Vagal mechanisms in respiratory control

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Vagal Mechanisms in Respiratory Control

VAGAL MECHANISMS IN RESPIRATORY CONTROL

Vagale Mechanismen voor de Regulatie van de Ademhaling

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
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CONTENTS

Chapter 1:	Introduction, summary of results and conclusions	7
Chapter 2:	Vagal amplification of inspiratory activity at different levels of ventilation	15
Chapter 3:	Vagally mediated modification of inspiratory activity induced by changes in airway pressure	33
Chapter 4:	Reflex mechanisms and vagal afferents involved in the response of inspiratory activity to changed airway pressure	51
Chapter 5:	Conventional versus slug CO ₂ loading and the control of breathing	63
Chapter 6:	General discussion: a model for the pulmonary contribution to the control of breathing	77
Chapter 7:	Samenvatting	87
	List of Abbreviations	92
	Curriculum Vitae	93
	List of Publications	94
	Nawoord	95

Chapter 1

Introduction, summary of results and conclusions

Introduction

The contribution of vagal afferents from the lung to the control of breathing has been a subject of study since the investigations of Hering and Breuer. Their report (Hering, 1868) already described the responses to end-inspiratory occlusion of the trachea and to forced deflation of the lung. These reflexes are now usually referred to as Breuer-Hering reflexes. The responses were shown to be mediated by afferents in the vagal nerves. For a long time the Breuer-Hering reflexes have been thought to function as a kind of safety mechanisms to protect the lungs from overinflation or collapse (Adrian 1933). The search for a "mixed venous CO_2 receptor" in the early sixties which would mediate the exercise hyperpnea (Dejours, 1964), renewed the interest for vagal receptors. Many experiments have been conducted since then which demonstrated a contribution of vagal mechanisms in the response of ventilation to metabolic CO_2 loads (Bartoli et al., 1974; Banzett et al., 1978). The most convincing experiments were carried out by Sheldon and Green (1982) and Green and Sheldon (1983). These two authors used anaesthetized dogs in which the systemic and the pulmonary circulation were completely separated and controlled. The arterial PCO_2 (P_aCO_2) in the systemic circulation was kept constant while the pulmonary blood flow or PCO_2 of the pulmonary arterial blood was varied. Increases and decreases in both variables caused appropriate changes in ventilation. All responses disappeared after transection of the cervical vagi. These findings suggest that vagal reflex mechanisms are involved in the chemical control of breathing.

The impact vagal reflexes may have on respiratory control depends on the properties of the receptors in the tracheobronchial tree that are involved. Three types of receptors are now commonly discerned, all of which have their afferents in the vagal nerves: the slowly adapting stretch receptor (SAR), the rapidly adapting stretch receptor (RAR) and the C-fiber (Coleridge and Coleridge, 1986). The properties of the lung receptors as described below are derived mainly from experiments in cats and dogs. When differences between these two experimental animals are important, the species is indicated.

SARs—also called pulmonary stretch receptors—are located in the smooth muscle layers of the trachea and bronchi up to the level of the terminal bronchioles and alveolar ducts. Their afferent fibers in the vagal nerves have myelin sheaths, allowing fast conduction ($12\text{--}59\text{ m s}^{-1}$, Sant'Ambrogio, 1982).

The RARs—also called irritant receptors—are found mainly in the larger bronchi, higher in concentration at bifurcations, especially at the carina. In smaller bronchi they are thought to be scarce. The endings of the receptor complexes are located in the epithelial layer, but they may also be present in less superficial layers (Coleridge and Coleridge, 1986). Their afferents are also myelinated. RARs are probably thinner than SARs. The conduction velocities of RARs are on average smaller than those of SARs, but they overlap extensively ($4\text{--}37\text{ m s}^{-1}$, Sant'Ambrogio, 1982).

C-fibers endings have been found all over the lung and extra thoracic airways. The intrapulmonary C-fibers—probably the type J receptors of Paintal (1973)—are more accessible by the pulmonary than by the bronchial circulation. In the hilar nerve, the

unmyelinated C-fibers outnumber the myelinated fibers of RARs and SARs, their ratio being between 4:1 and 10:1 in cats (Coleridge and Coleridge, 1986). The conduction velocities of the unmyelinated C-fibers are low ($<3 \text{ m s}^{-1}$, Jonzon et al. 1988).

The different types of receptors react to a wide variety of stimuli. Not all of the evidence is without controversies, due in part to species differences. The most important stimulus for SARs is stretch. Furthermore, SAR activity is increased considerably by lowering PCO_2 at the receptor site, especially between 0 and 3 kPa (Coleridge et al., 1978).

RARs are also sensitive to stretch and CO_2 , but besides that to a vast number of chemicals like histamine, SO_2 , ammonia, dust and cigarette smoke. It is not always clear whether RARs are stimulated directly or—also—as a result of changes in lung mechanics, especially broncho-constriction (Coleridge and Coleridge, 1986; Grunstein et al., 1977).

For C-fibers hyperinflation and increases in pulmonary blood volume are considered to be the most important mechanical stimuli. Among chemicals that evoke responses are capsaicin and phenyl diguanide (Coleridge and Coleridge, 1986), both of which lack any physiological function. As to yet, the role of these receptors in the control of breathing under normal or pathological conditions is not clear. The relatively large number of these fibers present in the hilar nerves of the cat suggests a more explicit function than has hitherto been found.

This thesis describes the studies of three vagal reflexes, all of which may be important for the control of breathing at increased ventilatory levels. Two of these reflexes—the vagal amplification of inspiratory activity by increased airflow and the modification of inspiratory activity by changes in end-expiratory transpulmonary pressure—form part of two different positive feedbacks. The third reflex studied is part of a negative feedback related to the inspiratory off-switch mechanism and demonstrates the contribution of the CO_2 sensitivity of vagal receptors to the control of minute ventilation ($\dot{V}_E$) and the accompanying breathing pattern. All experiments were carried out in spontaneously breathing, anaesthetized cats. The relationships observed are characterized quantitatively and their relative contributions to the control of hyperpneic breathing are discussed.

Summary of Results and Conclusions

The vagal amplification reflex

Chapter 2 describes the responses of inspiratory activity to changes in air flow to the lungs. Increasing the air flow to the lungs reinforces the rate of change of the stretch imposed upon the lung and its receptors. In an earlier study by Pack et al. (1981) a large increase in phrenic nerve (Phr) activity was found in response to a rapid inflation of the lung. Stopping the air flow at the start of neural inspiration lowered Phr activity. Even larger effects may be expected for intercostal muscle (IC) activity (DiMarco et al. 1981). These studies were performed in artificially ventilated animals. Further, inspiratory

activity measured during rapid inflations was compared to the corresponding activity during normal inflations. However, rapid inflations, besides an occasional sigh, occur only during hyperpnea in which case the inspiratory activity with occluded airways (no air flow) may be much different as well. Hence, we designed an experiment in spontaneously breathing anaesthetized cats in which ventilation was increased by infusion of 2,4-dinitrophenol into the femoral vein. This drug causes hypermetabolism resulting in increases in CO_2 production and O_2 uptake and, hence, ventilation. The inspiratory activity during the large inflations thus obtained were compared to the activity during breaths in which the airway opening was occluded. From the difference between the Phr and IC activity during normal breaths and occluded breaths, the amplification of the inspiratory activity was calculated (Figures 2-1 to 2-3). In this way we were able to quantify the actual contribution of this mechanism during spontaneous breathing instead of its potential contribution as had been investigated in earlier studies using artificial ventilation. From our experiments we concluded that the contribution of the vagal amplification to Phr activity was about 8% (Figures 2-4 and 2-5), which means that this mechanism is not important from a physiological point of view to reinforce Phr activity. The contribution of this reflex to IC activity could not be quantified adequately due to load compensating mechanisms. However, in some cats (3 out of 12) large values were found for the amplification of IC activity, indicating that this mechanism should not be discarded too rashly.

Reflex changes in inspiratory activity by changes in end-expiratory transpulmonary pressure

Changes in end-expiratory lung volume may be expressed by changes in end-expiratory transpulmonary pressure (P'_{ip}), which are much easier to measure. More importantly, P_{ip} correlates better with SAR activity than lung volume does (Davis et al., 1956). Changing P'_{ip} not only changes inspiratory and expiratory duration (T_I, T_E), which is known for more than 120 years now (the Breuer-Hering reflexes), but also the rate of rise of Phr (Bishop, 1963) and of IC activity (Figures 3-1 and 3-2). The contribution of this reflex in modulating inspiratory activity was studied by quantifying the changes in Phr and IC activity as a function of P'_{ip} . Changes in P'_{ip} ($\Delta P'_{ip}$) were imposed by applying stepwise changes from ambient pressure to continuous positive and negative airway pressures in the range between -1.2 and +1.2 kPa. This experiment is the subject of chapter 3. The results for both Phr and IC activity (Figures 3-3 and 3-4) could be described adequately by mono-exponential functions of the form:

$$\ln (\text{Phr}_p/\text{Phr}_C) = \text{Intercept} + \text{Slope} \cdot \Delta P'_{ip}$$

In this equation Phr_p is the amplitude of Phr activity at a certain point in time after the onset of its activity measured during an elevated or decreased airway pressure. Phr_C is the corresponding activity during control conditions (ambient pressure). The responses were almost entirely abolished by vagotomy (Figure 3-5). It was found that for a decrease in P'_{ip} of 0.5 kPa, which can be observed in exercising man, Phr activity

increased on average 2 times while IC activity increased 12 times. This means that this reflex may contribute substantially to inspiratory activity during hyperpnea.

Because this reflex was found to be important, we studied it in more detail to reveal the lung receptors mediating this reflex. To that end, we repeated the experiments as described in chapter 3, but now at many different temperatures of the cervical nervi vagi. This is described in chapter 4. Cooling a nerve impedes the conduction of action potentials in its fibers. The conduction of spikes is blocked at higher temperatures in myelinated than in unmyelinated fibers. In practice, this means that below 8-10 °C, activities of SARs and RARs no longer reach the respiratory centres in the brain stem, whereas activity in C-fibers still does. From the results of these experiments (Figure 4-3) and the properties of lung receptors known from the literature, it was concluded that the decreases in $\dot{P}_{hr}$ and IC activity when $\dot{P}'_{ip}$ is increased are mediated by SARs. Accordingly, when $\dot{P}'_{ip}$ is decreased disinhibition by SARs plays a role in increasing inspiratory activity, but the more important mechanism is likely to be excitation of inspiratory neurons by RARs. C-fibers did not contribute noticeably to any of the responses observed.

The influence of airway CO₂ on the inspiratory off-switch

Using a computer simulation Luijendijk (1983) showed that during hyperpnea the CO₂ diffusion front penetrates much deeper into the lung than during quiet breathing. Assuming that there are CO₂ sensitive lung receptors in the area that is not exposed to fresh air during quiet breathing but becomes exposed with increasing ventilation, these receptors may increase their inspiratory firing rate during hyperpnea due to the release of inhibition by CO₂.

To study the importance of airway CO₂ for the control of breathing, we designed an experiment using two different ways of CO₂ loading which differed in their ability to influence PCO₂ at the lung receptor sites. Because changing CO₂ at these receptor sites in an intact experimental animal invariably involves changes in $P_a\text{CO}_2$, we compared the results of the two methods for a number of respiratory parameters as a function of the change in $P_a\text{CO}_2$ ($\Delta P_a\text{CO}_2$). This is presented in chapter 5. The two methods used were: conventional CO₂ loading (CCL), in which loading is accomplished by inspiring CO₂ enriched air, and "slug" CO₂ loading (SCL) in which a slug or bolus of CO₂ (50% in air) is inspired at the start of each inspiration followed by room air.

The slope of the CO₂ response curve—i.e., the change in $\dot{V}_E$ divided by $\Delta P_a\text{CO}_2$ —for the SCL method was steeper than that obtained by the CCL method (Figure 5-1). On average, SCL slopes were 2.1 times larger than CCL slopes. Further, breathing frequency (f) was higher for SCL than for CCL at the same value for $\Delta P_a\text{CO}_2$, whereas tidal volume (V_T) was not significantly different for the two methods (Table 5-1). The larger values for f for SCL were due mainly to a decreased T_I . Therefore, mean inspiratory flow (V_T/T_I) as a function of $\Delta P_a\text{CO}_2$ was larger for SCL than for CCL, on average 1.15 times (Table 5-1). After bilateral transection of the cervical vagi the difference between the CCL and SCL response curves had almost disappeared (Figure

5-4). These results show that the inspiratory off-switch and the control of $\dot{V}_E$ are affected by the PCO_2 at the pulmonary receptor sites. Further experiments using vagal cooling should be conducted to reveal the lung receptors involved. Since SCL mimics a metabolic CO_2 load more closely than CCL does (Swanson, 1978), the finding that the more iso-capnic response was obtained by the SCL method, is of great physiological interest: pulmonary receptors may contribute to the (iso-capnic) hyperpnea of exercise.

In chapter 6, two positive feedbacks and one negative feedback are presented using the reflexes characterized in the previous chapters. One of the positive feedback includes the vagal amplification reflex (Figure 6-1). The appropriate stimulus and the receptors involved are discussed. Although this loop is of little importance for Phr activity during spontaneous breathing, its contribution to IC activity should be further investigated. The second positive feedback incorporates the influence of changes in P'_{ip} on Phr and IC activities (Figure 6-2). Other relations used in this feedback were derived from the literature. The negative feedback (Figure 6-3) describes the inverse relation between V_T and T_1 ($V_T \sim T_1^{-1}$) (Clark and von Euler, 1972). This relationship has been studied by many investigators (Coleridge & Coleridge, 1986), but the impact of PCO_2 at the lung receptor sites is an addition obtained from the experiments in chapter 5. How this negative feedback should be embedded in the overall scheme of the pulmonary contribution to the control of breathing is not clear, but a possible mechanism is proposed (Figure 6-4), using a part of the second feedback.

In conclusion, all three vagal reflexes described in this thesis contribute to respiratory control in spontaneously breathing, anaesthetized cats. The feedback related to the modification of inspiratory activity by changes in P'_{ip} may contribute considerably to the hyperpnea of exercise. The contribution of CO_2 sensitivity of pulmonary receptors to the control of $\dot{V}_E$ and to the pattern of breathing was demonstrated in this study. The role of end-expiratory lung volume in the control of breathing during exercise, hypoxia and hypercapnia may now be reconsidered in view of these results.

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Chapter 2

Vagal amplification of inspiratory activity at different levels of ventilation

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Abstract

The vagal amplifications of phrenic activity (A_{PHR}) and of inspiratory intercostal muscle activity (A_{IC}) were investigated as functions of minute ventilation ($\dot{V}_{\text{E}}$) in 12 spontaneously breathing, anaesthetized cats. Increasing levels of $\dot{V}_{\text{E}}$ were obtained by repeated venous administrations of 2,4-dinitrophenol. A_{PHR} was obtained from the ratio of the inspiratory phrenic activities in a normal and in an occluded breath. A_{IC} was calculated in a similar way. A_{PHR} and A_{IC} are thought to be mediated by slowly and/or rapidly adapting stretch receptors. Because airway CO_2 may inhibit the discharge of these receptors, we also investigated the influence of adding 1 and 2 vol% of CO_2 to inspired gas on A_{PHR} and A_{IC} .

The results showed that an increase in $\dot{V}_{\text{E}}$ had no influence on A_{PHR} . The value of A_{PHR} was about 1.08. The determination of A_{IC} was affected by a load compensating mechanism and, therefore, the impact of $\dot{V}_{\text{E}}$ on A_{IC} could not be quantified adequately. Nevertheless, in some cats high values for A_{IC} were obtained. Low levels of CO_2 in inspired gas influenced neither A_{PHR} nor A_{IC} .

Our findings suggest that the vagal amplification of phrenic nerve activity has a constant gain and it seems to play a relatively unimportant role in sustaining hyperpneic breathing. The contribution of A_{IC} may be large and needs further investigation.

Introduction

The primary mechanism by which ventilation increases with increasing physical exercise both in man and other species is still unknown (Wasserman et al., 1986). Much attention, however, has been drawn by mechanisms that mediate their effect via the vagal nerves (Dempsey et al., 1985). Pack et al. (1981) have suggested that the amplification of phrenic activity by increased airflow may play an important role in the sustenance of breathing at higher ventilatory levels. This inspiratory facilitation has been thought to be mediated by rapidly and/or slowly adapting stretch receptors (Pack, 1981). In dogs, these receptors are not only sensitive to stretch but also to CO_2 , and their activity decreases with increasing partial pressure of CO_2 in the airways, especially in the hypocapnic range (Coleridge et al., 1978). CO_2 sensitivity of pulmonary vagal afferents has also been found in cats (Kunz et al., 1976). Therefore, airway CO_2 may affect the amplification of inspiratory activity. The amplification of phrenic activity has been demonstrated in quietly breathing or artificially ventilated animals, such as dogs (Bartoli et al., 1975, Pack et al., 1981), cats (DiMarco et al., 1981) and pigs (Huszczuk et al., 1977). Data on spontaneously breathing animals at increased ventilatory levels are, however, lacking. Those data are needed to ascertain whether vagal amplification is really important in hyperpneic breathing. To that end, we designed an experiment to quantify the impact of different levels of minute ventilation ($\dot{V}_{\text{E}}$) on the vagal amplification of inspiratory activity in spontaneously breathing, anaesthetized cats. Vagal amplification has been attributed to changes in inspiratory volume and to changes in inspiratory flow (Pack et al., 1981, DiMarco et al., 1981). Leaving aside the problem of

the proper stimulus for this reflex, the increases in $\dot{V}_E$ in our experiments were accompanied by increases in tidal volume and inspiratory flow. $\dot{V}_E$ was raised by repeated intravenous infusions of 2,4-dinitrophenol (DNP). The amplification of phrenic activity (A_{PHR}) was quantified by comparing the integrated phrenic activity in a normal breath with the activity during an occluded breath when no inspiratory flow was allowed. Because bigger effects may be expected in the inspiratory intercostal muscle activity (DiMarco et al., 1981), the amplification of this muscle activity (A_{IC}) was determined as well.

In addition, we investigated the impact of low levels of CO_2 in inspired gas (1 and 2 vol%) on A_{PHR} and A_{IC} , because of the above-mentioned, possible modulation of stretch receptor activity by airway CO_2 .

Materials and Methods

The experiments were carried out in 12 spontaneously breathing adult cats (1.7 - 3.7 kg). Six cats (mean weight 3.1 kg) were anaesthetized with pentobarbital sodium (50 mg·kg⁻¹ ip). The remaining six cats (mean weight 2.8 kg) were anaesthetized with an initial dose of ketamine (10 mg·kg⁻¹ im) followed by intravenous administration of a mixture of urethane (100 mg·kg⁻¹) and chloralose (20 mg·kg⁻¹). Small supplemental doses were given when required. The cats were placed on an operating table in the supine position. The left femoral vein and artery were cannulated and a tracheal cannula (5 or 6 mm inner diameter) was inserted just below the larynx. The C₅ root of the left phrenic nerve was ventrally exposed and cut. The central stump was desheathed and placed on a pair of nickel silver electrodes immersed in a pool of paraffin oil. Another pair of nickel silver electrodes was placed parasternally in an intercostal muscle near the 8th rib, with the aim of recording inspiratory activity. The two signals from each pair of electrodes were differentially amplified, filtered, rectified and 'leakily' integrated with a time constant of 50 ms (Neurolog system, Digitimer).

The tracheal cannula was connected to the flow pressure transducer (Fleisch No. 0) of a pneumotachograph (Gould). During data processing the expiratory flow was integrated to obtain $\dot{V}_E$. The other side of the Fleisch head was connected to a main tube by means of a T-piece. Through this main tube a constant bias flow of 18 l·min⁻¹ was directed, which was sufficient to prevent rebreathing of expired gas. For the bias flow 3 different gas mixtures were used: air, 1 vol% of CO_2 in air and 2 vol% of CO_2 in air. The equipment dead space had a volume of 8.5 ml and a flow resistance of 0.55 kPa·s·l⁻¹. A valve mounted in the T-piece allowed the occlusion of the airway opening at the end of expiration. This valve was used to withhold the inspiration of gas for a single breath, which is called an occluded breath.

Different levels of $\dot{V}_E$, ranging from quiet breathing to nearly maximal ventilation, were obtained by repeated, intravenous infusions (5 mg·kg⁻¹) of 2,4-dinitrophenol (DNP) (Huch et al., 1969). This drug causes hypermetabolism with a concomitant increase in body temperature due to excess heat production. In practice, at low levels of DNP body temperature had still to be maintained by warming the operating table, while at high

levels of DNP the cats had to be cooled by an electrical fan. In this way body temperature was kept between 36.5 and 39 °C. The temperature was measured with a rectal thermistor (Newport).

Tracheal pressure (P_{tr}) and transpulmonary pressure (P_{ip}) were measured with differential pressure transducers (PM131TC, Statham). P_{tr} was measured near the connection of the Fleisch head with the T-piece. P_{ip} was obtained from the difference between P_{tr} and the pressure in an oesophageal balloon (length 5 cm, diameter 1 cm) filled with 0.8 ml of air and placed in the lower part of the oesophagus. The proper operation of the balloon was verified with the help of an occluded breath, during which no change in P_{ip} should occur.

To evaluate steady state conditions of ventilation and circulation the partial pressures of nitrogen (PN_2), oxygen (PO_2) and carbon dioxide (PCO_2) in inspired and expired gas were measured simultaneously by a mass spectrometer (Balzers, QMG511). Arterial blood pressure was measured with a differential pressure transducer (Gould, Statham, P23). Systolic, diastolic and mean values as well as heart rate were continuously displayed.

The signals were A/D converted by a computer (Zilog, MCZ1/05). Some of the signals (PN_2 , PO_2 , PCO_2 , blood pressure and rectal temperature) were digitized at a frequency of 25 Hz and the remaining signals (phrenic and intercostal activity, flow and P_{ip}) at a frequency of 50 Hz. Data were stored on floppy disk for off-line analysis. To allow on-line inspection of the data, most of the signals were also recorded on an 8 channel ink recorder (Elema-Schoenander). Arterial blood samples (1.5 ml) were analysed in duplicate at 37 °C for pH, PO_2 and PCO_2 (Instrumentation Laboratory, IL413). The values were corrected to body temperature (Ashwood et al., 1983).

The experimental procedure was as follows. A run of data sampling lasted 8 minutes and was divided into three periods. The first period was started in a steady state condition with 0 vol% of CO_2 in inspired air. The second period commenced at the end of the 2nd minute of data sampling by raising the CO_2 content in inspired gas from 0 to 1 vol% and the third period began at the end of the 5th minute by raising the CO_2 content from 1 to 2 vol%. During the last minute of each of these three periods, several occlusions were performed at intervals of 6 to 20 breaths. The quality of the occlusion was judged by its proper start at the end of expiration, the smooth decline of P_{tr} and a constant P_{ip} (Figures 2-2 and 2-3, P_{tr} not shown). The occlusion was cut off when its inspiratory time (T_i) was somewhat longer (<1 s) than the T_i of the preceding normal breaths, in order to prevent unwanted changes in the steady state condition of the experimental animal. Just before and after a period of data sampling an arterial blood sample was drawn and immediately analysed. After the latter blood sample had been taken the CO_2 content of inspired gas was readjusted to 0 vol%. Thereafter, a dose of DNP was given to increase ventilation. After a new steady state had been reached (15-20 minutes) the next cycle of data sampling was started. In each cat 4 to 6 doses of DNP were administered.

Figure 2-1 Schematic drawings of Phr (lower panel) and IC activity (upper panel) as functions of $\bar{T}_I$. A normal and an occluded breath (occ) are superimposed. The vertical dashed lines bound the ten time intervals of $\bar{T}_I$. The horizontal bars indicate the mean values of integrated Phr or IC activity in arbitrary units for the corresponding interval of $\bar{T}_I$. Dashed bars refer to the occluded breath, straight bars to the normal breath.

The vagal amplification of phrenic activity (A_{PHR}) was determined from the integrated phrenic activity measured in a number of normal breaths ($n=5-25$) and the activity measured in a number of occluded breaths ($n=3-8$). These breaths were taken from the same run of data sampling and, obviously, all of them were related to the same experimental conditions of V_{E} and CO_2 content in inspired gas. A_{PHR} was calculated in

the following way (Figure 2-1). First, the mean value of T_i ($\bar{T}_i$) of the normal breaths was determined. T_i was defined as the time elapsed between onset and rapid decay of integrated phrenic activity (T_i in Figure 2-1). Thereafter, the inspiratory phase of each breath—normal as well as occluded—was subdivided into 10 consecutive time intervals of equal duration ($0.1\bar{T}_i$), the beginning of the 1st interval coinciding with the onset of phrenic activity. In figure 2-1 dashed lines bound these 10 time intervals. Even in a normal breath the end of the 10th interval did not always coincide with the instant of rapid decay of phrenic activity due to the differences between T_i and $\bar{T}_i$ (Figure 2-1, lower panel). For each breath—normal or occluded—the average value of phrenic activity was calculated for each of the 10 intervals. Next, the values obtained for a particular interval were averaged for all breaths together, but separately for occluded breaths and normal breaths. This means that two mean values were obtained for the phrenic activity, one for the normal breaths and one for the occluded breaths, and the ratio of these two values represents A_{PIR} for that particular interval. In this way 10 values for A_{PIR} were obtained ($A_{PIR,1}$ to $A_{PIR,10}$), one for each of the 10 intervals. The vagal amplification of the intercostal activity (A_{IC}) was analysed in the same way using the same time intervals used for the determination of A_{PIR} (Figure 2-1 upper panel).

Student's t-test for correlated observations was used to ascertain the statistical significance of intra-individual differences. Linear regression analysis was applied to determine the impact of $\dot{V}_E$ on A_{PIR} .

Results

The impact of $\dot{V}_E$ on A_{PIR}

Typical registrations of phrenic activity are displayed in the upper traces of figures 2-2 and 2-3, which refer, respectively, to the resting condition (neither DNP nor CO_2 added) and nearly maximal $\dot{V}_E$. On the right hand sides of both figures the neurogram of a normal breath and that of an occluded breath are superimposed, showing the larger phrenic activity for the normal breath. At all levels of $\dot{V}_E$ the differences in phrenic activity between a normal and an occluded breath were small in the first part of the phrenic neurogram (Figures 2-2 and 2-3). The mean values of $A_{PIR,2}$ through $A_{PIR,5}$ (Materials and Methods) in 70 of the 219 observations were less than 1.00. This means that there was no clear tendency for phrenic activity to be larger in a normal breath than in an occluded breath for the first part of the neurograms.

Larger differences between the phrenic activities in a normal and an occluded breath were observed in the second part of inspiration (Figures 2-2 and 2-3). Significant differences ($P < 0.05$) were found between the values of $A_{PIR,5}$ and $A_{PIR,7}$, $A_{PIR,5}$ and $A_{PIR,9}$, and $A_{PIR,7}$ and $A_{PIR,9}$ in 6, 8 and 3 of the 12 cats, respectively. On average, however, the differences were small. This means that there was only a minor trend towards higher phrenic amplification in the range from 50% to 90% of T_i , and in some cats this trend was not found at all. For this reason, and for the sake of simplicity, we

will use the average value of $A_{\text{PHR},5}$ through $A_{\text{PHR},9}$ as A_{PHR} (Materials and Methods). For all measurements together A_{PHR} ranged from 0.95 to 1.31.

The impact of $\dot{V}_E$ on A_{PHR} is shown in figure 2-4 (panels A and D). The different bars in this figure correspond to different levels of $\dot{V}_E$ and the number of observations fitting within a certain range are given at the bottom of the corresponding bar. The height of a bar reflects the average value of A_{PHR} for that level of $\dot{V}_E$. Some cats had two ventilatory levels fitting in a particular range of $\dot{V}_E$ and, as a consequence, these cats contributed twice to the average value of A_{PHR} for that range. This figure was chosen for presentation and was not used in statistics. The data base for the panels A and D consisted of 72 values for A_{PHR} . Of these 72 values only 2 were below 1.00, whereas all others exceeded this value. As a consequence, the vagal amplification of phrenic activity contributed to inspiratory activity at all levels of $\dot{V}_E$. Regression lines for A_{PHR} as a function of $\dot{V}_E$ were determined separately for each cat and each CO_2 level for the purpose of analysing the impact of $\dot{V}_E$ on A_{PHR} . The values for the slope did not differ from zero ($P>0.10$). Hence, A_{PHR} appeared to be independent of $\dot{V}_E$.

The impact of $\dot{V}_E$ on A_{IC}

In 8 of the 12 cats the intercostal activity during the normal breaths was generally larger than the activity during the occluded breaths ($A_{\text{IC}}>1$) at all levels of $\dot{V}_E$. In the remaining 4 cats the intercostal activity during the occluded breaths was generally larger ($A_{\text{IC}}<1$). Examples of these opposite responses are shown in the second traces of figures 2-3 and 2-2, respectively. The values for A_{IC} ranged from 0.51 to 2.58.

The superimposed recordings on the right hand side of figure 2-2 show yet another phenomenon. The intercostal activity starts after the onset of phrenic activity. For an occluded breath the time difference between the onsets of intercostal and phrenic activity shortens. This change in time difference affected the results for A_{IC} , because the beginning of the first of the 10 time intervals used to calculate $A_{\text{IC},1}$ to $A_{\text{IC},10}$ coincided with the onset of phrenic activity (Materials and Methods). With increasing doses of DNP the time difference between the onsets of intercostal and phrenic activity shortened as well (Figure 2-3, right hand side). Apparently some mechanism interfered with the method used to determine A_{IC} . Therefore, the influence of different levels of $\dot{V}_E$ on A_{IC} could not be quantified adequately.

The impact of low levels of CO_2 on A_{PHR} and A_{IC}

The addition of 1 or 2 vol% of CO_2 to the inspired air did not systematically influence A_{PHR} at any ventilatory level. This result is shown in figure 2-4, where panels B and C refer to the experiments with cats anaesthetized with pentobarbital and panels E and F refer to the cats anaesthetized with chloralose and urethane. Only in 4 of the 12 cats were significant differences ($P<0.05$) found between the results for A_{PHR} of two different CO_2 levels. In none of these cases, however, was a significant difference between the values for A_{PHR} at 0 and 1 vol% of CO_2 attended by a significant difference between the values for A_{PHR} at 0 and 2 vol% of CO_2 . Therefore, these differences were

Figure 2-2 Comparison of the inspiratory activities in two consecutive breaths at resting conditions. The first breath is a normal one, the second breath is an occluded one. On the right hand side of the figure the traces corresponding to the normal and the occluded breaths are superimposed. The occluded breath (occ) is indicated by an arrow. From top to bottom: Phr , integrated efferent phrenic nerve activity in arbitrary units; IC , integrated efferent intercostal muscle activity in arbitrary units; $\dot{V}$, tracheal flow in $\text{ml} \cdot \text{s}^{-1}$ (inspiration upwards); P_{tp} , transpulmonary pressure in kPa .

not conclusive.

As explained above, the time difference between the onsets of intercostal and phrenic activity made it impossible to quantify the influence of $\dot{V}_E$ on A_{IC} . Assuming that at a certain dose of DNP this time difference was fairly constant, we can still analyse the influence of CO_2 addition to inspired air on A_{IC} . In none of the cats was a significant difference found between the results for A_{IC} at two different CO_2 levels ($P > 0.10$).

Pentobarbital versus chloralose/urethane anaesthesia

Since there was no influence of the level of CO_2 in inspired air on A_{PIR} , we averaged the data of A_{PIR} for the 3 CO_2 levels. However, this was done separately for the two anaesthetics. The mean values of A_{PIR} are displayed as a function of $\dot{V}_E$ in figure 2-5, which is essentially the cumulative of figure 2-4. Comparing the two anaesthetics, the mean values of A_{PIR} for urethane/ chloralose were larger, especially at ventilatory levels below $0.5 \text{ l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. From figure 2-5 it may be clear that A_{PIR} was fairly constant, while the average value of A_{PIR} was about 1.08.

Figure 2-3 Comparison of the inspiratory activities of Phr and IC activity in two consecutive breaths at a cumulative dose of $20 \text{ mg} \cdot \text{kg}^{-1}$ DNP and without a CO_2 load. For further explanation see figure 2-2. These two figures refer to different cats.

The influence of DNP on ventilatory and circulatory parameters

Nearly maximal $\dot{V}_E$ was reached at a cumulative dose of 20, 25 or $30 \text{ mg} \cdot \text{kg}^{-1}$ DNP. At that ventilatory level the mean value of $\dot{V}_E$ was $1.6 \text{ l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (range 1.33 to 2.38), which means an increase of about 4 times with respect to $\dot{V}_E$ at resting conditions (Tables 2-1 and 2-2). In these two tables the influence of DNP on other physiological parameters is given as well. PCO_2 in arterial blood (P_aCO_2) tended to decrease with increasing doses of DNP. In general, PO_2 in arterial blood (P_aO_2), pH, heart rate and blood pressures were only slightly affected. Because body temperature was controlled (Materials and Methods), the changes in rectal temperature were only small. Between the two types of anaesthesia only small differences in pH, P_aO_2 and blood pressures were found. Under resting conditions, the mean value of $\dot{V}_E$ was larger in the cats anaesthetized with pentobarbital. For both anaesthetics, however, the same maximal values for $\dot{V}_E$ were reached. With chloralose/urethane anaesthesia heart rate was lower and the decrease in P_aCO_2 with increasing levels of DNP was larger than with pentobarbital. The differences in $\dot{V}_E$ ($\Delta\dot{V}_E$) and P_aCO_2 ($\Delta\text{P}_a\text{CO}_2$) between breathing with 2 vol% of CO_2 in inspired gas and breathing without CO_2 addition, allowed us to calculate the slope of the CO_2 response curve ($\Delta\dot{V}_E/\Delta\text{P}_a\text{CO}_2$). For both anaesthetics $\Delta\dot{V}_E/\Delta\text{P}_a\text{CO}_2$ was almost the same at comparable levels of DNP, except for the two lowest levels of DNP, where the larger slope was found in cats anaesthetized with

Table 2-1 Influence of increasing levels of DNP on different physiological parameters in six cats anaesthetized with pentobarbital. Mean values and standard deviations (SD).

parameter [unit]	cumulative dose of DNP [$\text{mg}\cdot\text{kg}^{-1}$]					
	0 (control)	5	10	15	20	25/30
$\dot{V}_E$ [$\text{l}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$]	0.46	0.52	0.75	1.08	1.57	1.31*
SD	0.27	0.20	0.27	0.35	0.58	0.23
arterial						
pH	7.458	7.448	7.466	7.483	7.455	7.467*
SD	0.042	0.021	0.014	0.015	0.055	0.070
PO_2 [kPa]	12.9	12.9	12.5	12.5	12.5	12.2*
SD	1.1	0.9	1.3	0.9	1.2	0.7
PCO_2 [kPa]	4.12	4.18	4.05	3.81	3.88	3.85*
SD	0.87	0.59	0.47	0.59	0.93	0.68
$\Delta\dot{V}_E/\Delta P_a\text{CO}_2$ [$\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{kPa}^{-1}$]	881	1158	1076	846	429	303*
Heart rate [min^{-1}]	235	235	247	244	251	275*
SD	33	24	19	23	27	29
Blood pressure						
systolic [kPa]	17.7	17.9	18.8	19.2	19.3	20.6*
SD	1.7	1.3	2.8	2.5	2.4	2.1
diastolic [kPa]	11.2	10.5	11.1	10.7	9.2	9.5*
SD	2.0	1.9	2.5	2.9	2.7	0.9
Body temp. [$^{\circ}\text{C}$]	36.9	37.3	37.7	38.3	38.3	38.3*
SD	1.3	0.9	0.8	1.2	1.1	1.3

$\Delta\dot{V}_E/\Delta P_a\text{CO}_2$, slope of the CO_2 response curve. * n=3

pentobarbital. Thus, the cats anaesthetized with pentobarbital differed from the group of cats anaesthetized with chloralose/ urethane mainly at low levels of DNP.

Discussion

Bartoli and coworkers (1975) have demonstrated in artificially ventilated dogs that increasing the inspiratory flow to the lungs reinforced phrenic activity considerably. Bilateral vagotomy abolished the facilitation of phrenic activity. Intercoastal neural activity also increased in response to increased lung inflation (DiMarco et al., 1981). These facilitations of inspiratory activity are part of a positive feedback loop, which may be mediated by rapidly and/or slowly adapting stretch receptors (Pack, 1981). Because of the large increases in inspiratory activity found in the aforementioned experiments, it has been speculated that this feedback loop might be important in sustaining the inspiratory activity at high ventilatory levels, as in exercise (Pack et al., 1981). The aim of our study was to quantify the contribution of A_{PHR} and A_{IC} to inspiratory activity over a wide range of $\dot{V}_{\text{E}}$.

The impact of DNP on ventilation and circulation

In response to 4-6 cumulative doses of DNP of $5 \text{ mg} \cdot \text{kg}^{-1}$, $\dot{V}_{\text{E}}$ increased in all cats to about 4 times $\dot{V}_{\text{E}}$ at resting conditions; it could not be increased any further by infusing an extra dose of DNP. When such a situation was reached, the addition of 1 or 2 vol% of CO_2 to inspired gas did not increase $\dot{V}_{\text{E}}$ either. Apparently the cats reached nearly maximal $\dot{V}_{\text{E}}$ after 4-6 cumulative doses of DNP. At this level $\dot{V}_{\text{E}}$ is about $1.5 \text{ l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. This is comparable to a man of 70 kg breathing $100 \text{ l} \cdot \text{min}^{-1}$ which corresponds to breathing levels during heavy exercise (Henke et al., 1988). Huch et al. (1969) observed increases in $\dot{V}_{\text{E}}$ of about 8 times resting $\dot{V}_{\text{E}}$ at a cumulative dose of DNP of $20 \text{ mg} \cdot \text{kg}^{-1}$ in dogs. At that level $\dot{V}_{\text{E}}$ is $1.67 \text{ l} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ which is well comparable to our results in cats. Huch et al. (1969) also determined cardiac output, which was increased almost 3 times at a DNP level of $20 \text{ mg} \cdot \text{kg}^{-1}$. Although we did not measure cardiac output, the results regarding arterial blood pressures and heart rate (Tables 2-1 and 2-2) support the view that it increased.

Repeated infusions of DNP had hardly any influence on arterial PCO_2 , pH or PO_2 in both our experiments (Tables 2-1 and 2-2) and those of others (Huch et al., 1969, Levine, 1977). At high ventilatory levels P_aCO_2 decreased, which is generally found during exercise in man and in experimental animals (Dempsey et al., 1985). Further, Levine (1977) demonstrated in a cross-circulation experiment—in which the head of one dog was perfused with blood originating from a donor dog to which DNP was administered—that DNP had neither influence on central and peripheral chemoreceptors nor any other central toxic effect causing an increase in $\dot{V}_{\text{E}}$. This evidence suggests that DNP operates mainly by increasing CO_2 production. Moreover, it has been shown that an increase in CO_2 -load to the lung via mixed venous blood causes a vagally mediated increase in $\dot{V}_{\text{E}}$ (Sheldon and Green, 1982; Green and Sheldon,

Table 2-2 Influence of increasing levels of DNP on different physiological parameters in six cats anaesthetized with chloralose/urethane. Mean values and standard deviations (SD).

parameter [unit]	cumulative dose of DNP [$\text{mg}\cdot\text{kg}^{-1}$]					
	0 (control)	5	10	15	20	25/30
$\dot{V}_E$ [$\text{l}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$]	0.36	0.50	0.74	0.97	1.18	1.59
SD	0.07	0.09	0.10	0.12	0.15	0.28
arterial						
pH	7.421	7.433	7.452	7.473	7.469	7.469
SD	0.023	0.043	0.033	0.027	0.029	0.050
PO_2 [kPa]	12.3	12.3	12.5	12.8	13.0	13.2
SD	1.2	1.1	1.2	1.0	0.9	0.5
PCO_2 [kPa]	4.41	4.19	3.71	3.58	3.45	3.29
SD	0.32	0.50	0.43	0.39	0.40	0.49
$\Delta\dot{V}_E/\Delta P_a\text{CO}_2$ [$\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}\cdot\text{kPa}^{-1}$]	400	857	966	848	473	289
Heart rate [min^{-1}]	184	191	189	187	191	198
SD	46	44	51	51	49	41
Blood pressure						
systolic [kPa]	18.7	19.2	19.7	19.3	19.6	20.9
SD	3.9	3.7	4.1	3.2	3.1	4.1
diastolic [kPa]	12.4	12.7	12.7	11.3	10.5	10.7
SD	2.4	1.7	2.0	1.9	1.3	2.4
Body temp. [$^{\circ}\text{C}$]	37.0	37.2	37.5	37.6	37.5	37.6
SD	0.3	0.3	0.4	0.5	0.6	1.0

$\Delta\dot{V}_E/\Delta P_a\text{CO}_2$, slope of the CO_2 response curve.

Figure 2-4 Mean value of $A_{PHR} \pm SEM$ as a function of $\dot{V}_E$. The number of observations fitting in a range of $\dot{V}_E$ are displayed at the bottom of that bar. Upper panels (A,B,C) for six pentobarbital anaesthetized cats. Lower panels (D,E,F) for six cats anaesthetized with chloralose/urethane. The panels A and D, B and E, and C and F refer to the experiments with 0%, 1% and 2% of CO_2 in inspired gas, respectively

1983). Therefore, infusion of this drug simulates in many respects the effects observed during exercise tests.

The impact of $\dot{V}_E$ on A_{PHR}

Our study showed that A_{PHR} was independent of $\dot{V}_E$ and that A_{PHR} had a constant value of about 1.08 (Figure 2-5). Other authors (Pack et al., 1981), however, have found much larger differences in phrenic activity between occluded breaths and breaths with high inspiratory flows. In their artificially ventilated dogs phrenic activity was increased by

Figure 2-5 Mean value of $A_{PHR} \pm SEM$ as a function of $\dot{V}_E$ for all levels of CO_2 in inspired gas together. Panel A refers to the results of six cats anaesthetized with pentobarbital, panel B to the results of the six cats anaesthetized with chloralose/urethane. The numbers at the bottom of the bars indicate the number of observations contributing to that bar.

about 40% when the inspiratory flow was 2.5 to 3 times that of a normal breath. However, one should realize that this is a comparison between an occluded breath at resting condition and an inspiration forced by a flow that corresponds to an enhanced ventilatory level. In our experiments phrenic activity increased 3.2-6.0 times when $\dot{V}_E$ increased 2.9 times. This value was determined from 3 experiments in which the phrenic signal was stable over the entire recording time—about 2-3 hours. This means that an $A_{PHR}=1.08$ at a high ventilatory level corresponds to a 25-48% increase in phrenic activity when compared to the resting level, which is comparable to the aforementioned result of Pack et al. (1981). Accordingly, though increased airflow may have a large impact on phrenic activity, it is clear from our study that its relative contribution (8%) is fairly small during spontaneous breathing.

In comparison with chloralose/urethane anaesthesia we found lower values for A_{PHR} in cats anaesthetized with pentobarbital, especially at ventilatory levels below $0.5 \text{ l min}^{-1} \text{ kg}^{-1}$ (Figure 2-5). These cats received pentobarbital initially by an intraperitoneal injection and later on by intravenous infusions when required. A store of pentobarbital may, therefore, have existed in the abdominal cavity. According to DiMarco et al.

(1981), the vagal amplification of inspiratory activity may disappear by venous administration of a small dose ($2 \text{ mg} \cdot \text{kg}^{-1}$) of pentobarbital in addition to the dose of pentobarbital used for general anaesthesia. Therefore, the release from the abdominal cavity of small quantities of stored pentobarbital may have diminished A_{PIR} . Only in one experiment was it necessary to give an additional dose of pentobarbital once DNP administration had been started. This might explain that the lower values of A_{PIR} were especially found at lower levels of $\dot{V}_{\text{E}}$.

The impact of $\dot{V}_{\text{E}}$ on A_{IC}

The start of an inspiration was defined by the onset of phrenic activity, which only slightly preceded the onset of inspiratory flow (20-40 ms). At low ventilatory levels intercostal activity started up to 1.5 s later than phrenic activity. The onset of intercostal activity tended to start earlier in an occluded breath than in a normal breath (Figure 2-2). Because phrenic activity started earlier than intercostal activity, the contraction of the diaphragm in a normal breath may have lowered the intrapleural pressure while there was no intercostal activity yet. During an occluded breath, however, this pressure drop was much larger and the intercostal muscles may have been stretched (Decramer and De Troyer, 1984). By a load compensating mechanism these muscles would then have counteracted their extension, resulting in an earlier start of intercostal activity in an occluded than in a normal breath. This load compensating mechanism may also explain the fact that in some cats the intercostal activity during an occluded breath was enhanced as compared to that in a normal breath, i.e. $A_{\text{IC}} < 1$. In these cats $A_{\text{IC}} < 1$ cannot be caused exclusively by an earlier onset of intercostal activity in the occluded breaths, because these results were found at high $\dot{V}_{\text{E}}$ as well, where time delays were negligible. In these cats the load compensating mechanism appeared to contribute considerably to intercostal activity during occluded breaths, whereas in the other cats the vagal amplification predominated. DiMarco et al. (1981) also found occasionally larger intercostal neural activity in occluded breaths as compared to normal breaths in spontaneously breathing, vagotomized cats. In 3 cats A_{IC} values larger than 1.50 were generally found from the first dose of DNP on. In some cats vagal amplification of intercostal activity may, therefore, have contributed considerably to inspiratory activity. The interfering influence of the load compensating reflex on intercostal activity during occluded breaths scattered the data and made it impossible to quantify the influence of $\dot{V}_{\text{E}}$ on A_{IC} properly.

We found no evidence that phrenic activity was affected by a load compensating mechanism. Anyhow, such a mechanism would be unlikely since the diaphragm contains few muscle spindles (Shannon, 1986). If such a mechanism contributed to phrenic activity during an occluded breath, it would lower A_{PIR} , resulting in an underestimation of the values for A_{PIR} .

The impact of CO₂ on A_{PIR} and A_{IC}

Rapidly as well as slowly adapting receptors of the dog show decreasing activity when PCO₂ at the receptor site is increased. The most sensitive range is between 0 and 3 kPa (Coleridge et al., 1978). CO₂ sensitivity of vagal afferents was also found in cats (Kunz et al., 1976). Therefore, increases in airway CO₂ during inspiration may influence A_{PIR} and A_{IC}. Our study showed that 1 or 2 vol% of CO₂ in inspired gas did not systematically affect A_{PIR} and A_{IC} (Figure 2-4). The absence of influence of CO₂ on the amplifications is in agreement with the results of similar experiments conducted by DiMarco et al. (1981), who used artificially ventilated cats.

The location in the lung of the receptors that are involved in the amplification of inspiratory activity is crucial if we want to influence the activity of these receptors by adding CO₂ to inspired gas. If these receptors are located in an area of the lung that is exposed to CO₂ free air during inspiration (Sant'Ambrogio, 1982), low levels of CO₂ in inspired gas may influence the receptor activity and the expected effect may be found. If these receptors are located in the more distal parts of the lungs (Nilsestuen, 1981), where the PCO₂ does not change much during a breathing cycle, low levels of CO₂ in inspired gas will hardly affect the PCO₂ and, as a consequence, A_{PIR} and A_{IC} may not change either. At high levels of $\dot{V}_E$, CO₂ free air penetrates much deeper into the lung at the end of inspiration (about 3 airway generations in man) than at resting $\dot{V}_E$ (Luijendijk, 1983). In our experiments we found no systematic influence of CO₂ on A_{PIR} even at high ventilatory levels. Therefore, a more distal location of the receptors is unlikely to explain the lack of CO₂ influence. Other explanations may be that the values observed for A_{PIR} were too close to unity to detect a modulating effect of CO₂ on A_{PIR}, or that the modulating effect of CO₂ on lung stretch receptor output itself is too small in the cat.

In conclusion, an obvious contribution of the vagal amplification to phrenic activity was shown in spontaneously breathing anaesthetized cats at all levels of ventilation. The interpretation of the results for IC activity were complicated by contribution of a load compensating reflex. Since neither $\dot{V}_E$ nor CO₂ affected the values of A_{PIR}, this vagal amplification of inspiratory activity may be regarded as a constant amplification of central inspiratory output. Our results for A_{PIR} are consistent with those reported in the literature for artificially ventilated experimental animals. The amplification of phrenic activity observed during spontaneous breathing, however, appears to be small. Our data in cats and those of Pack et al. (1981) in dogs suggest that this reflex loop is of minor importance for the sustenance of the exercise hyperpnea in mammals. However, in three of the twelve cats a value for A_{IC} larger than 1.5 was found repeatedly. Therefore, the contribution of this reflex loop to A_{IC} needs further study.

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Chapter 3

Vagally mediated modification of inspiratory activity induced by changes in airway pressure

submitted

Abstract

The impact of varying the end-expiratory transpulmonary pressure (P'_{ip}) on phrenic (Phr) and inspiratory intercostal muscle (IC) activity early in inspiration was studied in 12 spontaneously breathing, anaesthetized cats. P'_{ip} was varied in a stepwise fashion by rapidly changing the tracheal pressure (P_{tr}) from ambient pressure to a pressure in the range between -1.2 and +1.2 kPa relative to ambient pressure, and vice versa. The amplitudes of integrated Phr and IC activity were determined in each breath at three particular points in time (t), corresponding to 0.1, 0.2 and 0.3 s after the onset of Phr activity, respectively. For all three values of t , Phr and IC activity increased with a decrease in P_{tr} and decreased with an increase in P_{tr} . The relationships between the changes in Phr activity and in P'_{ip} ($\Delta P'_{ip}$), and between the changes in IC activity and $\Delta P'_{ip}$ could be described satisfactorily by mono-exponential functions. An increase in P'_{ip} of 0.1 kPa resulted in a decrease in Phr activity of 16%, 13% and 12% for the 3 aforementioned values of t , respectively. For IC activity the corresponding values were 42%, 42% and 36%, respectively. After bilateral vagotomy no changes in Phr activity in response to changes in P_{tr} were observed any more, whereas the changes in IC activity had decreased to about one tenth of the corresponding values obtained before vagotomy. Apparently, extra-vagal mechanisms contribute for a small part to the effect of changes in P'_{ip} on IC activity. Our results indicate that relatively small changes in P'_{ip} may have a large impact on central inspiratory output.

Introduction

The preliminary study of Luijendijk et al. (1988) concerning central inspiratory output during positive and negative airway pressure in cats, demonstrated an inverse relationship between end-expiratory transpulmonary pressure (P'_{ip}) and phrenic (Phr) activity early in inspiration, which was ascribed to changes in the activity of slowly adapting stretch receptors. Changes in P'_{ip} ($\Delta P'_{ip}$) were induced by small changes in tracheal pressure (P_{tr}). The ratios of the amplitudes of integrated Phr activity during changed airway pressure and at control condition were described by a mono-exponential function of $\Delta P'_{ip}$.

In order to obtain more quantitative data on the relationship between inspiratory activity and P'_{ip} , we extended the original experiments of Luijendijk et al. (1988). Besides for Phr activity we also determined this relationship for inspiratory intercostal (IC) muscle activity. First and higher order regression analyses were done to ascertain that mono-exponential equations describe the impact of changes in P'_{ip} on Phr and IC activity. Further, we repeated a part of the experiments in the same animals after bilateral vagal transection in order to ascertain the dependence of the responses observed on lung receptor output. In the next chapter we will focus on the types of lung receptors that are involved in the vagal modification of inspiratory activity by changes in P'_{ip} .

Materials and Methods

Materials, surgery and equipment

The experiments were carried out in 12 spontaneously breathing adult cats (1.7 - 3.7 kg), anaesthetized with an initial dose of ketamine ($10 \text{ mg}\cdot\text{kg}^{-1} \text{ im}$) followed by intravenous administration of a mixture of urethane ($100 \text{ mg}\cdot\text{kg}^{-1}$) and chloralose ($20 \text{ mg}\cdot\text{kg}^{-1}$). Anaesthesia was maintained with small supplemental doses as needed. During the measurements the cats were lying in the supine position on an operating table.

Left femoral vein and artery were cannulated and a tracheal cannula was inserted just below the larynx. An oesophageal balloon was placed in the lower part of the oesophagus. Inspiratory activity was measured with two pairs of nickel silver electrodes. On the one pair the central stump of the cut C_5 root of the left Phr nerve was placed, and the second pair was inserted in an inspiratory IC muscle, located parasternally near the 4th rib. The two signals were differentially amplified and 'leakily' integrated with a time constant of 50 ms (Neurolog, Digitimer).

The tracheal cannula was connected to a flow pressure transducer (Fleisch No. 0) of a pneumotachograph (Gould). The other end of the Fleisch head was connected to a main tube by means of a T-piece.

P_{tr} and transpulmonary pressure (P_{tp}) were measured with differential pressure transducers (Statham, PM131TC). P_{tr} was measured near the connection of the Fleisch head with the T-piece (Figure 3-1) and it was expressed as the difference with ambient pressure. P_{tp} was obtained from the difference between P_{tr} and the pressure in the oesophageal balloon. Accordingly, P_{tp} increases during inspiration and decreases during expiration. The proper location of the balloon was verified by recording an inspiratory effort against an occluded trachea, whereby P_{tp} should remain constant.

The equipment dead space—Fleisch head with connecting tubes and the side-arm of the T-piece inclusive—had a volume of 8.5 ml and a flow resistance of $0.55 \text{ kPa}\cdot\text{s}\cdot\text{l}^{-1}$. In the main tube a constant bias flow of humidified air of $18 \text{ l}\cdot\text{min}^{-1}$ was maintained with the help of a high and a low pressure tank for changes to positive and negative P_{tr} , respectively. This bias flow was sufficient to prevent rebreathing of expired gas. By turning the 3-way tap (V_1), the connection to room air (RA) was occluded and the bias flow was then directed through the adjustable flow resistance (AFR) (Figure 3-1). At the same time the connection to the water manometer was accomplished. With the help of this manometer and the adjustable flow resistance a certain value of P_{tr} was preset. During an experimental run the preset P_{tr} could be established almost instantaneously by rapidly turning V_1 (Figure 3-1). A large bottle (50 l) and a water manometer with wide tubes ($\phi 9 \text{ cm}$) were used to minimize pressure fluctuations in the main tube due to the breathing of the cat. Turning the 3-way tap (V_2) for connection with the low pressure tank inverted the direction of the bias flow. When V_1 was set for connection with the manometer, a pressure drop behind the adjustable flow resistance (AFR) was obtained, allowing negative P_{tr} .

Figure 3-1 Diagram of the breathing circuit allowing positive and negative airway pressures. The position of the valves and the direction of the air stream are indicated for positive airway pressure.

To evaluate the conditions concerning ventilation and circulation the partial pressures of nitrogen (PN_2), oxygen (PO_2) and carbon dioxide (PCO_2) in inspired and expired gas were measured continuously by a mass spectrometer (Balzers, QMG511) as well as arterial blood pressure, which was measured with a differential pressure transducer (Gould-Statham, P23). In addition, body temperature was measured with a rectal thermistor (Newport) and kept constant ($37 - 38^\circ\text{C}$) by warming the operating table.

All signals were monitored on a polygraph and A/D converted by a computer (DEC, PDP11/73) at a frequency of 50 Hz. The data were stored on disk for off-line analysis. Arterial blood samples (0.14 ml) were collected in lithium heparin coated capillaries (Corning) and immediately analyzed at 37°C for pH, PO_2 and PCO_2 (Instrumentation Laboratory IL413). The obtained values were corrected to body temperature (Ashwood et al., 1983).

Experimental procedure

After P_{ir} had been preset, data sampling was started in a steady state condition with the cat breathing at ambient pressure. This is called the control (C) condition. After 10 breaths had been recorded, P_{ir} was changed in a stepwise fashion to a higher or lower value (P condition), which was maintained for 6 breaths. Thereafter, P_{ir} was switched to ambient pressure again and at least another 4 breaths were recorded. P_{ir} was always changed during an expiration. Such an experiment can be indicated by the sequence $C \rightarrow P \rightarrow C$. Experiments with the reverse pressure sequence, $P \rightarrow C \rightarrow P$, were carried out as well, starting from a steady state condition with $P_{ir} \neq 0$. The values used for P_{ir} were -1.2, -0.9, -0.6, -0.3, +0.3, +0.6, +0.9 and +1.2 kPa. After the experiments for the different values of P_{ir} had been completed, both vagi were transected. Thereafter, the experiments were repeated for $P_{ir} = -0.9$ and $P_{ir} = +0.9$ kPa.

Directly after a pressure sequence had been completed, the first part of the sequence ($C \rightarrow P$ or $P \rightarrow C$) was repeated, whereby arterial blood was sampled just before the change in P_{ir} and about 20 s thereafter. This procedure was carried out after each run of data sampling—both before and after vagotomy. The small size of the blood capillaries allowed frequent sampling while the cumulative amount of collected blood remained less than 10% of the animals blood volume.

Data handling

For each breath the amplitudes of integrated Phr and IC activity were determined at three preselected points in time (t)—i.e., 0.1, 0.2 and 0.3 s after the onset of Phr activity. The value of P_{ip} just before the start of a breath was defined as the P'_{ip} belonging to that breath. For these variables the values of 3 consecutive breaths were averaged, taking 3 breaths just before and 3 breaths after each change in P_{ir} . The first breath following a change in P_{ir} was discarded, because for this breath P'_{ip} could not be determined accurately due to the induced changes in lung volume during the preceding expiration. The obtained mean values for Phr activities at condition P (Phr_P) were divided by the corresponding values at condition C (Phr_C). In this way 3 ratios (Phr_P/Phr_C) were obtained for each change in P_{ir} , expressing the relative changes in Phr activity for each of the three values of t. In the same way, 3 ratios for IC activity (IC_P/IC_C) were calculated. Further, the difference between the mean values of P'_{ip} at conditions P and C was calculated ($=\Delta P'_{ip}$). Finally, the natural logarithms of Phr_P/Phr_C for the different values of P_{ir} were plotted as a function of the corresponding values of $\Delta P'_{ip}$ and a line was fitted on these data points by linear regression analysis (Luijendijk et al., 1988). In this way, three regression lines were obtained—one for each value of t. For the calculation of the lines the point (0,0) was also included. This trivial point refers to $\Delta P'_{ip}=0$ and $Phr_P/Phr_C=1$. IC_P/IC_C was analyzed in the same way.

The impacts of changes in P_{ir} on other physiological parameters (Table 3-3) were studied using the same breaths that were used in the calculations for Phr and IC. Student's t-test for paired observations was used for statistical analysis.

Results

Changing P_{ir} has important impacts on the signals measured. As shown in figure 3-2, the change to a continuous positive P_{ir} involves an increase in P'_{ip} and decreases in Phr and IC activity. From the lower traces of this figure, one can derive the changes in the breathing pattern accompanying the change to a positive P_{ir} : the duration of inspiration (T_I) is slightly shortened, whereas the duration of expiration (T_E) is lengthened considerably. The upper trace displays a decrease in arterial blood pressure.

Figure 3-3 shows the impacts of a change to a negative P_{ir} , which leads to a decrease in P'_{ip} and instant increases in Phr and IC activity. These integrated nerve and muscle activities do not return to zero levels: Phr as well as IC recordings show inspiratory activity even in late expiration (tonic activity). Note that the changes in Phr and IC activity are already seen during the first breath following a change in P_{ir} , indicating that they are due to a rapid, neural reflex regulation. A change to negative P_{ir} affects the pattern of breathing more or less in the opposite direction of the findings for a change to positive P_{ir} : T_I is hardly affected, whereas T_E is shortened considerably. Arterial blood pressure hardly changes.

In these two figures P_{ip} is sometimes negative. Since our cats were in the supine position, this is likely to be due to the weight of the mediastinum pressing on the oesophageal balloon. We did not correct for this shift in P_{ip} , for ΔP_{ip} is not affected by it (Milic-Emili et al., 1964).

Inspiratory activity during changed airway pressure

The natural logarithms of Phr_p/Phr_C and of IC_p/IC_C were plotted as functions of $\Delta P'_{ip}$ for each cat separately (Materials and Methods). Examples of such plots are shown in figure 3-4 for Phr activity for $t = 0.2$ s and in figure 3-5 for IC activity for $t = 0.3$ s. Phr_p/Phr_C as well as IC_p/IC_C are inversely related to $\Delta P'_{ip}$. Table 3-3 gives the mean differences in P'_{ip} between conditions P and C for all P_{ir} applied. The slope in figure 3-5 is much steeper than that in figure 3-4, showing that the effects for IC activity are more pronounced than those for Phr activity.

In table 3-1 the results of the regression analysis for Phr activity are presented. The magnitudes of the slopes are variable, but in all cats they are negative. The intercepts are in most cases close to zero, as expected, since no change in P'_{ip} should have no impact on Phr (or IC) activity. The slopes for the 3 sets of lines were significantly different from zero ($P < 0.01$). In general, the steepness of the slope decreased with increasing t . Significant differences ($P < 0.05$) were found between the slopes for $t = 0.1$ and $t = 0.2$ s, $t = 0.2$ and $t = 0.3$ s, and $t = 0.1$ and $t = 0.3$ s. For $t = 0.1$ s the

Figure 3-2 Effects of a step change in P_{tr} (trace 3) from 0 to +1.2 kPa. From top to bottom: blood pressure, P_{tp} , P_{tr} , integrated Phr and IC activity, air flow.

average slope was equal to -1.708 (Table 3-1), which is equivalent to a decrease in Phr activity of 16% per 0.1 kPa increase in P'_{tp} . The corresponding values for $t = 0.2$ s and $t = 0.3$ s were 13% and 12%, respectively.

The results for IC activity are shown in table 3-2. As for Phr activity, the slopes show large inter-individual variations but all slopes are negative. The intercepts are relatively close to zero and not systematically positive or negative. For each value of t , the slopes of the regression lines were—like those for Phr activity—significantly different from zero ($P < 0.01$).

Except for two slopes (cat No 51 for $t = 0.1$ s and $t = 0.2$ s), all slopes were more negative for IC than for Phr activity, showing that the changes were larger for IC activity than for Phr activity. Between $t = 0.1$ s and $t = 0.2$ s the differences in the slopes were not significant ($P > 0.10$), which contrasts with the findings for Phr activity. The steepness of the slopes decreased significantly between $t = 0.2$ and $t = 0.3$ s ($P < 0.01$). This means that in general the largest impact of P'_{tp} on

Figure 3-3 Effects of a step change in P_{tr} (trace 3) from 0 to -1.2 kPa and vice versa. Further explanation in figure 3-2. These two figures refer to different cats.

inspiratory activity was found early in inspiration.

Further, we tested whether quadratic equations would fit our data better than the above presented linear equations—i.e., we added a quadratic term to the linear regression equation. The addition of this quadratic term improved the fit only in 7 out of the 69 cases. In our opinion, this means that the relationships between $\Delta P'_{ip}$ and Phr_p/Phr_C and between $\Delta P'_{ip}$ and IC_p/IC_C can be described satisfactorily by mono-exponential functions.

Vagotomy and inspiratory activity during changed airway pressure

Figure 3-6 shows IC_p/IC_C as a function of $\Delta P'_{ip}$ for $t = 0.2$ s for all experiments conducted after bilateral vagal transection. After vagal transection, changed airway pressure impedes ventilation and circulation more than before transection. This is reflected in the increased changes in P'_{ip} observed after transection (compare figures

Table 3-1 Regression parameters for $\ln(\text{Phr}_p/\text{Phr}_c)$ as function of $\Delta P'_{ip}$. Individual and mean values ($\pm$ SD) for slopes, intercepts and residual SD.

Cat	t = 0.1 s			t = 0.2 s			t = 0.3 s		
	Slope	Intcpt	ResSD	Slope	Intcpt	ResSD	Slope	Intcpt	ResSD
51	-1.891	0.045	0.316	-1.393	0.037	0.221	-1.185	-0.009	0.194
53	-0.718	0.116	0.206	-0.568	0.104	0.163	-0.497	0.097	0.128
55	-3.018	0.338	0.376	-2.435	0.231	0.292	-2.037	0.182	0.214
56	-0.698	0.128	0.285	-0.438	0.163	0.236	-0.199	0.149	0.219
57	-1.291	0.040	0.138	-1.178	0.051	0.082	-1.024	0.014	0.126
58	-1.618	0.014	0.196	-1.431	0.001	0.158	-1.162	-0.044	0.113
59	-1.716	-0.024	0.198	-2.210	-0.095	0.164	-2.254	-0.123	0.206
60	-1.997	0.036	0.186	-1.420	0.036	0.224	-1.515	0.030	0.193
61	-0.847	0.216	0.267	-0.552	0.196	0.218	-0.334	0.176	0.188
62	-1.453	0.352	0.325	-1.472	0.290	0.259	-1.377	0.248	0.211
63	-4.454	0.158	0.258	-3.415	0.134	0.208	-2.648	0.110	0.173
64	-0.726	0.231	0.215	-0.811	0.186	0.174	-0.713	0.185	0.186
mean	-1.708	0.138		-1.444	0.111		-1.245	0.085	
SD	1.096	0.125		0.879	0.109		0.770	0.111	
VTs	-0.026	0.007	0.168	-0.028	0.006	0.165	-0.046	0.017	0.161

Slopes in kPa^{-1} ; VTs: results obtained after bilateral vagal transection.

Figure 3-4 Logarithm of $\text{Phr}_P/\text{Phr}_C$ as a function of $\Delta P'_{tp}$ for $t=0.2$ s. Symbols + and x refer to the first and second change in P_w in each pressure sequence.

3-5 and 3-6). Since only a limited number of observations was obtained, which did not allow individual data handling, the results of these experiments were pooled. Table 3-2 shows the slopes for IC activity obtained after vagal transection (VTS), which are all three negative and obviously different from 0. Their values are reduced to about 10% of the mean values of the corresponding slopes before vagal transection, indicating that, for a small part, the responses observed are due to extra-vagal mechanisms. For Phr activity the slopes after vagotomy were all close to 0 (Table 3-1), indicating that for this component of the inspiratory activity the responses are fully vagally mediated.

Table 3-2 Regression parameters for $\ln(IC_p/IC_c)$ as a function of $\Delta P'_{ip}$. Individual and mean values ($\pm SD$) of slopes, intercepts and residual SD.

Cat	t = 0.1 s			t = 0.2 s			t = 0.3 s		
	Slope	Intcpt	ResSD	Slope	Intcpt	ResSD	Slope	Intcpt	ResSD
51	-1.505	0.781	1.100	-1.329	0.372	0.843	-1.334	0.264	0.592
55	-7.404	0.438	0.624	-14.817	0.225	0.852	-13.264	-0.174	0.696
56	-5.780	0.230	1.669	-6.487	-0.227	1.091	-4.765	-0.396	0.669
57	-6.784	-0.402	0.762	-5.659	-0.451	0.657	-4.016	-0.276	0.466
58	-3.960	-0.218	0.506	-4.660	-0.308	0.497	-3.050	-0.166	0.381
59	-3.250	0.520	0.843	-4.415	0.452	0.861	-4.601	0.084	0.719
60	-8.401	-0.503	0.794	-5.965	-0.325	0.739	-4.571	-0.233	0.621
61	-2.310	0.271	0.589	-2.286	0.495	0.798	-1.770	0.449	0.749
62	-3.387	0.766	0.939	-3.526	0.609	0.660	-3.479	0.365	0.575
63	-13.789	0.531	0.934	-7.887	0.168	0.524	-4.765	0.166	0.352
64	-2.659	1.291	1.037	-3.663	0.574	1.299	-3.459	0.419	0.695
mean	-5.384	0.337		-5.518	0.144		-4.461	0.046	
SD	3.584	0.543		3.614	0.399		3.145	0.306	
VTS	-0.507	0.132	0.654	-0.812	0.026	0.615	-0.518	0.086	0.480

Slopes in kPa^{-1} ; VTS: results obtained after bilateral vagal transection.

Figure 3-5 The natural logarithm of IC_P/IC_C as a function of $\Delta P'_{tp}$ for $t = 0.3$ s. For further explanation see figure 3-4.

Impacts of changes in airway pressure on other physiological parameters

Changes in P_{ir} had considerable impacts on breathing pattern and circulation (Figures 3-2 and 3-3). In table 3-3 the mean changes in a number of physiological parameters are presented for the values of P_{ir} used. The average values ($\pm$ SD) of the ratios for these parameters during changed airway pressure as compared to control are shown for T_I and T_E , for frequency, for tidal volume, for minute ventilation and for heart rate. Changes in the other parameters are represented as average differences ($\pm$ SD) between the values at conditions P and C. This concerns P'_{ip} , blood pressure, partial pressures of O_2 and CO_2 in arterial blood, and arterial pH. Since only the first part of the pressure sequence (C $\rightarrow$ P or P $\rightarrow$ C) was applied for blood sampling, the number of observations for the last three parameters was halved. The application of positive pressures enhanced mean T_E by 31% and shortened mean T_I by 28% for $P_{ir} = +1.2$ kPa, resulting in minor changes in breathing frequency.

Tidal volumes changed inconsistently, resulting in positive or small negative changes in minute ventilation. Circulatory parameters changed considerably: the decrease in mean blood pressure amounted to 2.2 kPa (= 16 mm Hg) for the highest P_{ir} applied. The increases in heart rate were relatively small (13%). The mean changes in the partial pressures of O_2 and CO_2 in arterial blood at high P_{ir} had large SD's, indicating that the cats showed widely differing responses to the concomitant circulatory impediment.

Applying negative P_{ir} hardly affected T_I , while T_E was much reduced (Table 3-3), resulting in a considerable increase in breathing frequency, with an average value of 32% at $P_{ir} = -1.2$ kPa. This increase in frequency was accompanied by a relatively small decrease in tidal volume, resulting in an increase in minute ventilation. Circulation was hardly affected by negative P_{ir} and neither were arterial PCO_2 and pH. Arterial PO_2 , however, was reduced, but it never reached values where substantial ventilatory responses were to be expected.

Discussion

Hering and Breuer (Hering, 1868) were the first to demonstrate that inspiratory activity may be strongly affected by vagal afferent activity: a deep deflation initiates a rapid and large inspiration, whereas occluding the airway opening at the end of inspiration delays the following inspiratory effort considerably. These reflexes have been ascribed to changes in the activity of slowly adapting stretch receptors (SAR) as well as, for deflations, to rapidly adapting stretch receptors (Coleridge and Coleridge, 1986). To evaluate possible contributions of the vagal afferent activity to normal respiratory control it is necessary to obtain detailed quantitative data on the relationship between lung receptor activity and inspiratory drive. In this study we applied positive and negative P_{ir} during the entire breathing cycle, involving changes in end-expiratory lung volume, which in turn changed lung receptor activity. Since changes in SAR activity are best related to changes in P_{ip} (Sant'Ambrogio, 1982) we chose this parameter as the independent variable rather than using P_{ir} or lung volume. The relationship between P'_{ip} and inspiratory (Phr and IC) activity was determined only in early inspiration, in order to avoid any influence of the inspiratory off-switch mechanism on the results obtained.

Impact of vagotomy and extra-pulmonary receptors on Phr and IC activities

Studies of vagal reflexes are often hampered by unintended circulatory and respiratory changes. Raw data (Figures 3-2 and 3-3) as well as average values (Table 3-3) indicated that changes in airway pressure involved important changes in circulatory and ventilatory parameters such as blood pressure, heart rate, arterial pH and blood gases. Most of the receptors involved in the responses of these parameters do not mediate their afferent activity via the vagal nerves. As a consequence, their contribution to changes in Phr and IC activity may be estimated by the changes that

Table 3-3 The impact of step changes in P_{tr} on some physiological parameters. Mean values ($\pm$ SD) of ratios (no dimension) or differences (units) between step and control.

P_{tr} [kPa]	-1.2	-0.9	-0.6	-0.3	+0.3	+0.6	+0.9	+1.2
P'_{ip} [kPa]	-0.282	-0.228	-0.174	-0.087	0.123	0.232	0.301	0.420
SD	0.150	0.149	0.076	0.057	0.068	0.147	0.165	0.236
duration of inspiration	0.961	1.027	1.054	1.053	0.930	0.815	0.813	0.720
SD	0.260	0.238	0.184	0.118	0.128	0.183	0.272	0.363
duration of expiration	0.649	0.729	0.821	0.943	1.054	1.086	1.294	1.309
SD	0.136	0.150	0.127	0.088	0.112	0.156	0.512	0.371
breathing frequency	1.318	1.213	1.096	1.014	1.026	1.091	1.052	1.096
SD	0.295	0.308	0.187	0.093	0.144	0.162	0.244	0.329
tidal volume	0.873	0.979	1.017	1.020	0.954	0.899	1.084	0.935
SD	0.179	0.134	0.155	0.065	0.081	0.134	0.743	0.712
minute ventilation	1.129	1.173	1.108	1.031	0.981	0.969	1.144	1.050
SD	0.244	0.274	0.203	0.105	0.198	0.159	0.947	0.962
mean blood pressure [kPa]	0.254	0.140	0.099	0.077	-0.236	-0.787	-1.691	-2.222
SD	0.486	0.402	0.404	0.309	0.399	0.665	1.834	2.200
Heart Rate	0.993	0.981	0.994	0.999	1.022	1.039	1.078	1.133
SD	0.050	0.050	0.031	0.032	0.046	0.065	0.112	0.134
n	48	44	48	52	46	44	46	38
P_aCO_2 [kPa]	0.055	0.045	0.038	0.082	-0.030	-0.100	-0.188	-0.320
SD	0.350	0.233	0.164	0.180	0.276	0.270	0.898	0.719
P_aO_2 [kPa]	-1.103	-0.542	-0.334	-0.362	0.266	0.421	0.902	1.447
SD	0.957	0.911	0.746	0.644	1.057	1.002	2.906	2.171
arterial pH []	-0.003	-0.003	-0.001	0.004	0.002	0.006	0.011	0.022
SD	0.016	0.017	0.013	0.009	0.016	0.023	0.057	0.053
n	24	22	24	26	23	22	23	19

remain after vagotomy. The changes in ventilatory and circulatory parameters may be much larger after vagotomy—due to, for instance, the loss of vagal control of lung volume. After vagotomy Phr activity was found not to change at all in response to a change in P'_{ip} , and IC activity showed only small changes, which are likely to be due to a load compensating mechanism, as will be argued below.

It seems rather surprising that the changes in arterial PCO_2 , which occurred especially after positive changes in P'_{ip} (Table 3-3), did not influence Phr activity. As shown in figure 3-2, the Phr and IC activities of the second breath after the first change in P_{ir} (C→P), which is the first breath to be analyzed at condition P, are already different from those at condition C. The next four breaths show no tendency to increase, although arterial PCO_2 was increased by 0.4 kPa in the accompanying measurement, which was conducted for blood sampling purposes. This paradox may be due to a difference in timing between the measurements. The breaths to be analyzed at condition P fitted in a time interval of about 10 s after the change in P_{ir} (Figure 3-2). The collection of duplicate blood samples, however, took considerably more time (15-30 s) and, as a consequence, led to an overestimation of the changes in blood gases and pH for the breaths analysed.

Russell and Bishop (1976) have demonstrated that the activity of receptors that have their afferent fibres running along the hilar nerves, was necessary to elicit the responses of Phr and abdominal muscle activities to positive airway pressure. Nevertheless, they could not totally exclude the possibility that vagal afferents other than those that come from the lungs—for instance, from cardiovascular receptors—modulated the responses observed. Since in our study changes in blood pressure and heart rate were much more pronounced during positive than during negative airway pressure (Table 3-3), we may assume that these receptors will influence inspiratory activity less, if at all, during negative airway pressure.

In summary, the observed changes in Phr activity were most probably caused by changes in the activity of receptors in the lung that have their afferent fibres in the vagal nerves. For IC activity a small contribution was made by an extra-vagal mechanism, which evidently does not influence Phr activity. This mechanism might well be the load compensating reflex by afferent activity of muscle spindles. Our observations regarding this mechanism reflect the distribution of muscle spindles in diaphragm and intercostal muscles: the former is only poorly supplied whereas in the latter spindles are numerous (Cherniack and Altose, 1981).

P_{ip} and inspiratory activity

The results for Phr activity obtained in our study are in good agreement with those of the preliminary study of Luijendijk et al. (1988). For an increase in P'_{ip} of 0.1 kPa, they found relative decreases in Phr activity of 15% for $t = 0.2$ s and 12% for $t = 0.4$ s, whereas our values were 13% for $t = 0.2$ s and 12% for $t = 0.3$ s. This is a remarkable similarity, considering that they used a small number of cats and different anaesthetics. In their study either pentobarbital or ketamine was used, whereas anaesthesia in our study was invariably obtained by a mixture of urethane

Figure 3-6 The natural logarithm of IC_P/IC_C as a function of $\Delta P'_{tp}$ for $t = 0.2$ s after vagotomy. For further explanation see figure 3-4.

and chloralose. This means that the results of their as well as our experiments were independent of the anaesthetics used. Furthermore, our study confirmed the findings of Luijendijk et al. (1988) that the relationship between P'_{ip} and Phr activity could be described by mono-exponential functions. In addition, P'_{ip} and IC appeared to be related mono-exponentially as well.

The changes in P'_{ip} —on average -0.28 kPa for $P_{ir} = -1.2$ and 0.42 kPa for $P_{ir} = +1.2$ kPa—are small regarding the changes in P_{ir} (Table 3-3). For negative airway pressure this may be due to the tonic activity in the inspiratory muscles (Figure 3-3). During positive airway pressure expiratory activity has been proved (Russell and Bishop, 1976), which may reduce the increase in P_{ip} . After vagotomy, when these muscles have ceased being active, the changes in P'_{ip} are larger (compare figures 3-5 and 3-6). The described relationships between P'_{ip} and inspiratory activity may be part of a positive feedback loop (Luijendijk, 1986). Decreases in end-expiratory lung volume, which may be associated with active expiration as in exercise, will increase inspiratory

activity by reflex. The physiological importance of this reflex regulation of inspiratory activity may be illustrated by comparing the changes in Phr activity we found to the changes in Phr activity that resulted from experiments with CO₂ enriched inspiratory air—also in anaesthetized cats—conducted by Siafakas et al. (1981). Phr activity was raised by about 100% in response to an increase in alveolar PCO₂ of 1 kPa for both $t = 0.2$ s and $t = 0.3$ s. This value is an average value determined from an illustration of their experiments in hypercapnia. To obtain a comparable increase in Phr activity in our experiments for $t = 0.2$ and $t = 0.3$ s, P'_{ip} should decrease by 0.5 and 0.6 kPa, respectively. The accompanying changes in IC activity are 3 to 4 times larger. Although there are no data available on changes in end-expiratory lung volume or P'_{ip} in cats during exercise, breathing pressures are generally comparable between species. In man, decreases in P'_{ip} of about 0.6 kPa may be achieved even during moderate exercise (Henke et al., 1988).

In conclusion, mono-exponential relationships exist between P'_{ip} and Phr activity and between P'_{ip} and IC activity. The observed changes in Phr and IC activity during changed airway pressure are most probably caused by changes in the activity of receptors in the lung that have their afferents in the vagal nerves. The proposed positive feedback loop may play an important role in sustaining breathing in exercise. The gain of this feedback loop for IC activity is 3-4 times larger than that for Phr activity.

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Chapter 4

Reflex mechanisms and vagal afferents involved in the response of inspiratory activity to changed airway pressure

submitted

Abstract

This study is concerned with the determination of reflex mechanisms and the types of lung receptors that are involved in the response of inspiratory activity to increased and decreased airway pressure. To that end, we determined, in 14 spontaneously breathing, anaesthetized cats, the influence of vagal cooling on the responses of the diaphragm (Dia) and an inspiratory parasternal intercostal (IC) muscle to a change to continuous positive and negative airway pressures. The ratios in the amplitudes of Dia and IC activities during changed airway pressure and control conditions were plotted as a function of the changes in end-expiratory transpulmonary pressure (P'_{ip}). The resulting relationships were described by mono-exponential functions.

Dia and IC activities increased with decreasing P'_{ip} and decreased when P'_{ip} increased. In each cat the largest changes in Dia and IC activities were observed at a vagal temperature (T_v) of 37 °C. The changes in inspiratory activity decreased with decreasing T_v , and below $T_v = 10$ °C no further changes were observed. Apparently, unmyelinated fibre activity (C-fibres) does not contribute to the response of inspiratory activity to changed airway pressure. It is argued that the effect of positive airway pressure is due to increased inhibition of central inspiratory output by slowly adapting stretch receptors, whereas increased excitation by rapidly adapting stretch receptors is likely to be the most important mechanism increasing inspiratory activity during negative airway pressure.

Introduction

Chapter 3 quantifies the responses of phrenic and inspiratory parasternal intercostal (IC) activity to changes in end-expiratory transpulmonary pressure (P'_{ip}) early in inspiration. It was shown that both inspiratory activities decreased with increasing P'_{ip} and increased with decreasing P'_{ip} . Ratios were calculated between the inspiratory activities during increased or decreased airway pressure and under control conditions. The relation between these ratios and the change in P'_{ip} ($\Delta P'_{ip}$) could be described satisfactorily by mono-exponential functions. The material presented here quantifies the influence of vagal cooling on this relationship. Selective vagal cooling has proved to be a powerful tool in separating the contributions of different types of lung receptors to vagally mediated reflexes (Coleridge and Coleridge 1986). In our study, this technique was used to elucidate the question whether the increase in central inspiratory activity in response to a decrease in P'_{ip} is due solely to attenuation of the inhibitory effect of slowly adapting stretch receptors (SAR) (Luijendijk et al. 1988) or whether increasing excitation by rapidly adapting stretch receptors (RAR) and/or C-fibres may play an important role as well. The contribution of these other receptors is likely, because the inspiratory activity during negative airway pressure is much larger than that observed after vagotomy.

Materials and Methods

Materials, surgery and equipment

Experiments were carried out in 14 spontaneously breathing, adult cats (2.2 - 4.5 kg) anaesthetized with an initial dose of ketamine ($10 \text{ mg} \cdot \text{kg}^{-1} \text{ im}$) followed by intravenous administration of a mixture of urethane ($100 \text{ mg} \cdot \text{kg}^{-1}$) and chloralose ($20 \text{ mg} \cdot \text{kg}^{-1}$). Small supplemental doses were given when required to maintain anaesthesia. None of these cats was used in the companion study (Chapter 3). Cats lay on an operating table in the supine position.

Left femoral vein and artery were cannulated and a tracheostomy was performed. An oesophageal balloon was inserted in the lower part of the oesophagus for measurement of transpulmonary pressure (P_{ip}). The cervical vagi were dissected free over a distance of 3-4 cm and carefully placed in the gold-coated grooves of two cooling devices immersed in a pool of paraffin oil, formed by the folds of the skin. Using these cooling devices (Patberg et al. 1984), vagal temperature (T_v) could be varied rapidly between 0 and 37°C , with an accuracy of 0.2°C . Inspiratory activity was derived from the costal part of the diaphragm (Dia) and from an IC muscle located parasternally at the level of the 4th rib. In this study Dia activity was used instead of phrenic activity because the space occupied by the two cooling devices did not allow measurement of phrenic activity. The signals from Dia and IC muscles were differentially amplified, filtered between 200 and 3000 Hz and 'leakily' integrated with a time constant of 50 ms (Digitimer).

The tracheal cannula was connected to one end of the Fleisch head (No. 0) of a pneumotachograph (Gould). At the other end, the Fleisch head was connected to a main tube by means of a T-piece. A constant air flow of $18 \text{ l} \cdot \text{min}^{-1}$ was directed through the main tube. Using two taps, a low and a high pressure tank, and a water manometer, the tracheal pressure (P_{tr}) could be set in the range between -1.2 and +1.2 kPa relative to ambient pressure, as described in detail in chapter 3 (Figure 3-1).

P_{tr} and P_{ip} were measured using differential pressure transducers (PM131TC, Statham). P_{tr} was measured at the connection of the Fleisch head to the T-piece, and expressed as the difference with ambient pressure. P_{ip} was obtained from the difference between P_{tr} and the pressure in the oesophageal balloon. Arterial blood pressure was recorded with a differential pressure transducer (Gould, Statham, P23). Body temperature was monitored continuously by a rectal thermistor (Newport), and it was kept constant ($37 - 38^\circ\text{C}$) by warming the operating table.

In all, 12 signals were digitized by a computer (PDP11/73, DEC) at a frequency of 50 Hz: integrated Dia and IC muscle activities, tracheal air flow, P_{ip} , P_{tr} , partial pressures of N_2 , O_2 and CO_2 in inspiratory and expiratory gas—measured by a mass spectrometer (Balzers, QMG511)—blood pressure, rectal temperature and the temperatures of the 2 cooling devices. The data were stored on hard disk for off-line analysis.

Experimental procedure

Both cooling devices were set at a particular T_v . Data sampling was started in a steady state condition with the cat breathing at ambient pressure, which is the control (C) condition. After 10 breaths had been recorded, P_{ir} was changed stepwise to +0.9 kPa or -0.9 kPa (condition P) which was maintained for 7 breaths. Thereafter, P_{ir} was returned to control and at least another 4 breaths were recorded. P_{ir} was always changed during an expiration. This measurement can be indicated by the pressure sequence C→P→C. The reverse pressure sequence (P→C→P) was also performed, starting from a steady state condition at P_{ir} different from ambient pressure. In about half of the experiments the capnogram and the flow pattern were severely affected by positive airway pressure—especially at low T_v . Therefore, we decided to use $P_{ir} = +0.6$ and $P_{ir} = -0.6$ kPa in these cases. Thus, two levels of P_{ir} were applied and two pressure sequences, yielding four different measurements. These four measurements were performed at 9 different values of T_v : 37, 14, 12, 10, 8, 6, 4, 2, 0 °C, and—in order to obtain some intermediate data points—for four cats also at 18 °C. As a rule, T_v was changed from high to low, but other sequences were also followed. After each set of four measurements, T_v was reset to 37 °C. P_{ir} was then switched to a positive and a negative value, to ascertain that the effects of cooling were fully reversible. If the responses to these changes in P_{ir} were still satisfactory, the measurements were carried on at the next T_v . Only in one case had the experiment to be terminated after $T_v = 6$ °C had been applied.

Data analysis

For each breath the amplitudes of integrated Dia and IC activity were determined at three preselected points in time (t) after the onset of Dia activity ($t = 0.1$, $t = 0.2$ and $t = 0.3$ s, respectively). The value of P_{ip} just before the start of a breath was defined as the P'_{ip} belonging to that breath. For these three variables the values of 3 consecutive breaths were averaged, taking 3 breaths just before and 3 breaths after a change in P_{ir} . The breath just after a step change in P_{ir} was not used in the analyses because the volume change in the preceding expiration did not allow proper determination of the P'_{ip} of that breath. The mean value for Dia activity obtained at condition P was divided by the corresponding value at condition C (Dia_p/Dia_c). The same calculation was made for IC activity, yielding IC_p/IC_c . Accordingly, for each change in P_{ir} 3 values for Dia_p/Dia_c and 3 values for IC_p/IC_c were obtained, one for each of the values of t . The difference between the mean value of P'_{ip} at condition P and the mean value of P'_{ip} at condition C was calculated ($=\Delta P'_{ip}$). For all step changes in P_{ir} , the natural logarithms of Dia_p/Dia_c at a certain value of t were plotted against the corresponding $\Delta P'_{ip}$ (Figure 4-3). The same was done for IC_p/IC_c . Linear regression analysis was applied to the data points. In this way 6 regression lines were obtained for each cat at each level of T_v .

Student's *t*-test for paired observations was applied to ascertain differences between slopes obtained at different T_v .

Figure 4-1 Effects of a step change in P_{tr} from 0 to -0.6 kPa at $T_v=37^\circ\text{C}$. From top to bottom: blood pressure, P_{ip} , P_{tr} , Dia and IC activities, air flow.

Results

Experimental recordings of two measurements in the same cat during negative airway pressure are displayed in figures 4-1 and 4-2 for $T_v = 37^\circ\text{C}$ and $T_v = 6^\circ\text{C}$, respectively. The changes in P_{tr} applied are shown in the third trace of each figure, while the resulting changes in P_{ip} can be derived from the second trace. Note that the changes in P'_{ip} at 6°C are larger than those at 37°C , although the changes in P_{tr} are the same. Dia and IC activities increase in figure 4-1 in response to the decrease in P'_{ip} , illustrating the inverse relationship between inspiratory activity and P'_{ip} . Dia and IC activities in figure 4-2 hardly change in response to the change in P'_{ip} , indicating that at $T_v = 6^\circ\text{C}$ transmission of vagal afferent information with respect to these responses was largely blocked.

Figure 4-2 Effects of a step change in P_{tr} from 0 to -0.6 kPa at $T_v=6^\circ\text{C}$. Same cat as in figure 4-1, which supplies further explanation.

The natural logarithms of $\text{Dia}_p/\text{Dia}_c$ and IC_p/IC_c were plotted as functions of $\Delta P'_{ip}$, and regression lines were calculated. This was done separately for each cat, for each T_v , for each of the two muscle activities and for the three values of t (Materials and Methods). We have demonstrated previously that regression lines describe the relationship between $\Delta P'_{ip}$ and $\ln \text{Dia}_p/\text{Dia}_c$ (or $\ln \text{IC}_p/\text{IC}_c$) satisfactorily, when both vagi are at body temperature (Chapter 3). Each regression line was checked visually to ensure that this held also at lowered T_v (Figure 4-3). Since the intercepts of the regression lines were small (0.12 ± 0.18), the slopes of these lines can be used as their sole characteristic. The mean values for the slopes ($\pm \text{SEM}$) as a function of T_v are displayed in figure 4-4 for all three values of t and for both muscle activities separately. As described earlier (Chapter 3), the slopes for IC activity were more negative than those for diaphragm (phrenic) activity. The results obtained at $T_v = 0^\circ\text{C}$ were comparable to the results of our previous studies after vagotomy, in which the slopes for phrenic activity reached zero values and the slopes for IC activity retained a small negative value. The remaining

slopes for IC activity after vagotomy could be attributed to a load compensating mechanism by afferent activity from muscle spindles. This mechanism is hardly of any importance for the diaphragm (Cherniack and Altose 1981).

All three panels of figure 4-4 show that the slopes become less negative with decreasing T_v down to $T_v = 10^\circ\text{C}$, where they reach more or less their final values. This behaviour was supported by the results of the statistical analysis, from which the results for $T_v = 18^\circ\text{C}$ were excluded because only 4 observations were obtained at this T_v . The slopes at $T_v = 37^\circ\text{C}$ were significantly more negative than the slopes at all other T_v levels ($P < 0.005$) for both muscle activities and for all three values of t . In all cases the slopes at $T_v = 14^\circ\text{C}$ were significantly more negative ($P < 0.05$) than the corresponding slopes for $T_v \leq 10^\circ\text{C}$. For $T_v \leq 14^\circ\text{C}$ further significant differences ($P < 0.05$) were sometimes found between the values for the slopes at two particular levels of T_v , but there was no consistency in this pattern. All significant differences obtained from our analyses supported the observation that the values for the slopes became less negative with decreasing T_v .

Discussion

Vagal cooling is a widely used technique for determining the type(s) of lung receptors involved in a pulmonary reflex. The disadvantages of this technique have been reviewed by Paintal (1973), the most important being the impossibility to determine the contributions of the different afferents quantitatively, because the total blocking of one type of afferents leads to a reduction in the maximal transmissible frequency of the remaining fibres. Useful qualitative results may, however, be obtained.

For further discussion, it may be helpful to divide the responses described in this chapter into responses to negative and to positive P_{ir} . Negative P_{ir} , accompanied by decreases in P'_{ip} and end-expiratory lung volume, involves increases in inspiratory activity, while positive P_{ir} , accompanied by increases in P'_{ip} and end-expiratory lung volume, leads to decreases in Dia and IC activities. In chapter 3 it has been argued that both responses depend on intact intra-thoracic, vagal afferents. Thus, three types of lung receptors remain that can be involved. Two of these types—i.e., SAR and RAR—have myelinated afferents in the vagal nerves, while the third type has afferents without myelin sheaths—i.e., C-fibres.

C-fibres

The present study shows that no further changes in the responses of Dia or IC activities to changes in airway pressure could be observed at $T_v < 10^\circ\text{C}$ (Figure 4-4). This means that activity in C-fibres does not contribute to either response, since their activity—in dogs—is largely unimpaired at these T_v (Jonzon et al. 1988). In spontaneously breathing rabbits with both vagi cooled to 8°C , Patberg (1983) found that an increase or a decrease in P_{ir} involved an increase in tonic inspiratory activity, which was interpreted as a stimulating effect of lung receptors masked at higher temperatures by the inhibiting

Figure 4-3 Natural logarithm of IC_P/IC_C as function of $\Delta P'_{tp}$. $t=0.2$ s, $T_v=14^\circ\text{C}$, Slope = -2.51 kPa^{-1} . Corresponding slope at $37^\circ\text{C} = -3.62$.

influence of SAR activity. We never observed such a stimulating effect of a change in P_{tr} at low levels of T_v in the cat. Recordings at $T_v \leq 8^\circ\text{C}$ always resembled those obtained at $T_v = 0^\circ\text{C}$. This conflicting result is likely to be due to species differences. Delpierre et al. (1981) reported that hyperdeflation was one of the most potent stimuli to C-fibres in cats. The changes in lung volume (100 ml) in their experiments were much larger than those applied in our study. This might indicate that only extreme deflations evoke C-fibre activity in the cat (Coleridge and Coleridge 1986). This may explain why this type of fibres did not contribute to the responses observed in the present study.

SARs and RARs

Figure 4-4 shows that the slopes of the regression lines became gradually less negative with decreasing T_v . The largest changes were found between $T_v = 37^\circ\text{C}$ and $T_v = 14^\circ\text{C}$. This means that at relatively high temperatures ($T_v = 14 - 18^\circ\text{C}$) the responses of

Figure 4-4 Mean slopes ($\pm$ SEM) as a function of T_v . Different panels refer to different values of t . Δ for Dia, $\circ$ for IC. $n=11-14$ ($n=4$ for $T_v=18^\circ\text{C}$).

IC and Dia activity to changed airway pressure were already reduced. This was observed for both positive and negative P_{ir} . At these T_v low frequency receptor activity is transmitted completely (Jonzon et al. 1988). Thus, vagal cooling down to 18°C can only reduce the transmission of high frequency receptor activity. As a consequence, the responses of IC and Dia activity to both positive and negative P_{ir} should depend on high discharge frequencies in vagal afferents. The following reflex mechanisms may now be considered to account for our findings.

The decreased inspiratory activity observed during positive P_{ir} is most probably due to increased inhibition from SARs. Davis et al. (1956) showed that SAR activity increased in close relation to increases in P_{ip} . Increases in SAR activity lead to "inhibition of inspiration", commonly indicated by prolonged expiratory time (Coleridge and Coleridge 1986). The decreased inspiratory activity early in inspiration observed in the present study and in the accompanying study (Chapter 3), demonstrates that this "inhibition of inspiration" not only delays the onset of the next inspiration but also affects the central inspiratory output itself.

In part, decreased SAR activity may have accounted for the observed increases in the inspiratory activities during negative P_{ir} by disinhibition. The increases in Dia and IC activity were, however, much larger than the increases found after vagotomy (Chapter

3) or at $T_v = 0^\circ\text{C}$, in which cases transmission of SAR activity is completely blocked. This means that the mechanism of disinhibition by SARs should be supplemented by stimulating activity from other receptors during negative P_{ir} . This is in accordance with our previous conclusion that high frequency receptor activity should account for our findings for both positive and negative P_{ir} . It is well known that deflation of the lungs increases RAR activity in cats (Armstrong and Luck 1974) and rabbits (Sellick and Widdicombe 1970, D'Angelo et al. 1976), and that stimulation of RARs leads to increased inspiratory activity (Coleridge and Coleridge 1986). Therefore, increased RAR activity may be held responsible for the larger part of the increases in Dia and IC activity found during negative P_{ir} . This means that during negative airway pressure two mechanisms act together: disinhibition by SAR activity and excitation by RAR activity. Recently, Green et al. (1990) have reached about the same conclusion for dogs in a different experimental set-up.

In conclusion, C-fibres do not contribute to the responses of Dia and IC activity to changed airway pressure in the range of P_{ir} values used. Increased SAR activity causes the decrease in central inspiratory output observed during positive airway pressure, whereas increased activity of RARs is likely to be the most important mechanism to increase central inspiratory output during negative airway pressure.

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Chapter 5

Conventional versus slug CO_2 loading and the control of breathing

submitted

Abstract

Conventional inspiratory CO₂ loading (CCL) is accomplished by having subjects breathe CO₂ enriched air. An alternative method of CO₂ loading is to inject a bolus of CO₂ at the start of each inspiration into the inspired air: slug CO₂ loading (SCL). During SCL PCO₂ in the conducting airways declines quickly towards 0 kPa in the course of inspiration, whereas PCO₂ remains at the inspiratory PCO₂ level during CCL. We designed an experiment to find out whether CCL and SCL may stimulate the respiratory controller differently and whether a vagal mechanism contributed to the responses.

We compared the ventilatory responses to SCL and CCL in 14 anaesthetized, spontaneously breathing cats. In each experimental animal 5 CCL experiments ($F_i\text{CO}_2 = 0.01\text{--}0.05$), 6 SCL experiments (slugs of 50% CO₂ in air ranging from 0.5 to 6 ml) and 3 control experiments in which no CO₂ was loaded, were performed in a fixed randomized order. The partial pressure of CO₂ in arterial blood was determined from small blood samples (0.14 ml). In 3 cats we repeated the experiments after bilateral transection of the cervical vagi to evaluate the contribution of vagal receptors to the responses observed.

The average slope of the CO₂ response curve for SCL was 2 times steeper than the average slope for CCL ($P=0.009$). The differences in minute ventilation for a particular value of $P_a\text{CO}_2$ were due to differences in breathing frequency ($P=0.007$), whereas tidal volumes did not differ significantly ($P=0.25$). The increased breathing frequency for SCL compared to CCL was due mainly to a decreased duration of inspiration ($P=0.01$). After vagotomy the difference between the response curves for SCL and CCL was much reduced.

It is concluded that SCL and CCL affect the respiratory controller in a different way causing differences in breathing pattern and CO₂ sensitivity between the two methods. Evidently, a mechanism based on CO₂ sensitivity of pulmonary receptors is involved in the responses observed.

Introduction

During conventional CO₂ loading (CCL) gas mixtures are inspired with a constant concentration of CO₂. An alternative method has been suggested by Fenn and Craig (1963), in which CO₂ is loaded by adding a bolus or 'slug' of CO₂ to inspired air at the start of each inspiration: slug CO₂ loading (SCL) (Swanson, 1978). This method was originally designed to gain better control of the CO₂ load imposed upon the lungs, thus overcoming the problem of inspiring increasing amounts of CO₂ when ventilation is increased. These two techniques produce different levels of PCO₂ in the conducting airways during the course of inspiration. For CCL PCO₂ remains at inspiratory level during the entire inspiration, whereas it decreases to 0 kPa for SCL. We tested the hypothesis that this difference in PCO₂ is important for the control of breathing. We tried to maximize the difference between SCL and CCL by injecting a small bolus (0.5–6 ml) of 50% CO₂ in air directly into the tracheal cannula starting a few milliseconds after

the start of each inspiration. During CCL a constant fraction of CO_2 was inspired (1.5%).

Cummin et al. (1987) compared minute ventilation ($\dot{V}_E$) obtained by CCL to $\dot{V}_E$ obtained by the Fenn and Craig (1963) technique at the same CO_2 load in man and found higher $\dot{V}_E$ for the latter, which they ascribed to differences in dead space CO_2 concentration. We followed a different approach. We did not try to obtain comparable CO_2 loads for SCL and CCL, but measured $\dot{V}_E$ and arterial PCO_2 at several different CO_2 loads in the same animal and compared the slopes of the response curves obtained during SCL and CCL for each animal. For three cats we repeated the experiments after vagotomy to find out whether the activity of vagal receptors contributed to the responses observed.

The differences in the responses between SCL and CCL are discussed in the light of the CO_2 sensitivity of pulmonary receptors and the oscillations of CO_2 in arterial blood.

Materials and Methods

materials, surgery and equipment

The experiments were carried out in 14 spontaneously breathing adult cats (mean body mass 3.5 ± 0.87 kg). Cats were anaesthetized with an initial dose of ketamine ($10 \text{ mg} \cdot \text{kg}^{-1} \text{ im}$) followed by intravenous administration of a mixture of urethane ($100 \text{ mg} \cdot \text{kg}^{-1}$) and chloralose ($20 \text{ mg} \cdot \text{kg}^{-1}$). Small supplemental doses of this mixture were given when required. The cats were placed on an operating table in the supine position. The left femoral vein and artery were cannulated and a tracheal cannula was inserted just below the larynx.

The tracheal cannula was connected to the flow pressure transducer (Fleisch No. 0) of a pneumotachograph (Gould). The other side of the Fleisch head was connected to a main tube by means of a T-piece. Through this main tube a constant bias flow of $18 \text{ l} \cdot \text{min}^{-1}$ was directed, which was sufficient to prevent rebreathing of expired gas. For CCL, CO_2 (1.5 vol%) was added to this bias flow. For SCL, a bolus of CO_2 (50 vol% in air) was injected into the trachea at the distal end of the tracheal cannula using a fast opening ($<5 \text{ ms}$) electro-magnetic valve. This valve was triggered by the "zero crossing flow" of the pneumotachograph. The duration of the injection was set to 50 ms and the pressure used to inject the bolus was adjusted to obtain different levels of SCL. The injection was completed within 60 ms after the onset of inspiration.

The equipment dead space had a volume of 8.5 ml and a flow resistance of $0.6 \text{ kPa} \cdot \text{s} \cdot \text{l}^{-1}$. Body temperature was measured with a rectal thermistor (Newport) and kept between 37.5 and 38.5 °C by heating the operating table.

To evaluate steady state conditions of ventilation and circulation the partial pressure of carbon dioxide in inspired and expired gas was measured by a mass spectrometer (Balzers, QMG511) or a capnograph (Nellcor, N1000). Arterial blood pressure was measured with a differential pressure transducer (Statham, P23). Systolic, diastolic and mean values as well as heart rate were displayed continuously.

The signals were A/D converted by a computer. Data were stored on floppy disc for off-line analysis. To allow on-line inspection of the data, most of the signals were also recorded on an 8 channel ink recorder (Elema-Schoenander).

From the arterial cannula small blood samples (0.14 ml) were collected in heparin coated glass capillaries (Corning) and immediately analysed in duplicate at 37 °C for pH, PO₂ and PCO₂ (IL413 or ABL3). The values were corrected to body temperature (Ashwood et al., 1983). The total volume of blood collected during the entire experiment was less than 5% of the animals blood volume.

Experimental procedure

During each experimental run of CO₂ loading one data file and two pairs of blood samples were collected as follows. When the cat was in a stable condition regarding ventilation, end-tidal PCO₂ and blood pressure, a duplicate blood sample was drawn from the arterial cannula for control pH, PO₂ and PCO₂. Then CCL or SCL was started. After 3 minutes, data sampling was started and it was continued for at least one minute. Thereafter, another duplicate blood sample was collected and the CO₂ load was ended. At least 5 minutes were allowed for full recovery before the next run was started. In this way 6 SCL runs (0.5-6 ml of 50% CO₂ in air) and 5 CCL runs (1-5% of CO₂ in air) were carried out in each cat. Control P_aCO₂ levels for two consecutive runs seldom differed more than 0.15 kPa.

The procedure of the 3 control runs was simple: in a steady state condition—without CO₂ loads—a data file was gathered, whereafter two blood samples were taken. The runs were generally performed in a fixed randomized order. A control measurement preceded each sequence of 4 runs with CO₂ loading.

In three cats we repeated the entire sequence of SCL, CCL and control measurements after bilateral transection of the cervical vagi. Because breathing frequency (f) was lower after vagotomy, we used slugs of 100% CO₂ in stead of 50% in air to obtain comparable CO₂ loads.

We did several tests to exclude unwanted side-effects of the SCL technique, the results of which will be discussed below (section critique of method in discussion).

Data processing and statistics

From the flow signal tidal volume (V_T), $\dot{V}_E$, f and the durations of inspiration (T_I) and expiration (T_E) were determined. For all these quantities we used the mean value obtained from the last 30 breaths of each data file. From P_aCO₂ obtained from the blood samples just before and at the end of a CCL or SCL run we calculated ΔP_aCO_2 for that run. $\Delta \dot{V}_E$ was derived from the difference in $\dot{V}_E$ between a CO₂ loaded run (SCL or CCL) and the preceding run at control conditions. The slopes of the CO₂ response curves— $\Delta \dot{V}_E$ as a function of ΔP_aCO_2 —were estimated by $\Sigma y \cdot (\Sigma x)^{-1}$ (Mardia et al., 1983). For other parameters we used normal linear regression analysis. Student's t-test for paired observations was used to ascertain the statistical significance of the differences in the slopes of the regression lines between CCL and SCL.

Figure 5-1 CO₂ response curves of 14 cats, separately for CCL (circles, solid lines) and SCL (plus signs, dashed lines). Right hand bottom panel (all) displays the average slopes.

Results

Figure 5-1 displays $\Delta \dot{V}_E$ as a function of $\Delta P_a\text{CO}_2$, separately for CCL and SCL, and for each cat a separate panel is used. All data points are indicated. In all of the cats the lines fitted through the data points for SCL (dashed lines) are steeper than the lines through the data points for CCL (solid lines). The slopes of the lines for CCL and SCL were significantly different ($P=0.0088$). In five cats hypocapnic responses were sometimes observed during the SCL experiments (Figure 5-1, cats 2, 5, 7, 8 and 14) and in one cat (7) also during CCL. The ratio between the slopes for SCL and CCL showed some scatter between the cats. The largest value was 8.65 for cat 13 and the smallest was 1.10 for cat 14, and on average the ratio was $2.06 (\pm 2.88)$. The apnoea points calculated from the $\dot{V}_E - P_a\text{CO}_2$ plots were $3.22 (\pm 0.29)$ and $3.33 (\pm 0.43)$ kPa for CCL and SCL, respectively, and they were not significantly different ($P=0.33$).

The mean values ($\pm$ SD) for $P_a\text{CO}_2$, arterial pH, $\dot{V}_E$, V_T , T_I and T_{E_i} in the control condition—before and after vagotomy—are given in Table 5-1. After vagotomy the breathing pattern was clearly changed, but $P_a\text{CO}_2$ was not. The decreased $\dot{V}_E$ observed after vagotomy may be caused by decreased dead space ventilation and increased efficiency of gas mixing.

Besides the response of $\dot{V}_E$ to $P_a\text{CO}_2$, we analysed several other relationships between respiratory variables. The results are shown in table 5-2 in terms of the regression parameters slope and intercept. The differences between the values of the intercepts for SCL and CCL were on average small (Table 5-2), and none were significant. Therefore, we will now focus on the difference between the slopes of the regression lines for SCL and CCL.

Table 5-1 Mean values of some ventilatory variables in control conditions. For the 3 cats which were also studied after vagotomy, the average values before and after vagotomy are given.

	all cats			cats 12,13 and 14					
	(intact)			intact			vagotomized		
	mean	SD	n	mean	SD	n	mean	SD	n
$P_a\text{CO}_2$	4.11	0.37	14	4.47	0.42	3	4.40	0.08	3
pH _a	7.43	0.04	11						
$\dot{V}_E$	1.04	0.29	14	1.31	0.37	3	0.99	0.08	3
V_T	30.5	8.0	14	52.6	3.8	3	67.2	18.8	3
f	30.4	7.8	14	26.7	10.7	3	15.1	3.3	3
T_I	1.12	0.35	14	1.45	0.60	3	2.24	0.30	3
T_{E_i}	1.02	0.23	14	1.10	0.15	3	1.90	0.64	3

$P_a\text{CO}_2$ in kPa, pH_a in units, $\dot{V}_E$ in l·min⁻¹, V_T in ml, f in s⁻¹, T_I and T_{E_i} in s. Values for arterial pH in cats 12-14 are not available.

Figure 5-2 Hey-plots— $\dot{V}_E$ as a function of V_T —for CCL and SCL experiments for each cat separately. For further explanation see figure 6-1.

The 'Hey-plots'— $\dot{V}_E$ as a function of V_I —are given in figure 5-2. Except for cats 6, 13 and 14, the regression lines for SCL are steeper than for CCL. The larger values for the slopes in the Hey-plots ($P=0.0023$) were the result of higher f for SCL than for CCL experiments. The slopes for f as a function of $\Delta P_a\text{CO}_2$ were significantly different ($P=0.0066$) between SCL and CCL (Table 5-2). The slopes for V_T as a function of $\Delta P_a\text{CO}_2$ did not differ between SCL and CCL ($P=0.25$). The larger values for f during SCL compared to CCL were due to decreased T_I . The values for the slopes for T_I as a function of $\Delta P_a\text{CO}_2$ were significantly different between SCL and CCL ($P=0.0096$), while they were not for expiratory duration (T_E) as a function of $\Delta P_a\text{CO}_2$ ($P=0.18$). Hence, the slopes for T_I/T_E as a function of $\Delta P_a\text{CO}_2$ for SCL were generally smaller than those for CCL (Figure 5-3, Table 5-2). Accordingly, the slopes for inspiratory flow ($\dot{V}_I$) as a function of $\Delta P_a\text{CO}_2$ were larger for SCL than for CCL. On average, the ratio of these slopes (SCL/CCL) was 1.56 (range 0.68 to 3.30).

Figure 5-4 displays the response curves for 3 cats before and after vagotomy. The differences between the response curves for SCL and CCL before vagotomy are representative for the whole group (Figure 5-1): cat 13 had the largest ratio of all 14 cats between the slopes for SCL and CCL (8.65), and cat 14 had the smallest value (1.10). For cat 12 the ratio (1.49) was somewhat below average (2.06). After vagotomy the ratio between SCL and CCL slopes was strongly reduced for cat 13 (1.15) and also the other two cats showed somewhat lower values (1.29 and 1.08 for cat 12 and 14, respectively). The difference between SCL and CCL after vagotomy just failed to reach significance ($P=0.0622$). The mean values for the slopes after vagotomy (2.0 and 2.3 $\text{l}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$, for CCL and SCL, respectively) were comparable to the CCL slopes before vagotomy (2.4 $\text{l}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$). This means that vagotomy reduced the response to SCL, whereas a vagal mechanism did not contribute importantly for CCL. An extra-vagal mechanism might be considered for the small difference between SCL and CCL which remained after vagotomy.

Discussion

Critique of methods

The slopes of the CO_2 response curves for SCL were two times larger than the slopes for CCL (Table 5-2). The slopes of the CO_2 response curves for CCL ($2.0 \text{ l}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$) were steeper than those described by Gautier (1976) for awake cats—about $1.1 \text{ l}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}$. We used bigger cats in our study and, further, we estimated the slopes of the response curves according to Mardia et al. (1983) which both may lead to an increase in the values for the slopes.

We did the following tests to ascertain that our results were not influenced by unintended effects during the experiments and subsequent data analysis. Firstly, we introduced slugs of N_2 , O_2 or air into the trachea which had no effect on $\dot{V}_E$ or the breathing pattern, suggesting that there was no mechanical stimulus involved in SCL.

Figure 5-3 The ratio T_1/T_2 as a function of ΔP_{CO_2} for CCL and SCL experiments for each cat separately. For further explanation see figure 6-1.

Further, the blood gas values obtained from the low volume capillaries (0.14 ml) were not systematically different from those of simultaneously obtained larger blood samples in heparinized glass syringes of 1.5 ml. The small size of the samples allowed us to obtain a value for $P_a\text{CO}_2$ before and after each SCL or CCL experiment, without depriving the experimental animal of more than 5% of its blood volume. Calculating $\Delta P_a\text{CO}_2$ in stead of using $P_a\text{CO}_2$ directly, corrected for gradual changes in $P_a\text{CO}_2$ during the day e.g. resulting from small changes in the depth of anaesthesia. To ascertain that measurements were done in a steady state condition we examined each data file for a trend in the breath-to-breath calculated value for $\dot{V}_E$, which was absent. The $P_a\text{CO}_2$ levels obtained before each experimental run seldom differed more than 0.15 kPa between consecutive runs, suggesting that all runs were started from a steady state condition.

Techniques for loading CO_2 at the start of inspiration

Loading the respiratory system with CO_2 at the start of inspiration mimics metabolic CO_2 loading more closely than CCL does (Swanson, 1978). This has been done basically by two methods: the Fenn and Craig technique (1963) and the tube breathing technique. Fenn and Craig (1963) added a constant flow of CO_2 to the inspiratory limb of a valve system. During expiration CO_2 accumulates in front of the inspiratory valve, and the next inspiration starts with a high CO_2 content in inspired air, declining in the course of inspiration towards lower levels, although never reaching zero values. End-inspiratory CO_2 concentrations may be 0.5-1.0% (Cummin et al., 1984, 1987).

With the tube breathing technique, an external dead space is added. At the start of inspiration the content of the external dead space—i.e., alveolar gas—is inspired first, followed by fresh air. In fact this is a kind of rebreathing technique, causing not only an increase in $P_a\text{CO}_2$ but also a decrease in $P_a\text{O}_2$. Both these techniques, however, may not be very effective in mimicking metabolic loading: longitudinal dispersion of a gas bolus in the airways is considerable, especially when the mouth and larynx are not bypassed (Ultman and Thomas, 1979). During tube breathing, the volume of the tube is larger than half V_T (Masuyama and Honda, 1984). For both these techniques the difference with CCL is, therefore, likely to be small.

Concerning our method CO_2 is loaded as early in inspiration as possible. CO_2 is injected directly into the tracheal cannula, thus bypassing the Fleisch head and other "mixing chambers" in the breathing circuit. The bolus may spread over the tracheal volume and enter in part the equipment dead space. This is, however, unlikely since the largest flow used to inject a bolus was $120 \text{ ml}\cdot\text{s}^{-1}$, which is a normal inspiratory flow for a SCL experiment with a 6 ml bolus. In conclusion, the technique for SCL used in our study may be much more effective in mimicking a metabolic CO_2 load of breathing than the methods used so far.

Table 5-2 Mean regression coefficients ($\pm$ SD) for CCL and SCL experiments. The last column gives the *P* values of Student's *t*-test for the 14 paired values of the slopes for SCL and CCL.

variables		CCL		SCL		P
dep	indep	Slope	Intercept	Slope	Intercept	
$\Delta \dot{V}_E$	$\Delta P_a\text{CO}_2$	2032 (728)		4188 (3861)		0.0088
V_T	$\Delta P_a\text{CO}_2$	44.5 (21.5)	40.3 (11.5)	54.1 (30.8)	43.9 (16.2)	0.2527
<i>f</i>	$\Delta P_a\text{CO}_2$	2.9 (3.2)	30.5 (7.8)	5.7 (8.5)	31.4 (7.5)	0.0066
T_I	$\Delta P_a\text{CO}_2$	-0.17 (0.15)	1.09 (0.32)	-0.28 (0.26)	1.05 (0.20)	0.0096
T_{I_E}	$\Delta P_a\text{CO}_2$	-0.08 (0.10)	1.02 (0.23)	-0.03 (0.33)	0.98 (0.21)	0.1777
T_I/T_{I_E}	$\Delta P_a\text{CO}_2$	-0.08 (0.12)	1.08 (0.20)	-0.26 (0.33)	1.08 (0.21)	0.0025
$\dot{V}_I$	$\Delta P_a\text{CO}_2$	56.0 (26.2)	38.2 (9.9)	79.1 (28.7)	43.9 (16.3)	0.0051
$\dot{V}_{I_E}$	V_T	37.6 (5.2)	-332 (323)	43.3 (9.6)	-479 (494)	0.0023

dep: dependant, indep: independent variable, $\Delta \dot{V}_E$ in $\text{ml}\cdot\text{min}^{-1}$, $\Delta P_a\text{CO}_2$ in kPa, V_T in ml, *f* in min^{-1} , T_I, T_{I_E} in s, $\dot{V}_I$ in $\text{ml}\cdot\text{s}^{-1}$.

The effect of different loading techniques on ventilatory parameters

Our results show higher $\dot{V}_E$ for SCL compared to CCL. Using tube breathing, Masuyama and Honda (1984) found comparable increases (about 100%) in the slopes of the CO_2 response curve in man. In contrast to our results, they only found significant differences in V_T and not in *f*. In a more recent paper of the same group (Maruyama et al., 1988) these results could apparently not be reproduced: a small difference in the slope of the CO_2 response curve was found only in hypoxia. Studies in human subjects comparing different methods for CO_2 loading often require a background of hypoxia to obtain different results for the two methods (see Cummin et al., 1987). Our results were obtained in normoxaemia: $P_a\text{O}_2$ ranged between 11.2 kPa for a control experiment up to 16.5 kPa for the highest CO_2 loads. Also in cats a small increase in $\dot{V}_E$ (11%) has been reported during tube breathing as compared to CCL, and this result was also obtained in normoxia (Marsh and Nye, 1982).

Mechanisms for the control of ventilation during CCL and SCL

Marsh and Nye (1982), comparing tube breathing to CCL at the same CO_2 load, obtained about the same increases in $\dot{V}_E$ before and after vagotomy, whereas the differences between CCL and SCL had largely disappeared after section of the sinus nerves. From these findings they concluded that oscillations in arterial PCO_2 were the most important mechanism causing differences between CCL and tube breathing. In our experiments the slopes of the response curves for SCL were diminished after vagotomy (Figure 5-4). The magnitude of the change in $\dot{V}_E$ obtained in their experiments (11%) is, however, comparable to the mean change between SCL and CCL slopes we obtained after vagotomy (17%). This brings us to the following hypothesis: tube breathing is hardly capable of stimulating a pulmonary mechanism and most of the effects on $\dot{V}_E$ are caused by oscillations of CO_2 in arterial blood. Our technique may deliver comparable oscillations in arterial PCO_2 and their effects on $\dot{V}_E$ may be estimated from the difference in the response curves for SCL and CCL obtained after vagotomy. Evidently, the larger part of the difference in $\dot{V}_E$ between SCL and CCL in our experiments may be caused by a vagal mechanism. Why this mechanism is not active during tube breathing will be discussed below.

Vagal mechanisms for the control of breathing during SCL

During the larger part of inspiration the PCO_2 level at the pulmonary receptor site is different between SCL and CCL. During SCL, PCO_2 in the conducting airways rapidly decreases to 0 kPa, whereas during CCL PCO_2 equals the levels in inspired gas. The tissue PCO_2 at the receptor site may follow the variations in airway PCO_2 rapidly (Luijendijk, 1983) and pulmonary receptors in cats are sensitive to changes in airway CO_2 (Kunz et al., 1976). The activity of both slowly and rapidly adapting stretch receptors is reduced by increasing airway CO_2 , especially in the range between 0 and 3 kPa (Coleridge et al., 1978). Hence, the variations in airway PCO_2 may modulate the firing pattern of the receptors. During tube breathing the firing pattern of the receptors may not be very different from that during CCL, for the CO_2 free air reaches the receptor site only at the end of inspiration. Inhaling CO_2 decreases the activity of slowly adapting stretch receptors (Bradley et al., 1976). As a result, T_I may increase and f decrease. Accordingly, we found higher slopes for T_I as a function $\Delta P_a \text{CO}_2$ for CCL than for SCL experiments, whereas slopes for f as a function of $\Delta P_a \text{CO}_2$ were lower. The question how this mechanism causes increases in $\dot{V}_I$ during SCL remains to be solved.

SCL mimics a metabolic CO_2 load more closely than CCL does (Swanson, 1978). Accordingly, we found the more isocapnic response for SCL compared to CCL. The same vagal mechanism stimulating ventilation may be underlying the observation in the experiments of Sheldon and Green (1982). They found in dogs in which the systemic and pulmonary circulation were completely separated that increases in pulmonary arterial CO_2 content increased ventilation, while systemic conditions were kept constant. After vagotomy this response disappeared. It has been demonstrated that in response

Figure 5-4 CO_2 response curves for three cats before (upper panels) and after vagotomy (lower panels). For further explanation see figure 6-1.

to changes in pulmonary arterial PCO_2 the activity of slowly adapting stretch receptors could be influenced in this kind of experiment (Green et al., 1986).

In conclusion, we found two times higher slopes for the CO_2 response curve for SCL compared to CCL during spontaneous, normoxic breathing. These differences were due to higher f for SCL, predominantly caused by shortening of inspiratory time. It is shown that the differences in $\dot{V}_E$ are largely caused by a vagal mechanism. The way in which slug CO_2 loading is implemented may be important to stimulate this vagal mechanism. Further studies in conscious man injecting CO_2 at the start of inspiration directly into the naso-pharynx should elucidate the importance of our findings for human respiratory control, whereas animal studies may further clarify the underlying vagal mechanisms.

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Chapter 6

General discussion: a model for the pulmonary contribution to the control of breathing

Introduction

Experiments in dogs on complete cardiopulmonary by-pass have shown that minute ventilation ($\dot{V}_E$) increases when the CO_2 content of the pulmonary arterial blood is increased, while systemic arterial PCO_2 is kept constant. Similarly, increases in pulmonary blood flow at constant PCO_2 also increased $\dot{V}_E$. The responses to these "metabolic" CO_2 loads were abolished after bilateral vagal transection (Sheldon and Green, 1982; Green & Sheldon, 1983). In addition, Bartoli et al. (1974) using a comparable preparation found increases in $\dot{V}_E$ as a result of inspiratory CO_2 loading. This chapter presents two positive feedbacks which may account for the increases in $\dot{V}_E$ observed in these experiments. Further, a negative feedback is presented describing the CO_2 -sensitivity of the inspiratory off-switch mechanism.

The Feedbacks

The inspiratory amplification reflex

Figure 6-1 describes a positive feedback loop which includes the inspiratory amplification reflex. The first relationship in this loop—increasing the output of inspiratory motoneurons leads to increased inspiratory flow ($\dot{V}_I$)—is trivial. This direct relation is almost proportional in cats (Siafakas et al., 1981 a,b; Zin et al., 1982). The rest of the loop is generally studied as a whole: manipulating $\dot{V}_I$ alters the output of inspiratory motoneurons. In these studies, fully paralysed animals have been ventilated mechanically using a pump triggered by the start of phrenic activity. Inflating dogs thus prepared with high $\dot{V}_I$ increased phrenic activity considerably (Pack et al., 1981). In our study (Chapter 2) we showed that during spontaneous breathing in anaesthetized cats the gain of this reflex loop is more or less constant over the entire range of ventilation from quiet breathing up to maximal ventilation, irrespective of the addition of CO_2 to inspired air. The gain was on average 1.08 for phrenic activity, which means that the inspiratory amplification reflex does not contribute substantially to phrenic activity. Although we were not able to quantify the amplification of intercostal activity properly, it may have a larger value (DiMarco et al., 1981). The contribution of load compensating mechanisms—increasing the intercostal activity during occluded breaths—can lead to underestimations of the actual amplification. Since we found values up to 2.5, this reflex loop may still be important for intercostal muscle activity (IC)—at least in some cats. During unoccluded breaths load compensation may also contribute to IC activity, especially when $\dot{V}_I$ is large.

Although there is general agreement on the existence of this positive feedback loop, the mechanism by which it functions is highly controversial. Two hypotheses prevail (Figure 6-1). From his experiments in dogs, Pack favours the view that flow is the appropriate stimulus, while RARs mediate the response (Pack et al., 1981; Pack, 1981). There is a vast body of evidence that RARs are sensitive to flow but rapidly adapt to maintained inflations, whereas SARs are less affected by flow and adapt slowly to maintained

Figure 6-1 Positive feedback loop containing the vagal amplification reflex. Two hypotheses (see text) are shown. $\dot{V}_I$: inspiratory flow, V : inspired volume.

inflations (Pack & DeLaney, 1983; Pack et al., 1986). Increasing RAR activity enhances inspiratory activity (Chapter 4). On the other hand, the hypothesis that volume is the appropriate stimulus for this reflex loop while SARs mediate the response (Figure 6-1), originates from experiments in cats (DiMarco et al., 1981). A neural substrate has been described that could subserve this hypothesis (Averill et al., 1984; 1985). One might suggest species differences here. However, there are as far as we know no reports on stimulation of inspiratory activity by increased SAR activity. Also our results in cats using vagal cooling (Chapter 4, Meessen et al., 1990) never revealed any stimulating activity of SARs on phrenic or intercostal activity, whereas inhibition was the normal response. Therefore, the view that flow is the proper stimulus and RARs mediate the response seems to be more likely. Further evidence should be awaited to decide for either of these two views.

End-expiratory lung volume (V_{EEL}) and the inspiratory activity of the subsequent breath

Figure 6-2 describes the feedback loop containing this relation. Starting from the output of inspiratory motoneurons, the relation with $\dot{V}_I$ is the same as in figure 6-1: increases in neural output will lead to increased air flow. The next relation between $\dot{V}_I$ and tidal volume (V_T) is rather complicated: with low to moderate $\dot{V}_I$, V_T is proportional to $\dot{V}_I$ because the duration of inspiration (T_I) does not change. This is called range 1. In range 2, T_I shortens with increasing air flow (Cunningham et al., 1986) and, as a result, V_T is proportional to $\dot{V}_I^a$ with $a < 1$. Clark and von Euler (1972) found no range 1 in cats. In their study V_T was proportional to $\dot{V}_I^{0.5}$.

The inverse relationship between V_T and V_{EEL} depends on the respiratory stimulus used. In exercise V_{EEL} decreases with increasing $\dot{V}_E$, whereas V_{EEL} is nearly constant during CO_2 loading (Henke et al., 1988). A hypothesis how this difference might be caused is

Figure 6-2 Positive feedback loop containing influences of changes in $V_{EE,L}$ on inspiratory activity. + indicates positive, - negative correlation between two consecutive quantities.

also indicated in figure 6-2: increased V_T is accompanied by increased SAR activity. High SAR activity elicits expiratory activity in abdominal muscles (Bishop, 1963), resulting in lowered $V_{EE,L}$. During CO_2 loading the activity of pulmonary receptors may be reduced when PCO_2 at the receptor sites is larger than 3 kPa (Coleridge et al., 1978), and as a result, $V_{EE,L}$ may be less reduced than during exercise.

The inverse relationship between V_T and end-expiratory SAR activity has been described by Mustafa and Purves (1972). Since these authors used artificially ventilated rabbits, the changes in end-expiratory SAR activity could not have been caused by changes in $V_{EE,L}$.

Changes in $V_{EE,L}$ may be estimated from changes in P'_{tp} . They are proportional to each other, the ratio being static lung compliance. In our experiments (Chapters 3 and 4) we manipulated P'_{tp} and measured phrenic and intercostal activities, thus quantifying the remaining relations in figure 6-2. The resulting inverse relationships could be described by a mono-exponential function: $\ln(\text{Phr}_p/\text{Phr}_c) = \text{Slope} \cdot \Delta P'_{tp} + \text{Intercept}$. In this equation Phr_p is the phrenic activity following a change in P'_{tp} and Phr_c is the corresponding activity in control conditions. A similar equation described the responses for intercostal activity. The (negative) slopes were steeper for phrenic activity, while intercepts were relatively close to zero (Tables 3-1 and 3-2).

Chapter 4 describes experiments which show that the relationship between P'_{tp} and inspiratory activity is due to inhibition of this activity by SARs when P'_{tp} is increased, whereas with decreasing P'_{tp} excitation by RARs becomes more important.

The importance of the entire feedback loop for hyperpneic breathing may be estimated by comparing the results of Henke et al. (1988) obtained in humans with our results in cats, assuming that pressures are roughly comparable between species. They showed that $\dot{V}_I$ increased 3 times between rest and moderate exercise, while P_{ip} decreased by 0.2 kPa. In cats, such a decrease in P'_{ip} would have increased phrenic activity by 35% and intercostal activity by 270% (on average). This means that this feedback loop may contribute considerably to the sustenance of hyperpneic breathing.

The influence of airway CO_2 on the inspiratory off-switch mechanism

In figure 6-3 two feedbacks are shown, describing the dual relationship between inspiratory motoneuron activity and T_I and the impact of the PCO_2 at the SAR sites on one of these feedbacks. Starting again from the inspiratory motoneuron activity and going upwards, the first relation with $\dot{V}_I$ is trivial. Increasing $\dot{V}_I$ increases inspired volume (V) and P_{ip} at a certain point in time during inspiration and so is the concomitant SAR output. Increased SAR output results in shortening of T_I . This part of figure 6-3 is in fact the classical Breuer-Hering inspiration-inhibitory inflation reflex (Hering, 1868). The addition of the PCO_2 at the SAR sites to this feedback is derived from the experiments in chapter 5 and will be discussed below. The other feedback in figure 6-3 is derived from the work of von Euler and Trippenbach (1976). The hypothetical volume threshold has proved to be a very stimulating concept for research in this field. The threshold is thought to be set by the central inspiratory activity and is proportional to the inspiratory motoneuron activity. This positive feedback may explain why in long registrations of the flow pattern under constant conditions $\dot{V}_T$ is positively correlated with T_I , while the mean inspiratory flow is almost constant (Cunningham et al., 1986). Under such circumstances small breath-to-breath variations in the inspiratory threshold may cause these changes in T_I and $\dot{V}_T$. However, $\dot{V}_T$ and T_I are negatively correlated when $\dot{V}_I$ is increased—by exercise or hypercapnia. In the previous section we already discussed the mathematical relation between $\dot{V}_T$ and T_I : in cats $\dot{V}_T$ may be proportional to T_I^{-1} (Clark and von Euler, 1972). Accordingly, we found in the experiments of chapter 5 where ventilation was increased by CO_2 loading, for most of our cats (11 out of 14) only a range 2, whereas in the remaining 3 cats only a range 1 could be discerned.

The addition of the CO_2 sensitivity to the relations in figure 6-3 is new. In chapter 5 we described a series of experiments in which we compared two loading techniques: conventional CO_2 loading (CCL) in which a constant level of CO_2 is inspired and a technique in which CO_2 is injected at the start of inspiration: slug CO_2 loading (SCL). It was demonstrated that T_I was longer for CCL than for SCL at the same P_aCO_2 . This can be explained by assuming that for CCL the PCO_2 at the receptor sites is high during the entire inspiration, whereas it is low during the larger part of inspiration for SCL. Since CO_2 inhibits SAR activity (Coleridge et al., 1978), the decay of the inspiratory threshold will be slower when the PCO_2 is high and as a result T_I is increased at the same $\dot{V}_T$. When $\dot{V}_I$ is increased by exercise, PCO_2 at the receptor site will be decreased (Luijendijk, 1983), which leads to shortening of T_I and increased breathing frequency.

Figure 6-3 Negative feedback containing the inspiratory off-switch mechanism. Changing PCO_2 at the SAR sites influences T_I .

In the experiments of chapter 5 a large difference in $\dot{V}_E$ was found: the slopes of the CO_2 response curves for SCL were—on average—two times larger than the slopes for the CCL response curves. This means that also $\dot{V}_I$ is higher during SCL compared to CCL at the same $P_a\text{CO}_2$. To explain this finding we have to connect the feedback of figure 6-3 to that of figure 6-2. This is done in figure 6-4, in which all feedbacks are shown together. The inspiratory off-switch is part of feedback C in this diagram. By the box containing "PCO₂ at the SAR sites" this feedback is connected to B. At the end of inspiration PCO₂ at the SAR sites is lower and, hence, SAR activity will be higher during SCL than during CCL at the same V_T . Expiratory activity of abdominal muscles is increased when end-inspiratory SAR activity is increased (Bishop and Bachofen, 1972; Russel and Bishop, 1976). This may cause a decrease in V_{HLL} . Following the rest of loop B, this will lead to an increase in $\dot{V}_I$.

Concluding remarks

In the loops of figures 6-1 and 6-2 inspiratory motoneuron output, which is almost proportional to $\dot{V}_I$, has a central position. However, the ventilatory level is normally expressed in terms of $\dot{V}_E$. These variables are related by:

Figure 6-4 Connection between the 3 feedbacks (A,B,C). Loops have positive feedback when the number of - signs in the loop is even and negative feedback when the number is odd.

$$\dot{V}_E = V_T \cdot f = 60 \cdot \dot{V}_I \cdot T_I \cdot T_{tot}^{-1}$$

In this equation T_{tot} is the duration of one breathing cycle. Thus, $\dot{V}_E$ depends on $\dot{V}_I$ and the duty cycle (T_I/T_{tot}). In the experiments described in chapter 5, the duty cycle varied—for all cats together—between 0.4 and 0.6, whereas $\dot{V}_I$ varied between 0.020 and 0.165 l·s⁻¹. This means that flow is a more important determinant of $\dot{V}_E$ than the duty cycle is.

Since loop A of figure 6-4 is possibly of little significance, the most important vagal contribution to enhance $\dot{V}_I$ is related to the mechanism of loop B. In this light, the importance of V_{EEL} for the control of breathing during different ventilatory stimuli like exercise, hypoxia or hypercapnia may be reconsidered.

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Chapter 7

Samenvatting

Ademhalen is een bezigheid die ons allen (letterlijk en figuurlijk) na aan het hart ligt. Onze eerste ademdeug na de geboorte zorgt ervoor dat alle omstanders weer opgelucht adem kunnen halen en we gaan door met ademen tot we de laatste adem uitblazen. Tussen deze twee momenten in moet de ademhaling zich steeds aanpassen aan onze behoeften. We kunnen hard lopen, klimmen tot grote hoogte waar de lucht meer dan twee maal zo ijl is als op zee niveau, en ondertussen nog eten en praten. Dit alles vereist een zeer complex regelsysteem voor de ademhaling. Daartoe bezit het lichaam op diverse plaatsen receptoren die veranderingen in de chemische samenstelling van het bloed en b.v. informatie over het volume van de longen doorgeven aan de hersenen. Dit proefschrift houdt zich bezig met een heel klein stukje van dit complexe regelsysteem: n.l. wat is de bijdrage van de receptoren in de longen aan de regulatie van de ademhaling ?

In de volgende alinea's zal ik U eerst iets vertellen over de longen, dan zal ik uit leggen waarom de experimenten die in dit proefschrift beschreven worden gedaan zijn en de conclusies geven die daaruit getrokken zijn. Hierna mag U beoordelen of het antwoord op de hierboven genoemde vraagstelling iets dichterbij is gekomen.

De longen vormen een complex orgaan: vanaf de luchtpijp vertakt de bronchiaal-boom zich in twee hoofdbronchiën en blijft zich verder vertakken tot er zo'n 30.000 terminale bronchiolen zijn. Op elk van deze terminale bronchiolen sluit een ruimte aan, acinus genoemd, waar de uitwisseling tussen gas in de longen en gas in het bloed plaatsvindt. In de acini bevinden zich de longblaasjes, in totaal zo'n 300 miljoen voor beide longen. Het totale binnenoppervlak van de longen is zo'n 100 m^2 en het longvolume is ongeveer 8 liter bij een volwassen man na volledige inademing.

Via de longen vindt er uitwisseling plaats van zuurstof en kooldioxide tussen het bloed en de omgeving. Zuurstofmoleculen die zich in de longen bevinden gaan d.m.v. diffusie naar het bloed. Het bloed vervoert de zuurstof o.a. naar de spieren, waar het, wederom via diffusie, de spiercel inkomt. Daar wordt het gebruikt om er vetzuren en suikers mee te verbranden. Bij deze verbranding komt kooldioxide vrij en de benodigde energie voor de spierfunctie. De vrijgekomen kooldioxide diffundeert naar het bloed dat het naar de longen vervoert waar zorg wordt gedragen voor verdere afvoer uit het lichaam. In deze laatste paar zinnen komt voortdurend het woord diffusie terug. Dit is een passief proces (d.w.z. het kost geen energie), waarbij moleculen van een stof b.v. zuurstof van het ene compartiment (de longen) door een scheidingswand heen gaan naar het andere compartiment (het bloed) en in feite net zo makkelijk ook weer terug. Het proces stopt als de druk van het gas aan beide zijden van de scheidingswand gelijk is. Er is dan een evenwicht bereikt waarbij per tijdseenheid evenveel moleculen van de longenlucht naar het bloed gaan als andersom. Het zal een ieder intuïtief duidelijk zijn, dat een groot oppervlak waarover de diffusie kan plaatsvinden en een dunne scheidingswand tussen longenlucht en bloed een grote transportcapaciteit d.m.v. diffusie mogelijk maken. Dit verklaart grotendeels de ingewikkelde bouw van de longen.

Het lichaam kent verschillende systemen om de ademhaling te reguleren. Langs de slagaders in de hals en in het weefsel van de hersenstam bevinden zich receptoren die

de gasdruk van zuurstof en kooldioxide (CO_2) in het bloed meten. Het ademcentrum in de hersenen kan op basis van deze gegevens het ademhalingsniveau bijregelen. Behalve door deze receptoren kan de ademhaling ook beïnvloed worden door receptoren die zich in de longen zelf bevinden. De bijdrage van deze longreceptoren tot de regulatie van de ademhaling is het onderwerp van dit proefschrift. Er bestaan drie typen longreceptoren: de langzaam adapterende rekreceptoren, de snel adapterende rekreceptoren en C-vezels. De signalen, die deze receptoren produceren, worden door zenuwvezels in de beide vaguszenuwen getransporteerd naar het centrum voor de regulatie van de ademhaling in de hersenen. Mechanismen die van deze zenuwbaan gebruik maken kan men dan ook aanduiden als "vagale mechanismen".

Voor onze vraagstelling was het van belang een aantal variabelen, die samenhangen met de regulatie van de ademhaling, nauwkeurig te kunnen meten, terwijl de omstandigheden waaronder gemeten werd goed gecontroleerd moesten zijn. Het nauwkeurig meten van elektrische signalen van zenuwen en spieren vereist het vrijprepareren van deze structuren. Deze overwegingen samen met nog een aantal andere hebben tot de keuze geleid om alle experimenten uit te voeren met volledig genarcotiseerde proefdieren, in dit geval katten. Wij hebben geprobeerd een aantal experimenten te ontwerpen waarbij de meeste variabelen die de ademregulatie beïnvloeden constant bleven en alleen die variabelen gewijzigd werden die invloed hebben op de longreceptoren. Op deze manier konden wij met een vrijwel intact proefdier de bijdrage van de longreceptoren tot de ademregulatie bestuderen.

In hoofdstuk 2 wordt het experiment beschreven waarmee onderzocht is of de snelheid van de ademstroom die de longen ingaat een (belangrijke) stimulus is de ademhaling (verder) te doen toenemen. Het lijkt een vreemde gedachte dat als je krachtiger inademt (en dus je ademstroom vergroot) je daardoor de behoefte zou krijgen om je ademstroom nog verder te verhogen. Als we echter bedenken dat de ademregulatie nog vele andere mechanismen kent die een te hoog ademhalingsniveau juist voorkomen, dan zou een dergelijk mechanisme nuttig kunnen zijn om b.v. als we gaan hard lopen, de ademhaling net zo lang op te voeren tot een ander onderdeel van het regelsysteem gaat tegenwerken. Dit laatste is dan een remmend systeem en staat bekend als een regelsysteem met negatieve terugkoppeling, terwijl het te bestuderen systeem een positieve terugkoppeling heeft. Uit literatuuronderzoek was naar voren gekomen dat vergroting van de ademstroom tijdens een geforceerde inademing (de testteug), een sterke toeneming van de ademactiviteit veroorzaakte. Uit ons experiment blijkt echter, dat dit voornamelijk komt, omdat men deze testteugen vergeleek met de normale ademdeugen in rust. Voer je (zoals wij gedaan hebben) eerst de ademhaling op tot het niveau van de testteug en vergelijk je de ademdeugen dan met een teug waarbij geen ademstroom is (door afsluiting van de luchtpijp voor één teug), dan vind je een toeneming van slechts 8%. Tijdens normaal ademen lijkt de positieve terugkoppeling van dit mechanisme dus van weinig belang te zijn.

In hoofdstuk 3 wordt bekeken of het longvolume aan het eind van de uitademing van belang is voor de inademiingsactiviteit van de daaropvolgende teug. Dat een dergelijk systeem bestaat kunt U makkelijk bij uzelf testen: na een maximale uitademing is de volgende inademing snel en krachtig. Het is uit ander onderzoek bekend, dat tijdens inspanning een verkleining van het longvolume aan het einde van de uitademing optreedt. In de longen zitten receptoren, die gevoelig zijn voor de rekkingstoestand van de longen. Stimulatie van deze receptoren door het vergroten van het longvolume leidt tot een afnemng van de inademiingsactiviteit. Andersom bleek een geringe verkleining van het longvolume al een forse toeneming van de inademiingsactiviteit te geven. Dit mechanisme blijkt dus een zeer belangrijke bijdrage te kunnen leveren aan de stimulatie van de ademhaling tijdens inspanning.

Omdat dit mechanisme zo belangrijk bleek te zijn, besloten we verder uit te zoeken welke receptoren in de longen daarbij betrokken zijn. De resultaten hiervan worden beschreven in hoofdstuk 4. De vraagstelling is onderzocht door de zenuwvezels van de longreceptoren, die in de beide vaguszenuwen verlopen, in stapjes af te koelen. Dikke zenuwvezels stoppen met het transport van de signalen bij 10-14 °C, terwijl dunne vezels pas bij 0 °C volledig stoppen. Op grond van dit experiment en literatuur op dit gebied kon geconcludeerd worden, dat receptoren in de longen met hele dunne vezels (de zogenaamde C-vezels of J-receptoren) geen enkele rol spelen. Bij vergroting van het longvolume zijn het de langzaam adapterende rekreceptoren die voor afnemng van de inademiings-activiteit zorg dragen, terwijl bij verkleining van het longvolume de snel adapterende receptoren de belangrijkste bijdrage leveren aan toeneming van de ademactiviteit.

Na twee mechanische stimuli bestudeerd te hebben, wilden we ook één chemische stimulus bekijken en wel de invloed van kooldioxide (CO₂) in de luchtwegen op de ademhaling. De activiteit van sommige longreceptoren neemt namelijk af bij verhoging van de concentratie van CO₂ in de luchtwegen. Aangezien we zelf doorlopend CO₂ produceren en er ook andere receptoren in het lichaam zijn die gevoelig zijn voor CO₂, is dit een zeer complex experiment geworden. Als je CO₂ inademt, komt dit via diffusie ook in het bloed terecht, waar het de bestaande CO₂-concentratie en druk doet toenemen. Deze toeneming leidt via receptoren in de bloedbaan tot een verhoogde ademactiviteit. We hebben zowel de concentratie in het bloed gemeten als de hoeveelheid lucht die in- en uitgeademd wordt (= ventilatie). Als je verschillende hoeveelheden CO₂ aan de inademiingslucht toevoegt, ontstaan er ook verschillende combinaties van CO₂-concentratie in het bloed met een daarbij verkregen ventilatie. In een plaatje waarin de ventilatie uitgezet wordt als functie van de CO₂-concentratie in het bloed, kun je dan een lijn trekken door de punten: dat heet een CO₂-responsecurve. De helling van deze lijn is een maat voor de gevoeligheid van het regelsysteem van de ademhaling voor CO₂. Door nu de CO₂ op twee verschillende manieren aan te bieden, zodat de longreceptoren er een verschil in zien maar de receptoren in de bloedbaan niet, kun je bekijken of er een bijdrage van de longreceptoren is. In dat geval moet de helling van de response curve anders verlopen. In hoofdstuk 5 kunt U verschillende plaatjes zien waarin deze lijnen voor elke kat steeds duidelijk van elkaar verschillen, wat

een bewijs is voor een belangrijke bijdrage van CO₂ gevoelige longreceptoren aan de regulatie van ademhaling. Als we bedenken dat we tijdens inspanning veel meer CO₂ gaan produceren, kunnen de receptoren in de longen mogelijk een bijdrage leveren aan de toeneming van de ventilatie die dan nodig is. Hoe een dergelijk mechanisme er precies uit ziet moet nog verder onderzocht worden.

In hoofdstuk 6 wordt tenslotte een aantal schema's gepresenteerd waarin aangegeven wordt hoe de experimenten in positief en negatief teruggekoppelde regelsystemen ingepast kunnen worden en hoe deze systemen zouden kunnen samenwerken.

Na het bovenstaande gelezen te hebben zult U zich misschien afvragen: wat is nu het belang van dit onderzoek? Hoewel een zeer terechte vraag is deze niet zo makkelijk te beantwoorden. Fundamenteel onderzoek tracht feiten te verzamelen waar andere onderzoekers met een meer praktische vraagstelling op kunnen voortbouwen. Sinds enige jaren is men in staat de longen te transplanteren. De longreceptoren van de donor-longen zijn hierna niet meer verbonden met de hersenen van de ontvangende patiënt. Met de in dit proefschrift beschreven relaties zou men meer inzicht kunnen krijgen in de gevolgen van deze behandeling voor de regulatie van de ademhaling bij dit soort patiënten. Ook kan men met de schema's van hoofdstuk 6 in de hand eens proberen te verklaren waarom een patiënt(e) met hyperventilatie baat heeft bij het (tijdelijk) in- en uitademen in een plastic zakje: in feite voegt hij/zij dan nog eens extra CO₂ toe, wat tot een nog hogere ventilatie zou moeten leiden, wat juist niet gebeurt! Evengoed is het mogelijk dat de door mij vastgestelde relaties hier geen enkele rol bij spelen en is dit onderzoek voorlopig alleen van "academisch" belang. Het is echter goed te beseffen, dat het meer toegepaste onderzoek naar verloop van tijd zou opdrogen bij gebrek aan kennis over fundamentele processen.

List of Abbreviations

A_{IC}	[]	Amplification of intercostal activity
A_{PHR}	[]	Amplification of phrenic activity
CCL		Conventional CO ₂ loading
Dia	[a.u.]	Integrated muscle activity of the diaphragm
DNP	[mg·kg ⁻¹]	2,4-dinitrophenol
IC	[a.u.]	Integrated inspiratory activity of intercostal muscles
PCO ₂	[kPa]	Partial pressure of carbon dioxide
P _a CO ₂	[kPa]	Partial pressure of carbon dioxide in arterial blood
Phr	[a.u.]	Integrated efferent, phrenic nerve activity
PN ₂	[kPa]	Partial pressure of nitrogen
PO ₂	[kPa]	Partial pressure of oxygen
P _a O ₂	[kPa]	Partial pressure of oxygen in arterial blood
P _{oes}	[kPa]	Oesophageal pressure
P _{tp}	[kPa]	Transpulmonary pressure
P' _{tp}	[kPa]	End-expiratory transpulmonary pressure
P _{tr}	[kPa]	Tracheal pressure
RAR		Rapidly adapting stretch receptor
SAR		Slowly adapting stretch receptor
SCL		Slug CO ₂ loading
T _E	[s]	Duration of expiration
T _I	[s]	Duration of inspiration
T _{tot}	[s]	Total duration of a breathing cycle
T _v	[°C]	Temperature of the vagal nerves
$\dot{V}$	[ml·s ⁻¹]	Airflow
$\dot{V}_E$	[l·min ⁻¹]	Minute ventilation
V _{HEEL}	[ml]	End-expiratory lung volume
$\dot{V}_I$	[ml·s ⁻¹]	Inspiratory flow
V _T	[ml]	Tidal volume

Curriculum Vitae

De auteur van dit proefschrift werd geboren in Rotterdam op 20 december 1958. In 1977 werd met goed gevolg het gymnasium β afgesloten. Daarna studeerde hij biologie aan de Landbouw Hogeschool te Wageningen, waar hij in januari 1985 het doctoraal diploma (met lof) ontving. Daarna werkte hij drie en half jaar aan de vakgroep Medische Fysiologie van de Rijksuniversiteit te Utrecht ten laste van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO). Binnen de werkgroep "Ademhalingsfysiologie", geleid door Dr. S.C.M. Luijendijk, werden de in dit proefschrift beschreven experimenten uitgevoerd. Sinds 1 januari 1989 werkt hij aan de vakgroep Pulmonologie van de Rijksuniversiteit Limburg in het Academisch Ziekenhuis van Maastricht, waar dit proefschrift werd afgerond.

List of Publications

1. Rombout, J.H.W.M., C.P.M. van der Grinten, F.M. Peeze Binkhorst, J.J. Taverne-Thiele and H. Schooneveld. Immunocytochemical identification and localization of peptide hormones in the gastro-entero-pancreatic (GEP) endocrine system of the mouse and a stomachless fish, *Barbus conchoni*us. *Histochemistry* (1986) **84**: 471-483.
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3. Luijtelaa, E.L.J.M. van, C.P.M. van der Grinten, H.J. Blokhuis and A.L.M. Coenen. Sleep in the domestic hen (*Gallus domesticus*). *Physiology and Behaviour* (1987) **41**: 409-414
4. Grinten C.P.M. van der, W.R. de Vries, S.C.M. Luijendijk. Contribution of vagal inspiratory facilitation to efferent phrenic activity at different levels of ventilation. *Clin. Respir. Physiol.* **23** Suppl. 12 (1987) 392S
5. Grinten C.P.M. van der, W.R. de Vries, S.C.M. Luijendijk. Vagal modification of inspiratory activity during pressure breathing in cats. *Europ. Respir. J.* **2** Suppl 5 (1989) 311S
6. Meessen, N.E.L., C.P.M. van der Grinten, H.Th.M. Folgering, S.C.M. Luijendijk. Role of lung receptors in the generation of histamine-induced tonic inspiratory activity. *Europ. Respir. J.* **3** Suppl 10 (1990) 140S
7. Grinten C.P.M. van der, W.R. de Vries, S.C.M. Luijendijk. Vagal amplification of phrenic activity at different levels of ventilation in spontaneously breathing cats. *Europ. J. Appl. Physiol.* **62** (1991) 49-55
8. Grinten C.P.M. van der, W.R. de Vries, S.C.M. Luijendijk. Vagally mediated modification of inspiratory activity by changes to continuous positive and negative airway pressure in cats. (submitted)
9. Grinten C.P.M. van der, W.R. de Vries, S.C.M. Luijendijk. Reflex mechanisms and vagal afferents involved in the response of inspiratory activity to changed airway pressure in cats. (submitted)
10. Grinten C.P.M. van der, E. Schoute, W.R. de Vries, S.C.M. Luijendijk. Conventional versus slug CO₂ loading and the control of breathing in anaesthetized cats (submitted)
11. Grinten C.P.M. van der, E. Schoute, W.R. de Vries, S.C.M. Luijendijk. Conventional versus slug CO₂ loading in anaesthetized cats (abstract, submitted)
12. Grinten C.P.M. van der, S.C.M. Luijendijk. A model for the contribution of lung receptors to the control of breathing: positive and negative feed-backs (abstract,submitted)

Nawoord

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