muscular fatigue (Sjøgaard, 1986). The largest predicted decrease in diaphragmatic RMP presented in this study was by 25.5 mV. Such a decrease in RMP would surely have hastened the onset of diaphragmatic fatigue in that rabbit. A constant depolarization of the muscle by 20 to 30 mV inactivates 50% of the Na^+ channels (Hodgkin & Huxley, 1952), making the membrane completely inactive. Because interstitial $[K^+]$ was probably higher than that in venous and arterial circulation during IRL, a much larger depolarization of diaphragmatic RMP might have occurred than predicted from $[K^+]$ in the blood.

Conclusion

Inspiratory resistive loading induced a combined metabolic and respiratory acidosis which was associated with a significant rise in arterial potassium concentration. All these changes occurred when diaphragm fatigue was indicated. Theoretically at least, the increased arterial $[K^+]$ was of a magnitude to induce fatigue. Because of the design of this experiment, the effect of K^+ and other blood chemistry variables on diaphragm contractility cannot be separated. In all probability, the effects of hypercapnia, hypoxia, increased K^+ and increased diaphragmatic exertion in response to IRL on diaphragm contractility may be additive or synergistic, and together may have hastened the onset of contractile failure.

This study has not provided any direct evidence to identify the source or sources responsible for K^+ secretion. Since the only muscles probably being fatigued were the

inspiratory muscles, the increase in K^+ could be attributed to K^+ secretion from these muscles. Obviously, further study will be required to test this hypothesis. Because this question was periphery to the thesis, it was not investigated. However, in the subsequent two chapters an assessment is made of the relative contribution made by a metabolic and respiratory acidosis to the rise in arterial K^+ noted during IRL.

CHAPTER 6

EFFECT OF AN ACUTE METABOLIC AND RESPIRATORY ACIDOSIS ON ARTERIAL POTASSIUM CONCENTRATION

Introduction

This study was undertaken to investigate the independent effects of an acute metabolic and an acute respiratory acidosis on potassium secretion. It is anticipated that this investigation would give an insight into how each type of acidosis may have contributed to the rise in arterial $[K^+]$ reported in the previous chapter.

Methods

In this study six male New Zealand white rabbits were anaesthetized with pentobarbitone (60 mg IV) followed by a continuous infusion of ketamine (12.5 mg/kg/hr). Each animal was paralysed with flaxedil (4 mg/kg) and then mechanically ventilated. During the control, the inspired O_2 was increased to 50% O_2 in N_2 to prevent hypoxia, and respiratory rate and tidal volume were adjusted to give Pa_{CO_2} and acid-base values similar to the mean control values reported in the rabbits in Chapter 5.

Hypoxia-induced metabolic acidosis (HMA) and hypoventilation-induced respiratory acidosis (HRA) were compared in each rabbit. The order in which they were administered was varied in case one form of acidosis influenced the response to the other. In three rabbits HRA preceded HMA (Protocol 1) and in two rabbits HMA preceded HRA (Protocol 2). Details of the two protocols are given on subsequent pages. In a sixth rabbit (241), only a respiratory acidosis was induced for 30 minutes with

arterial blood samples taken before and at intervals during acidosis (5, 10, 20 and 30 minutes).

Protocol 1

An arterial blood sample was taken immediately before induction of respiratory acidosis to determine control values for acid-base status, blood gases composition, and $[K^+]$. To induce a respiratory acidosis the tidal volume was halved from 28.8 ± 0.9 ml to 14 ± 0.6 ml while ventilatory frequency was kept constant at 67 ± 1.6 breaths/min. Inspired O_2 was also keep constant at 50% in N_2. The level of ventilation was maintained for 30 minutes while arterial blood samples were taken at five minute intervals for potassium, blood gases and acid-base analysis. Tidal volume was returned to control value after 30 minutes. Arterial blood samples were taken at 5 minute intervals to determine when blood chemistry variables had returned to a control level. Variables were considered to be at a control level when arterial $[K^+]$ was within ± 0.5 mmol/l of the pre-control value. If arterial $[K^+]$ did not return to control levels within 30 minutes, the experiment was stopped. Once an animal had recovered from the effects of a respiratory acidosis, a metabolic acidosis was induced by reducing the inspired O_2 to give an end tidal oxygen of 7% to 9%. This level of end-tidal O_2 was chosen to produce an arterial P_{O_2} similar to that observed at diaphragm fatigue (see Chapter 5). The low inspired O_2 gas mixture was maintained for 30 minutes and once again arterial blood samples for analysis were taken at five minute intervals. For an outline of Protocol 1 refer to Table 6.1.

Protocol 2

Arterial blood samples were taken to establish control values for pH, blood gases, base-excess and arterial $[K^+]$. A metabolic acidosis was induced as outlined in Protocol 1. Arterial blood samples were taken at 5 minute intervals for analysis during acidosis, and then at 5 minute intervals during recovery. Variables were considered to be at a control level when arterial $[K^+]$ was within ± 0.5 mmol/l of the pre-control value. If arterial $[K^+]$ did not return to control levels within 30 minutes, the experiment was stopped. A respiratory acidosis was produced as described in Protocol 1 once a new control level for $[K^+]$ was established. During respiratory acidosis blood samples were withdrawn at intervals of five minutes for analysis. For an outline of Protocol 2 refer to Table 6.1.

Table 6.1.

Protocols to determine the contribution made by an acute respiratory acidosis and an acute metabolic acidosis to an increase in arterial potassium concentration ($[K^+]$). HMA denotes hypoxia-induced metabolic acidosis and HRA, respiratory acidosis. SV represents spontaneous ventilation and MV, mechanical ventilation.

Protocol 1

Condition		Control 1	HRA	Control 2	HMA
Manoeuvre	SV +50% O_2	MV +50% O_2 to give ET^{CO_2} 4% ±0.5%	1/2MV +50% O_2	MV +50% O_2 to give ET^{CO_2} 4%±0.5%	MV +<21% O_2 to give ET^{O_2} 8% ±1%
Duration			30 min.	K^+ ±0.5 mmol/l of control 1	30 min.
K^+ analysis	at least 1	at least 2	5 min-intervals	5 min-intervals	5 min-intervals

Protocol 2

Condition		Control 1	HMA.	Control 2	HRA
Manoeuvre	SV +50% O_2	MV +50% O_2 to give ET^{CO_2} 4%±0.5%	MV +<21% O_2 to give ET^{O_2} 8% ±1%	MV +50% O_2 to give ET^{CO_2} 4%±0.5%	1/2MV +50% O_2
Duration			30 min.	K^+ ±0.5 mmol/l of control 1	30 min.
K^+ analysis	at least 1	at least 2	5 min-intervals	5 min-intervals	5 min-intervals

Control experiment

Two of the six rabbits were mechanically ventilated with 50% O_2 in N_2 for 30 minutes at a tidal volume of 28 ml and rate of 60 breaths/min. Arterial blood samples were taken for measurements of pH, actual base-excess, blood gas tensions, and $[K^+]$ at ten minute intervals. This experiment was carried out to determine whether there was any time-dependent variation in arterial acid-base status, blood gas tensions, or $[K^+]$ with mechanical ventilation in the absence of hypoventilation or hypoxia. The control experiments were performed before the induction of HMA and HRA.

Analysis of data

For HMA and HRA data for analysis were obtained from four and five rabbits respectively. Data from both protocols were pooled to assess the mean effects of HMA and HRA on arterial acid-base, blood gases, and $[K^+]$. All data were considered when averaging changes in variables. All results are expressed as means ± SE. Changes in variables were assessed by analysis of variance. In addition, the correlation between HMA and HRA data was analyzed by linear regression using the method of least squares. For simple linear regression analysis, pH values were converted to $[H^+]$ as described in Chapter 5. A change in a variable and the slope of the regression line were deemed significant at $P < 0.05$ level.

Results

Control arterial blood variables before induction of a metabolic and respiratory acidosis

There was no significant difference in mean control pH between HMA and HRA (7.41 ± 0.04 units vs 7.40 ± 0.02 units respectively). The control arterial [K^+] for HMA was 2.4 ± 0.03 mmol/l whereas the control value for HRA was 2.9 ± 0.5 mmol/l. This difference was not statistically significant. No significant difference was found in values of either arterial P_{CO_2} (HMA, 27.0 ± 1.3 mmHg vs HRA, 29.8 ± 1.0) or base-excess (HMA, -5.78 ± 1.7 mmol/l vs HRA, -4.52 ± 0.7 mmol/l).

Effect of an acute metabolic and respiratory acidosis on arterial acid-base, blood gas tensions, and potassium

The effect of HMA on arterial blood chemistry is summarized in Table 6.2. During HMA, arterial pH decreased steadily. Arterial P_{CO_2} remained fairly constant whereas Pa_{O_2} decreased substantially after five minutes of ventilation with a hypoxic gas mixture before stabilizing. Arterial [K^+] increased steadily before peaking at 20 minutes. Base-excess decreased sharply after five minutes and then continued to decrease at a reduced rate. All variables except Pa_{CO_2} and Pa_{O_2} were statistically different from control throughout. The change in Pa_{O_2} was significant after 10 minutes whereas Pa_{CO_2} changed insignificantly.

The effect of HRA on arterial blood chemistry is summarized in Table 6.3. During the first 20 minutes of HRA, arterial pH decreased progressively before stabilizing. Arterial P_{CO_2} almost doubled after five minutes of HRA, and then continued to increase at a slower rate. Both arterial pH and P_{CO_2} were significantly altered throughout HRA. Base-excess was significantly different from 5 minutes onwards. In contrast, Pa_{O_2}, and K^+ were not significantly altered. Arterial P_{O_2} remained relatively constant whereas $[K^+]$ fluctuated.

Time (min)	pH (units)	Pa_{CO2} (mmHg)	Pa_{O2} (mmHg)	K^+ (mmol/l)	Base-excess (mmol/l)
0	7.41	27.0	307.8	2.4	-5.8
	(0.03)	(1.3)	(76.6)	(0.1)	(1.7)
5	7.36	24.6	43.7	3.1	-10.4
	(0.02)	(1.9)	(2.3)	(0.2)	(1.3)
10	7.30	24.3	42.3	3.2	-12.6
	(0.01)	(1.9)	(2.6)	(0.2)	(1.2)
15	7.27	26.0	41.7	3.5	-14.2
	(0.00)	(1.8)	(3.2)	(0.2)	(0.5)
20	7.22	25.7	39.9	3.6	-15.9
	(0.02)	(1.9)	(3.6)	(0.2)	(0.7)
25	7.18	23.8	41.5	3.4	-18.5
	(0.03)	(2.6)	(3.4)	(0.2)	(0.2)
30	7.14	22.9	42.3	3.6	-20.1
	(0.02)	(2.9)	(4.2)	(0.2)	(0.5)

Table 6.2. The effect of hypoxia-induced metabolic acidosis on arterial blood variables. Results are expressed as means of four rabbits with SE in brackets.

Time (min)	pH (units)	Pa_{CO2} (mmHg)	Pa_{O2} (mmHg)	K^+ (mmol/l)	Base-excess (mmol/l)
0	7.40	29.8	326.5	2.7	-4.5
	(0.02)	(1.0)	(51.6)	(0.3)	(0.7)
5	7.19	59.0	339.5	2.6	-6.7
	(0.01)	(1.6)	(43.5)	(0.3)	(0.5)
10	7.22	66.6	348.0	2.8	-7.6
	(0.08)	(3.5)	(53.2)	(0.4)	(0.7)
15	7.11	73.3	345.5	2.8	-7.6
	(0.01)	(3.5)	(66.8)	(0.5)	(0.6)
20	7.09	74.4	329.3	2.8	-8.8
	(0.02)	(5.9)	(57.6)	(0.3)	(1.4)
25	7.09	79.6	352.3	3.0	-8.2
	(0.02)	(8.1)	(87.4)	(0.7)	(1.5)
30	7.09	78.3	299.2	3.2	-8.7
	(0.02)	(8.2)	(44.8)	(0.4)	(1.4)

Table 6.3. The effect of hypoventilation-induced respiratory acidosis on arterial blood variables. Results are expressed as means of five rabbits with SE in brackets.

Relationship between [H+] and determinants of acid-base balance

During HMA, there was a strong negative and highly significant correlation between [H+] and base-excess (see Fig. 6.1). However, there was no correlation between [H+] and Pa_{CO_2} (r = 0.008, P = 0.969) during HMA. During HRA, there was a highly significant positive correlation between arterial [H+] and Pa_{CO_2} (see Fig. 6.2). There was also a significant correlation between [H+] and base-excess during HRA (r = 0.61, P = 0.004)

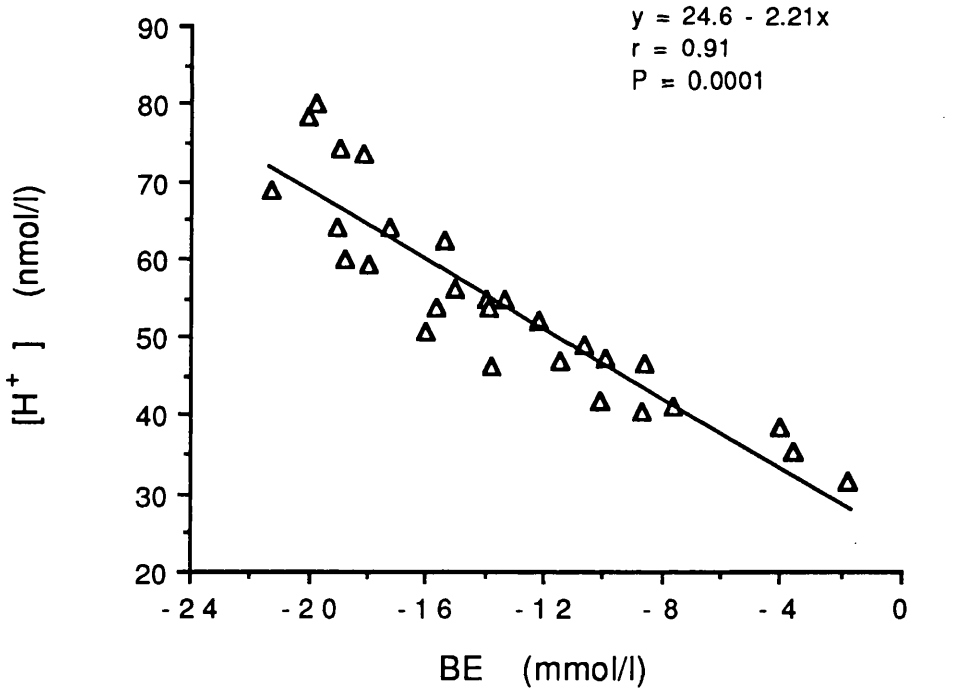


Fig. 6.1. Relationship between arterial [H+] and base-excess (BE) during an acute metabolic acidosis. All control measurements and measurements obtained during hypoxia from four rabbits are presented.

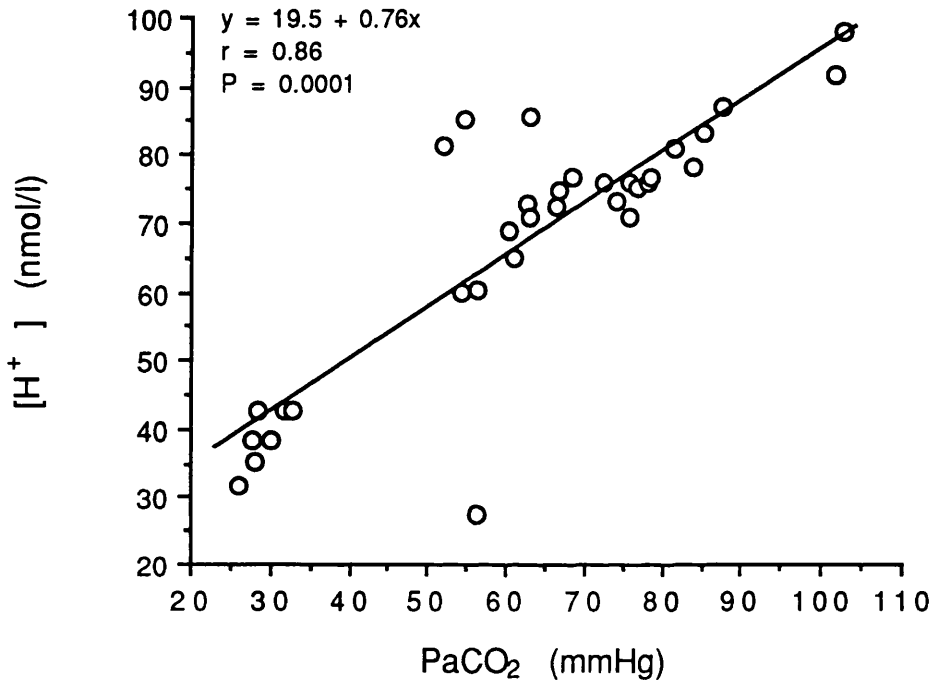


Fig 6.2. Relationship between arterial $[H^+]$ and Pa_{CO_2} during an acute respiratory acidosis. All control measurements, made during normocapnia, and measurements made during hypercapnia from five rabbits are presented.

Comparison of the effects of HMA and HRA on arterial potassium

Arterial [K^+] rose steadily during HMA whereas it fluctuated during HRA as shown in Fig. 6.3. The peak rise in [K^+] produced by HMA occurred 20 minutes into hypoxic gas breathing and the peak rise produced by HRA occurred earlier at 10 minutes of HRA. The maximum rise in arterial [K^+] induced by a HMA averaged 1.3 mmol/l whereas the maximum rise in [K^+] induced by a HRA averaged only 0.3 mmol/l. Interestingly, mean arterial [K^+] fell below control twice during a HRA.

HRA produced a greater reduction in arterial pH than a HMA. Arterial pH decreased by an average of 0.31 units for HRA while it decreased by an average of 0.27 units for HMA. Despite having a smaller reduction in arterial pH, HMA produced a mean increase in arterial [K^+] of 1.2 mmol/l which was significantly greater than an average rise in arterial K^+ of 0.2 mmol/l produced by HRA.

In addition, there was a highly significant positive correlation between arterial [K^+] and arterial [H^+] for HMA (see Fig. 6.4). In contrast, this was not the case for HRA (see Fig. 6.5).

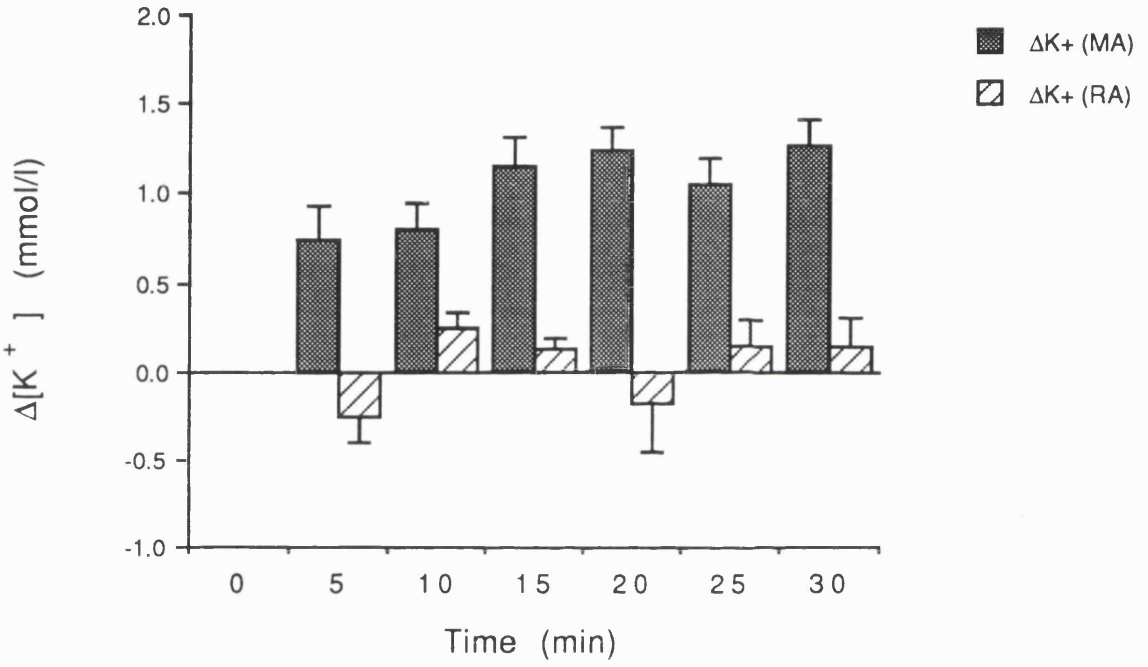


Fig. 6.3. The time course of changes in arterial [K^+] (ΔK^+) produced by a metabolic (MA) and respiratory acidosis (RA). The values are means changes ± SE of control value. The MA represents data from four rabbits and the RA from five rabbits. Time 0 minutes is control.

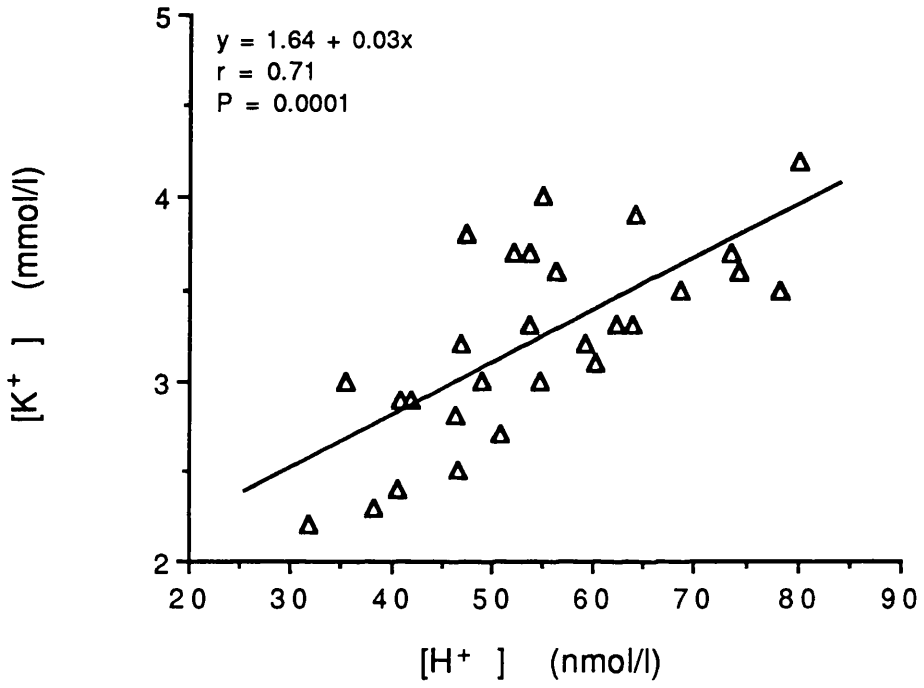


Fig. 6.4. Relationship between arterial [H^+] and [K^+] during an acute metabolic acidosis. Both control values and those measured during a metabolic acidosis for four rabbits are presented.

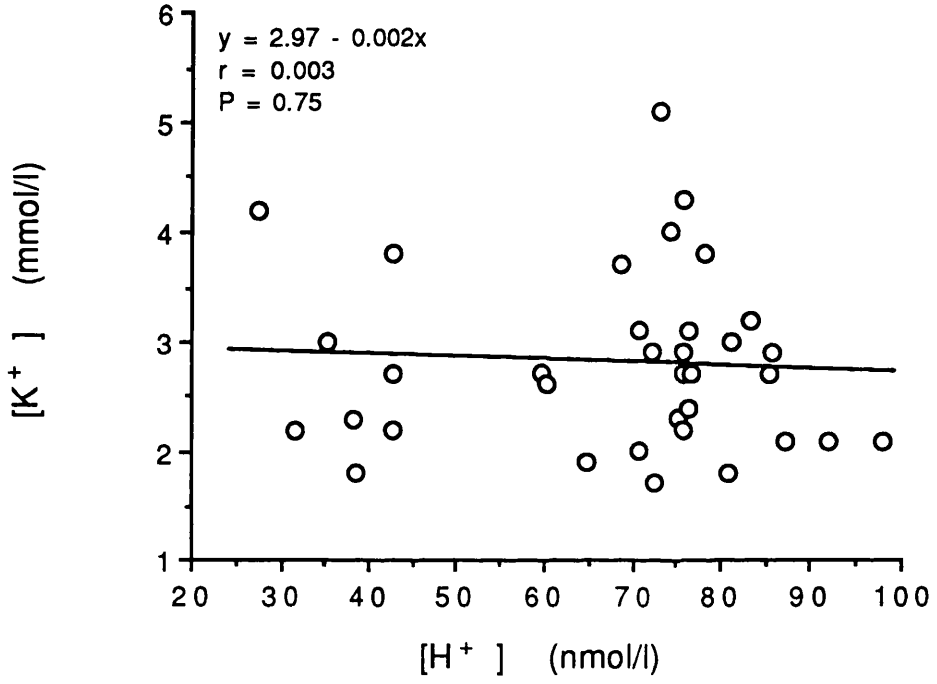


Fig. 6.5. Relationship between arterial [H^+] and [K^+] during an acute respiratory acidosis. Both control values and those measured during a respiratory acidosis for five rabbits are presented.

Control experiment

During the control study, arterial pH decreased by 0.04 units at 30 minutes. The changes in the other variables showed no obvious trends. Both arterial P_{CO_2} and base-excess were lower by 1.4 mmHg and 2.9 mmol/l respectively after 30 minutes of mechanical ventilation; whereas Pa_{O_2} was higher by 19.1 mmHg. Arterial [K^+] remained constant. None of the measured blood chemistry variables changed significantly during the 30 minutes of mechanical ventilation. The results of the control study are summarized in Table 6.3.

Time (min)	pH (units)	Pa_{CO2} (mmHg)	Pa_{O2} (mmHg)	K^+ (mmol/l)	Base-excess (mmol/l)
0	7.44 (0.01)	26.2 (0.4)	328.7 (21.7)	2.5 (0.1)	-5.0 (0.6)
10	7.41 (0.01)	25.1 (4.3)	310.9 (9.9)	2.5 (0.3)	-7.00 (3.2)
20	7.39 (0.02)	25.8 (0.7)	340.5 (12.5)	2.6 (0.2)	-6.85 (1.7)
30	7.40 (0.01)	24.8 (0.6)	347.8 (20.7)	2.5 (0.1)	-7.90 (0.8)

Table 6.3. Arterial pH, K^+ and blood gases in two animals during 30 minutes of continuous mechanical ventilation with 50% O_2. Results are presented as means with SE in brackets.

Discussion

Both HRA and HMA produced changes in arterial blood acid-base composition and [K^+]. HRA was characterized by hypercapnia, low pH, and a modest rise in arterial [K^+]. In contrast, HMA was associated with a low pH, low base-excess, and a substantial rise in arterial [K^+]. For HMA, arterial [K^+] was related to the severity of acidosis. This was not the case for HRA.

Control experiment

The control study indicates that there were no time dependent changes in arterial acid-base status, blood gas tensions, or [K^+] associated with mechanical ventilation in the absence of hypoventilation or hypoxia. It is worthy of note that arterial [K^+] was not influenced by administration of flaxedil. Any changes observed during HMA and HRA were therefore due to these interventions.

Effect of HRA and HMA on K^+ release

Despite only a small difference in pH between HMA and HRA, the average rise in arterial [K^+] produced by HMA was six times that produced by HRA. The body of evidence available indicates that the rise in [K^+] per change in pH during an acute respiratory acidosis is lower than that during mineral acid acidosis but higher than that during organic acid acidosis (Adrogue & Madias, 1981). In this study, a metabolic acidosis was probably induced because of increased production of organic acids. Therefore, the rise in arterial [K^+] during HRA should have been greater than a rise in arterial [K^+] induced by HMA.

Although lactic acid concentrations were not measured in the present study, it is highly probable that the production of this acid increased in response to tissue hypoxia. A number

of studies have shown that infusion of lactic acid does not raise the plasma potassium concentration (Oster et al., 1978; Oster et al., 1980; Tobin, 1958). In view of these studies, the progressive increase in arterial potassium observed during HMA was probably not induced by increased lactic acid concentrations alone.

In previous studies, an organic metabolic acidosis was induced by infusion of lactic acid, acetic acid, or β-hydroxy-butyric acid (Oster et al., 1978; Oster et al., 1980; Tobin, 1958). In contrast, a metabolic acidosis was induced by hypoxia in this study. There is evidence to show that hypoxia can produce an increase in plasma [K^+] (Estavillo et al., 1988). Estavillo and colleagues reported that in artificially ventilated cats a reduction in end-tidal O_2 to around 40 mmHg produced an increase in arterial K^+ by as much as 2 mmol/l. During HMA, Pa_{O_2} averaged 40 mmHg and arterial [K^+] increased on average by 1.2 mmol/l. They did not report the effect hypoxia had on the acid-base status of their cats. However, it is highly probable that a metabolic acidosis was induced.

Both this study and the investigation by Estavillo and colleagues (1988) have indicated that severe hypoxia may induce an appreciably increase in arterial [K^+] in the rabbit and the cat respectively. The mechanisms responsible for hypoxia-induced hyperkalemia have not been elucidated. However, possible mechanisms might involve increased production of metabolic acids induced by tissue hypoxia or hypoxia-induced release of catecholamines from the adrenals. Both mechanisms could increase plasma [K^+]. But the hyperkalemic response to organic metabolic acidosis is at best small (Oster et al., 1980; Tobin, 1958) and that induced by increased catecholamine levels is short lived less than 10 minutes (Brewer et al., 1939; Todd & Vick, 1971). It would appear, therefore, that other mechanisms may have been responsible for hyperkalemic response to hypoxia.

The consensus is that an acute respiratory acidosis results in an increase in plasma potassium concentration, and evidence in support of this view is presented in an excellent review by Adrogue and Madias (1981). A number of studies have reported no increase in extracellular [K^+] during a respiratory acidosis (Kilburn, 1965; Poole-Wilson & Cameron, 1975). Interestingly, in the present study, arterial [K^+] fell below control value twice during HRA.

It is not certain whether the second hypokalemic response was a real phenomenon as it only occurred in two rabbits with the decrease in arterial [K^+] in one rabbit being within the error of measurement of K^+. However, the initial hypokalemic response is probably a real phenomenon as it occurred in four of the five rabbits. In two of the four rabbits, arterial [K^+] decreased by more than 0.1 mmol/l. Furthermore, the control study indicates that changes in arterial [K^+] which occurred during HRA are a consequence of this intervention, and Hughes and colleagues (1990) have also observed a significant hypokalemic response after 5 minutes of HRA in the cat.

This present study and that of Hughes differed from others in terms of the method used to induce a respiratory acidosis and the time that plasma K^+ was first measured during a respiratory acidosis. In other studies, respiratory acidosis was produced by getting the subject or animal to breathe CO_2-enriched gases and the first blood sample was not taken until 10 minutes to an hour following the start of CO_2 loading (Adrogue & Madias, 1981; Burnell et al., 1956; Bracket et al., 1965; Held et al., 1971; Javaheri et al., 1989). Why the methods of CO_2 loading and mechanical hypoventilation should have produced different responses cannot be readily explained. The hypokalemic response may have been missed by other researchers because they did not measure plasma K^+ within the first five minutes of CO_2 loading. This explanation is supported by the results in this study where plasma [K^+] generally increased after 5

minutes of HRA and that of Hughes and colleagues who also noted a rise in arterial K^+ when HRA was continued beyond 5 minutes (unpublished results).

In conclusion, hypoventilation-induced respiratory acidosis produced a more substantial decrease in arterial pH than hypoxia-induced metabolic acidosis. However, HRA resulted on average in a modest and insignificant rise in arterial [K^+] compared to a significant rise in arterial [K^+] produced by a HMA. There was a strong positive correlation between arterial [H^+] and [K^+] during an acute metabolic acidosis. This is in contrast with a poor correlation between arterial [H^+] and [K^+] during an acute respiratory acidosis.

All the evidence provided by this study indicates that the metabolic component of the acidosis may have contributed substantially more than the respiratory component of the acidosis to the rise in arterial [K^+] reported in the previous chapter. The next chapter will investigate: (1) whether a simple arithmetic sum of the response to a metabolic acidosis and a respiratory acidosis can completely account for the rise in arterial [K^+] observed during IRL (see Chapter 5); (2) whether interaction between a metabolic acidosis and a respiratory acidosis is more complex; (3) whether a third factor, such as release of K^+ from active muscles, may have also contributed to that rise.

CHAPTER 7

COMPARISON OF THE EFFECT OF A METABOLIC AND RESPIRATORY ACIDOSIS, AND INSPIRATORY RESISTIVE LOADING ON POTASSIUM RELEASE

Introduction

It has been well documented that exercising limb muscles release potassium (Hnik et al., 1976; Hirche et al., 1980; Linton et al, 1984, Sjøgaard, 1986). At present no information is available about whether inspiratory muscles, in particular diaphragm, release K^+ during inspiratory resistive loaded breathing. Unlike limb exercise, exercising the diaphragm by applying an inspiratory load to breathing invariably produces a systemic hypoxemia and hypercapnemia resulting in an acidosis (Chapter 5). These changes in blood acid-base status during IRL could induce hyperkalemia. To determine the effect of hypoxia and hypercapnia-induced acidosis on arterial $[K^+]$, the experiments in Chapter 6 were carried out. The results of those experiments indicate that hypercapnia-induced acidosis had minimal effect upon arterial $[K^+]$ whereas hypoxia-induced acidosis caused a significant rise in arterial $[K^+]$. In this chapter, the results from the previous two chapters will be compared in order to determine the relative contribution made by a metabolic and a respiratory acidosis to the rise in arterial $[K^+]$ observed during inspiratory resistive loaded breathing, thereby estimate the possible contribution made by the loaded inspiratory muscles.

Analysis

The results obtained in Chapters 5 and 6 were used to compare the effects of inspiratory resistive loading-induced acidosis (IRLA) with hypoxia-induced metabolic acidosis (HMA) and hypoventilation-induced respiratory acidosis (HRA) on arterial [K^+]. Sometimes, the methods employed to induce an acidosis in the three groups of rabbits will be referred to as treatments.

Comparison of blood chemistry between IRLA, HMA, and HRA

Control values of arterial pH, Pa_{CO_2}, Pa_{O_2}, base-excess, and [K^+] were averaged for each of the three treatments. For HMA and HRA, peak arterial [K^+] was averaged and values for arterial pH, Pa_{CO_2}, Pa_{O_2}, base-excess coinciding with peak [K^+] were also averaged. In the case of IRLA, the measurements of the these variables at fatigue were averaged. In two experiments, serial samples of arterial blood were taken at 5 minute intervals throughout IRL. In these experiments, arterial [K^+] peaked when the diaphragm fatigued. In view of this finding, it is probable that arterial [K^+] at fatigue represents the peak value for the other rabbits.

Estimating potassium secretion

To allow a fair comparison of the rate of potassium released by the three treatments, only rabbits whose arterial pH did not decrease by more than 0.4 units during IRLA, HMA or HRA were selected for the analysis. Peak arterial [K^+] values for HMA and HRA while values measured at fatigue for IRLA were used to estimate the rate of potassium release. To make allowances for differences in body weight, and duration of the treatment, potassium released by active muscles or the liver or both will be expressed as μmol/min/kg.

To do this, all animals were weighed and total plasma volume of the rabbit was assumed to be 50 ml/kg of body weight (Courtice, 1943). The net gain or loss of arterial $[K^+]$ was determined by subtracting arterial $[K^+]$ measured before treatment ($[K^+]_{PT}$) from that measured during treatment ($[K^+]_T$). This value was multiplied by total plasma volume to give the net change in plasma $[K^+]$. The net gain or loss of arterial potassium was expressed as potassium change per total body wet weight (wwt). This was done because it was not clear which muscles were releasing or taking up K^+. The rate of change in net plasma $[K^+]$ was calculated by dividing the duration of the treatment into net change in plasma K^+. This result was then divided by body wet weight to relate the net change in plasma $[K^+]$ to total body muscle mass, i.e. body size.

The formula used to calculate the rate of change of plasma $[K^+]$ per total body wet weight is as follows:

$$\frac{50 \times wwt \times ([K^+]_T - [K^+]_{PT})}{wwt \times t}$$

The rate of change of plasma $[K^+]$ per total body weighy was then averaged for each treatment.

Unless otherwise stated, the means of the different treatments were compared by a one factor analysis of variance (ANOVA) to assess whether there was a difference between them. A post-hoc Scheffé F-test was performed to identify which treatments were different (Hicks, 1990). A difference between treatments was deemed significant when $P < 0.05$.

Results

Comparison of control arterial blood variables of IRLA with HMA, and HRA

HMA versus IRLA controls

There was no significant difference in mean control pH between IRLA and HMA (7.45 ± 0.04 units vs 7.41 ± 0.04 units). Similarly, there was no significant difference in control arterial P_{CO2} (IRLA, 28 ± 2.2 mmHg; HMA, 27 ± 1.27 mmHg), or base-excess measurements (HMA, -5.78 ± 1.72; IRLA, -2.8 ± 1.8). The only variable that showed any significant difference was arterial potassium which was significantly higher (P = 0.003) for IRLA (2.88 ± 0.12 mmol/l) than HMA (2.35 ± 0.03 mmol/l).

HRA versus IRLA controls

The control pH values were significantly different (HRA, 7.40 ± 0.02 units; IRLA 7.45 ± 0.04 units; P = 0.031). The control arterial K^+ for HRA (2.9 ± 0.5) was not significantly different from that of IRLA. No significant difference was found in values of either arterial P_{CO2} (HRA, 29.8 ± 1.0 vs IRLA, 28.0 ± 2.2 mmHg) or base-excess (HRA, -4.52 ± 0.7 mmol/l vs IRLA, -2.8 ± 1.8 mmol/l).

Comparison of acid-base status and blood gas tensions between IRLA, HMA, and HRA

IRLA produced the biggest fall in pH value followed by HRA then HMA (see Table 7.1). However, the differences in pH between the treatments were not significant. HRA and IRLA produced comparable degrees of hypercapnia. In contrast, HMA was associated with hypocapnia. Arterial P_{O2} values for IRLA and HMA were not significantly different, both were less than 50 mmHg. For HRA Pa_{O2} was six times greater. Base-excess measurements for IRLA and HRA were not significantly different. For HMA was significantly lower.

	pH (units)	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	Base-excess (mmol/l)
IRLA	7.04 ± 0.04	76.0 ± 7.5	46.1 ± 9.1	-11.8 ± 2.3
HMA	7.14 ± 0.02	22.9 ± 2.9	42.3 ± 4.2	-20.1 ± 0.5
HRA	7.07 ± 0.02	86.4 ± 3.9	308.2 ± 69.8	-7.8 ± 1.0

Table 7.1. Comparison of the effects of IRLA, HMA, and HRA on arterial blood acid-base and blood gas tensions. Measurements for IRLA were made at fatigue (n = 6). Those for HMA (n = 4) and HRA (n = 5) were made at peak arterial potassium concentration.

Comparison of changes in arterial potassium

The peak values for arterial [K^+] produced by HMA and HRA were 1.27 ± 0.14 mmol/l (n = 4) and 0.43 ± 0.07 (n = 5) mmol/l respectively. In contrast, the rise in arterial [K^+] at diaphragm fatigue in response to IRLA was 2.31 ± 0.68 mmol/l (n = 6). A significant difference in the rise of arterial [K^+] was found between the following: HMA vs HRA, $P < 0.001$; IRLA vs HRA, $P < 0.05$. These results are graphically presented in Fig. 7.1.

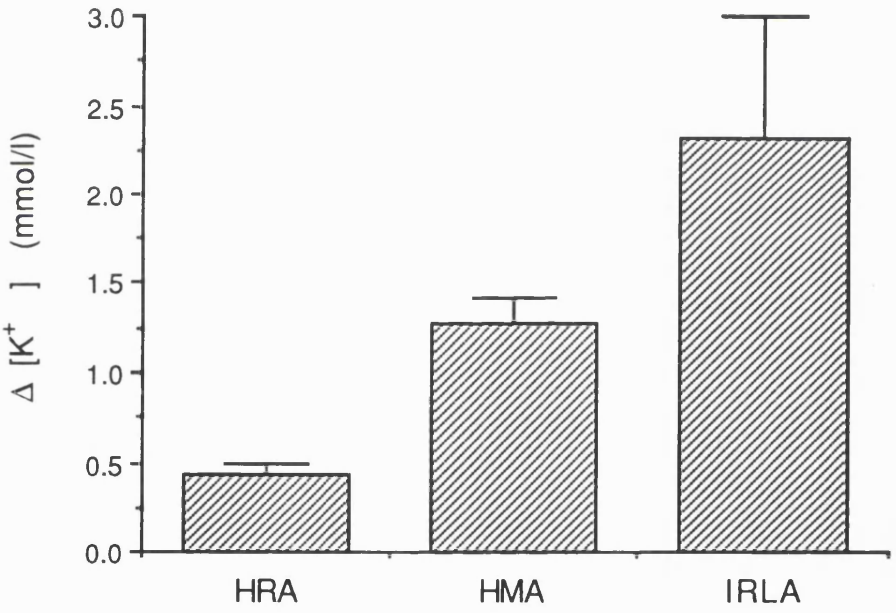


Fig. 7.1. Effect of hypoventilation-induced respiratory acidosis (HRA), hypoxia-induced metabolic acidosis (HMA) and inspiratory resistive loading-induced acidosis (IRLA) on arterial [K^+]. The results for a metabolic and respiratory acidosis are mean peak values ± SE in 4, and 5 rabbits respectively. Those for IRLA are means of arterial [K^+] measured at diaphragm fatigue ± SE in 6 rabbits.

Excluding data where pH changes were greater than 0.40 units, the average increase in [K^+] was reduced to 1.27 ±

0.70 mmol/l and 0.43 ± 0.07 for IRLA (n = 3) and HRA (n = 3) respectively. The average increase in [K^+] remained the same at 1.27 ± 0.14 mmol/l for HMA. An unpaired t-test revealed that the difference existed between HMA and HRA, and between HRA and IRLA.

Comparison of weight of rabbits and duration of treatments

The weights of the three groups of rabbits were similar; 2.78 ± 0.21 kg for IRL group, 2.70 ± 0.35 kg for HMA group and 2.99 ± 0.08 kg for HRA group. The average time at which arterial blood [K^+] measurements peaked during HMA and HRA were 30 minutes and 28.3 ± 1.67 minutes respectively. The average endurance of the diaphragm to inspiratory resistive loading (IRL) for the three rabbits was 34.2 ± 9.26 minutes. Difference in weight and duration between groups were not statistically significant.

Estimate of potassium secretion

The rate of rise in arterial [K^+] induced by IRLA was 1.95 ± 0.58 μmol/kg/min for three rabbits. By contrast, it was 2.12 ± 0.45 μmol/kg/min and 0.76 ± 0.13 μmol/kg/min for HMA (n = 4) and HRA (n = 3) respectively. An ANOVA showed no significant difference between each treatment. However, an unpaired t-test indicated a difference between HMA and HRA.

Discussion

The severity of the acidosis produced by IRLA, HMA, and HRA was similar. Furthermore the degree of hypoxia and hypercapnia observed during IRLA was very much the same as peak changes observed during HMA and HRA respectively. The mean rise in arterial [K^+] during IRLA was higher than would have been predicted from the sum of the rise in arterial [K^+] resulting from HMA and HRA (HMA + HRA = 1.1 vs IRLA =

2.3 mmol/l). However, when data from rabbits with pH changes greater than 0.40 units were excluded, the rise in arterial [K^+] produced by IRLA was less than the sum of the rise in [K^+] produced by HMA and HRA.

By taking the magnitude of pH fall into consideration, four rabbits were eliminated from the analysis. In case of IRLA, the three observations used in this analysis might not have been representative of the effects of IRLA on arterial [K^+] and thus the contribution of exercise-induced K^+ release to the rise in plasma K^+ may not have been expressed in the results.

The hyperkalemic response observed during IRLA, HMA and HRA may have resulted from release of K^+ not only from active muscles but also from other sources. Increased arterial [K^+] from haemolyzis is improbable because there was no overt evidence of the presence of haemoglobin in blood samples and because measured arterial [K^+] remained constant during the control experiments. Release of K^+ from erythrocytes in response to changes in pH, Pa_{CO_2} and Pa_{O_2} is also unlikely to have been responsible for hyperkalemic responses reported in this chapter. During an acute respiratory acidosis in humans, Kilburn (1965) found that K^+ content in erythrocytes remained relatively constant. Rolett and colleagues (1990) measuring K^+ content of erythrocytes, also, found that it remained constant during limb exercise.

In this thesis, cell damage as a result of surgery or IRL can be ruled out as a cause of the hyperkalemia. During the control periods, arterial [K^+] remained fairly steady not changing by more than 0.1 mmol/l. Furthermore, increased arterial [K^+] observed during IRLA, should have been maintained following stopping inspiratory resistive loading but arterial [K^+] decreased to control levels after inspiratory resistive loaded breathing

Undoubtedly, the liver could have contributed to the rise in arterial [K^+] observed in IRLA and HRA. It has been shown that hypercapnia and asphyxia can induce release of K^+ from this organ as a consequence of activation of sympathetic efferent and increased catecholamine output from the adrenals (Fenn & Asano, 1956). Since the results presented in this chapter showed that HRA produced a rise in arterial [K^+] by no more than 0.5 mmol/l, it could be argued that only a fifth of the total rise in arterial [K^+] observed during IRLA, averaging 2.3 mmol/l, can be attributed to K^+ release from the liver.

On the basis of the discussion so far, the probable source of K^+ release was the skeletal muscles which include the inspiratory muscles. For both HMA and HRA, the net efflux of K^+ from these muscles cannot be attributed to contraction of muscles as all skeletal muscles were paralysed, and therefore could be attributed to an exchange of extracellular H^+ for intracellular K^+. However, it is not totally clear whether the release of K^+ from skeletal muscles during IRLA occurred as result of an exchange of extracellular H^+ for intracellular K^+ or increased neuromuscular activity.

Based on experimental evidence, it is clear that a net efflux of [K^+] from exercising limb muscles is responsible for the rise in plasma [K^+] during exercise (Hnik et al., 1976; Hirche et al., 1980; Linton et al, 1984, Sjøgaard G, 1986, Medbø & Sejersted, 1990). Two studies showed while exercising leg released K^+, the contralateral resting leg would take up K^+ (Sjøgaard, 1986; Rolett et al., 1990). In view of this observation, it could be argued that the only muscles that could have lost K^+ during IRLA experiments presented in this thesis were the loaded inspiratory muscles as all other muscle groups were inactive. Electrical stimulation of phrenico-diaphragm preparation has been shown to induce a substantial movement of K^+ out of the diaphragm (Lade & Brown, 1963). This indicates that, like other

skeletal muscles, the diaphragm may release K^+ during intense diaphragm activity such as severe inspiratory resistive loading.

In conclusion, this chapter present evidence that indicates that hyperkalemia observed during IRLA may be due to the additive effect of an acute metabolic and respiratory acidoses. Although the contribution of the exercising inspiratory muscles to the rise in arterial $[K^+]$ was not demonstrated, the possibility that exercising muscles did contribute cannot be ignored. The source of the increased plasma $[K^+]$ cannot be identified with confidence. However, regardless of the source of K^+ release, the studies presented in the last three chapters have indicated that potassium and hydrogen ions could be potential stimuli of diaphragmatic chemoreceptors.

Potassium and hydrogen have been shown to activate group IV afferents in nerves arising from limb skeletal muscles (Rybicki et al., 1985; Kaufman & Rybicki, 1987). Sjøgaard (1990) has proposed that K^+ release from exercising muscles may initiate reflexes that would control the pattern of contraction of the muscle. Since the diaphragm is a skeletal muscle and plenty of small afferents arising from it, the increased extracellular K^+ observed during inspiratory resistive loading may have excited type III and IV diaphragmatic receptors, initiating a reflex, which regulated the activity of the diaphragm, and in so doing influenced ventilation. This hypothesis is tested in Chapters 9 and 10.

NEURAL FACTORS RELATED TO DIAPHRAGM FATIGUE

CHAPTERS 8 TO 10

CHAPTER 8

THE EFFECT OF ELECTRICAL STIMULATION OF PHRENIC AFFERENTS ON VENTILATION IN RABBITS

Introduction

Very little is known about the possible role played by phrenic afferent activity in the control of breathing. The number of group I and II diaphragmatic receptors is few (Duron, 1981) and it is believed that feedback from these receptors does not participate in the control of breathing. However, compared to other skeletal muscles, the diaphragm is relatively rich in type III and IV receptors (Duron et al., 1980). The group of afferent fibres that innervate them have been studied extensively in the last few years (Jammes et al., 1986; Road et al., 1987; Marlot et al., 1987; Revelette et al., 1988; Hussain et al., 1990).

In these recent studies either electrical stimulation or capsaicin was used to activate the small-phrenic afferents. The ventilatory responses reported varied, even with the same stimulus. Jammes et al. (1986) have reported inhibition of phrenic efferent activity, whereas Road and colleagues (1987) indicated that activation of small-phrenic afferents enhances the activity of phrenic efferent. In contrast, Marlot and colleagues (1987) reported both an initial inhibition and a subsequent excitation in neural output to the diaphragm.

The studies, investigating the role played by activation of small-phrenic afferent fibres in the regulation of breathing, have concentrated on either the cat or the dog. Furthermore, Sant' Ambrogio and Widdicombe (1965) have speculated that in the rabbit activation of phrenic afferents has no significant influence on the control of eupneic breathing. However, activation of phrenic afferents in this animal might have a part to play during development of diaphragm fatigue. If this

is true, then the likely candidates would be group III and IV phrenic afferents; in limb skeletal muscles, afferents of these types are activated by metabolites of muscle contraction (Kaufman & Rybicki, 1987; Rotto et al., 1988). Moreover, group III and IV phrenic afferents in the cat have been shown to be excited by metabolites of muscle contraction (Graham et al., 1986; Jammes et al., 1986). The purpose of this study was to determine whether electrical stimulation of group III and IV in the phrenic influence neural output to the diaphragm in the rabbit and whether the effect is excitatory or inhibitory.

Methods

Five male New Zealand White rabbits, weighing 2.3-3.0 kg, were anaesthetized with pentobarbitone. They were bilaterally vagotomized. All rabbits breathed spontaneously 40% O_2 in N_2. The experimental set-up is shown in Fig. 8.1.

The right phrenic nerve was isolated in the neck and dissected away from connective tissue as previously described in Chapter 2. A bipolar stimulating electrode was placed under the middle root of the phrenic nerve, which contains fibres from C_5 and C_6. The electrode was encased in perspex to prevent spread of stimulus current to other tissues. The twitch threshold was determined as the minimal voltage to visibly twitch the diaphragm. Due to limitations of the stimulator, the twitch threshold voltage was measured to an accuracy of 0.1 volts. Thus the minimum voltage ranged from 0.1 to 0.2 volts. The nerve was cut distally to the stimulating electrode. The portion of nerve on the electrode was then covered with a piece of cotton wool soaked in mineral oil to prevent it drying out.

Protocol A

To assess the effects of activation of group I-IV afferents on minute ventilation, the different group of fibres were selectively recruited by electrical stimulation. Arterial blood pressure, end-tidal CO_2, and ventilation were recorded before stimulation for at least ten breaths. The proximal end of phrenic nerve was electrically stimulated continuously for one minute in four of the rabbits. The stimulus parameters were set at a pulse width of 2 ms, a discharge frequency of 40 Hz, and variable voltages. The voltage studied included 1, 4, and 10 volts. These voltages equated to on average 8, 32, and 80 times twitch threshold.

Protocol B

A close examination of the effects of activation of group III and IV phrenic afferents on the components of ventilation was also carried out. For this study, the stimulus strength 80 times twitch threshold was used: a 10 V rectangular pulse of 2 ms at 40 Hz. Further recordings of the ventilatory and cardiovascular variables were made before and during phrenic nerve stimulation, and at least for 10 breaths 2-4 minutes after the end of stimulation. In each case, near-control state, as judged by respiratory rate and tidal volume, was reached within a minute after stimulation. A period of 4 to 10 minutes was allowed between stimulations. This manoeuvre was performed twice or three times.

Protocol C

On one occasion, the electrical stimulation was continuously applied for two minutes while recording ventilation.

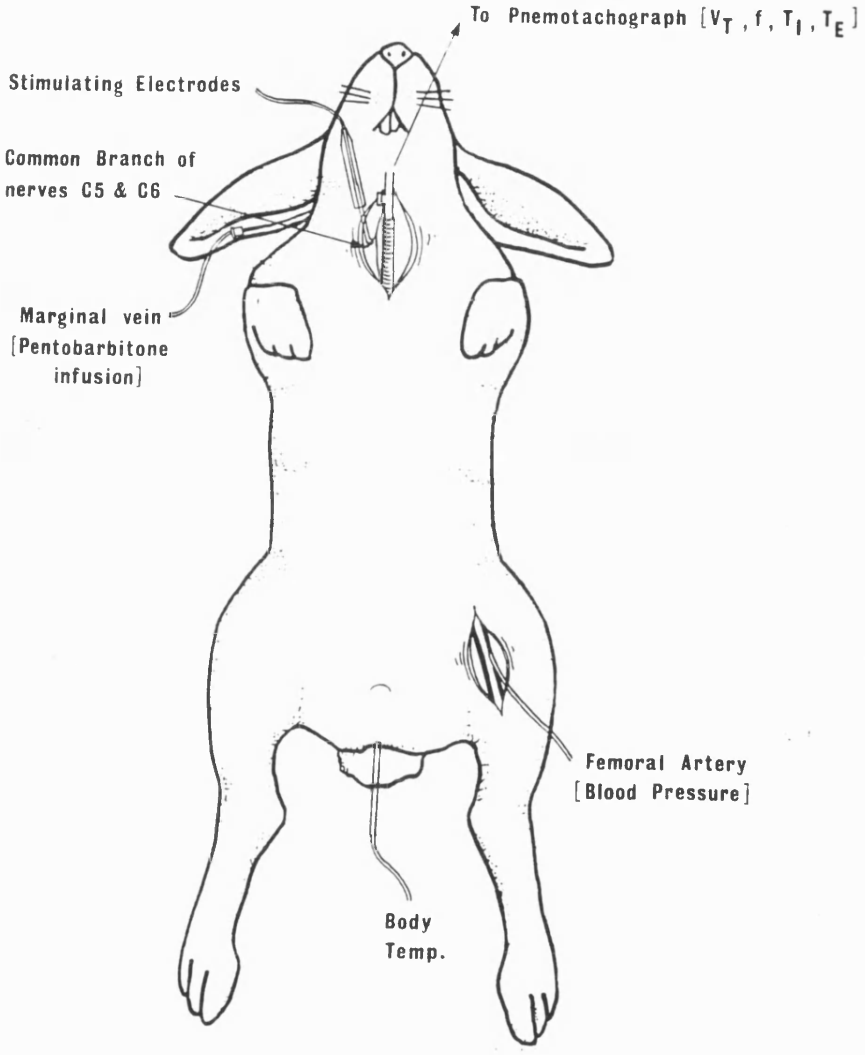


Fig. 8.1. Diagram of the experimental preparation used in neurophysiological studies.

Data analysis

The data from protocol A experiments were analyzed to give minute ventilation (V_I) before and during stimulation. Minute ventilation was averaged for each stimulus strength and then expressed as a percentage of their control value.

The data from protocol B were analyzed to give tidal volume (V_T), respiratory frequency (f_R), minute ventilation (V_I), expiratory time (T_E), inspiratory time (T_I), mean inspiratory flow (V_T/T_I), and respiratory duty cycle (T_I/T_{TOT}). A mean of 10 breaths measured immediately before stimulation was the control. To assess the ventilatory response to continuous stimulation of a phrenic nerve for one minute, the minute period was divided into 4 bins of 15 seconds, each bin contained about 6-16 breaths. The respiratory variables of each bin were averaged and expressed as percentages of control values. The variables for ten recovery breaths measured two to four minutes after stimulation were also averaged and expressed as a percentage of control.

All results are expressed as mean ± SE. To assess the significance of the changes in respiratory variables over the period of stimulation for both sets of data, the variables were averaged over the minute of stimulation and then compared with pre-control values using a paired t-test. $P < 0.05$ was accepted as the level of significance.

Results

Initial ventilatory response to stimulation

Electrical stimulation at 32 or 80 times twitch threshold of the central end of a cut phrenic nerve resulted in a substantial reduction of tidal volume and prolongation of breathing cycle for the first two to six breaths. Subsequently, ventilation was increased. If the stimulation

started during inspiration, inspiration was stopped abruptly (see Fig 8.2). The initial ventilatory response to stimulation was not quantified on a breath-by-breath basis as the primary interest was to assess the longer term effect of stimulation of phrenic afferents on ventilation.

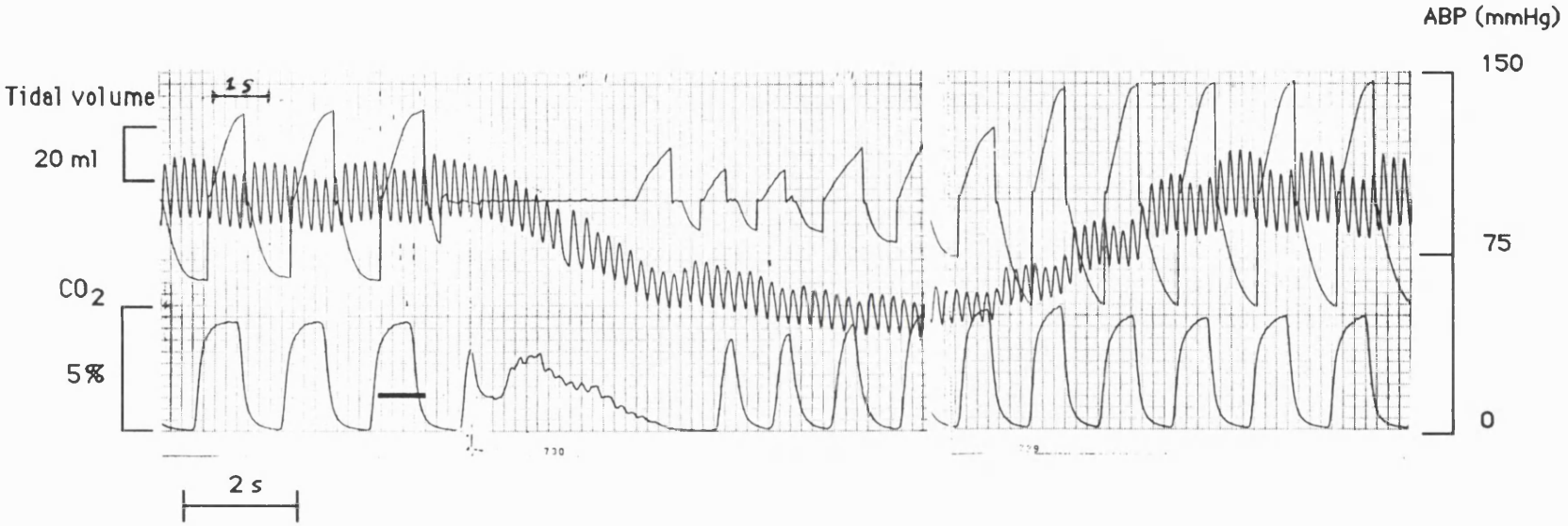


Fig. 8.2. Initial effect of stimulation of phrenic afferents on ventilation. Trace showing pneumotachogram, end-tidal CO_2, and mean arterial blood pressure. The black marker corresponds with the point at which the electrical stimulator was switched on.

Effect of varying stimulus strength on ventilation.

Using a stimulus voltage eight times twitch threshold resulted in an insignificant decrease in inspired minute ventilation (1029 vs 1016 ml, P = 0.59, n = 6). A stimulus voltage of 32 times twitch threshold produced a highly significant increase in minute ventilation (1054 vs 1112 ml/min, P = 0.003, n = 6). A significant increase in ventilation was also obtained at voltages of greater than 80 times twitch threshold (996 vs 1264 ml/min, P = 0.05, n = 6).

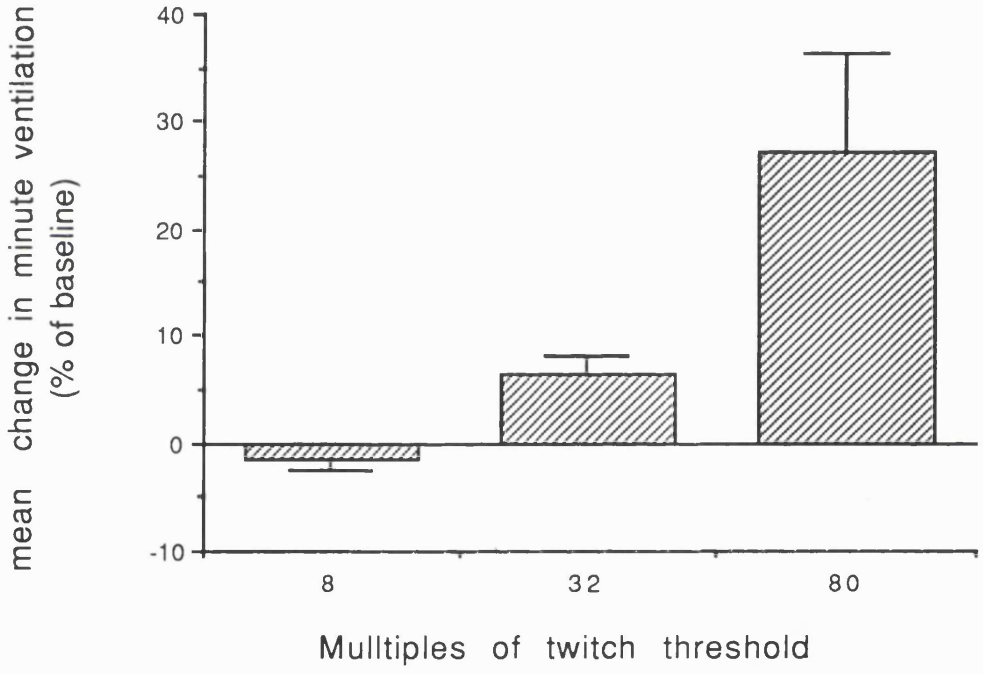


Fig 8.3 Histogram showing changes in inspired minute ventilation induced by electrical stimulation of right phrenic nerve afferents at 8, 32, and 80 times twitch threshold. Values are means ± SE for 6 observations in four rabbits

Effect of stimulation of group III and IV afferents on ventilation

Continuous electrical stimulation of phrenic afferents at 10 V for one minute invariably altered the pattern of breathing. Average changes of respiratory variables, expressed as percentages of the control values are summarized in Table 8.1. The response to electrical stimulation was an increase in V_I achieved by a significant increase in f_R. The increase in f_R was achieved by a decrease in both T_E and T_I in every rabbit. V_T changed inconsistently; in three rabbits it decreased whereas in two it increased. The mean change in V_T was not significantly different from control (Table 8.1). During stimulation, end-tidal CO_2 decreased as a result of hyperventilation. Both V_T/T_I and T_I/T_{TOT} increased whereas T_I/T_{TOT} remained constant. The tachypnea induced by phrenic nerve stimulation started after a delay of an average of six stimulated breaths (Fig. 8.3). By 30 seconds of phrenic nerve stimulation, all respiratory variables had changed maximally (Fig. 8.4 to 8.6). All respiratory variables recovered rapidly to control values within 20 seconds of stopping stimulation.

The cardiovascular and ventilatory responses to continuous electrical stimulation for two minutes are shown in Figure 8.7. Over the first minute of stimulation, the pattern of the response is typical of that observed when the phrenic nerve was stimulated only for a minute. It is noteworthy that end-tidal CO_2 remained below the control level for the duration of the stimulation. Although not readily apparent from the pneumotachogram, this would indicate that ventilation remained above control level during stimulation.

TABLE 8.1.

Changes in respiratory variables induced by phrenic nerve stimulation in spontaneously breathing rabbits. Values are means ± SE for 12 observations in 5 rabbits before (control) and during one minute of electrical stimulation with 10 V, 2 ms at 40 Hz. Electrical stimulation induced ($P < 0.05$) or did not induce (NS) a significant change in a respiratory variable.

	V_I (l/min)	V_T (ml)	f_R (b/min)	T_I (sec)	T_E (sec)	V_T/T_I (ml/sec)	T_I/T_{TOT}
Control	0.935	31.8	29.9	0.72	1.35	44.8	0.35
SE ±	0.388	5.74	2.60	0.06	0.13	8.59	0.02
Stimulation	1.150	30.8	38.6	0.60	1.03	51.5	0.37
SE ±	0.046	0.49	1.92	0.03	0.05	2.38	0.003
Δ%	23	-3	29	-17	-24	15	7
P	<0.05	NS	<0.05	<0.05	<0.05	<0.05	NS

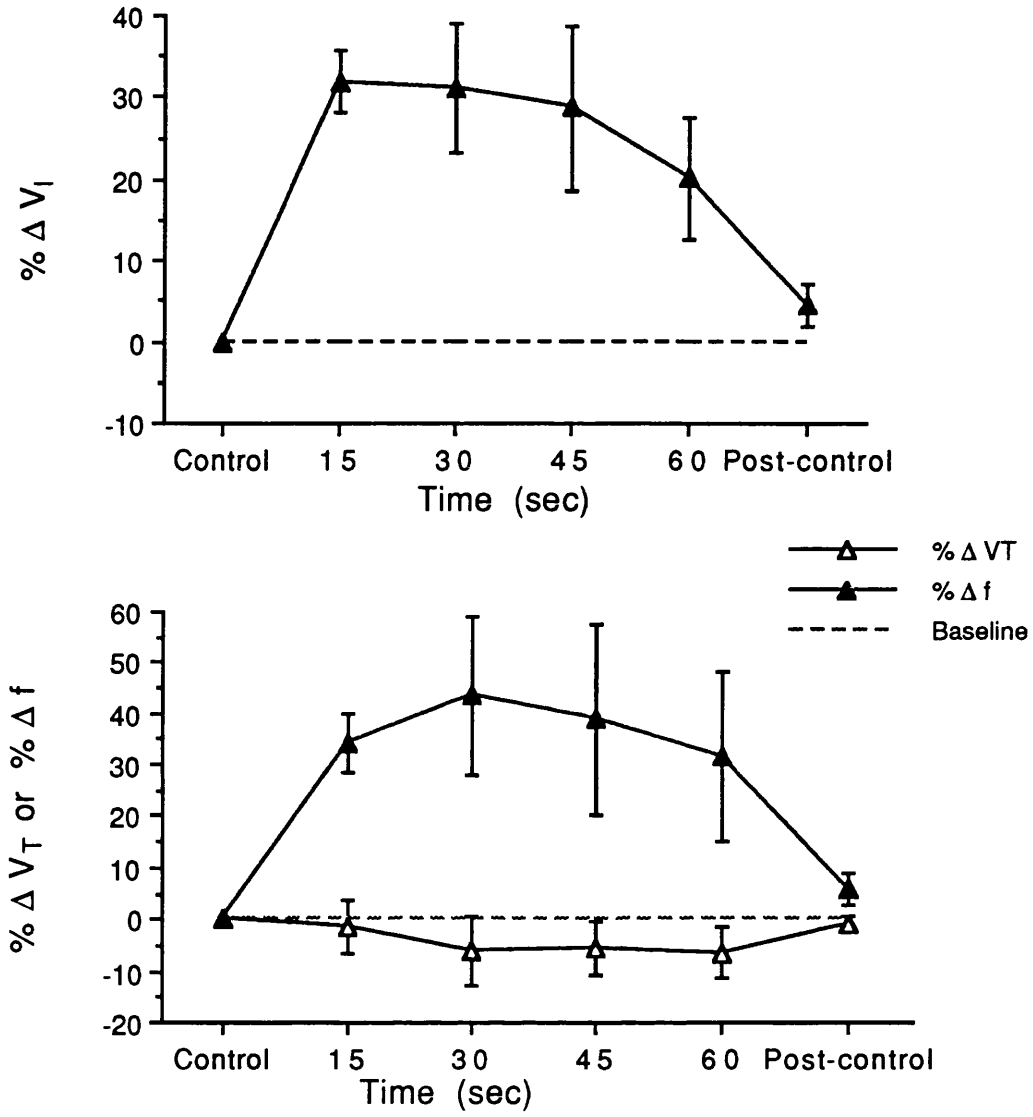


Fig. 8.4 Changes in inspired minute ventilation (V_I), respiratory frequency (f_R), and tidal volume (V_T), expressed as percentages of their control values, during phrenic nerve stimulation (10 V, 2 ms at 40 Hz) and 2-4 minutes after stimulation (post-control). Values are means ± SE for 12 observations in 5 rabbits

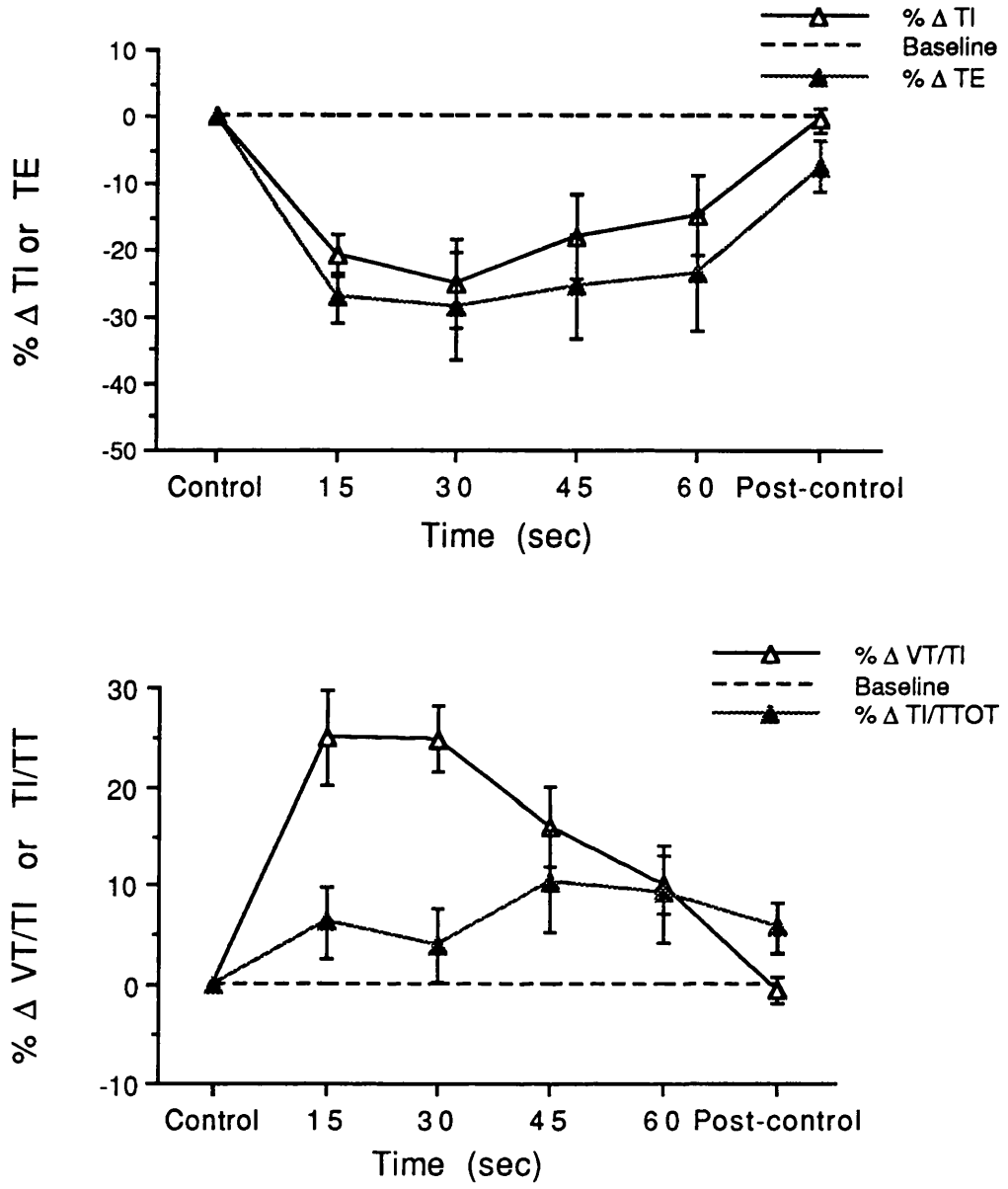


Fig. 8 5. Changes in expiratory time (T_E), inspiratory time (T_I), duty cycle (T_I/T_T) and mean inspiratory flow (V_T/T_I), expressed as percentages of their control values, during phrenic nerve stimulation (10 V, 2 ms at 40 Hz) and 2-4 minutes after stimulation (post-control). Values are means ± SE for 12 observations in 5 rabbits

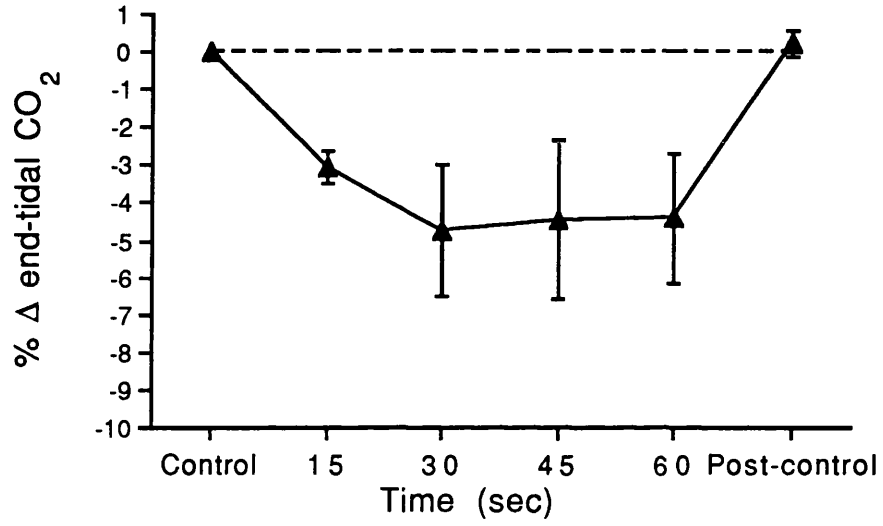


Fig. 8.6. Changes in end-tidal CO_2, expressed as percentages of their control values, during phrenic nerve stimulation (10 V, 2 ms at 40 Hz) and 2-4 minutes after stimulation (post-control). Values are means ± SE for 12 observations in 5 rabbits

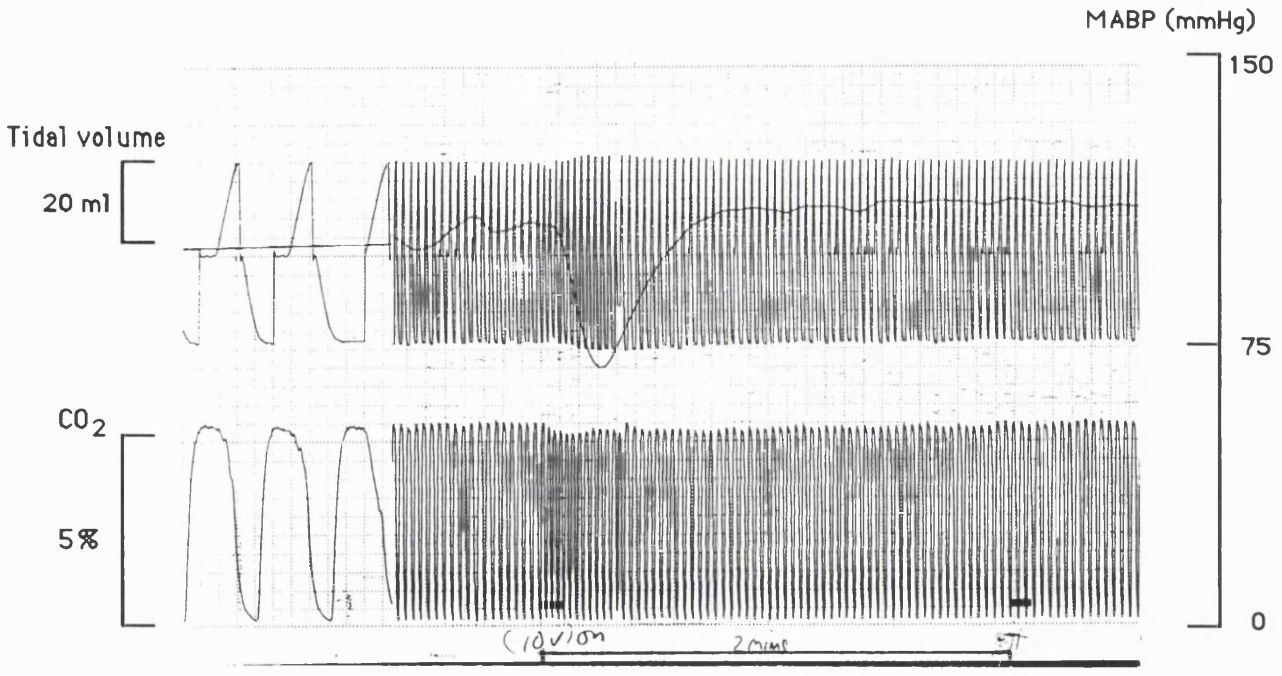


Fig. 8.7. Effect of electrical stimulation (10 V, 2 ms at 40 Hz) of the central end of the right phrenic nerve on tidal volume, mean arterial blood pressure, and end-tidal CO_2 in a spontaneously breathing rabbit. The trace shows changes in the variables over two minutes of stimulation.

Cardiovascular Response To Phrenic Afferent Stimulation

In all rabbits, electrical stimulation of phrenic afferents at 10 V caused a maximum reduction in mean arterial blood pressure (MABP) by 26.74% (± 2.51%). More detailed analysis of cardiovascular variables in two rabbits revealed that systolic pressure of both animals decreased maximally by 23.98% (± 9.36%) and diastolic by 34.59% (± 7.77%), whereas heart rate decreased from 360 beats/minutes to 300 beats/minutes. The decrease in MABP always followed the alterations in breathing pattern. The dip in ABP occurred within 10 seconds of the onset of stimulation, and reached a maximum fall by 17.5 sec (± 5.59 sec). Arterial blood pressure recovered within 50 seconds of the onset of stimulation.

Table 8.2. Maximum changes in mean arterial blood pressure, systolic and diastolic arterial blood pressure in response to 10 V electrical stimulation of the middle cervical root of the phrenic nerve.

	Control	Stimulation	P
MABP (mmHg)	100.0 ± 1.2	73.7 ± 2.5	< 0.05
SABP (mmHg)	117.5 ± 12.5	90.5 ± 20.5	NS
DABP (mmHg)	83.5 ± 1.5	54.5 ± 5.5	NS

Values are means ± SE of mean arterial blood pressure (MABP) in four rabbits (n = 7), systolic arterial blood pressure (SABP) and diastolic arterial blood (DABP) for two rabbits (n = 2). Stimulation induced a significant change when $P < 0.05$.

Discussion

The main ventilatory effect of stimulation of phrenic afferents with 10 V in this study was an increase in minute ventilation as a result of an increase in respiratory rate. Minute ventilation increased in all five vagotomized rabbits anaesthetized with pentobarbitone. The increase in respiratory rate was due to a reduction in both T_I and T_E. This increase in minute ventilation was associated with a decrease in $_{ET}CO_2$ and a transient decrease in mean arterial blood pressure. An increase in ventilation was also observed at high intensity electrical stimulation at 32 times threshold.

Electrical stimulation was chosen for use in this study because different groups of afferents fibres can be selectively recruited by using the appropriate stimulus parameters. The threshold of an afferent fibre to electrical stimulation varies inversely with its diameter; progressive increase in stimulus strength applied a nerve activates progressively thinner fibres. For example, all group I and II afferent fibres are recruited at 10 times twitch threshold, whereas group III afferents are activated at 10-34 times (Eccles et al., 1954) and group IV afferents are activated at intensities greater than 45 times the threshold (Gasser, 1950). Thus any reflex elicited by weakest stimuli can be attributed to large diameter fibres and any new response appearing with increased stimulus strength may be attributed to smaller fibres. A high electrical stimulus threshold was required to evoke an increase in ventilation in this study.

The portions of the diaphragm containing receptors innervated by afferent fibres travelling in roots C_5 and C_6 of the phrenic nerve is not known. If the afferent fibres in these roots innervate the same portion of the diaphragm as their corresponding efferent fibres, then these afferent fibres would arise from diaphragmatic receptors in the ipsilateral

costal and crural regions. The two muscles of the diaphragm, costa and crura, have separate segmental innervation. In the dog, efferent fibres in C_5 innervate anterior and part of the posterior portion of the diaphragm, digits 1, 2, and 8. Those in C_6, on the other hand, innervate middle and posterior portion, digits 3 to 8 (Landau et al., 1962).

Several studies have shown that activation of phrenic afferents III and IV can influence respiratory drive in the cat and the dog (Jammes et al., 1986; Marlot et al., 1987; Road et al., 1987; Revelette et al., 1988; Hussain et al., 1990). In the cat, Jammes and colleagues (1986) measured the phrenic motor response to electrical stimulation of phrenic afferent fibres with either nerve cooling to block the conduction of large diameter fibres or the application of procaine to interrupt the conduction in small diameter fibres. Stimulation of the afferent fibres under either condition evoked a reduction in phrenic efferent discharge frequency and a decrease in the inspiratory time. Jammes and colleagues (1986) proposed that activation of the phrenic afferents depresses diaphragm activity. In contrast, most other studies reported an increase in ventilation or diaphragmatic electromyogram activity (dEMG) in response to electrical stimulation of small-phrenic afferents in the cat and the dog (Marlot et al., 1987; Road et al., 1987).

The difference in ventilatory response to stimulation of small-phrenic afferents between Jammes and colleagues (1986) and other researchers has been attributed to interruption of group III fibres by procaine (Marlot et al., 1987) and ineffective blocking of conduction in large fibres by cooling (Road et al., 1987). It has been argued that if conduction of impulses in group III fibres were not blocked by procaine or the impulses in the large afferents were more effectively interrupted by cooling, Jammes and colleagues would have observed an increase in ventilation in response to electrical stimulation of the cut central end of the phrenic nerve. In support of this view, Revelette and colleagues (1988) showed

an increase in ventilation when the small-phrenic afferents were selectively stimulated by capsaicin injections into the phrenic artery. The results presented here also support the prevailing view.

Minute ventilation in this study increased because of increased respiratory frequency (f_R). In studies by Marlot (1987) in cats and by Road (1987) in dogs, an increase in ventilation in response to activation of small-phrenic afferents by electrical stimulation was also achieved by an increase in f_R. The increase in f_R reported in this study resulted from a decrease in both T_I and T_E in all rabbits. In Marlot's cats, the increase in respiratory rate induced by stimulation of phrenic afferents was mainly due to a shortening of T_I in all animals. In ten cats, T_E was prolonged and in the other five cats it was reduced. Road and colleagues, studying the effect of electrical stimulation of the phrenic nerve on ventilation in dogs anaesthetized by either α-chloralose or pentobarbitone, found a significant reduction in T_E while T_I remained unchanged during stimulation in both groups of dogs. The results presented here and by Marlot are different from results reported by Road. In Road's experiments, the animals were not vagotomized, whereas in this study and that of Marlot, they were vagotomized. Thus presence of vagal activity may have predominated over phrenic afferent activity in the control of T_I.

The increased minute ventilation evoked by activation of small-phrenic afferents in the cat and the dog was also achieved by increased tidal volume (Marlot et al., 1987; Road et al., 1987). In contrast, tidal volume remained constant during hyperventilation induced by electrical stimulation of phrenic afferents in the rabbit. Unlike the cat and dog, the tidal volume of the rabbit at rest is greater than its functional residual capacity (Crosfill & Widdicombe, 1961). This means that resting tidal volume is closer to vital capacity in the rabbit than in cats and

dogs. Therefore, the rabbit can only increase its minute ventilation substantially by breathing at a faster rate.

The present study was not designed to determine whether the reflex initiated by phrenic afferent activation was supraspinal or spinal. The first response to stimulation of phrenic afferents in this study, was an immediate reduction in tidal volume for the first 2-3 breaths. This response has been observed in cats with stimulation of phrenic afferents (Marlot et al., 1987) and limb muscle afferents (Kumazawa et al., 1983). Both research teams attributed the observed ventilatory response to activation of group III afferent fibres. Such an inhibitory response is believed to be due to direct spinal inhibition of phrenic motoneurons (Eldridge et al., 1981). There is also indication that the reduction in tidal volume for the first couple of breaths during phrenic nerve stimulation may have a supraspinal component involving medullary respiratory related neurons. Marlot and colleagues (1987) observed a slight reduction in activity in medullary respiratory related neurons when tidal volume was reduced.

Because stimulation of the phrenic afferent in the rabbit produced an increase in breathing frequency, these afferents probably project to the brain. Areas in the brain to which phrenic nerve afferents projections have been located include the cerebellum (Marlot et al., 1984), dorsal and, lateral reticular formation (Macron et al., 1985), medullary respiratory areas (Macron et al., 1986; Speck & Revelette, 1987) and sensorimotor cortex (Davenport et al., 1985).

In the rabbit, both arterial blood pressure and heart rate decreased during electrical stimulation of phrenic afferents. Results published by other researchers so far on the subject are contradictory. For example, electrical stimulation of phrenic afferents has produced a decrease, an increase, or no change in blood pressure (Kohram et al., 1947; Road et al., 1987). Kohram and colleagues occasionally observed an immediate decrease in blood pressure in response to

stimulation of the central ends of both phrenic nerves in the cat. This decrease in blood pressure was not always associated with a slowing of the heart. These responses are the same as those reported in the rabbit. Despite using similar stimulation parameters as Road, Kohram and colleagues found that stimulation of phrenic afferents in the dog caused a 17% increase in blood pressure. They deduced that the pressor response was due to activation of group III and IV phrenic afferents.

In a recent study, injection of capsaicin into the phrenic arteries of anaesthetized dogs evoked an increase in arterial blood pressure and heart rate (Hussain et al., 1991). Since capsaicin specifically activate group III and IV afferent fibres, Hussain and colleagues attributed this pressor response to activation of thin-fibre phrenic afferents. The clearest evidence that ventilatory responses induced by stimulation of afferent fibres were mediated by group III and IV fibres has been provided by Waldrop and colleagues (1984). They showed that chemical activation of group I and II afferents by succinylcholine produced no significant change in blood pressure and respiratory drive.

Other studies on the influence of group III and IV arising from limb muscles on cardiovascular system have revealed the following. Electrical stimulation of 5-20 Hz and intensity less than 15 times the threshold of muscle afferents produces a decline in blood pressure and heart rate (Gordon, 1943; Katz & Perryman, 1965; Johansson, 1962). This depressor response has been attributed to group III afferents and a few group II afferents (Johansson, 1962). Whereas stimulation at frequencies 40-100 Hz and at intensity of greater than 15 times threshold (Fell, 1968; Johansson, 1962) or intra-arterial injection of capsaicin induces an increase in both blood pressure and heart rate. This pressor response has been attributed to group IV afferents (Johansson, 1962). With stimulation parameters (10 V, 40 Hz, and 2 ms) used in the present study, a pressor response was anticipated

Failure to evoke a pressor response could be attributed to insufficient activation of group IV afferents as the stimulus current was too weak. Since the stimulus current was not measured in this or in Road's experiment, it is not known whether the stimulus current delivered to phrenic nerve was the same. Although stimulus voltages were the same, higher resistance of the nerve or electrode in the present study could have given a smaller stimulus current according to Ohm's law. As the vagi was cut, the cardio-inhibitory response could only have been mediated by central inhibition of sympathetic tone to the heart.

In conclusion, electrical stimulation of phrenic afferents at more than 32 times stimulus threshold produces a transient decrease in ventilation followed by an increase in ventilation and a transient decrease in blood pressure. The increase in ventilation suggests a positive reflex which under non-experimental conditions might be initiated by changes in concentration of metabolites in the loaded diaphragm. The decrease in blood pressure could be a nociceptive response rather than a specific cardiovascular adjustment to meet the needs of the diaphragm.

CHAPTER 9

EFFECTS OF POTASSIUM-INDUCED ACTIVATION OF DIAPHRAGMATIC RECEPTORS ON VENTILATION

Introduction

In Chapter 8, it was shown that electrical stimulation of phrenic afferents caused a reflex increase in ventilation and transient decrease in mean arterial blood pressure (MABP). Such changes in ventilation and blood pressure may occur when the phrenic afferents are physiologically activated. This thesis has shown that arterial potassium concentration increases during inspiratory resistive loading (see Chapter 5). The purpose of this investigation, therefore, was to determine whether K^+ can activate diaphragmatic receptors, initiating reflex changes in ventilation and MABP.

Protocol

Four male New Zealand White rabbits were used in this study. They were anaesthetized as described in Chapter 2. The abdominal surface of their diaphragm was exposed by retracting and covering the abdominal contents with moistened gauze and a plastic sheet.

The test potassium concentrations were made by diluting a stock solution of 20% (2 M) potassium chloride (KCl) in 0.9% sodium chloride (NaCl). These KCl solutions were made up as tabulated below.

Test [KCl] (M)	Stock 2% KCl (ml)	0.9% NaCl (ml)
0.5	2.5	7.5
1.0	5.0	5.0
1.5	7.5	2.5

Table 9.1. Dilution of 20% KCl to give KCl concentrations of 0.5, 1.0, and 1.5 M.

The test potassium solutions were in turn applied to the right sternal and right costal digits using a cotton bud. Because the right crural digit was inaccessible, potassium solutions were not applied to it. The order, in which the three potassium concentrations were applied to the diaphragm, was randomized. A recovery period of a minimum of four minutes was allowed between applications. Ventilation, end-tidal CO_2 and MABP were continuously recorded for 30 seconds before and for 60 seconds after applications. For each rabbit, the number of applications ranged from 6 to 9.

Control experiment

A control test was carried out either two minutes before potassium was applied to the diaphragm or after recovery. The control test involved touching or brushing the diaphragm with a dry cotton bud or one soaked in normal saline while recording ventilation and MABP.

Data analysis

Only the data for successful potassium applications were analyzed. A successful application is defined as one producing a perceptible change in ventilation. Respiratory variables were averaged over 10 breaths immediately before the application of potassium, and 10 consecutive breaths during which the maximum response to potassium was indicated. Results are presented as means ± SE.

Because blood pressure measurements from rabbit C14 were unreliable, only measurements from rabbit A19 is reported. The peak measurement in mean arterial blood pressure over a bin of the ten control breaths was taken as the control value. The maximum fluctuation in MABP over a bin was no more than 5 mmHg. To assess the maximum cardiovascular response to potassium application, the maximum reduction in mean arterial blood pressure was determined.

To determine whether mean arterial blood pressure or a respiratory variable had changed significantly in response to potassium, the values measured before and those measured after application of potassium were compared by analysis of variance. A change in mean arterial blood pressure or a respiratory variable was deemed significant when $P < 0.05$.

Results

Potassium applied to the diaphragm did not produce any respiratory or cardiovascular changes in two rabbits. For two others, rabbits A19 and C14, there were 9 out of 9 and 2 out of 9 successful applications respectively. The success of an application was not dependent upon the concentration of potassium used nor the site of application in rabbit A19.

MABP and ventilation started to change simultaneously in response to potassium. However, the maximum change in MABP occurred before ventilation peaked in rabbit A19.

Successful applications

In rabbits A19 and C14, brushing or touching the diaphragm with a cotton bud soaked in potassium solution produced changes in tidal volume (V_T), respiratory rate (f_R) (Table 9.1 to 9.3), and MABP (Fig. 9.1). Tidal volume increased by 13.8% ± 1.5% while f_R increased by 6.9% ± 1.2%. This increase in f_R was achieved by decrease in both expiratory time (T_E) and inspiratory time (T_I) by 7.5% ± 1.3% and 6.2% ± 1.5% respectively. The net effect of changes in V_T and f_R was an increase in inspired minute ventilation by 21.7% ± 2.0%.

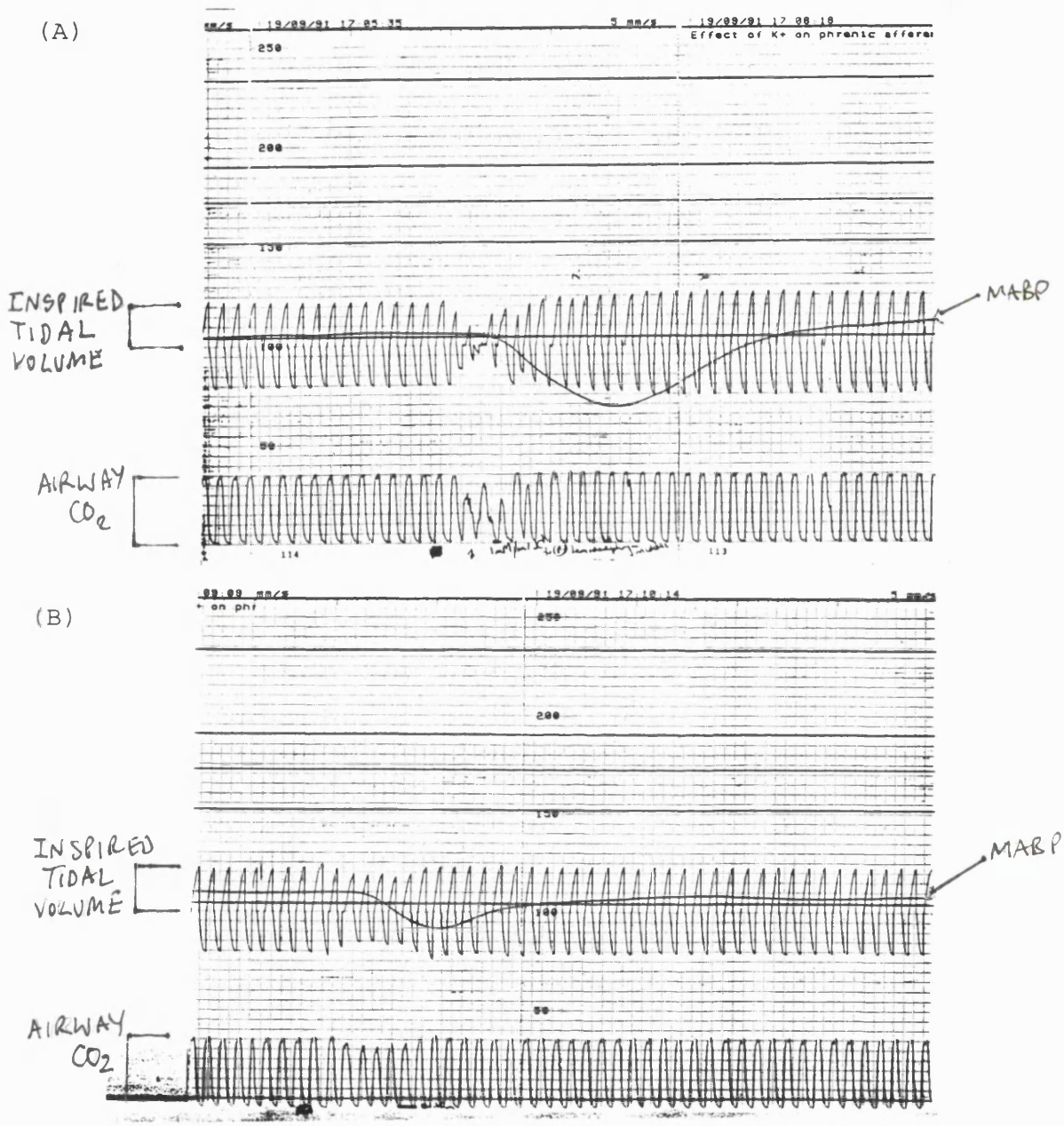


Fig. 9.1. Comparison between the effect of 1 M KCl (A) and the effects of normal saline (B) on ventilation and arterial blood pressure in the same rabbit. The test solutions were applied to the same site on the diaphragm and for the same duration. The black marker indicates the start of application.

Rabbit		V_T (ml)		T_I (s)		T_E (s)		f_R (breaths/min)		V_I (ml/min)		ET^{CO_2} (%)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A19	1	22.8 ± 0.1	26.7 ± 0.1 ***	0.80 ± 0.0	0.80 ± 0.0	0.76 ± 0.02	0.68 ± 0.03	38.5 ± 0.4	40.7 ± 0.03 *	877.5 ± 7.4	1087.5 ± 26.1 ***	4.2 ± 0.0	4.2 ± 0.02
A19	2	22.2 ± 0.6	24.4 ± 0.2 ***	0.84 ± 0.02	0.79 ± 0.01 *	0.76 ± 0.02	0.69 ± 0.02	37.5 ± 0.0	40.6 ± 0.7 **	832.5 ± 21.9	991.2 ± 15.7 ***	4.4 ± 0	4.3 ± 0.02
A19	3	21.0 ± 0.15	23.0 ± 0.0 ***	0.80 ± 0.02	0.77 ± 0.01 *	0.80 ± 0.02	0.77 ± 0.01	37.5 ± 0.0	40.1 ± 0.89 ***	787.5 ± 0.0	924.1 ± 20.5 ***	4.4 ± 0.02	4.2 ± 0.0
A19	4	22	26.4 ± 0.4 ***	0.80 ± 0.0	0.77 ± 0.01	0.80 ± 0.0	0.74 ± 0.03	37.5 ± 0.0	39.8 ± 0.6 **	825 ± 0.0	1051.1 ± 22.6 ***	4.5 ± 0.02	4.3 ± 0.02

Table 9.2. Changes in respiratory variables corresponding to maximum increase in minute ventilation induced by potassium application to the diaphragm. Respiratory variables were averaged over 10 breaths before the application of potassium (Pre), and the changes in these variables in response to potassium were averaged over 10 consecutive breaths (Post). Results are presented as means ± SE. * denotes that the change respiratory variable was significantly different at $P < 0.05$ level. ** and *** denote highly significant changes in variables at $P < 0.01$ and $P < 0.001$ level respectively.

Rabbit		V_T (ml)		T_I (s)		T_E (s)		f_R (breaths/min)		V_I (ml/min)		$_{ET}CO_2$ (%)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A19	5	21.0 ± 0.1	24.0 ± 0.0 ***	0.81 ± 0.01	0.75 ± 0.02 **	0.72 ± 0.01	0.69 ± 0.01	39.2 ± 0.4	41.7 ± 0.5 ***	831.7 ± 5.3	1001.1 ± 11.2 ***	4.5 ± 0.02	6.2 ± 0.02
A19	6	22.0 ± 0.2	24.9 ± 0.2 ***	0.80 ± 0.01	0.74 ± 0.02 *	0.69 ± 0.02	0.67 ± 0.01 *	40.3 ± 0.6	42.6 ± 0.3 **	887.1 ± 12.1	1060 ± 11.6 ***	4.3 ± 0.02	4.2 ± 0.02 ***
A19	7	21.6 ± 2.0	23.9 ± 0.5 ***	0.75 ± 0.02	0.74 ± 0.02	0.73 ± 0.01	0.68 ± 0.01 *	40.6 ± 0.5	42.3 ± 0.4 *	876.5 ± 8.0	1012.8 ± 10.6 ***	4.2 ± 0.0	4.2 ± 0.0
A19	8	22.1 ± 0.2	23.2 ± 0.2 ***	0.82 ± 0.02	0.78 ± 0.01	0.74 ± 0.02	0.69 ± 0.02	38.6 ± 0.7	40.8 ± 0.4 *	851.2 ± 9.4	952.4 ± 15.9 ***	4.5 ± 0.0	4.3 ± 0.02 ***

Table 9.3. Changes in respiratory variables corresponding to maximum increase in minute ventilation induced by potassium application to the diaphragm. Respiratory variables were averaged over 10 breaths before the application of potassium (Pre), and the changes in these variables in response to potassium were averaged over 10 consecutive breaths (Post). Results are presented as means ± SE. * denotes that the change respiratory variable was significantly different at $P < 0.05$ level. ** and *** denote highly significant changes in variables at $P < 0.01$ and $P < 0.001$ level respectively.

Rabbit		V_T (ml)		T_I (s)		T_E (s)		f_R (breaths/min)		V_I (ml/min)		ET^{CO_2} (%)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
A19	9	21.6 ± 0.3	25.1 ± 0.1 ***	0.72 ± 0.02	0.61 ± 0.01 ***	0.71 ± 0.02	0.60 ± 0.0 ***	42.0 ± 0.4	49.6 ± 0.4 ***	906.6 ± 9.4	1247.5 ± 7.9 ***	4.3 ± 0.02	4.3 ± 0.02
C14	1	25.5 ± 0.2	29.1 ± 0.5 ***	0.47 ± 0.01	0.42 ± 0.02	0.51 ± 0.01	0.50 ± 0.02	61.3 ± 0.9	65.8 ± 2.0	1566.3 ± 19.4	1912.3 ± 61.9 ***	3.5 ± 0.0	3.3 ± 0.06 ***
C14	2	20.0 ± 0.15	24.5 ± 0.15 ***	0.42 ± 0.01	0.42 ± 0.01	0.61 ± 0.02	0.59 ± 0.00 *	58.3 ± 1.0	59.1 ± 0.6 *	1166.7 ± 21.9	1449.5 ± 15.7 ***	4.2 ± 0.0	4.0 ± 0.04 ***

Table 9.4. Changes in respiratory variables corresponding to maximum increase in minute ventilation induced by potassium application to the diaphragm. Respiratory variables were averaged over 10 breaths before the application of potassium (Pre), and the changes in these variables in response to potassium were averaged over 10 consecutive breaths (Post). Results are presented as means ± SE. * denotes that the change respiratory variable was significantly different at $P < 0.05$ level. ** and *** denote highly significant changes in variables at $P < 0.01$ and $P < 0.001$ level respectively.

	Mean arterial blood pressure (mmHg)	
Trial number	Pre	Post
1	110	70
2	110	95
3	107	95
4	105	70
5	107	88
6	105	83
7	108	79
8	100	85
9	100	75

Table 9.5. Maximum changes in mean arterial blood pressure induced by potassium application to the diaphragm. Blood pressure measured before application is denoted by Pre and measured after application is denoted by Post. The results are from rabbit A19.

Control experiment

Generally, brushing or touching the diaphragm with either a dry cotton bud or one soaked in normal saline did not induce a change in ventilation or MABP. On two occasions in rabbit A19, however, brushing the diaphragm with saline did produce ventilatory and cardiovascular responses which were less pronounced than that produced in response to potassium application (Fig. 9.1). The changes in ventilation and blood pressure only occurred while saline was being applied. This indicates that these changes were caused by the applied pressure.

Discussion

This study demonstrates that potassium applied to the diaphragm can initiate respiratory and cardiovascular responses similar to those associated with nociception.

Technical considerations

Potassium was applied topically to activate diaphragmatic receptors for several reasons. In the rabbit, the arteries feeding the diaphragm were too small to cannulate successfully. In preliminary studies, attempts were made to cannulate a phrenic artery or the internal diaphragmatic circular artery but this usually resulted in haemorrhage from a torn vessel or a dislodged cannula. If KCl was injected into a main artery, it would travel to anywhere in the body. The circulating K^+ would activate nociceptors in limb muscles (Wildenthal et al., 1968) or carotid chemoreceptors (Linton & Band, 1985). Therefore, any changes in ventilation in response to intra-arterial injection of K^+ could not be attributed to phrenic afferent activation alone. To ensure that only the diaphragmatic receptors were exposed to potassium, K^+ was applied topically to the diaphragm.

Control experiment

Although firm pressure applied to the diaphragm did produce ventilatory and cardiovascular responses, these responses were less pronounced than those produced in response to potassium application and were only evident for the duration of the application of pressure. It is conceivable that firm pressure activated high threshold pressure receptors, innervated by group III phrenic afferents resulting in ventilatory and cardiovascular changes. Anyway, K^+ was usually applied to the diaphragm by gently brushing or touching its surface with a cotton bud soaked in potassium solution. While light brushing or touching of the diaphragm surface with a cotton bud soaked in potassium produced ventilatory and cardiovascular changes, repeating the same manoeuvre with a dry bud or one soaked normal saline did not produce any changes in ventilation or MABP. The ventilatory and cardiovascular responses to K^+ are unlikely to have been initiated by activation of extra-diaphragmatic receptors as the abdominal contents were protected under a plastic sheet and the cotton buds applied to the diaphragm were not dripping wet but damp. In view of the evidence outlined here, it is apparent that K^+ activated diaphragmatic receptors, initiating an increase in ventilation and a decrease in MABP. Two interrelated questions arise when attempting to interpret the physiological significance of this result: first, is K^+ the normal physiological stimulant of this reflex, and second, are the reflexes active during the development of diaphragm fatigue?

Physiological significance

It was shown in Chapter 5 that loaded inspiratory resistive breathing resulted in an increase in arterial K^+. Furthermore, potassium release from contracting limb muscles has been established. Acute hyperkalemia during exercise and perhaps acute respiratory failure is a normal physiological response. Teleologically, therefore, changes in K^+ should act

as a natural stimulant. The following works provide evidence in support of the notion that K^+ is a natural noxious stimulant. Rybicki and colleagues (1985) found that increasing interstitial potassium concentrations to levels similar to those seen during static contractions excited group III and IV afferents in the gracilis nerve of the dog. Infusion of KCl into the femoral artery of the dog has also been shown to increased ventilation, heart rate, and blood pressure (Wildenthal et al, 1968). The potassium concentrations (0.3 to 1 M) infused in the study of Wildenthal and colleagues were similar to those used Rybicki and colleagues (1985) and the experiments presented in this chapter.

Unlike the aforementioned studies, in the rabbit experiments K^+ was applied topically to the diaphragm instead of being injected into an artery. Because K^+ was applied topically, the amount reaching the receptors varied. This variation in amount of K^+ applied to the receptive field may partly explain the lack of consistency in ventilatory and cardiovascular responses to K^+ application. There are two other plausible explanations for the lack of response and the variability of the response to K^+ observed in the rabbit. First, either diaphragmatic receptors were not activated or an insufficient number was activated when potassium was applied to the diaphragm. Second, the level of anaesthesia may have influenced the ventilatory and cardiovascular response to K^+. In support of the second explanation, Wildenthal and others (1968) found that if the dog was deeply anaesthetized, subcutaneous and intracutaneous KCl injections would not produce changes in ventilation and circulation.

Conclusion

In the anaesthetized rabbit, potassium produces an increase in ventilation as well as a decrease in MABP. The ventilatory and circulatory responses to K^+ are probably dependant upon the level of anaesthesia. These responses could therefore

have been mediated by pain and are likely to be supra-spinal in nature. The final set of experiments in Chapter 10 will try to establish whether diaphragmatic receptors were activated and whether the afferent limb of these reflexes is the phrenic nerve.

CHAPTER 10

RESPONSE OF PHRENIC AFFERENT FIBRES TO POTASSIUM

Introduction

The consensus view is that muscle afferents participate in the neural regulation of the cardiovascular and respiratory responses to muscular exercise. These responses are mediated by activation of group III and IV afferents (McCloskey & Mitchell, 1972; Alam & Smirk, 1937). Recent studies have shown that activation of the phrenic afferents, arising from the diaphragm muscle, can alter cardiovascular and respiratory functions in the cat and the dog (Jammes et al., 1986; Hussain et al., 1991). In the preceding two chapters, it was shown that activation of group III and IV phrenic afferents evoked an increase in ventilation and transient decrease in mean arterial blood pressure in the rabbit.

The natural stimuli to these muscle afferents are not known but believed to be mechanical deformation of their receptive field or metabolites produced by contracting muscles or both. Potential metabolites are lactic acid, phosphates, and adenosine; these metabolites have been reported to activate group III and IV afferents arising from limb muscles and the diaphragm (Rotto & Kaufman, 1988; Thim & Baum, 1987; Graham et al., 1986; Jammes et al, 1986). Another potential chemical stimulus, although not strictly speaking a metabolite, is potassium. Recent studies have shown that group III and IV afferents arising from gracilis muscle could be excited by potassium (Rybicki et al., 1985; Kaufman & Rybicki, 1987). The effect of K^+ on phrenic afferent fibres is not known.

During exercise K^+ is released from active muscles, increasing extracellular K^+ levels. In Chapter eight, it has been established that severe inspiratory resistive loading induces an increase in arterial K^+ concentration. This study

indicated that K^+ might be a potential stimulus of small-phrenic afferents. The purpose of this chapter is to examine the effect of K^+ on the activity of phrenic nerve afferents.

Methods

Three rabbits were anaesthetized with pentobarbitone. Anaesthesia was maintained with ketamine infusion. They were tracheostomized. One femoral artery was cannulated for monitoring blood pressure and arterial blood sampling. All rabbits received 50% O_2 in 50% N_2. The diaphragm was exposed as outlined in Chapter 2.

The skin of the neck was incised and tied to the table with ties passed over a metal bar to form a pool. The pool was filled with mineral oil at room temperature; mineral oil protects the nerve from desiccation. The middle root of the right phrenic nerve, containing fibres from C_5 and C_6, was isolated as previously described in Chapter 2. The peripheral end of the nerve was cut and desheathed to minimize the noise to signal ratio. The cut nerve was then divided into several filaments. Each filament, in turn, was placed on one hook of a pair of stainless steel electrodes to record phrenic afferent activity. The other hook was earthed by attaching connective tissue or adipose tissue to it. The nerve was prepared for recording of afferent activity on a dental mirror 12.5 mm in diameter while viewing through a Zeiss binocular microscope at 25 or 40 times magnification.

The AC preamp (NL 105) was set at gain 5K and the AC amp (NL 106) at x 10: the total amplification of the signal was 50K. The NL filter was set at high cut off 900 Hz and low cut off 65 Hz. A pulse height analyzer (PHA) was used to discriminate between action potentials of varying amplitudes. The upper and lower windows were adjusted to select action potentials of a particular amplitude for

counting. The action potential impulses were counted by a frequency counter set at 1 or 2 sec bins. The raw phrenic afferent activity, discriminated signal, frequency count signal, mean arterial blood pressure and pneumotachogram were recorded on a Gould ES 2000 electrostatic recorder.

Each filament consisted of many fibres. Some of the filaments had spontaneous activity. Others did not. The spontaneously active filaments were classified as being either tonic or phasic units; tonic units discharge randomly whereas phasic ones discharge in phase with either expiration or inspiration. Group III and IV afferents are reported to discharge tonically whereas group I and II phasically (Graham et al, 1986; Jammes et al., 1986). Only filaments that displayed tonic activity or no spontaneous activity were studied. Those with phasic activity were discarded. The right hemi-diaphragm was probed with a cotton bud to identify the receptive field of the fibres under investigation. When the receptive field was identified, the activity of the fibres increased momentarily.

The test potassium concentrations were made by diluting stock solution of 20% (2 M) potassium chloride (KCl) in 0.9% sodium chloride (NaCl). These KCl solutions were made up as tabulated below.

Test [KCl] (M)	Stock 2% KCl (ml)	0.9% NaCl (ml)
0.25	1.25	8.75
0.5	2.5	7.5
1.0	5.0	5.0

Table 10.1. Dilution of 20% KCl to give KCl concentrations of 0.25, 0.5, and 1.0 M.

Control afferent activity in the phrenic nerve was recorded for 30 to 60 seconds. To determine the effect of K^+ on tonic afferent activity, test KCl concentrations were applied to

the diaphragm with a cotton bud or a paint brush. Latency of response was determined. It was the time interval between the onset of application of test solution and the onset of the burst of afferent activity. The activity of phrenic afferents was recorded until it had returned to its control level or stabilized at new low level of activity.

The discharge frequency of a fibre was obtained by manually counting either the action potentials of the same amplitude or the recorded output from the PHA. All results are expressed as means ± SE. Control discharge frequency was averaged over 30 seconds immediately before the application of KCl to the diaphragm. Peak discharge rates of the afferents were averaged over a 2 second period. One-way analysis of variance was used to determine the statistical significance of changes in discharge frequency induced by K^+. $P < 0.05$ was accepted as the level of significance.

Control experiment

Sometimes a control test was carried out two minutes before the application of potassium to the diaphragm. The control test involved touching or brushing the diaphragm with a dry cotton bud or one soaked in normal saline while recording phrenic afferent nerve activity.

Results

Effect of diaphragmatic probing

The receptive fields of only three of the fifteen filaments were located by probing the diaphragm. A firm pressure to the receptive field was required to activate fibres in these filaments (Fig. 10.1). The receptive fields were located in the middle segment of the diaphragm, digits 4 or 5. Light probing, which did not produce deformation of the surface of the diaphragm, did not induce a burst of activity in the

phrenic afferents. The increased activity of the phrenic afferents in response to firm pressure was not produced as a result of muscle damage as the activity of the fibres returned to basal level as soon as the pressure was released. Because firm pressure was needed to activate the fibres, they were probably group III and/or IV afferents.

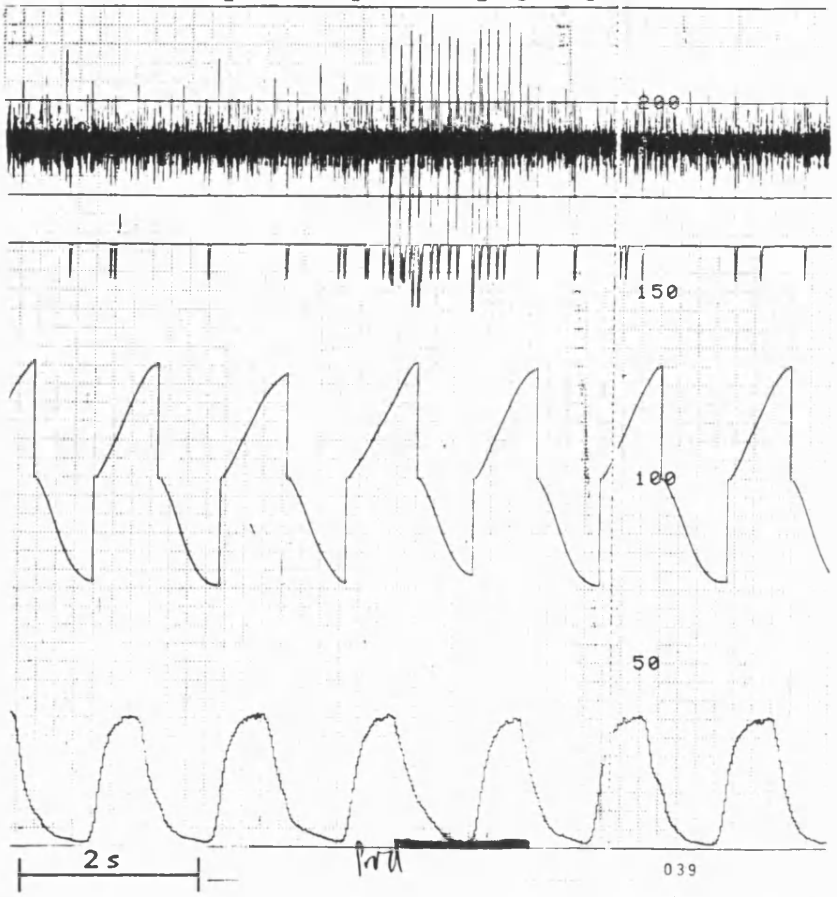


Fig. 10.1. Trace showing the effect of firm pressure on the activity of phrenic afferents. The black marker at bottom of the trace corresponds with the duration of the applied pressure to the diaphragm.

Effect of potassium on phrenic afferents

A total of 15 filaments was studied, which were either tonically active or quiescent before the application of K^+ to the diaphragm. Six of these showed no change in activity in response to K^+. The rest were excited by K^+ and from these twelve individual fibres were selected according to the amplitude of their action potential impulses. Eight of the fibres activated by K^+ had no spontaneous activity. The four others were tonically active with an average discharge

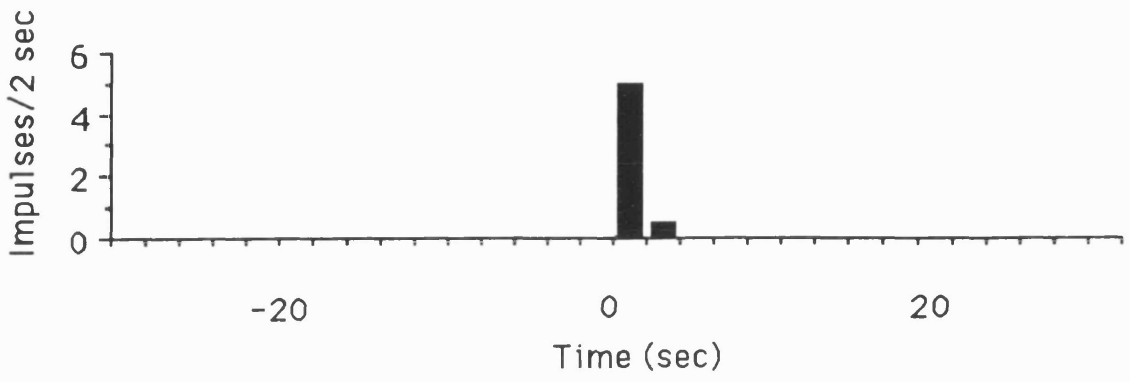
frequency of 0.76 ± 0.41 impulses/2 seconds over thirty seconds immediately before the application of K^+.

Because the receptive field of many of the filaments was not located, latency of response to K^+ could not be determined with confidence for these filaments; the receptive fields of only three filaments were identified by probing digit 4 or 5. The latency for these units was 0.4 seconds in each case.

The fibres, excited by K^+, displayed two patterns of response. The first pattern, displayed by five fibres, consisted of a low-frequency burst of impulses, remaining above control levels for 3.7 ± 0.6 seconds. Two of these five fibres were quiescent before the application of K^+. The next pattern, exhibited by seven fibres, consisted of low-frequency burst, which remained above control for 28.3 ± 5.09 seconds. Two out of the seven fibres were spontaneously active before the application of K^+. For all twelve fibres, the peak discharge frequency was 3.92 ± 0.45 impulses/2 seconds occurring after 4 ± 1.23 seconds the start of the change in fibre activity in response to K^+. The peak increase in activity was highly significant ($P < 0.01$) when compared to control discharge frequency (0.32 ± 0.18 impulses/2 sec). The pattern of response exhibited by all fibres appeared not to be dependent upon the KCl concentration applied to the diaphragm.

For three filaments, the firing rate increased initially then partially adapted but never returned to control levels even after half an hour. Because there was much nerve activity in these filaments, individual fibres could not be identified with confidence. Inability to identify individual fibres meant that assessment of discharge frequency in response to potassium was not possible for these three filaments.

(A)



(B)

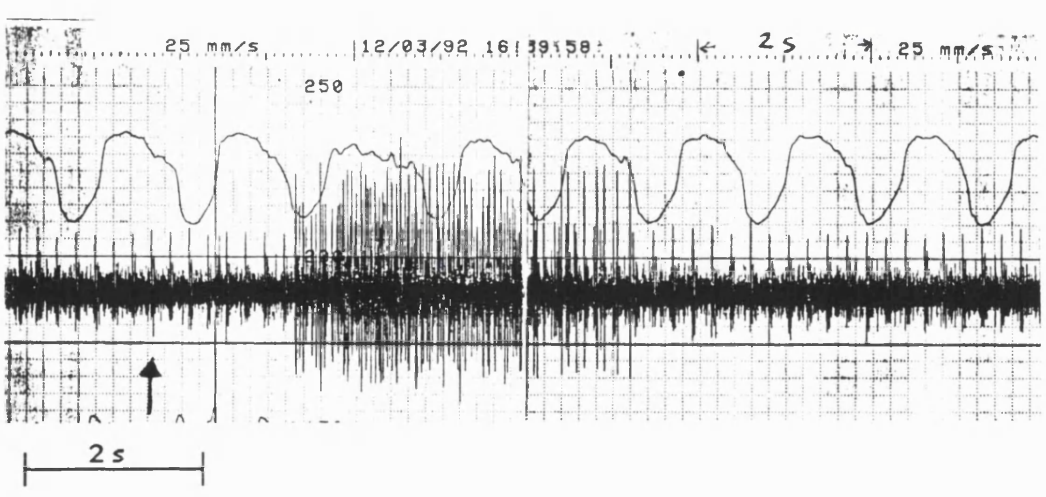
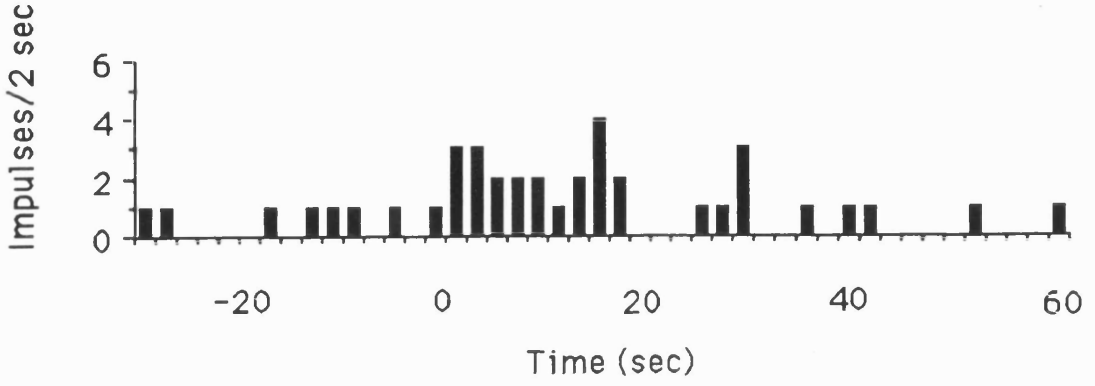


Fig. 10.2. Stimulation of phrenic afferents by topical application of potassium to the diaphragm. (A) Histogram showing rapid response to potassium by an individual fibre. Time 0 sec is the onset of stimulation. (B) Recording of action potentials of rapidly adapting afferent fibres from a different preparation: neurogram (bottom) and airflow signal (above). The arrow indicates the point of application of potassium.

(A)



(B)

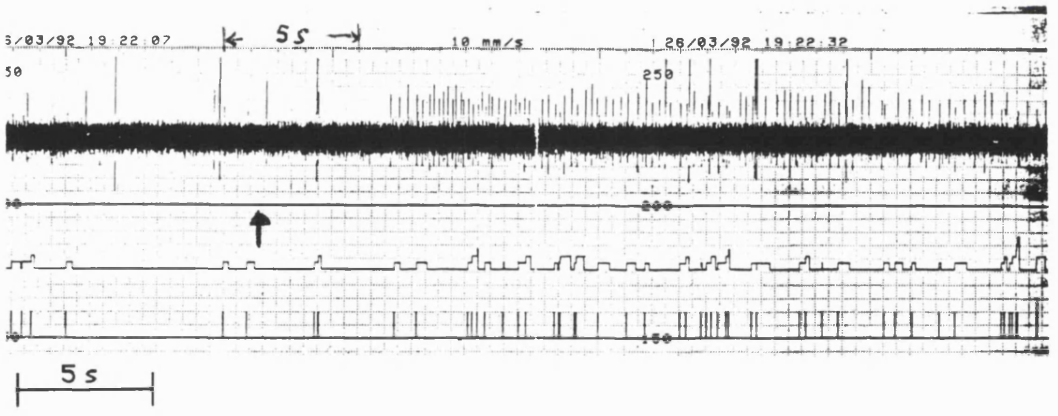


Fig. 10.3. Stimulation of phrenic afferents by topical application of potassium to the diaphragm. (A) Histogram showing a prolonged response to potassium by an individual fibre. Time 0 is the onset of stimulation. (B) Recording of action potentials of slowly adapting afferent fibres from a different preparation: neurogram (top), frequency counter output (middle) and PHA output (below). The arrow indicates the point of application of potassium.

Control experiment

The potassium solutions used in this study were made up with isotonic saline. To investigate the effect of saline or light pressure on the activity of afferent fibres, the receptive field of the afferent fibres was brushed with normal saline. In every case, no change in afferent fibre activity was seen as a consequence of saline application to the diaphragm. Evidently, therefore, neither the application of saline nor light pressure to receptive field was able to excite the phrenic afferent fibres studied.

Discussion

This study has shown that potassium can activate phrenic afferents. The time course of the two sets of fibres based on amplitude of action potential varied. Those with large impulses adapted more rapidly than those with small impulses.

Some of the filaments could not be excited by either probing or potassium. It is not clear whether the sensory endings were chemically inexcitable for technical reasons, such as damage or failure to locate their receptive field or for the physiological reason that their endings were specialized to receive a different form of stimulus. Failure to identify the receptive field may be the main reason for not exciting many of the fibres. For three filaments whose receptive fields were not located by probing, application of potassium over the whole ipsilateral hemi-diaphragm evoked an increase in their activity.

Attempts were made to measure conduction velocities for some of the nerve fibres. However, electrical stimulation of the cut phrenic nerve caused the diaphragm to twitch, pulling the fibres off the electrode and occasionally the fibres

were damaged. With a multi-fibre preparation, the recorded compound action potential would identify the group of afferents in the preparation; the compound action potential could not, however, identify the group of fibres excited by potassium unless the preparation contained one group of afferent fibres. For these reasons, recording of action potential and measurements of conduction velocities, necessary to identify groups of afferent fibres studied, were not obtained.

The tonic pattern of afferent discharge is characteristic of phrenic fibre groups III and IV. Jammes and colleagues (1984 & 1986) showed that the afferent tonic activity recorded in the phrenic nerve of a cat arose from small fibres with low conduction velocities (group III and IV), whereas phasic afferent activity arose from large fibres with high conduction velocities (group I and II). The small afferent fibres in the phrenic nerve are more numerous than large afferents; about 65% of the afferents in the phrenic nerve are group III and IV fibres (Duron, 1981). It is therefore probable that the fibres studied were small afferents. Furthermore, unlike group III and IV muscle afferents, group I and II are not usually excited by algesic substances such as potassium (Mense, 1977).

The concentration of K^+ applied to the diaphragm was similar to concentrations used by Rybicki and colleagues (1985). The design of the current experiment was such that the concentration of K^+ reaching diaphragmatic receptors could not be determined. The minimum increase in interstitial K^+ concentration required to activate group III and IV afferents in the gracilis nerve is on average 1.3 and 1.1 mmol/l respectively (Rybicki et al., 1985; Kaufman & Rybicki, 1987). Therefore it is reasonable to assume that application of K^+ to the diaphragm resulted in an increase in interstitial K^+ concentration to at least 1.1 mmol/l. Since the increase in activity of phrenic afferents was not concentration dependent, each K^+ concentration must have

resulted in maximal activation of these afferents. The amount of increase in interstitial [K^+] required to maximally activate the afferent fibres in a nerve has not been determined. However, Rybicki and colleagues (1985) have shown that group III and IV afferent in gracilis nerve increased their peak firing rate progressively with increasing interstitial potassium concentration, with peak firing rate occurring when interstitial potassium concentration was increased by about 5 mmol/l.

The latency of response of 0.4 seconds reported here is short compared to that reported by others which averaged 6 seconds (Rybicki et al., 1985; Kaufman & Rybicki, 1987). The difference in latency is related to the mode of application of K^+. In the studies by other researchers K^+ was injected intra-arterially. Their latency includes injection and circulatory time. In contrast, in this study K^+ was applied directly to muscle and as a consequence potassium had a shorter distance to travel. It had to diffuse across the abdominal surface of the diaphragm into the interstitial space before reaching a sufficient local concentration to excite the sensory endings.

An important question that should be answered is what types of diaphragmatic receptors were excited by potassium. The pattern of discharge seen and the fact they were activated by K^+ suggest that group III and IV fibres were studied. Sensory endings of these phrenic afferents have been shown to be activated by noxious chemical and mechanical stimuli (Mense, 1977; Graham et al., 1986; Jammes et al., 1986). Some of the chemical stimuli were metabolites of muscle contraction such as lactic acid. These observations indicate that sensory endings of group III and IV phrenic afferents may be metaboreceptors sensitive to chemical and mechanical stimuli. Such receptors may have been excited by K^+ in this study.

For most fibres, the increase in frequency of discharge persisted for less than 30 seconds. This finding agrees with those of Rybicki and colleagues (1985) and Kaufman and Rybicki (1987). Both research groups reported that group III and IV afferents exhibited a burst of activity to potassium stimulation which lasts up to 40 seconds. In this chapter, three filaments responded differently, a burst of activity in response to K^+ stimulation was not followed by a rapid decrease in activity to control levels. For these preparations, the increased frequency of discharge continued for several minutes. To explain this, the type of preparations used here and by other researchers must be considered.

In both Rybicki and Kaufman's studies, the activities of single afferent fibres were investigated. In contrast, the activities of multi-fibre preparations were investigated in this study. With a multi-fibre preparation, recruitment of previously quiescent fibres will be recorded on application of the stimulus to the receptive field. In this study, it is possible that fibres of similar diameter were recruited at different times. An explanation for this would be that receptors of some of the afferent fibres may have been located deep in the diaphragm or on its thoracic surface and therefore not readily exposed to K^+ on abdominal surface; over time K^+ may penetrate the muscle reaching previously inaccessible sensory endings, exciting them.

The possibility that the receptive field of these three preparations may have been damaged cannot be ignored. Increased neural activities in these preparations may have been prolonged by noxious chemicals released by damage tissue exciting their sensory endings. An example of a noxious chemical released by damage tissue is K^+.

To conclude, the diaphragm possesses free sensory endings identical to those of other skeletal muscles, which are sensitive to changes in extracellular K^+ concentration. A

third of the diaphragmatic receptors showed rapid adaptation to K^+ whereas the remainder took a long time to adapt to the stimulus. The group of afferent fibres transmitting the sensory information from the diaphragm to CNS appear to be III and IV.

CONCLUSION

CHAPTER 11

Implications and future Research

This thesis has raised more questions than it has answered. However, some important questions related to diaphragmatic nociception were answered. Moreover, potential models to explain the role played by phrenic afferent activity and changes in extracellular potassium concentration ($[K^+]$) in the control of breathing during diaphragm fatigue and exercise have emerged.

In the rabbit, the cause or causes of diaphragm fatigue were not established. Diaphragm fatigue may have been precipitated by increased arterial K^+ concentration, changes in blood chemistry during IRL as reported in Chapter 5 or because of a mismatch between the energy supply to the diaphragm and its energy demand. Indeed, the effects of hypercapnia, hypoxia, increased plasma K+ concentration and increased diaphragmatic activity on diaphragm contractility may be additive or synergistic, and together could precipitate a rapid onset of contractile fatigue. Clearly, research will be required to establish whether these combined changes in blood chemistry and diaphragmatic contraction can accelerate diaphragm fatigue. The mechanisms responsible for diaphragm fatigue remain as elusive as ever. The elucidation of the mechanisms underlying diaphragmatic fatigue was not aim of the thesis. The purpose of the thesis was to gain an insight into the role played by phrenic afferents in the control of breathing during diaphragm fatigue.

The increased arterial $[K^+]$ which occurred during severe inspiratory resistive loading (IRL) was associated with a combined metabolic and respiratory acidosis. In Chapters 6 and 7, it was shown that bulk of this rise in arterial $[K^+]$ was probably due to the metabolic component of the acidosis.

However, a contribution made by K^+ secreted from the active inspiratory muscles cannot be ruled out. If $[K^+]$ in the arterial and venous circulation from the diaphragm had been measured, a release of K^+ from the diaphragm may have been identified. A rise in arterial $[K^+]$ during IRL raises important medical questions. Does a rise in arterial $[K^+]$ occur during a severe asthmatic attack, and if so could it precipitate diaphragm fatigue in asthmatic patients?

To answer the first part of the question, an investigation into the effect of severe asthmatic attack on arterial potassium will be required as no data is available (Prof. A Dornhorst, personal communication). In case of the second part of the question, there is evidence to indicate that indeed an increase in arterial $[K^+]$ could precipitate diaphragm fatigue (Sjøgaard, 1986 & 1990). In Chapter 5, it was shown that based on theoretical considerations the magnitude of the rise in arterial $[K^+]$ during IRL for each rabbit was sufficient to induce diaphragm fatigue by lowering the resting membrane potential. Here, there is a need to establish whether hyperkalemia can reduce the diaphragmatic resting membrane potential.

Because the rise in arterial $[K^+]$ in the rabbit occurred with an acid-base disturbance similar to that of patients in respiratory failure, K^+ could be a natural stimulus of diaphragmatic nociceptors. Chapters 9 and 10 showed that the diaphragmatic receptors can be excited by K^+ and that K^+-induced activation of the phrenic afferents, innervating the diaphragmatic receptors, can produce an increase in ventilation.

Interestingly, both electrical and potassium stimulation of phrenic afferents produced tachypnea. This ventilatory response was probably mediated by group III and IV phrenic afferent fibres because only the electrical stimulation parameter that is known recruit group III and IV afferents

produced a ventilatory response and only group III and IV afferents are excited by K^+ (Mense, 1977).

The pattern of breathing associated with both increased phrenic afferent activity and inspiratory resistive loaded breathing in the vagotomized rabbit was characterized by tachypnea. However, it is not possible to conclude that the increase in breathing frequency in the vagotomized rabbit was initiated by K^+-induced activation of phrenic afferents. To investigate whether tachypnea was produced by K^+-induced activation of phrenic afferents during IRL, will require complex experiments involving the elimination of the phrenic afferents alone or all other afferent inputs that could influence breathing. Indeed, the pattern of breathing adopted during IRL may have been due to the activity of afferents other than those of the phrenic nerve.

According to Hussain and colleagues (1985), tachypnea, as observed during IRL and when the phrenic afferents were stimulated, could minimize the work of breathing during the early stages of IRL. More generally, it has been reported that for a given minute ventilation the work of breathing decreases as frequency increases (Otis et al., 1950). It is tempting to conclude that responses to both IRL and stimulation observed in this thesis minimize the work of breathing. However, it should be realized that deviation from resting minute ventilation would make ventilation less economical, and furthermore, when the phrenic afferents were stimulated, minute ventilation increased, indicating an increase in the work of the diaphragm.

The hyperventilation produced by electrical and chemical stimulation probably does not minimize the work of breathing. Such a ventilatory response mediated by phrenic afferents could ensure that alveolar ventilation increases in exact proportion to CO_2 production during exercise. Indeed, hyperventilation produced by K^+-induced activation of phrenic afferents may provide a model to explain how

ventilation is increased at a constant Pa_{CO_2} during exercise. During limb muscle exercise, K^+ is released from the active muscles and it may be that the resultant hyperkalemia may initiate reflexes arising from the diaphragmatic nociceptors and mediated by phrenic afferents, which elicit an increase in ventilation.

In conclusion, from this investigation into neural and humoral factors related to diaphragm fatigue, a model has emerged which could explain the roles of phrenic afferent activity and hyperkalemia in the development of diaphragm fatigue. During fatiguing IRL, arterial K^+ concentration is increased because of a metabolic acidosis and perhaps as a result of K^+ release from the fatiguing diaphragm. Initially, the resultant hyperkalemia initiates reflexes arising from the diaphragmatic nociceptors and mediated by phrenic afferents. This reflex culminates in an increase in inspiratory drive to overcome the obstruction and/or to prevent central fatigue. If the phrenic afferent feedback to the respiratory controller predominates, the diaphragm could be driven to exhaustion. However, in time, the hyperkalemia may become severe enough to impair diaphragmatic function, thereby preventing the diaphragm from being driven to exhaustion; that is exhaustion of diaphragmatic ATP is prevented which could have resulted in rigor mortis. This model provides the basis for future investigations.

Because the rabbits were anaesthetized, the contribution of noxious sensation arising from the diaphragm on the pattern breathing could not be assessed. However, circumstantial evidence in Chapter 9 indicates that rabbits may sense noxious stimuli applied to the diaphragm. In conscious humans, sensations arising from the diaphragm have been suggested to influence both the drive to breathe and the pattern of breathing (Guz, 1977 and 1979). Future investigations, therefore, could be directed at determining whether noxious sensation does arise from the diaphragm during fatiguing of the diaphragm.

REFERENCES

REFERENCES

Adams JM, Farkas GA, Rochester DF (1988) Vagal afferent, diaphragm fatigue and inspiratory resistance in anaesthetized dogs. J Appl Physiol 64: 2279-2286

Adrogue HJ, Madias NE (1981) Changes in plasma potassium concentration during acute acid-base disturbances. Am. J. Med. 71: 456-467

Alam M, Smirk FH (1937) Observation in man upon a blood pressure raising reflex arising from voluntary muscle. J Physiol. 89: 372-383

Aldrich TK (1987) Transmission fatigue of the rabbit diaphragm. Respir Physiol 69: 307-319

Aldrich TK (1988) Central fatigue of the rabbit diaphragm. Lung 166: 233-241

Aldrich TK, Appel D (1985) Diaphragm fatigue induced by inspiratory resistive loading in spontaneously breathing rabbits. J Appl Physiol 59: 1527-1532

Aldrich TK, Appel D (1987) Diaphragm blood flow during diaphragm fatigue induced by inspiratory resistive loading in the rabbit. (Abstract) Am Rev Respir Dis 135: A328

Asmussen E (1979) Muscle fatigue. Med Sci Sport 11: 313-321

Aubier M, Farkas G, DeTroyer A, Mozes R, Roussos C (1981a) Detection of diaphragmatic fatigue in man by phrenic nerve stimulation. J Appl Physiol 50(3): 538-544

Aubier MT, Trippenbach T, Roussos C (1981b) Respiratory muscle fatigue during cardiogenic shock. J Appl Physiol 51: 499-508

Bellemare F, Bigland-Ritchie B (1987) Central component of diaphragmatic fatigue assessed by phrenic nerve stimulation. J Appl Physiol 62: 1307-1316

Bigland-Ritchie B, Cafarelli E, Vollestad NK (1986) Fatigue of submaximal static contractions. Acta Physiol Scand 128 (Suppl 556) 137-148

Biscoe TJ, Purves MJ (1967) Observations on the rhythmic variation in the cat carotid body. J Physiol 190: 389-412

Bourgrey JM (1845) Mémoire sur les nerfs des membranes séreuses en général, et sur ceux du péritoine en particulier chez l'homme. CR Soc Biol, Paris 21: 566-570 (Cited by Hinsey JC & Philips RA, 1940)

Brackett NC, Cohen JJ, Schwartz WB (1965) Carbon dioxide titration curve of normal man. N Engl J Med 272: 6-12

Brewer G, Larson PS, Schroeder AR (1939) On the effect of epinephrine on potassium. Am J Physiol 122: 708-712

Brown EB Jr, Goott B (1963) Intracellular hydrogen ion changes and potassium movement. Am J Physiol 204: 765-770

Bruce EN, Smith JD, Grodins FS (1974) Chemical and reflex drives to breathing during resistance loading in cats. J Appl Physiol 37(2): 176-182

Burnell JM, Villami NF, Uyeno BT, Scribner BH (1956) The effect in humans of extracellular pH change on the relationship between serum potassium concentration and intracellular potassium. J Clin Invest 35: 935-939

Chance B, Boveris A (1978) Hyperoxia and hydroperoxide metabolism. In Robin (ed): Extrapulmonary Manifestations of Respiratory Disease. New York, Marcel Dekker, Inc: 185-237

Clausen T (1986) Regulation of active Na^+-K^+ transport in skeletal muscle. Physiol Rev 66(3): 542-580

Claustre J, Peyrin L (1982) Free and conjugated catecholamines and metabolites in the cat urine after hypoxia. J. Appl. Physiol. 52: 304-308

Cohen CA, G, Gross D, Roussos CS, Macklem PT (1982) Clinical manifestations of inspiratory muscle fatigue. Am J Med 73: 308-316

Comroe JH, Schmidt CF (1943) Reflexes from the limbs as a factor in the hyperpnea of muscular exercise, Am J Physiol 138: 536-547

Corda M, Von-Euler C, Lennerstrand G (1965) Proprioceptive innervation of the diaphragm. J Physiol 178: 161-177

Courtice FC (1943) The blood volume of normal animals. J Physiol 102: 290-305

Craig AB, Honig CR (1963) Hepatic metabolic and vascular responses to epinephrine: a unifying hypothesis. Am J Physiol 205: 1132-1138

Creese R, Scholes NW, Whelan WJ (1958) Resting potentials of the diaphragm muscle after prolonged anoxia. J Physiol 140: 301-317

Crosfill ML, Widdicombe JG (1961) Physical characteristics of the chest and lungs and work of breathing in different mammalian species. J Physiol 158: 1-14

Cuenod M (1961) Réflexes proprioceptifs du diaphragme chez le lapin. Helv Physiol Acta 19: 360-372

Cunningham WL, Becker EJ, Kreuzer F (1965) Catecholamines in plasma and urine at high altitude. J. Appl. Physiol. 607-6110

D'Silva JL (1936) Action of adrenaline on the perfused liver. J Physiol 87: 181-188

Davenport PW, Thompson FJ, Reep RL, Freed AN (1985) Projection of phrenic nerve afferent to the cat sensorimotor cortex. Brain Res 328: 150-153

Davies HW, Haldane JS, Priestly JG (1919) The response to respiratory resistance. J Physiol 53: 60-69

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107(4): 1198-1205

Dawson MJ, Gardian DG, Wilkie DR (1978) Muscular fatigue investigation by phosphorous nuclear magnetic resonance. Nature 274: 861-866

Dogiel AS (1902) Nervenendigungen in Bauchfell, in dem Schnen, Den Muskelspindelnund dem Centrum Tendineum des diaphragma beim Menschen und bei Saügethieren. Arch Mikroskop Anat Entwicklungsmech 59: 1-31 (Cited by Duron B, 1981)

Donaldson SKB, Hermansen L, Bolles L (1978) Differential direct effects of H^+ on Ca^{2+} -activated force of skinned fibers from soleus, cardiac, adductor magnus muscles of rabbits. Pflügers Arch 376: 55-65

Dornhorst A (1991) Personal communication.

Duron B (1981) Intercostal and diaphragmatic muscle endings and afferents. In: Thomas F Hornbein (ed) Lung biology in health and disease: The regulation of breathing, vol 17, pt 1, chapt 7, Marcel Dekker, New York, pp 473-540

Duron B,Jung-Caillal MC, Marlot D (1978) Myelinated nerve fibres supply and muscle spindles in the respiratory muscles of cat: quantitative study. Anat Embroyl 152: 171-192

Duron B, Marlot D (1980) The non-myelinated fibres of the phrenic and intercostal nerves in the cat. Z Mikrosk Anat Forsch Leipzig 94: 257-268

Eccles RM, Lundeberg A (1959) Synaptic actions in motoneurons by afferents which may evoke the flexion reflex. Rev Arch Ital Biol 97: 199-221

Edwards RHT (1979) The diaphragm as a muscle the mechanisms underlying fatigue. Am Rev Respir Dis 119 (Part 2): 81-84

Edwards RHT (1981) Human muscle function and fatigue. In Human Muscle Fatigue: Physiological Mechanisms. London. Pitmam Medical (Ciba Foundation Symposium 82): 1-18

Eldridge FL, Gill-Kumar P, Millhorn DE, Waldrop TG (1981) Spinal inhibition of phrenic motoneurones by stimulation of afferents from peripheral muscles. J Physiol 311: 67-79

Esau SA (1991) The effect of theophylline on hypoxic, hypercapnic hamster diaphragm muscle in vitro. Am Rev Respir Dis 143: 954-959

Estavillo JA, Nye PCG, Paterson DJ, Terry M (1988) The effect of hypoxia on arterial potassium concentration in the cat. J Physiol 403: 97P

Fabiato A, Fabiato F (1978) Effect of pH on myofilaments and sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. J. Physiol. 276: 233-255

Faulkner JA, Maxwell LC, Ruff GL, White TP (1979) The diaphragm as a muscle. Contractile properties. Am Rev Respir Dis 119 (Part 2): 89-92

Fell C (1968) Changes in blood flow distribution produced by central sciatic nerves. Am J Physiol 214: 561-565

Fenn WO, Asano T (1956) Effects of carbon dioxide inhalation on potassium liberation from the liver. Am J Physiol 185: 567-576

Fenn WO, Cobb DM (1934) The potassium equilibrium in muscle. J Gen Physiol 17: 629-656

Fitzgerald RS, Hauer MC, Bierkamper GG, Raff H (1983) Rat diaphragm response to unbuffered acidosis/alkalosis (Abstract) Fed Proc 42: 422

Fitzgerald RS, Hauer MC, Bierkamper GG, Raff H (1984) Responses of in vitro rat diaphragm to changes in acid-base environment. J Appl. Physiol. 57: 1202-1210

Fitzgerald RS, Howell S, Jacobus W (1986) 31-P NMR studies of intracellular pH of the in vitro resting rat diaphragm exposed to hypercapnia plus amiloride (Abstract). Federation Proc 45: 1021

Fraley DS, Alder S (1977) Correction of hyperkalemia by bicarbonate despite constant blood pH. Kidney Int 12: 354-360

Franz DN, Iggo A (1968) Conduction failure in myelinated axons at low temperatures. J Physiol 199: 319-345

Friedland JS, Paterson DJ (1988) Potassium and fatigue. Lancet 2: 961-962

Fuch F, Reddy Y, Briggs FN (1970) The interaction of cations with calcium-binding site of troponin. Biochem Biophys Acta 221: 407-409

Gandevia SC, McKenzie DK (1985) Activation of the human diaphragm during maximal static efforts. J Physiol 367: 45-56

Gernandt B (1946) Pain conduction in the phrenic nerve. Acta Physiol Scand 12: 225-260

Gasser H (1950) Unmedullated fibres originating in dosal root ganglion. J Gen Physiol 33: 651-690

Gautier H, Remmers JE, Bartlett (1973) Control of duration of expiration. Respir Physiol 18: 205-221

Graham R, Jammes Y, Delpierre S, Grimaud C, Roussos C (1986) The effects of ischaemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. Neurosci Lett 67: 257-262

Greene C (1935) Control of coronary blood flow by reflexes arising in widely distributed regions of the body. Amer J Physiol 113: 399-415

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

Heisler N (1975) Intracellular pH isolated rat diaphragm muscle with metabolic and respiratory changes of extracellular pH. Respir. Physiol. 23: 243-255

Held DR, Steiner CA (1971) Effect of acute respiratory acidosis on arterial plasma osmolality. Respir Physiol 12: 25-35

Hermansen L, Osnes JB (1972) Blood and muscle pH after maximal exercise in man. J. Appl. Physiol. 32: 304-308

Hicks CM (1990) Research and statistics. A practical introduction for nurses : Prentice Hall, pp 189-193

Hinsey JC, Hare K, Philips RA (1939) Sensory components of phrenic nerve of the cat. Proc Soc Exp Biol Med 4: 411-414

Hinsey JC, Philips RA (1940) Observation upon diaphragm sensation. J Neurophysiol 3: 175-181

Hirche H, Schumacher, Hagerman H (1980) Extracellular K^+ concentration and K^+ balance of the gastronemius muscle of the dog during exercise. Pfluegers Arch 387: 231-237

Hnik P, Holas M, Krekule I, Kriz N, Mejsnar J, Smiesko V, Ujec E, Vyskocil F (1976) Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. Pfluegers Arch 362: 85-94

Hodgkin A, Horowicz P (1959) The influence of potassium on single muscle fibre. J. Physiol. 148: 127-160

Hodgkin A, Huxley A (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117: 500-544

Hof VI, Dubo H, Daniels V, Younes M (1986) Steady-state response of quadriplegic subjects to inspiratory resistive load. J Appl Physiol 60(5): 1482-1492

Hudson B (1966) Afferent discharge from the phrenic nerve a rat diaphragm preparation. J Physiol 184: 9-10

Hughes K, Semple S, Stidwell R, Treasure T (1990) The effects of acute alteration in ventilation on arterial potassium in anaesthetized cat. J Physiol 422: 46P

Hussain S, Chatillion A, Roussos C, Magder S (1991) Chemical activation of thin-fiber phrenic afferents: 2 Cardiovascular responses. J Appl Physiol 70: 77-86

Hussain S, Magder S, Chatillion A, Roussos C (1990) Chemical activation of thin-fiber phrenic afferents: respiratory responses. J Appl Physiol 63: 1002-1011

Hussain S, Simkus G, Roussos C (1985) Respiratory muscle fatigue: a cause of ventilatory failure in septic shock. J Appl Physiol 58: 2033-2040

Inesi G, Hill TL (1983) Calcium and proton dependence of sacroplasmic reticulum ATPase. Biophys J 44: 274-280

Isaza GD, Ponser JD, Altose MD, Kelsen SG, Cherniack NS (1976) Airway occlusion pressures in awake and anaesthetized goats. Respir Physiol 27: 87-98

Jammes Y, Buchler B, Delpierre S, Grimaud C, Rassidakis A, Roussos C (1984) Investigation of the presence of small afferent fibres in the cat phrenic nerve and their possible functional role. Clin Respir Physiol 20: 4A

Jammes Y, Buchler B, Delpierre S, Rassidakis A, Grimaud C, Roussos C (1986) Phrenic afferent and their role in inspiratory control. J Appl Physiol 60: 854-867

Jardim J, Rarkas G, Prefault C, Thomas D, Macklem PT, Roussos C (1981) The failing inspiratory muscles under normoxic and hypoxic conditions. Am Rev Respir Dis 124: 274-279

Jarisch A, Landgen S, Neil E, Zotterman Y (1952) Impulse activity in the carotid sinus nerve following intra-carotid injection of potassium chloride, veratrine, sodium citrate, adenosinetriphosphate and alpha dinitrophenol. Acta Physiol Scand 25: 195-211

Javaheri S, Freidel J, Davis PJ (1989) Furosemide and cerebrospinal fluid ions during acute respiratory acidosis. J Appl Physiol 67(2): 563-569

Johannson B (1962) Circulatory responses to stimulation of somatic afferent (with special reference to depressor effects from muscle nerves) Acta Physiol Scand 54 (suppl 198): 1-95

Jones D (1981) Muscle fatigue due to changes beyond the neuromuscular junction. In Human Muscle Fatigue: Physiological Mechanisms. London. Pitmam Medical (Ciba Foundation Symposium 82): 178-196

Katz S, Perryman J (1965) Respiratory and blood pressure responses to stimulation of peripheral afferent nerves. Am J Physiol 208: 993-999

Kaufman M, Rybicki K (1987) Discharge properties of group III and IV afferents: their responses to mechanical and metabolic stimuli. Circ Res 61(suppl): I60-I65

Kohram R, Nolasco J, Wiggers C (1947) Types of afferents fibers in the phrenic nerve. Am J Physiol 151: 547-553

Kilburn K (1965) Movement of potassium during acute respiratory acidosis and recovery. J Appl Physiol 21: 679-684

Kumazawa T, Tadakai K, Mizumura K, Kim K (1983) Post-stimulus facilitatory and inhibitory effects on respiration induced by chemical and electrical stimulation of thin fibre muscular afferent in dogs. Neurosci Lett 35: 283

Krnjevic K, Miledi R (1958) Failure of neuromuscular propagation in rats. J Physiol 140: 440-461

Kugelberg E, Lindegren B (1979) Transmission and contraction fatigue of rat motor unit fibers. J Physiol 288: 285-300

Kurihara T, Brooks JE (1975) The mechanism of neuromuscular fatigue. Arch Neurol 32: 168-174

Lade RL, Roswith I, Brown E (1963) Movement of potassium between muscle and blood in response to respiratory acidosis. Am J Physiol 204: 761-764

Landau B, Alkert K, Roberts TS (1962) Studies on the innervation of the diaphragm. J Comp Neurol 119: 1-10

Langford LA, Schmidt RF (1983) An electron microscopic analysis of the left phrenic nerve in the rat. Anat Rec 205: 207-213

Linton R, Band D (1985) The effect of potassium on carotid chemoreceptor activity and ventilation in the cat. Respir Physiol 59: 65-70

Linton R, Lim M, Wolff, Wilmhurst P, Band DM (1984) Arterial plasma potassium measured continuously during exercise in man. Clin Sci 67: 427-431

Little M, McSwiney B (1938) Afferent fibres from the diaphragm. J Physiol 94: 2P-3P

Lockhat, Roussos C, Ianuzzo CD (1988) Metabolite changes in loaded hypoperfused and failing diaphragm. J Appl Physiol 65: 1563-1571

Luschka H Von (1853) Der Nervus phrenicus des Menschen. Tübingen, H Laupp: 72 (Cited by Hinsey JC & Philips RA, 1940)

Luschka H Von (1862) Die anatomie des Menschen. Tübingen, H Laupp. (Cited by Hinsey JC & Philips RA, 1940)

Macron JM, Marlot D, Duron B (1985) Phrenic afferent input to lateral medullary reticular formation of the cat. Respir Physiol 59: 155-167

Macron JM, Marlot D (1986) Effect of stimulation of the phrenic afferent fibres on medullary respiratory neurons in cat. Neurosci Lett 63: 231-236

Marlot D, Macron JM, Duron B (1984) Projections of phrenic afferences to the cat cerebellar cortex. Neurosci Lett 44: 95-98

Marlot D, Macron JM, Duron B (1987) Inhibitory and excitatory effects on respiration by phrenic nerve afferent stimulation in cats. Respir Physiol 69: 321-333

Mathiot MJ, Jammes Y, Grimaud C (1987) Role of vagal and spinal sensory pathways in diaphragmatic response to resistive loading. Neurosci Lett 73: 131-136

McCallister LW, McCoy KW, Connelly JC, Kaufman MP (1986) Stimulation of group III and IV afferents reflexly decrease total lung resistance in dogs. J Appl Physiol 61: 1346-1351

McCloskey DL, Mitchell JH (1972) Reflex cardiovascular and respiratory responses originating in exercising muscle. J Physiol 224: 173-186

Medbø JI, Sejersted OM (1990) Plasma potassium changes with high intensity exercise. J Physiol 421: 105-122

Mense S (1977) Nervous outflow from skeletal muscle following chemical noxious stimulation. J Physiol 267: 75-88

Merton PA (1954) Voluntary strength and fatigue. J Physiol 123: 553-564

Metzger JM, Fitts RH (1986) Contractile and biochemical properties of the diaphragm. Effect of exercise training and fatigue. J Appl Physiol 60: 1752-1758

Metzger JM, Fitts RH (1987) Role of intracellular pH in muscle fatigue. J Appl Physiol 62: 1392-1397

Nakamaru Y, Schwartz A (1972) The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J Gen Physiol 59: 22-32

Nassar-Gentina V, Passonneau JV, Vergara JL, Rapoport SI (1975) Metabolic correlates of fatigue and recovery from fatigue in single frog muscle fibers. J Gen Physiol 72: 539-606

Nye PCG, Paterson DJ (1987) The effect of hyperkalaemia on activity of pulmonary stretch receptors in the cat. J Physiol 391: 64P

Oster JR, Alpert HC, Rodriguez GR, Vaamonde CA (1988) Effect of acute reversal of experimentally-induced ketoacidosis with sodium bicarbonate on the plasma concentrations of phosphorous and potassium. Life Sciences 42: 811-819

Oster JR, Perez GO, Castro A, Vaamonde CA (1980) Plasma potassium response to acute metabolic acidosis induced by mineral nonmineral acids. Mineral Electrolyte Metab. 4: 28-36

Oster JR, Perez GO, Vaamonde CA (1978) Relationship between blood pH and K^+ and phosphorus during acute metabolic acidosis. Am J Physiol 235: F345-F351

Otis AB, Fenn WO, Rahn (1950) Mechanics of breathing in man. J Appl Physiol 2: 592-607

Phillipson E (1974) Vagal control of breathing pattern independent of lung inflation in conscious dogs. J Appl Physiol 37: 183-189

Poole-Wilson PA, Cameron IR (1975) Intracellular pH and K^+ of cardiac and skeletal muscle in acidosis and alkalosis. Am J Physiol 229: 1305-1310

Remmers JE, Martilla I (1975) Action of intercostal muscle afferents on the respiratory rhythm of the anaesthetized cats. Respir Physiol 24: 31-41

Revelette WR, Jewell LA, Frazier DT (1988) Effect of the diaphragm small-fibre afferent stimulation on ventilation in dogs J Appl Physiol 65(5): 2097-2106

Road JD (1990) Phrenic afferent and ventilatory control. Lung 168: 137-149

Road JD, West NH, Van Vliet BN (1987) Ventilatory effects of stimulation of phrenic afferents. J Appl Physiol 63(3): 1063-1069

Rochester DF, Bettini G (1976) Diaphragmatic blood flow and energy expenditure in the dog. Effects of inspiratory airflow resistance and hypercapnia. J Clin Invest 57: 661-672

Rogers TA (1957) Tissue buffering in the rat diaphragm. Am Physiol 191: 363-366

Rogers TA, Wachenfield AE (1958) Effect of physiologic acid of electrolyte in rat diaphragm. Am J Physiol 193: 623-626

Rolett EL, Stange S, Sjøgaard G, Kiens B, Saltin B (1990) β_2-adrenergic stimulation does not prevent potassium loss from exercising quadricep muscle. Am J Physiol 258: R1192-1200

Rotto DM, Kaufman MP (1988) Effects of metabolic products of muscular contraction on the discharge of group III and IV afferents. J Appl Physiol 64: 2306-2313

Roussos C (1979) Respiratory muscle in the hypercapnic patient. Bull Eur Physiopathol Respir 15: 117-123

Roussos C (1984) Ventilatory muscle fatigue governs breathing frequency. Clin Respir Physiol 20: 445-451

Roussos C (1990) Respiratory muscle fatigue and ventilatory failure. Chest 97 (3): 89S-96S

Roussos C, Macklem PT (1977) Diaphragmatic fatigue in man. J Appl Physiol 43: 189-197

Rybicki KJ, Waldrop TG, Kaufman MP (1985) Increasing gracilis muscle interstitial potassium concentrations stimulated group III and IV afferents. J Appl Physiol 58: 936-941

Sahlin K, Broberg S (1989) Release of potassium from muscle during prolonged dynamic exercise. Acta Physiol Scand 136: 293-294

Sant' Ambrogio G, Remmers JE (1985) Reflex influences acting on the respiratory muscles of the chest wall. In: The thorax, edited by C Roussos and Macklem PT,. New York: Dekke, 531-594

Sant' Ambrogio G, Widdicombe JG (1965) Respiratory reflexes acting on the diaphragm and inspiratory intercostal muscles of the rabbit. J Physiol 180: 766-779

Santiago TV, Remolina C, Soles V 3rd, Edelman NH (1981) Endorphins and control of breathing. Ability of naloxone to restore flow-resistive load compensation in chronic obstructive disease. N Engl J Med 304(20): 1190-1195

Scardella AT, Parisi RA, Phair DK, Santiago TV, Edelman NH (1986) The role of endogenous opioids in the ventilatory response to acute flow-resistive loads. Am Rev Respir Dis 133: 26-31

Schnader JY, Juan G, Howell S, Fitzgerald RS, Roussos C (1985) Arterial CO_2 partial pressure affects diaphragmatic function. J, Appl. Physiol. 58: 823-829

Schreider J (1883) Über die Funktionen des Nervus Phrenicus. Pflüg Arch Ges Physiol 31: 577-600 (Cited by Hinsey JC & Philips RA, 1940)

Senapati JM (1966) Effect of stimulation of muscle afferents on ventilation in dogs. J Appl Physiol 21: 242-246

Shannon R, Shear WT, Mercak AR, Bolser DC, Lindsey BG (1985) Non-vagal reflex effects on medullary inspiratory neurons during inspiratory loading. Respir Physiol 60: 193-204

Shee C, Cameron IR (1990) The effect of pH and hypoxia on function and intracellular pH of the rat diaphragm. Respir Physiol 79: 57-68

Simonsen E, Weiser P (1976) Physiological aspects and physiological correlates of work capacity and fatigue. Charles C Thomas, Springfield, IL

Sjøgaard G (1986) Water and electrolyte fluxes during balance during exercise and their relation to muscle fatigue. Acta Physiol. Scand. 128, suppl. 556: 129-138

Sjøgaard G (1990). Exercise-induced muscle fatigue - the significance of K^+. Acta Physiol. Scand. 140 (S593): 5-63.

Speck DF, Revelette WR (1987) Excitation of dorsal and ventral respiratory group neurons by phrenic nerve afferents. J Appl Physiol 62(3): 946-951

Stacey MJ (1969) Free nerve endings in skeletal muscle of the cat. J Anat 10: 231-254

Thimm F, Baum K (1987) Response of chemosensitive nerve fibers of group III and IV to metabolic changes in rat muscles. Pflüg Arch 410: 143-152

Thorton JW (1937) Broncho-dilation by stimulation of the phrenic nerve. J Physiol 90: 85-87P

Tobin RB (1958) Varying role extracellular electrolyte in metabolic acidosis and alkalosis. Am J Physiol 195: 685-692

Todd EP, Vicki RL (1971) Kalemotrophic effect of epinephrine analysis with adrenergic agonists and antagonists, Am J Physiol 220: 1964-1969

Trivedi B, Danforth WH (1966) Effect of pH on kinetics of frog muscle phosphofructokinase. J Biol Chem 241: 4110-4114

Waldrop TG, Rybicki KJ, Kaufman MP (1984) Chemical activation of group I and II muscle afferents has no cardiorespiratory effects. J Appl Physiol 56: 1223-1228

Watchko JF, LaFramboise WA, Standaert TA, Woodrum DE (1986) Diaphragmatic function during hypoxemia: neonatal and developmental aspects. J Appl Physiol 60: 1599-1604

Watchko JF, Standaert TA, Mayock DE, Twiggs G, Woodrum DE (1988) Ventilatory failure during loaded breathing: the role of central neural drive. J Appl Physiol 65: 249-255

Winkler G, Delaloye B (1957) A propos de la presence de fuseaux neuro-musculaires dans le diaphragm humain. Acta Anat 29: 114-116

Wildenthal K, Mierzwiak DS, Skinner NS, Mitchelll JH (1968). Potassium-induced cardiovascular and ventilatory reflexes from the dog hindlimb. Am J Physiol 215: 542-545.

OMISSIONS

Edwards RHT (1983) Biochemical basis of fatigue in exercise performance: Castrophe theory in muscular fatigue. In: Biochemistry of Exercise, Inter Ser Sports Sci vol. 13.edited by Vogel HG, Kruttgen HG and Poortmans J. Illinois: Human Kinetic Publ, 3-28

Guz A (1977) Respiratory sensations in man. Br Med Bull 33(2): 175-177

Guz A (1979) Sensory aspect of the diaphragm. Amer Rev Resp Dis 119 (2 Pt): 65-66

Schnader J, Howell S, Fitzgerald RS, Roussos C (1988) Interactions of fatigue and hypercapnia in canine diaphragm. J Appl Physiol 64: 1636-1643

Wanner A, Sackner MA (1973) Transvenous stimulation in anaesthetized dogs. J Appl Physiol 34(4): 489-494

Yasargil GM (1967) Systematische Untersuchung der motorischen Innervation des Zwerchfells beim Kaninchen. Helv Physiol Pharmacol Acta Suppl 18: 1-63

APPENDICES

APPENDIX A TO D

APPENDIX A

CALIBRATION OF ACID-BASE LABORATORY 4 POTASSIUM ELECTRODE- A COMPARISON BETWEEN POTENTIOMETRY (ABL 4) AND FLAME PHOTOMETRY.

Introduction

The Acid-base laboratory 4 (ABL 4) is designed to measure and calculate pH, blood gases tensions and concentration of potassium on a single sample of whole blood or plasma. It measures these parameters by electrodes. The ABL 4 has also a photometer which measures haemoglobin concentration. This blood-gas machine automatically calibrates its electrodes using two calibration solutions, carbon dioxide from a cylinder and from air at predetermined intervals.

The accuracy of the ABL 4 oxygen and carbon dioxide electrodes were checked monthly using blood tonometered with gas whose CO_2 and O_2 concentrations were predetermined by Lloyd Haldane gas analysis. The electrodes measure oxygen and carbon dioxide accurately. Repeatability of oxygen electrode is reported to be ± 0.7 at 30 mmHg and ± 4.0 at 376 mmHg. For carbon dioxide electrode, it is reported to be ± 0.3 at 40 mmHg and 1.0 at 144 mmHg.

The K^+ electrode reportedly can measure potassium to an accuracy of 0.1 mmol/l over a range of 4.4 to 10 mmol/l (ABL 4 user's manual, Radiometer Copenhagen, Feb. 1985). However, it was necessary to check that the K^+ electrode was measuring potassium concentration accurately and consistently. To this end, values of $[K^+]$ measured by the ABL 4 was compared with those obtained from flame photometry.

Methods

Four calibration solutions of potassium were made by serial dilution of a stock solution of 2 mmol/ml potassium chloride. Before using the flame photometer to analyze the calibration solutions, the flame photometer was calibrated against commercially prepared potassium solutions of 5 mmol/l and then 100 mmol/l. The lower $[K^+]$ is normally used to calibrate the flame photometer for plasma $[K^+]$ analysis, and the higher $[K^+]$ calibrates the flame photometer for urine $[K^+]$ analysis. The concentration of each calibration solution was measured twice by the flame photometer. These were then used to check the accuracy of the ABL 4 potassium electrode. The concentration of each calibration solution was then measured by the ABL 4. Each measurement of $[K^+]$ was duplicated. The results are presented below.

The sensitivity, S, of the ABL K^+ electrode was calculated from ABL 4 and flame photometry measurements:

$$S\ (\%) = \frac{\text{ABL 4 } [K^+]}{\text{FP } [K^+]} * 100 \qquad (A.1)$$

where
ABL 4 $[K^+]$ = potassium concentration measured by the ABL 4 electrode.
FP $[K^+]$ = potassium concentration measured by a flame photometer

Results

Table A.1. Potassium measurements for four calibration solutions. Plasma and urine denote when the flame photometer was calibrated for low (with 5 mmol/l [K^+]) and high (with 100 mmol/l [K^+]) potassium concentration respectively.

	Flame Photometer (plasma) [K^+] (mmol/l)	Flame Photometer (urine) [K^+] (mmol/l)	ABL 4 [K^+] (mmol/l)
1	14.4 ± 0.1	12.9 ± 0.3	13.0 ± 0.05
2	6.6 ± 0.08	5.9 ± 0.1	6.2 ± 0.05
3	3.3 ± 0.02	2.6 ± 0.05	2.9 ± 0.0
4	1.6 ± 0.01	1.0 ± 0.05	1.5 ± 0.0

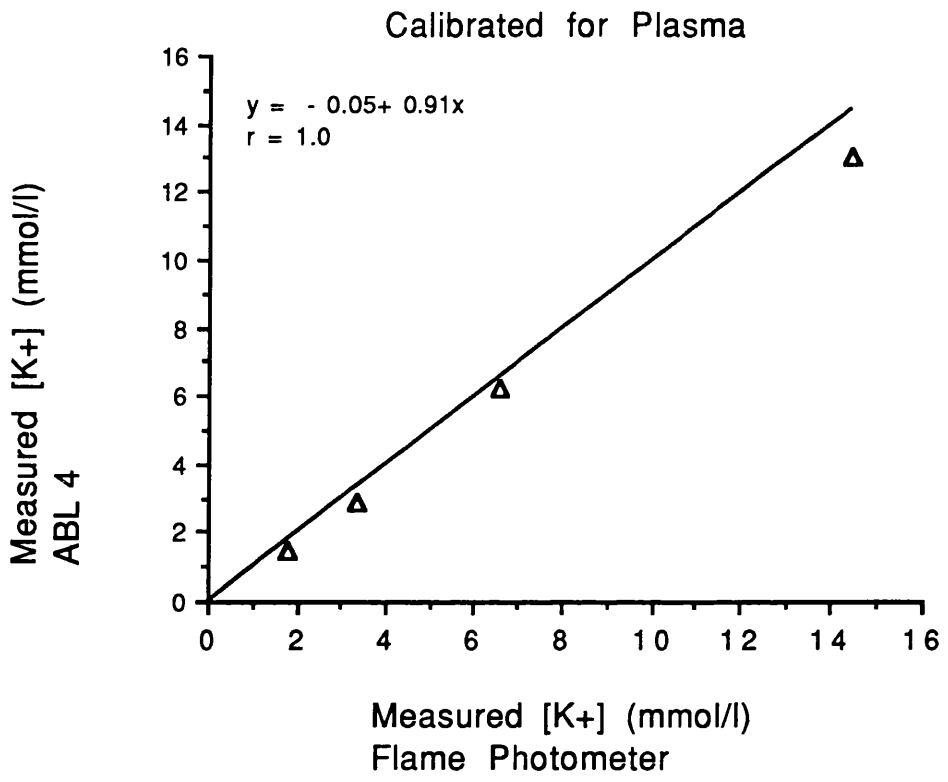


Fig. A.1. Relationship between potassium concentrations measured using ABL 4 and concentrations measured using a flame photometer. The flame photometer was calibrated for measuring plasma [K^+]. The straight line is the line of identity but the equation refers to the line of best fit. The equation indicates a strong positive correlation between [K^+] measured by flame photometry and that measured by potentiometry.

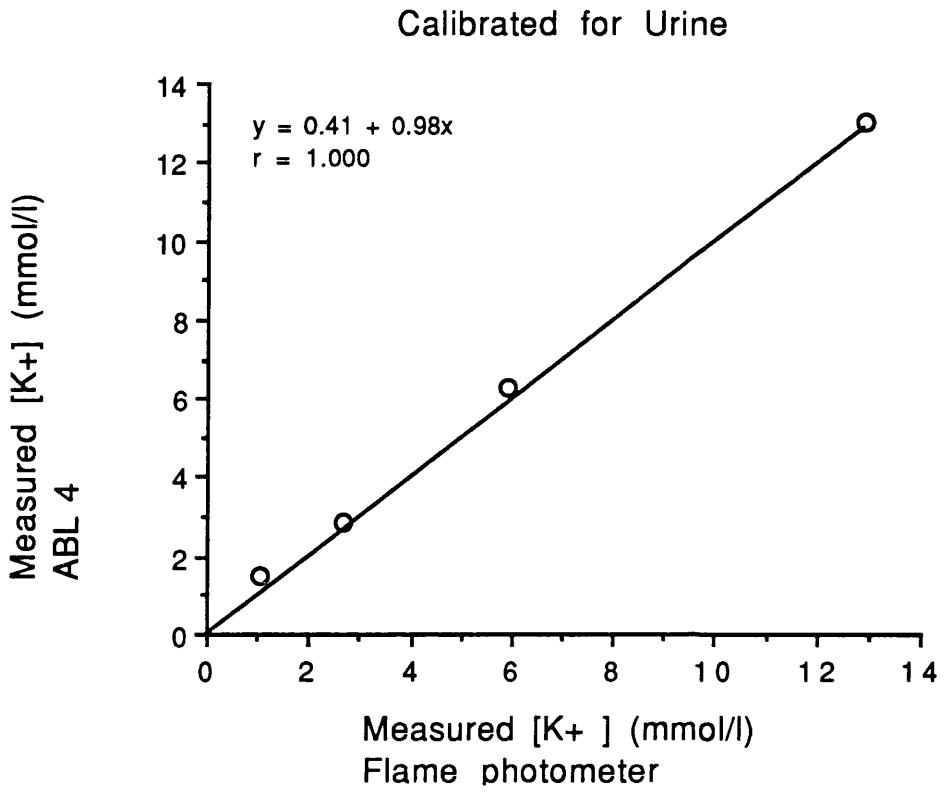


Fig. A.2. Relationship between potassium concentrations measured using ABL 4 and concentrations measured using a flame photometer. Flame photometer was calibrated for measuring urine [K^+]. The straight line is the line of identity but the equation refers to the line of best fit. The equation indicates a strong positive correlation between [K^+] measured by flame photometry and that measured by potentiometry.

Conclusion

The relationship between potassium measurements made by the ABL 4 and by a flame photometer is linear over the range of 1 to 14 mmol/l. This covers the physiological range for potassium concentration. The ABL 4 K^+ electrode was either 91% or 98% as sensitive as the flame photometer depending on whether the flame photometer was calibrated for measuring plasma or urine potassium concentrations respectively.

APPENDIX B

CALIBRATION OF GAELTEC PRESSURE TRANSDUCER

Introduction

The manufactures of Gaeltec transducers report that the transducers behave linearly over the pressure range of 0 to +300 mmHg. To ensure the Gaeltec pressure transducers linearly covered the range of inspiratory pressure ranged in rabbits, they were calibrated against a water manometer over a range of -60 to +50 cmH_2O.

The Principles of The U-Tube Manometer

A simple manometer can be used to measure pressure both above and below atmospheric pressure in a container. In this study, a manometer consists of water in a U-tube opened on one side to the atmosphere, with the other side closed to the atmosphere (see Fig. B.1). Some dry gas (D) is trapped in the closed end. The pressure of D is equal to the liquid pressure (p) at C. This in turn is equal to $A + P_h$, where A is the atmospheric pressure and P_h is the head of pressure of water above B.

$$p = A + P_h \qquad \text{(B.1)}$$

where $P_h = h \rho g$

h is height of the column of water above or below C, ρ is the density of water and g is the acceleration of the column of water due to gravity. Because both ρ and g are constants, they can be ignored to simplify equation B.1 to give thus:

$$p = A + h \qquad \text{(B.2)}$$

Methods

Construction of U-tube water manometer

The manometer was constructed from two glass tubes 51 cm length and a rubber tubing 120 cm in length. The glass tubes had an internal diameter of 0.45 cm and an external diameter of 0.60 cm. The rubber tubing internal and external diameters were 0.65 and 0.80 cm respectively. The tubes and tubing were fitted together as shown in the figure below. The joints between them were sealed with strips of waterproof elastoplast. The total length of the manometer was 215 cm. The manometer was carefully filled with water to ensure a continuous column of water.

Protocol

Before checking whether Gaeltec transducer can measure sub-atmospheric pressure, it was calibrated against pressure generated by a mercury sphygmomanometer.

One end of the manometer was connected to a Gaeltec pressure transducer while the other end was open to air. The closed column was fixed whereas the open column was moved up or down with respect to the closed column. To increase pressure in D, the opened column was raised so that B was above C. The pressure in D was decreased by lowering the opened column. This opened column was moved so that B was from 50 cm above to 60 cm below C. The *h* was measured using a metre rule. The pressure of D was measured by the Gaeltec transducer and recorded on electrostatic recorder (Gould ES 2000). The recorded deflections were measured.

The results of this investigation have been tabulated in Tab. B.1 as well as presented graphically below in Fig B.1. The head of pressure measurements (h) are presented below as

gauge pressures (pressures above and below atmospheric pressure) and not as absolute pressures.

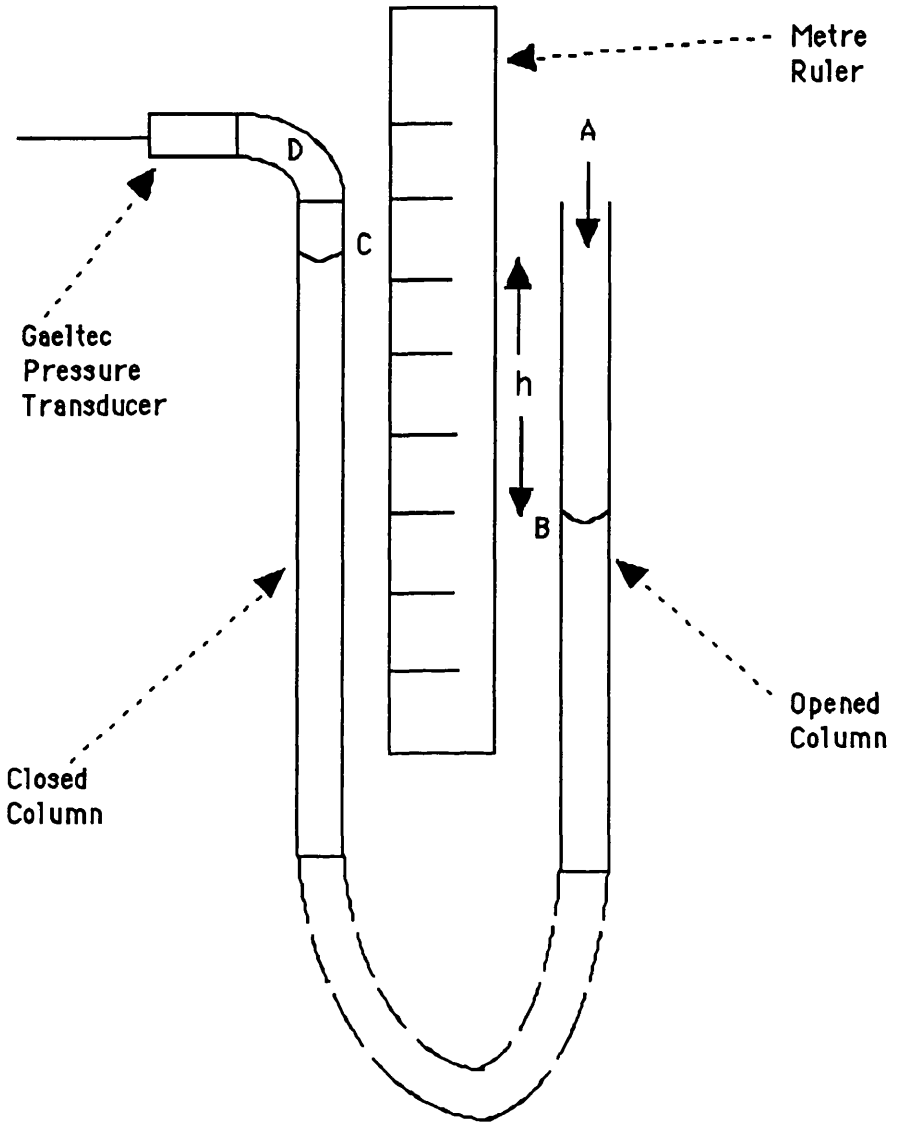


Fig. B.1. Diagram of the experimental set-up used to calibrate the Gaeltec pressure transducer. Figure is not drawn to scale.

Results

TABLE. B.1. Gaeltec Pressure transducer calibration at various pressures.

Pressure in D (mmHg)	Head of pressure h (cmH_2O)
38	50
30	39
24	31.5
15	20
7	10
0	0
-3	-4
-4	-5
-5	-7
-7	-9
-15	-20
-24	-32
-31	-41
-35	-45
-40	-52
-44	-57
-47	-61

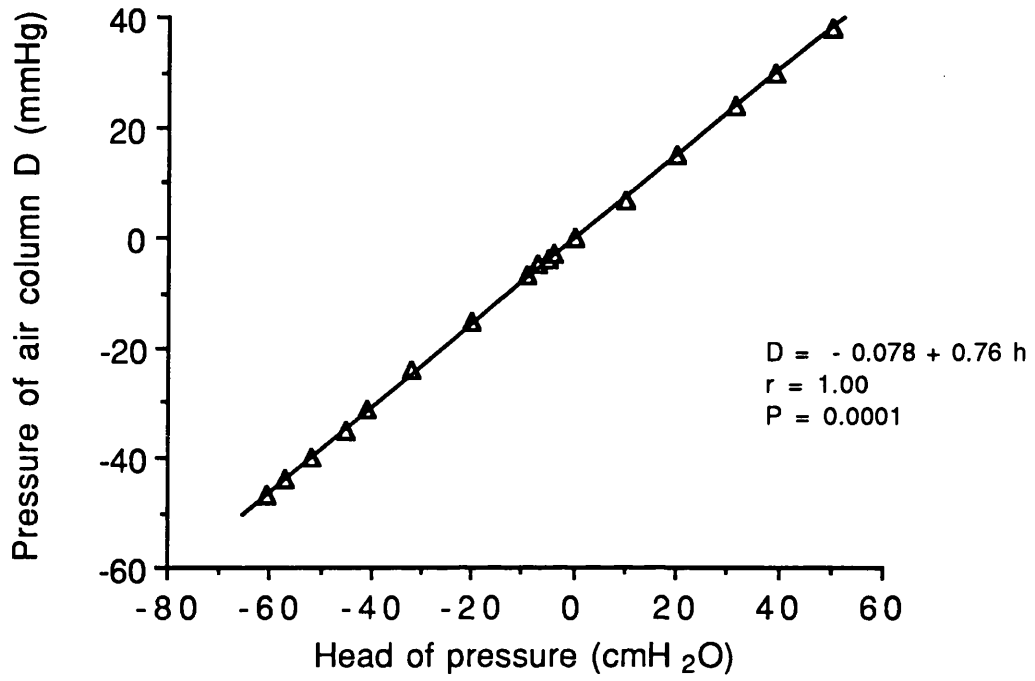


Fig. B.2. Gaeltec pressure measurements over a range of pressures. The graph shows strong positive correlation between head of pressure and measured Gaeltec pressure.

Conclusion

The Gaeltec pressure transducers behave linearly over the range of inspiratory pressures studied in this thesis.

APPENDIX C

CALIBRATION OF INSPIRATORY RESISTOR

Introduction

The pressure difference due to laminar flow in a straight, rigid tube is described by Poiseuille's law:

$$\Delta P = 8\ l\ \mu\ f/\pi\ r^4 \qquad (C.1)$$

where ΔP = pressure drop in the tube (P1-P2), l = length of the tube, r = tube radius, μ = viscosity of the fluid, f = volume flow through the tube.

Resistance to laminar flow is given by equation C.2. Dividing through by f, C.1 becomes $\Delta P/f = 8\ l\ \mu\ /\pi\ r^4$. Resistance (R) is equal to $8\ l\ \mu\ /\pi\ r^4$. Hence

$$R = \Delta P/f \qquad (C.2)$$

Methods

The ends of the resistor were each connected to a T-piece (Fig. C.1). Gaeltec pressure transducers were connected to each T-piece. An air cylinder was attached to one end of the set-up. Airflow between rates of 0.5-2.0 l/min was passed through the resistor. The flow meter of the air cylinder was previously calibrated by measuring air collected by spirometer over a minute at three flow settings. Measurements for each setting were made three times and there was no variation among the three measurements.

The order of the airflow rates was randomized. The pressure either side of the resistor was measured by Gaeltec pressure

transducers. Pressure signals were electronically summed to give pressure difference across the resistor. Both the summed and the individual pressure signals were recorded on Gould ES2000. Pressure measurements at each airflow rate were duplicated. The results are presented graphically below in Fig C.2.

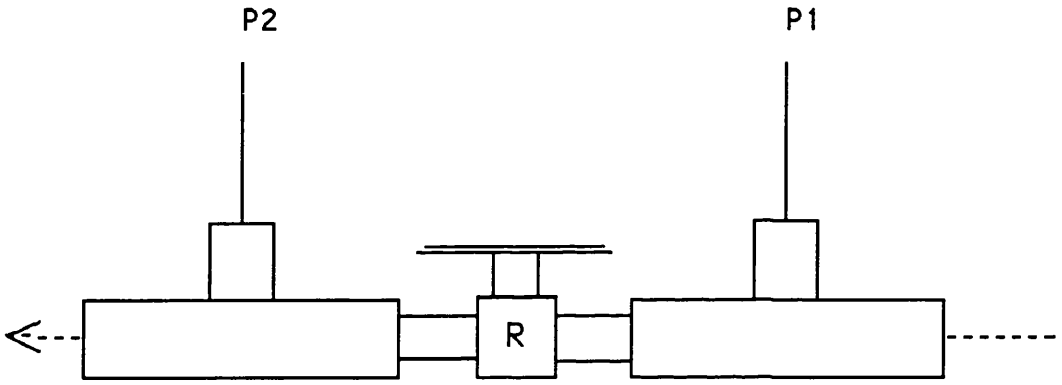


Fig. C.1. Diagram of the experimental set-up for determining the resistance of the resistor. Dotted arrow indicates direction of airflow. P1 and P2 are pressures measured by separate Gaeltec transducers. R represents the stopcock resistor. The resistance of R is measured by this experimental set-up.

Results

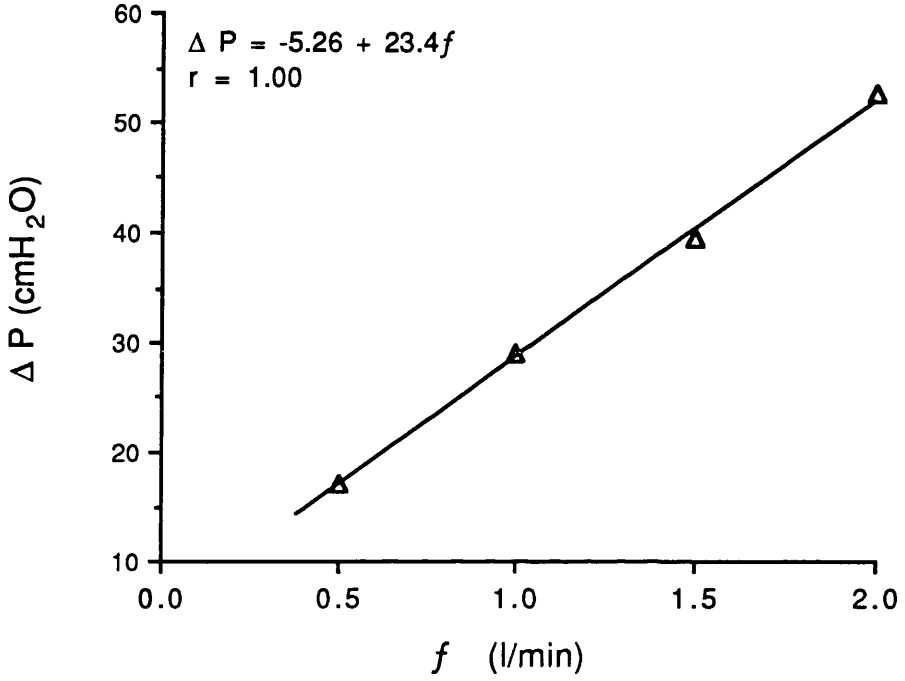


Fig. C.2. Relationship between airflow (f) and pressure gradient (ΔP) across the resistor. Each point represents the mean of two pressure measurements. The plot shows a good linear correlation. This indicates that airflow through the resistor was laminar over the flow range studied. From slope of the graph, the resistance of the resistor to airflow was determined. The resistance was 23.4 $cmH_2O/l/min$.

Conclusion

The resistor had a resistance of 23.4 $cmH_2O/l/min$. Air flow through the resistor appeared to be laminar over range of ventilation in the rabbit.

APPENDIX D

CALIBRATION OF THE CONTROL DIALS OF THE ALEC ELECTRICAL STIMULATOR

Introduction

Practical requirements for a good stimulator include: a defined shape stimulus, independently variable parameters, and low internal impedance. Another important requirement is that rise and the fall of the stimulus should be independent of the amplitude and frequency of the pulse. With a harmonic stimulus, a change in amplitude and frequency will alter the shape of the slopes Moreover, the effect of the stimulus may depend upon the phase of the wave at which stimulus is switched on and off. In contrast, the slopes of rectangular pulse are affected by changes in amplitude or frequency. Consequently a rectangular stimulus is preferred to harmonic or more complex or indefinable stimuli. The Alec stimulator was built with these requirements in mind by Alex Birkett (University College London Medical Physics Dept.). Besides the above requirements, this stimulator needed to have two identical, independently controlled output amplifiers.

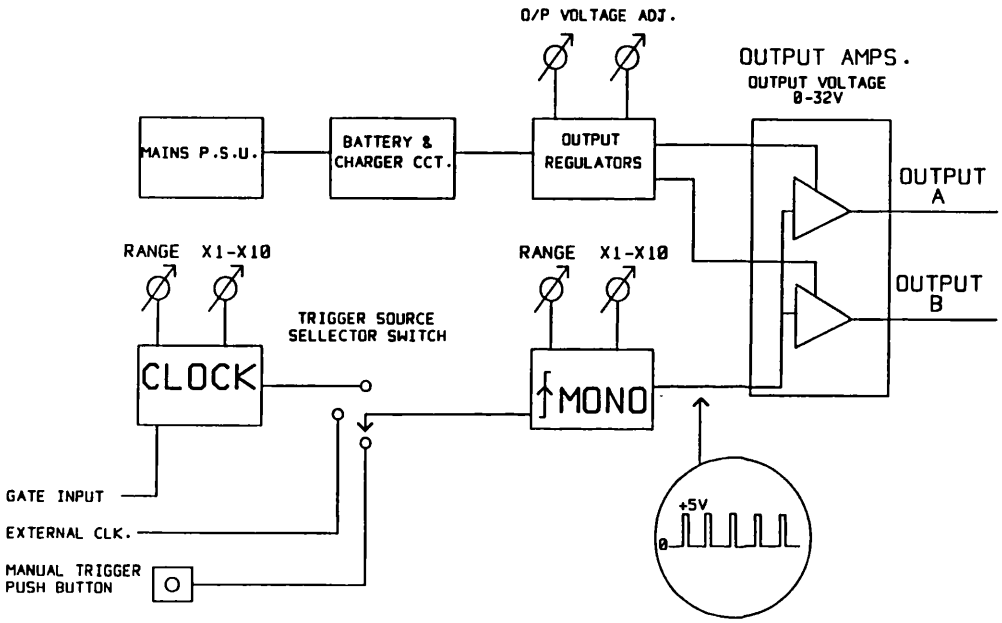


Fig. D 1. Schematic diagram of the Alec stimulator. Explanation of the diagram is given in the text below.

The Alec stimulator generates rectangular pulses. A train of rectangular pulses is determined by four parameters: the amplitude, the width of each pulse, the repetition frequency, and the duration of the train. A pulse or train of pulses is generated when triggered by internal clock oscillator, gating device, external clock, or manual trigger. The manual switch causes a single pulse to be generated. Each rising edge of logic signals greater than 3 V and less than 15 V arising from an external clock causes a single pulse. Pulses generated by internal clock are produced at a fix rate determined by the pulse repetition frequency control. Internal clock oscillator has a range control and a x1, x10, and x100 multiplier switch. The width of each pulse is defined by the pulse width control (Mono-stable). Mono-stable is triggered by the rising edge of a pulse. It has a range control and a x1 and x10 multiplier switch.

Power is supplied by four 9 V nickel cadmium rechargeable batteries or mains power supply. When the mains power supply is used, the batteries are charged. The stimulator can be used as an isolated pulse generator when the mains charger head is removed and external power supply is not in use. The output voltage to amplifiers A and B can be controlled independently at the output regulators (10 turn potentiometers). The output voltage ranges from 0 V to 36 V. It can be amplified to a maximum of a 100 V by an electrical mains powered pulse amplifier. Both the pulse generator and amplifier were designed to give an output current between 0 and 25 mA.

Technical specification

Power requirement	:	220 volts (± 11%), 50-60 Hz
Width	:	22 cm
Depth	:	23.5 cm
Height	:	10 cm
Total weight including batteries	:	3075 g

Description of front panel

Power switch	: on/off
Mode selection	: Internal, External, and Manual triggers
Frequency	: Range + x1, x10, x100 Hz
Pulse Width	: Range + 0.01, 0.1, 1 ms
Output A and B	: Voltage control
Yellow LED	: Power on indicator
Red LED	: Battery indicator

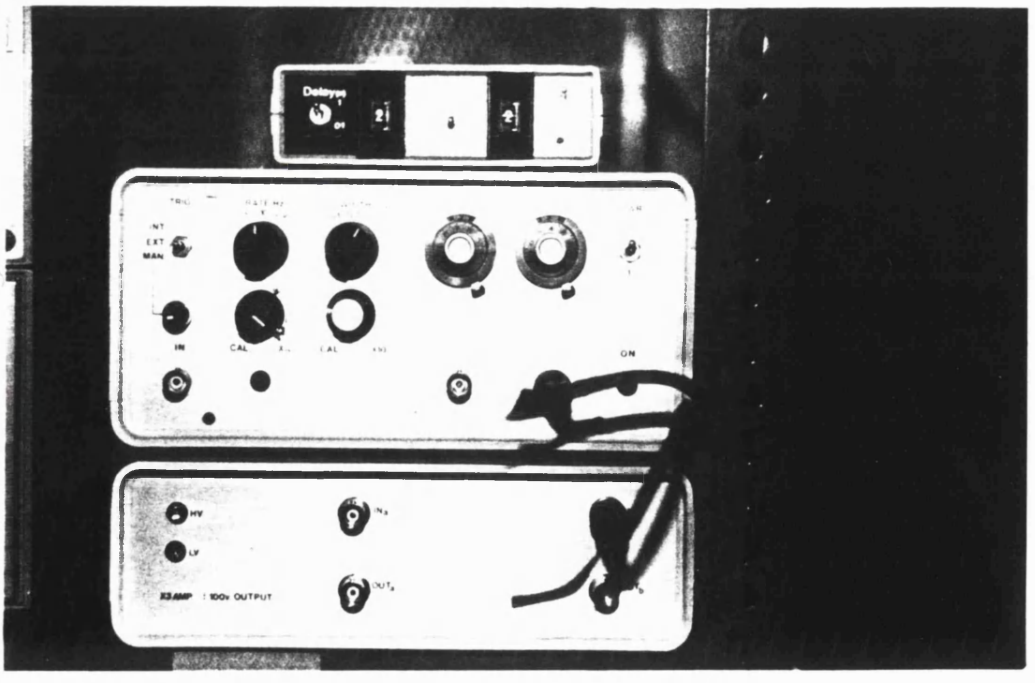


Photo. D.1. Gating device (top), Alec electrical stimulator (middle), and pulse amplifier (bottom). On the front panel of Alec stimulator, dials A and B are right and left respectively. To the right of the stimulator is the Tektronix storage oscilloscope.

Methods

The dials on Alec electrical stimulator regulating pulse width, pulse amplitude (volts), and frequency were calibrated. Both outputs were displayed on a four channel storage oscilloscope (Tektronix) to verify that the outputs were identical. The potentiometer controlling pulse amplitude was also calibrated with either the attenuator or pulse amplifier connected to its output.

To measure amplitude of pulse, the signal amplitude of the oscilloscope was set as follows: calibrating the potentiometer with attenuator connected, it was 0.1 volts per vertical divisions and with pulse amplifier connected 5 or 15 volts per vertical division; when calibrating stimulator without attachments, it was 5 volts per division. The time base was set at 1 milliseconds per horizontal division. To calibrate the potentiometers, they were adjusted while the number on them and the pulse amplitude were noted.

CALCULATION OF PULSE AMPLITUDE AND WIDTH

To measure the pulse width, the time base was set at 0.1 to 5 ms/division, and the dials moved positions noted and width of pulse measured. The signal amplitude of the oscilloscope was kept constant at 5 V/division and pulse amplitude at 10 V. Sometimes the storage facility of the oscilloscope was used to facilitate measurements of pulse width, and pulses per second.

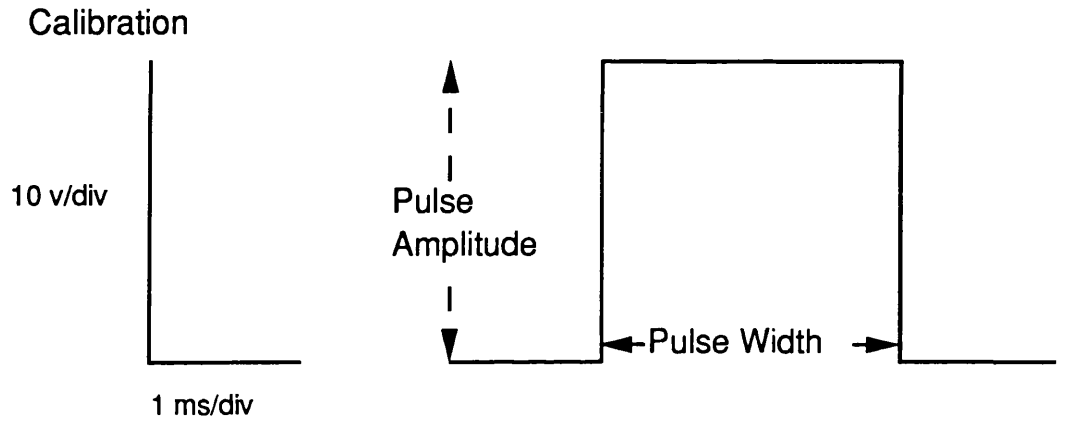


Fig. D.2. Determination of pulse amplitude and width.

Pulse amplitude

The pulse amplitude was calculated using this formula:

$$A = h \times cal_V \qquad (1)$$

The symbol A denotes pulse amplitude in volts and h denotes the high of the rectangular pulse in millimetres. Cal_V is the vertical calibration of the oscilloscope in volts per millimetre.

Pulse width

The pulse width was calculated using this formula:

$$W = d \times cal_H \qquad (2)$$

The symbol W denotes pulse width in ms and d denotes the width of the rectangular pulse in mm. Cal_H is the horizontal calibration of the oscilloscope in ms/mm.

CALCULATION OF PULSE FREQUENCY

To measure frequency of the pulses, the time base of the oscilloscope was set at 1 second per division for low frequencies through to 1 millisecond per division for high frequencies. The position of frequency control adjusted and the number of pulses per unit time recorded.

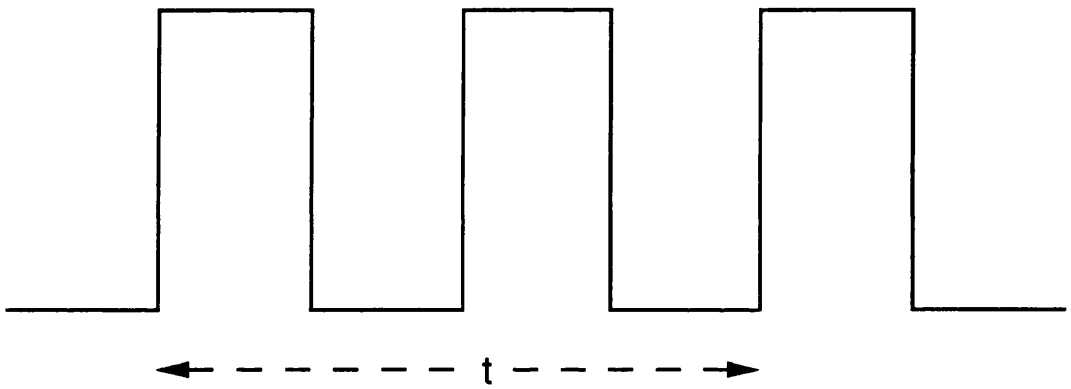


Fig. D.3. Determination of pulse frequency. Two pulses are counted between distance t.

Pulse frequency

The pulse frequency was calculated using formula (3):

$$H = n/t \qquad (3)$$

The frequency of pulse (H) is measured in pulse per seconds (Hz). The symbol n denotes the number of pulses counted in a unit time (t) in sec.

For example

If the number pulses counted in 50 ms is two, n will equal 2 and t will equal 50/100 sec.

Thus from equation (3) H = 2/0.05

The calculated frequency is 40 Hz.

DETERMINATION OF RISE AND FALL TIME OF THE RECTANGULAR PULSE

The rise and fall time of the rectangular pulse generated by Alec stimulator was measured by Alex Birkett.

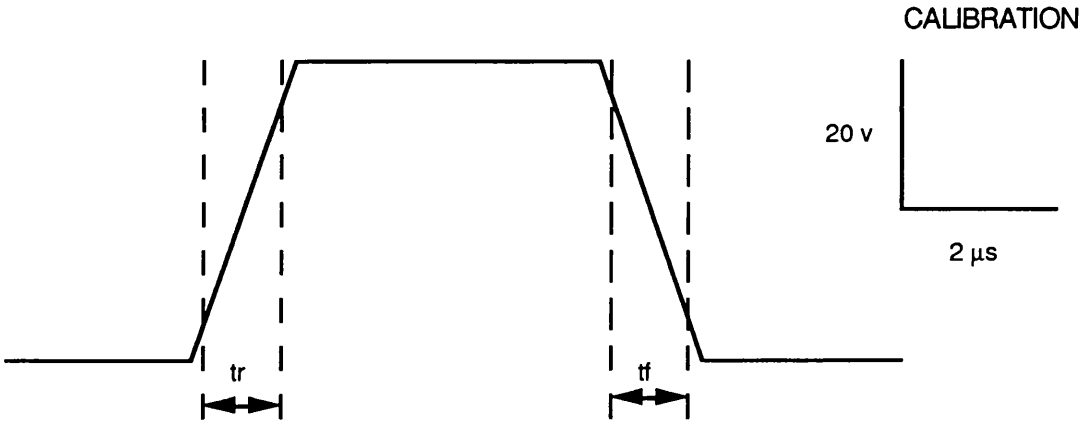


Fig. D.4. Schematic diagram of a rectangular pulse. The slopes are exaggerated to show how the rise time (tr) and fall time (tf) are determined. A pairs of dotted lines transecting the slopes mark the 10% and 90% rise and fall time.

Results

Pulse amplitude (volts), frequency, and width settings on dial A and B

Table D.1. Showing the range of frequencies, pulse width, and voltage generated by the Alec electrical stimulator.

PARAMETER	RANGE
Frequency	2-370 Hz
Pulse width	0.1-12 ms
Voltage	0-36 V (without pulse amp.) 0-100 V (with pulse amp.)

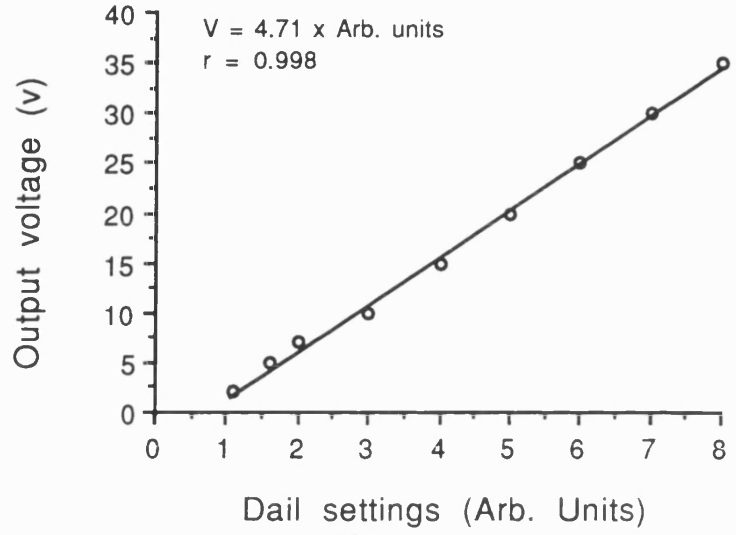


Fig. D.5. Calibration of the potentiometer of the Alec electrical stimulator.

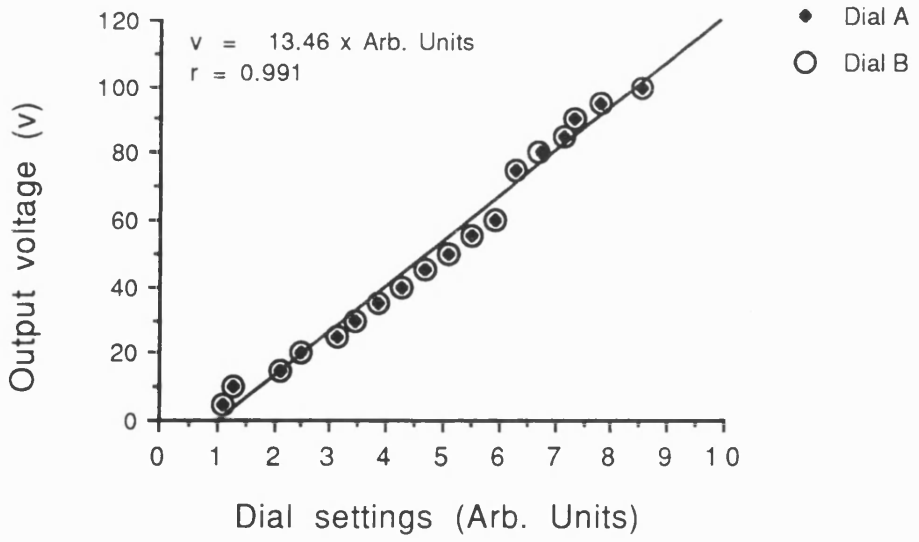


Fig. D.6. Calibration of the potentiometer of the Alec electrical stimulator when connected to pulse amplifier.

Dial A and B settings with attenuator connected

Because of the design of the dials, it was not possible to have consistent set for voltage outputs less than one volt. The attenuate was built to ensure that stimulator could consistently deliver one or fewer volts at a particular setting. The attenuator has three settings 0, 20 decibel (dB), and 40 dB. At setting 0, the voltage is not attenuated. Settings of 20 and 40 dB reduce the voltage to a tenth and hundredth respectively of the voltage output from the pulse generator.

The calibration curve when the attenuator was set at 40 dB shows a better correlation between voltage output and step changes in dial settings than calibration performed when it was set at 20 dB (see figs D.7 and D.8). For this reason, the attenuator was set at 40 dB when twitch thresholds were being determined in Chapter 8. However, when one volt output was required the attenuator was set at 20 dB and the dial at 2.3 Arb. Units. These results were identical for both dials A and B.

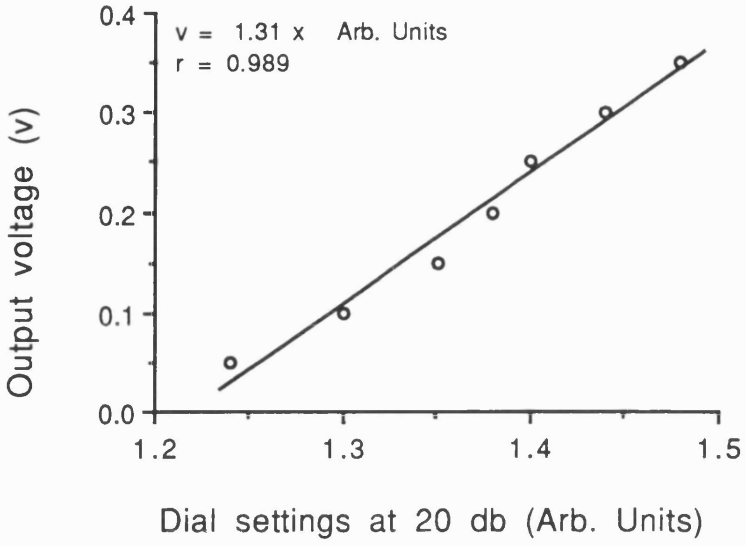


Fig. D.7. Calibration of the potentiometer of the Alec electrical stimulator when connected to attenuator set at 20 dB.

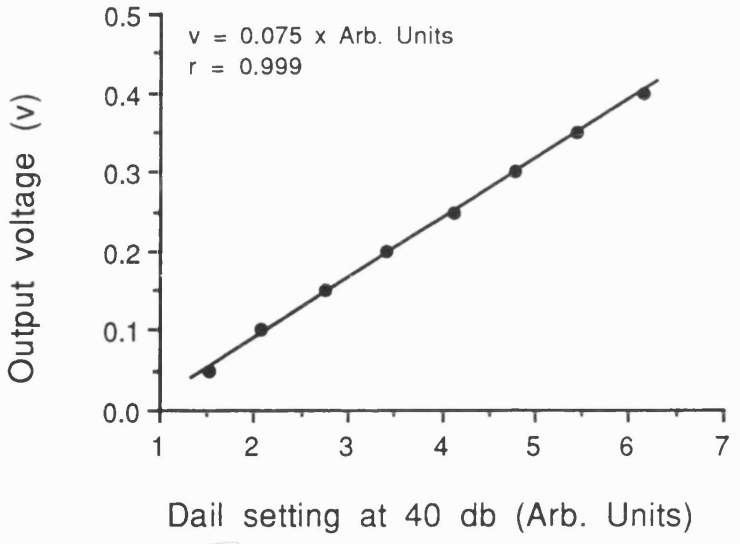


Fig. D.8. Calibration of the potentiometer of the Alec electrical stimulator when connected to attenuator set at 40 dB.

Rise and fall time of the rectangular pulse

The rise time and fall time were calculated to be ≤ 1 μs and were independent of the amplitude and stimulation frequency.