Ventilatory stimulation by acetazolamide and medroxyprogesterone acetate

A study in cats and COPD patients

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op het gebied van de Medische Wetenschappen

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Part II

Studies in human subjects

Abbreviations

ACET	Acetazolamide
B	The apneic threshold or extrapolated end-tidal carbon dioxide tension at zero ventilation
BE	Base excess
b i d	twice a day
BMI	Body mass index
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease
DEF	Dynamic End-tidal Forcing
ERS	European Respiratory Society
FEV_1	Forced expiratory volume in one second
HCVR	Hypercapnic Ventilatory Response
HVR	Hypoxic Ventilatory Response
IVC	Inspiratory Vital Capacity
MPA	Medroxyprogesterone Acetate
O_2	Oxygen
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen tension
$P_{ET}CO_2$	End-tidal carbon dioxide tension
$P(a\text{-}_{ET})CO_2$	Arterial-to-end-tidal carbon dioxide tension gradient
$P_{0.1}$	Mouth occlusion pressure at 100 msec
pH	Negative logarithm of the effective hydrogen-ion concentration
%pred	Percentage of predicted value
Sa_{O_2}	Arterial oxygen saturation
S	Carbon dioxide sensitivity of the peripheral (Sp) and central (Sc) chemoreflex loops
sem	Standard error of the mean
SD	Standard deviation
TLC	Total lung capacity
V'_{CO_2}	Carbon dioxide excretion
V'/Q'	Ventilation-perfusion ratio
V'	Inspiratory (V'_I) and expiratory (V'_E) minute ventilation
V_T	Tidal volume

CHAPTER 1

INTRODUCTION

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1.1. Control of breathing

The main purpose of the respiratory control system is to achieve and maintain arterial blood gas homeostasis, so that the oxygen demands of the organs are met and the metabolic end-product, carbon dioxide, is produced and exhaled.

Two major groups of ventilatory stimuli can be distinguished:

1 the **feedback** stimuli, as part of the ventilatory control system, which can be divided into:

- **chemical feedback / metabolic control** which consists of the arterial and cerebrospinal fluid pH (pHa, pH_{CSF}), arterial and cerebrospinal fluid carbon dioxide tension ($PaCO_2$, $P_{CSF}CO_2$) and arterial oxygen tension (PaO_2), detected by chemoreceptors.
- **non chemical feedback stimuli**, in which *mechanical* (e.g. airway resistance (R_{aw}); compliance of the thoracic wall (C_{th}) and compliance of lung parenchyma (C_L)) and *neuronal* (e g. pulmonary stretch receptors, hypothalamus, cortex and motor neurones) stimuli are involved.

2 the **non feedback** stimuli, such as *physiological, hormonal* (e g. adrenalin, thyroxin, progesterone), *physical* (*e.g* temperature and pain) and *iatrogenic* (*e.g.* intrinsic muscle training, drug therapy) stimuli.

Both types of stimuli may interact with each other.

Figure 1 1. shows a diagram of the ventilatory control system. It can be divided into two sub-systems: the controlling system and the controlled system.

The controlling system is the sub-system with blood gas values as input parameter and minute ventilation or the neuronal equivalent as output parameter. The controlled system has the ventilation as input and blood gas values as output. This occurs in ventilated patients The output parameter of the controlling system is the input parameter of the controlled system and vice versa [1-3]

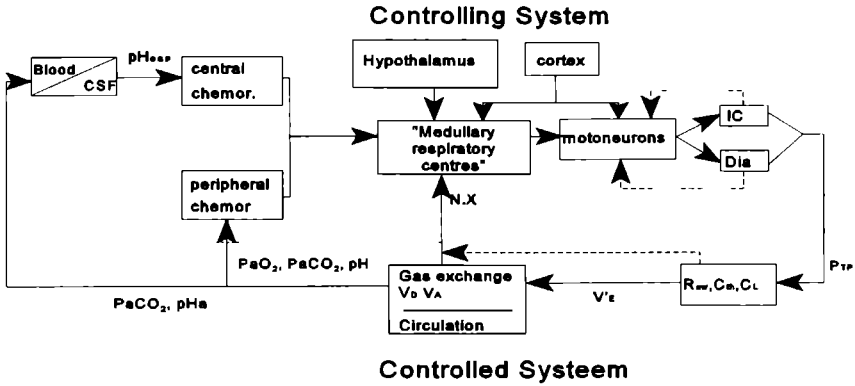

Figure 1.1. The ventilatory chemical control system (simplified).
CSF = cerebrospinal fluid; central chemor.= central chemoreceptors; peripheral chemor.= peripheral chemoreceptors; motorneurons = spinal motor neurons of the inspiratory muscles; IC = intercostal inspiratory muscles; DIA = the diaphragm; P_{TP} = transpulmonary pressure; R_{aw} = airway resistance; C_{th} = compliance of the thoracic wall; C_L = compliance of lung parenchyma; V'_E = minute ventilation ;V_D = anatomical dead space; V_A = alveolar ventilation; PaO_2 = arterial oxygen tension; $PaCO_2$ = arterial carbon dioxide tension; pHa = arterial pH; pH_{CSF}=cerebrospinal fluid pH. Above dashed line: controlling system, below: controlled system.

1.1.1. Feedback stimuli

1.1.1.1. Chemical feedback stimuli

The 'respiratory centers' are located in the medulla oblongata and pons [4] Gas exchange in the lungs will result in certain levels of $PaCO_2$, PaO_2 and pHa. The peripheral chemoreceptors, located at the carotid bifurcations (the carotid bodies) and in the aortic arch (the aortic bodies), sense the level of PaO_2, pHa and $PaCO_2$, convert them into neuronal signals, which are transmitted to the respiratory centers via carotid sinus nerves, glossopharyngeal nerves (NIX) and via vagal nerves (NX). The central chemoreceptors are located at the ventral medullary surface, including the retrotrapezoid nucleus, the region of the nucleus tractus solitarius, the region of the locus ceruleus, the rostral aspect of the ventral respiratory group and the medullary raphé [5, 6]. They are sensitive to local changes

in pH and PCO_2 in the extracellular fluid of the brain [4, 5, 7-9] In the respiratory regulation mechanism, the blood brain barrier (BBB) and the cerebral blood flow play an important role in modulating arterial blood gas stimuli for the central chemoreceptors Cerebral blood flow varies with arterial blood gas tension The BBB has a high permeability to lipid-soluble molecules (such as CO_2), whereas the ionic bicarbonate and hydrogen ions are probably impeded to pass this barrier [10] When arterial hydrogen ion (H^+-concentration) is elevated, ventilation is increased by (peripheral) chemoreceptor stimulation which leads to a fall in arterial PCO_2 As a consequence the H^+-concentration within the brain's extracellular fluid will fall

Apart from this 'chemical drive', the respiratory centers receive many other specific and non-specific input signals, such as input from hypothalamus, cortex and pulmonary stretch receptors via the vagal nerve (see below) The output of the medullary respiratory centers is transmitted to the motor neurons of the respiratory muscles (diaphragm and intercostal muscles) which are located in the spinal cord The changes in activity of these muscles will change the configuration of the thoracic wall and pressure in the thorax, resulting in a change in minute ventilation, the input stimuli of the controlled system

The performance of the ventilatory control system can be studied by measurements on the intact system, the so-called *closed loop* and in the *open loop* situation

In the *closed loop* situation, the controlled and the controlling system are functionally connected The effects of external disturbances, such as drugs, can be investigated In the *open loop* situation, the controlled system gives no input to the controlling system The chemical feedback stimuli can be manipulated in order to measure the ventilatory responses to changes in arterial carbon dioxide (CO_2), and oxygen (O_2), the so-called hypercapnic ventilatory response and hypoxic ventilatory response, respectively The level of ventilation, manipulated by the percentages of CO_2 and O_2 in the inspiratory air is measured as an output parameter of the controlling system

Graphically, the functioning of the ventilatory control system can be described by two curves (a) the hypercapnic ventilatory response that represents the controlling system The slope of the curve reflects the CO_2 sensitivity of the peripheral and central chemoreflex loops, whereas the (extrapolated) intercept with the x-axis is the apnoeic threshold and (b) the metabolic hyperbola that represents the characteristics of the controlled system, since

during mechanical ventilation, such as in paralysed patients, minute ventilation is imposed and arterial blood gases are the dependent variables Its shape is determined by the metabolic rate and CO_2 production These characteristics of the two subsystems together will inform us about the total control system The intersection of the ventilatory response to CO_2 and the metabolic hyperbola is the 'working point' of the total control system Any change in one of these subsystems will change the working point (figure 1 2)

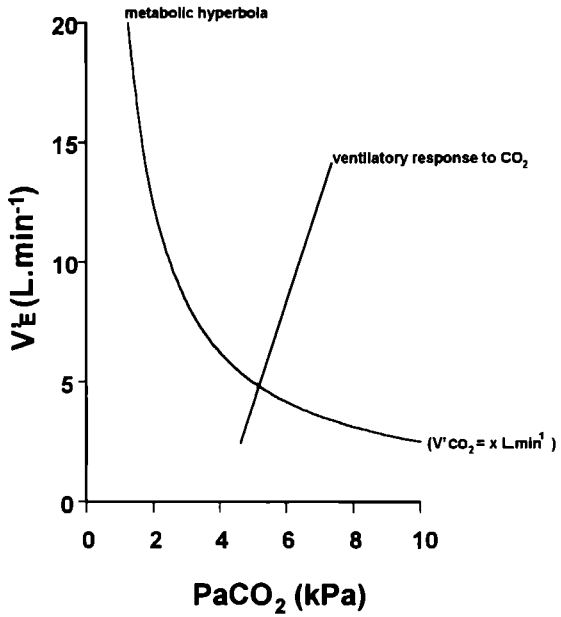

Figure 1 2 Characteristics of the ventilatory chemical control system
The controlled system is represented by the metabolic hyperbola, whose position is influenced by the metabolic rate and CO_2 -production The controlled system is represented by the ventilatory CO_2 response curve

1.1.1.2. Non-chemical feedback stimuli

The control of breathing can be influenced by several non-chemical feed back stimuli *Neuronal stimuli* influence the ventilatory control Afferent vagal activity generates non chemical feedback information to the controlling part of the ventilatory control system It also

plays a role in modulating the breathing pattern At least three types of receptors in the lung can be distinguished, which have afferent fibers in the vagal nerve stretch receptors, irritant receptors and juxtapulmonary capillary (J) receptors Stimulating the stretch receptors, during inspiration, increases afferent vagus nerve activity, which in turn stops inspiratory activity in the medullary centers This is known as the Breuer-Hering reflex [25] The irritant receptors are stimulated by specific irritants (*e g* cigarette smoke, ozone, noxious gases) and non-specific irritants (cold, fog) and induce hyperpnea, vasoconstriction and bronchoconstriction Finally, the juxtacapillary-pulmonary receptors play a role in dyspnea and tachypnea due to heart failure and pulmonary edema [25-28]

1.1.2. Non-feedback stimuli

An important stimulus to the respiratory control system is *psychological* influence, mediated via the cortex, the 'emotional brain' (limbic system) and sympathetic fibers A high activity of the emotional brain, psychological stress, together with a high sympathetic-adrenergic tone, all may contribute to the hyperventilation syndrome In this syndrome the metabolic feedback control is modified by psychological factors

Neurological syndromes such as cerebrovascular accidents, cerebral tumours, brain stem lesions etc , may result in dysregulation of ventilatory control [2, 29, 30]

The withdrawal of the 'wakefulness drive' results in unstable, periodic breathing in stage I sleep [27, 31] During the stages II, III and IV of slow wave sleep the wakefulness drive of breathing is diminished even more, resulting in a decreased ventilation and in a reduction in chemoresponsiveness The hypoventilation in non-REM sleep is associated with a small increase in $PaCO_2$ and a fall in PaO_2 During rapid eye movement (REM) sleep, the behavioural control system probably affects ventilatory control Furthermore, during REM sleep hypoventilation occurs due to the inactivity of the intercostal and accessory respiratory muscles In COPD patients hypoventilation may occur more prominently

Hormones and neurotransmitters affect the ventilatory control system Ventilation is increased by progesterone, resulting in a decrease in $PaCO_2$ probably by interaction with peripheral chemoreception and central hypothalamic interaction [11] Estrogens may facilitate this action [12] Hypothyroidism is accompanied by a ventilatory depression,

whereas hyperthyroidism increases ventilation mainly by an increase in tidal volume The thyroid hormone does not affect the slope of the ventilatory CO_2 curve, but induces a shift to the left of the curve [11, 13, 14] Examples of neurotransmitters affecting ventilation are acetylcholine, amino acids (GABA), aromatic monoamines (epinephrine, norepinephrine, dopamine) and peptides (substance-P, β-endorphines) [11, 15-24]

Physical stimuli such as pain, temperature and movement may change ventilation Pain, for example increases breathing probably without modifying the central and peripheral chemoreflex loop and the central modulation of the hypoxia-related output of the peripheral chemoreflex loop [32]

Iatrogenic stimuli such as drugs affect ventilatory control They can be divided into depressants and stimulants and may act on the chemoreception This will result in a decrease or an increase in slope and in shifts of the ventilatory CO_2 response curve and some are centrally acting drugs Anaesthetics, sedatives, alcohol and opoids are examples of depressants, acetazolamide, progesterone, almitrine bismyselate, theophylline and caffeine are stimulants (paragraph 1 5)

1.2. Measurements on ventilatory control

1.2.1. Input parameters - Blood gas values

The input parameters for the controlling system, *i e* PO_2 and PCO_2/pH (input parameters of the peripheral chemoreceptors) can be measured directly via an arterial blood gas sample, whereas the input for the central chemoreceptors, $P_{CSF}CO_2$, can be obtained by sampling CSF fluid by suboccipital or lumbar puncture However, less invasive techniques or even non-invasive techniques are desired The use of arterialized blood samples from the earlobe or fingertip (capillary samples) are accepted for estimating pH and $PaCO_2$, yet PaO_2 is not estimated correctly [33-35] End-tidal measurements of PCO_2 ($P_{ET}CO_2$) and PO_2 ($P_{ET}O_2$) can be used to approximate arterial values in healthy subjects However, in COPD patients end-tidal values of PCO_2 and PO_2 do not adequately reflect arterial values because of substantial arterial-to-end-tidal PCO_2 and PO_2 gradients Non-invasive techniques of measuring $P_{CSF}CO_2$ in humans are not described in the literature

1.2.2. Output parameters - Minute ventilation and mouth occlusion pressure

In healthy subjects, minute ventilation can be used as an output parameter of the controlling system. However, in COPD patients increased airway resistance, reduced compliance of the hyperinflated thorax and impaired function of respiratory muscles may modify the ventilation (figure 1 1.) In this situation, the ventilatory responses to chemical stimuli may be reduced, while more direct central output parameters such as $P_{0.1}$ or electromyogram (EMG) of respiratory muscles may not be affected [36, 37]. Therefore, the use of the ventilation as output parameter in COPD patients of the central respiratory control is limited.
A more direct output parameter of the controlling system in COPD patients is the mouth occlusion pressure. The mouth pressure 100 ms after onset of inspiration from an occluded mouthpiece ($P_{0.1}$) has been proposed as a more accurate index of respiratory center output. The $P_{0.1}$ is not influenced by airway resistance since there is no airflow during the occluded period [38-43]. $P_{0.1}$ is independent from changes in chemical drive during the occlusion, because there is not enough time for arterial gas tensions to change. Furthermore it is unlikely that conscious reactions will interfere with $P_{0.1}$ [38]. This method is only valid if the patient' s nervous system, spinal cord, peripheral nerves and respiratory muscles are not diseased, weakened or fatigued. Consequently, the difficulty in interpretation of the $P_{0.1}$ measurements in these patients will also depend on the function of the respiratory muscles and on the shape of the chest wall. Although these factors are often impaired in COPD patients, $P_{0.1}$ is a closer approximation of the respiratory centre output activity than minute ventilation [2, 38].

1.2.3. Ventilatory responses

1.2.3.1. Hypercapnic ventilatory response (HCVR)

The ventilatory response to CO_2 can be assessed by several methods At least three techniques are available

The Read rebreathing method

This method is based on rebreathing a 7% carbon dioxide mixture in oxygen from a small bag (4-6 litres) [44-47] With this design the open loop situation can be assessed the input parameter of the controlling system, $P_{ET}CO_2$, will increase in time while rebreathing, resulting in a change in V'_E, the output parameter of the controlling system The validity of Read's rebreathing method depends on the rapid equilibrium between alveolar gas, blood, brain and cerebrospinal fluid PCO_2 This means that a constant relationship is established between arterial PCO_2 and $P_{CSF}CO_2$ is not influenced by the level of ventilation [44, 48] In hypercapnic patients with chronic obstructive pulmonary disease (COPD) this method is less suitable, since no equilibrium will occur between $PaCO_2$ and $P_{ET}CO_2$ due to both a large dead space ventilation and an already high $PaCO_2$ Furthermore, it has been shown by Linton et al [48] and Berkenbosch et al [49] that applying the Read method in conditions of metabolic acidosis (*e g* during acetazolamide therapy) may result in a considerable overestimation of the slope of the CO_2 response curve The difference was explained by the rate of change in cerebral blood flow upon increasing carbon dioxide tension during the rebreathing and with the steady state method (see chapter 2)

The dynamic end-tidal forcing technique

The technique of dynamic end-tidal forcing (DEF) was introduced by Swanson and Bellville [50] The relative contributions of the peripheral and central chemoreceptors can be separated by a two-compartment analysis of dynamics of the ventilatory responses, using

the differences in time constants and in delay times Peripheral chemoreceptors are fast responding in contrast to the slow responding central chemoreceptors With this technique it is possible to force the end-tidal PCO_2 ($P_{ET}CO_2$) and PO_2 ($P_{ET}O_2$) to follow any desired pattern, e g step-changes, by manipulating the inspired CO_2 and O_2 concentrations with a computer on a breath by breath basis The $P_{ET}CO_2$ and $P_{ET}O_2$ can be, separately from each other, maintained at any desired level, independent of the ventilatory response and the gas tensions in the mixed venous return [51] (see chapter 2, 3 and 4) A two-compartment analysis of the ventilatory response to step changes in $P_{ET}CO_2$ allows the separation of a fast (peripheral chemoreceptors), and slow (central chemoreceptors) component [50, 52] (figure 1 3)

This technique has been validated by Berkenbosch et al [53], who artificially perfused the vertebral arteries and the ponto-medullary region in cats In this way, the contribution of the central and peripheral chemoreceptors to the total ventilation and their interaction was validated

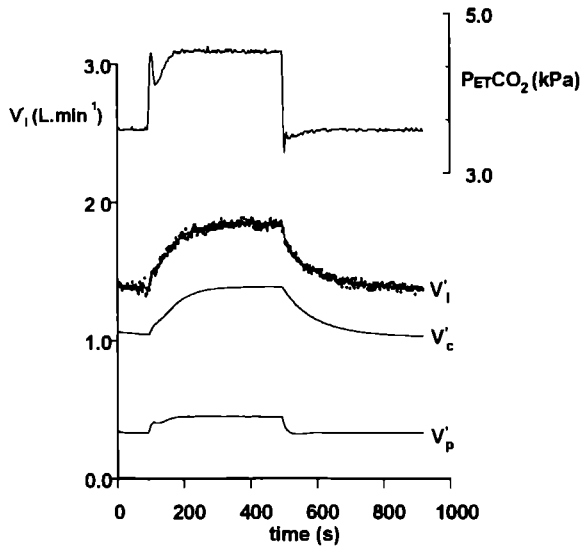

Figure 1 3 DEF-run
Example of a representative DEF run in a cat The upper part of the panel shows the $P_{ET}CO_2$ (scale right upper corner) The dots in the second trace represent breath by breath ventilation (V'_I) The curve through these data points is the model fit, the sum of the central component V'c (third trace) and the peripheral component V'p (lower trace)

The steady state method

This method is based on an elevation of alveolar PCO_2 (P_ACO_2) by breathing a gas mixture with elevated CO_2 concentrations The measurement of ventilation is made when a steady state is reached, i e after ~5-7 minutes in equilibration of alveolar and end-tidal PCO_2, and equilibrium of CO_2 between the arterial and CSF compartments With this technique no separation can be made between the sensitivities of the central and peripheral chemoreflex loops The steady state method can be used in COPD patients to obtain reliable data about the CO_2 sensitivity of the ventilatory control system (see chapter 5 and 6) [2, 48, 54, 55]

1.2.3.2. Hypoxic ventilatory response (HVR)

Assessment of ventilatory responses to *hypoxia* may be carried out using a variety of procedures When applying a step change into hypoxia, the HVR shows a biphasic response *e g* [56-58] There is an initial stimulating component arising from the peripheral chemoreceptors (*acute* response) followed by a centrally mediated hypoxic ventilatory depression The latter component probably arises from central neuronal structures, although changes in cerebral blood flow may also be involved, *e g* [59-61] It is important that HVR-responses are carried out under conditions of constant $P_{ET}CO_2$, since different levels of CO_2 influence this response at the peripheral chemoreceptor (interaction) (figure 1 4) The steady state relation between V'_I and PaO_2 is nonlinear and can be described by either hyperbolic or exponential functions, whereas a linear function can be used to describe the relation between V'_I and SaO_2 [61, 62] There is no physiologic reason to select one over the other However, with the linear response, the sensitivity S simplifies this method as only a limited number of data points is required [63]

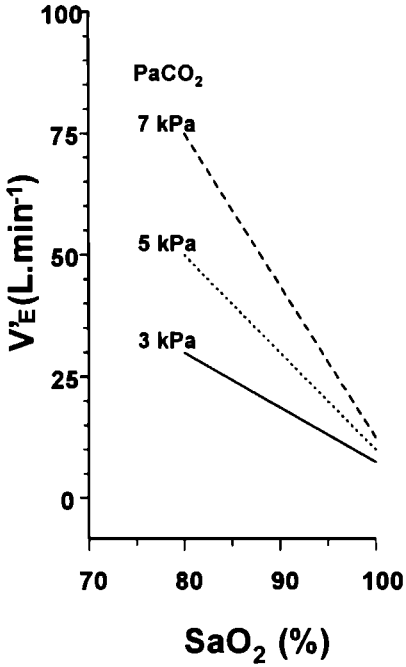

Figure 1.4. The hypoxic ventilatory response
An example of three hypoxic ventilatory responses at different levels of PCO_2.

1.3. Sleep and control of breathing

One of the non-feedback inputs for the ventilatory control system is the so-called 'wakefulness drive' Most probably this is reflected by the neural activity in the reticular activating system (RAS) combined with input from the cortex and thalamus During sleep the activity within this RAS-system is diminished, consequently the control of breathing will change (for references see Longobardo et al , 2002 [64])

The ventilatory characteristics of Non Rapid Eye Movement (NREM) sleep and Rapid Eye Movement (REM) sleep are shown in table 1 1 NREM sleep can be subdivided into (light) NREM 1 and 2 stages and the (deep) stages NREM 3 and 4 In REM sleep a 'tonic' and 'phasic' part can be discerned During NREM sleep 'metabolic' control of breathing is intact This means that the ventilation is mainly driven by the CO_2 production and the stimulation of the chemoreceptors by $PaCO_2$ Ventilatory responses to hypoxia and hypercapnia are lowered During NREM 1, which is short in duration, periodic breathing may occur Breathing becomes regular during NREM 2, 3 and 4 and during tonic REM sleep During phasic REM sleep, ventilation becomes irregular again and is probably somewhat influenced by 'behavioural mechanisms' which occur during dreaming [8, 65-70]

During REM sleep, the gamma motoneuron innervation of all muscles (except facial muscles) is virtually non-existent This gives rise to a 'gamma paralysis' of the muscles, including the intercostal and accessory muscles As the diaphragm has few muscle spindles, this muscle is hardly affected by the gamma paralysis The diaphragm is virtually the sole respiratory muscle in REM sleep

In COPD patients several studies have shown that during REM sleep hypoventilation is more prominent as compared to healthy subjects, which leads to a nocturnal deterioration of hypoxia and hypercapnia in these patients [66, 71] This increased hypoventilation is mainly caused by the inability of a flattened diaphragm to compensate the diminished intercostal and accessory respiratory muscle function and, in some patients, by a diminution of the already low hypoxic and hypercapnic ventilatory response

Sleep stages	$NREM_{1+2}$	$NREM_{3+4}$	REM (tonic)	REM (phasic)
Regulation	metabolic control	metabolic control	behavioural and metabolic control	behavioural control
Breathing	periodic, regular apnoeas possible	regular	mostly regular	irregular
Ventilatory Response to CO_2 and O_2	↓	↓	↓	↓↓
Upper airway resistance	↑	↑	↑↑	variable
Rib cage movement	↑	↑	↓	↓↓
Abdominal movement	↓	↓	↑	↑↑
minute ventilation	↓	↓↓	↓↓	variable
$PaCO_2$	↑	↑↑	↑↑	variable
PaO_2	↓	↓↓	↓↓	variable

Table 1 1 Characteristics of the NREM and REM sleep stages (Adapted from Vos PJE with permission [122])

1.4. COPD

COPD is a disease state characterized by airflow limitation that is not fully reversible The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases [72]

A subgroup of patients with severe COPD is unable to maintain their normal blood gas values, the hallmark of respiratory failure Two major types of respiratory failure can be distinguished gas exchange and ventilatory pump failure, manifested primarily by hypoxaemia and hypercapnia, respectively Generally, hypoxia can be the result of ventilation-perfusion (V'/Q') mismatch, right-left shunting, low F_IO_2 or impaired diffusion

Hypercapnia results from ventilatory pump failure, which was the selection criterion in the studied group of COPD patients in the current thesis

There are several causes of ventilatory pump failure inadequate ventilatory drive, excessive respiratory workload and inadequate inspiratory muscle function (Fig 1 5) [73-75]

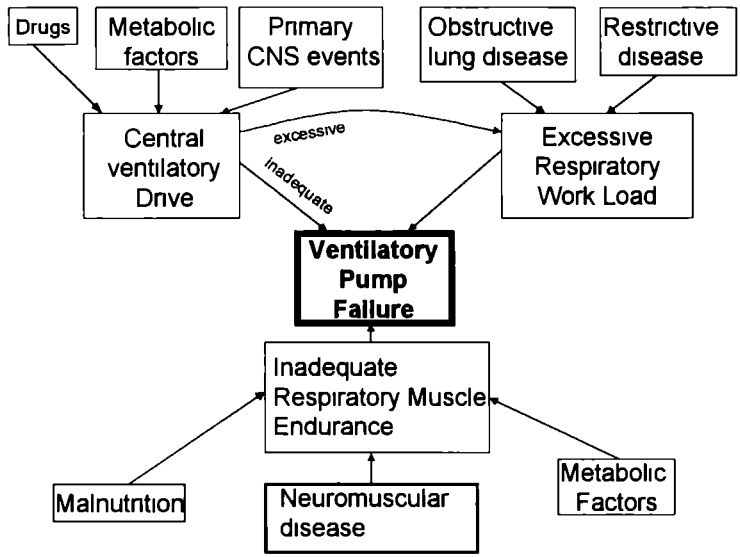

Figure 1 5 Ventilatory pump failure contributing factors (Adapted from Karpel J and Aldrich T , with permission [73])

Inadequate ventilatory drive will lead to CO_2 retention In hypercapnic COPD, an adaptation of chemoreceptors to chronic hypercapnia is postulated [76, 77]

Excessive respiratory workload due to expiratory flow limitation in patients with severe COPD can also lead to ventilatory pump failure Hyperinflation of the chest wall and airway obstruction play a major role in this situation [78, 79]

Inadequate inspiratory muscle function due to a flattening of the diaphragm is a major problem in COPD patients Furthermore, respiratory muscle function can be impaired by malnutrition and corticosteroid therapy Hyperinflation of the lungs may contribute to the excessive shortening during precontraction of the diaphragm, putting it in a unfavourable position on the length-tension curve [80]

The goals of treatment for COPD patients are to prevent symptoms of the disease and recurrent exacerbations, and to preserve optimal lung function and gas exchange In the light of the above mentioned factors that may contribute to ventilatory pump failure, treatment may aim at the correction of impaired *ventilatory drive*, reducing and/or overcoming the *respiratory load* and improve *respiratory muscle strength and endurance*

The *ventilatory drive* can be increased by respiratory stimulants This is described in paragraph 1.5.

The *respiratory load* can be decreased by drugs such as β_2-agonists, anticholinergic drugs, methylxanthines, corticosteroids, anti-oxidative agents and antibiotics and by non-invasive ventilation *e.g.*[81]. *Respiratory muscle strength and endurance* can be increased by inspiratory muscle training (TF-IMT) [82-85].

The prognosis of COPD patients is influenced adversely by hypoxia, malnutrition, lungfunction and most probably by hypercapnia. Treatment of hypoxia by long term oxygen supplementation improves survival [86-92]. However, some of the patients treated with oxygen supplementation will develop hypercapnia or existing hypercapnia may worsen [75, 93]. Some studies suggest that hypercapnia is an independent factor in determining the prognosis of COPD patients [89, 90, 92, 94]. However this is still under debate [88, 91, 95]. This validates the investigation into means of diminishing this hypercapnia.

1.5. Respiratory stimulants

1.5.1. Carbonic anhydrase inhibitors

Acetazolamide (ACET) and other carbonic anhydrase inhibitors (CAI) are potent respiratory stimulants *e.g.* [96-99]. In humans ACET inhibits hydrogen-ion ($[H^+]$) secretion by the renal tubuli resulting in a metabolic acidosis. Due to the metabolic acidosis arterial $[H^+]$ rises which is a stimulus (via peripheral chemoreceptors) to increase ventilation This will in turn cause a decrease in $PaCO_2$ and eventually an increase in cerebrospinal fluid $[H^+]$[1, 100, 101] The enzyme carbonic anhydrase is present in many tissues. Current literature has suggested that CAI will reduce cerebral blood flow, and thus contribute to CO_2 retention in the CSF and increase the central chemoreceptor stimulus Table 1.2 shows a list of organs and tissues involved in the ventilatory control that contain carbonic anhydrase. In chapter 2 and 3 the mechanism by which ACET stimulates ventilation and improves arterial blood gases will be further discussed

ORGAN/TISSUE	references
Brain astroglia choroid plexus dorsal medullary neurons hypothalamus Rostro ventrolateral medullary superficial neurons cerebral vessels	*e g* [100, 123-128]
Peripheral nervous system carotid bodies aortic bodies	*e g* [97, 129-132]
Lungs capillary endothelium alveolar epithelium	*e g* [133]
Blood erythrocytes	*e g* [100, 134]
Muscle Type 1, 2a and 2b (cytosolic) extracellular, sarcolemmar bound to Sarcoplasmatic Reticulum (SR) capillary endothelium	*e g* [135, 136]
Kidneys proximal tubuli	*e g* [100]

Table 1 2 Location and indications of physiological / functional activity of carbonic anhydrase in various tissues playing a role in the control of breathing

1.5.2. Progesterone

During the progestational phase of the menstrual cycle and during pregnancy the progesterone level is increased, which results in an alveolar hyperventilation and a fall in PCO_2 of about 1 kPa [102] Progesterone, the synthetic progesterone medroxyprogestone acetate (MPA) and its metabolites, act mainly via hypothalamic stimulating effects [11, 12, 68, 101, 103-107] Some studies report that MPA acts also via peripheral and/or central chemoreceptor pathways [11, 108] In chapter 4 the mechanism by which MPA influences the control of breathing will be discussed further

1.5.3. Other respiratory stimulants

Almitrine bismesylate causes a substantial and long-lasting increase of pulmonary ventilation by an action on the peripheral chemoreceptors In this way almitrine improves oxygen tension to a similar degree as does oxygen supplementation [109-112] Side effects, such as peripheral neuropathy, are described when higher doses are given [113] Almitrine is not registered in most European countries, including the Netherlands

Ammonium Chloride induces a metabolic acidosis [114-116] Minute ventilation is increased due to an increase in breathing frequency, suggesting that ammonium chloride acts via chemoreceptors

Doxapram is a central nervous system respiratory stimulant In low doses respiratory stimulation is mainly mediated through an effect on the peripheral chemoreceptors and on the brain stem respiratory neurons [101, 110, 117]

Methylxanthines are potent central nervous system stimulants and they have a direct effect on brainstem respiratory centers Theophylline and caffeine are used for children in the management of idiopathic apnea of prematurity [68, 118]

The rationale for prescribing theophyllines to COPD patients is primarily for their bronchodilator action [68]

Finally, *Prethcamid* is used as a respiratory stimulant Prethcamid (i v) lowers the stimulus threshold of the respiratory centers, which results in increase in tidal volume and frequency [119, 120] Brewis et al [121] reported no long-term effects of the drug and doubted whether the short term response is large enough to be clinically useful

1.6. General aims of the thesis

A subgroup of patients with severe chronic obstructive pulmonary disease (COPD) is hypercapnic and hypoxic It has been shown that long-term treatment with supplemental oxygen improves survival [86, 87] However, in some of the COPD patients hypercapnia may worsen with oxygen supplementation There are indications in current literature that hypercapnia itself may adversely influence the prognosis [89, 90, 92, 94] However, the role of hypercapnia and moreover, the effect of treatment of hypercapnia is still under debate [88, 91, 95] Nevertheless, it seems important to investigate means of diminishing hypercapnia In this thesis the effect of two respiratory stimulants, acetazolamide (ACET) and medroxyprogesterone acetate (MPA) are studied

This thesis can be divided into two parts

Part I consists of three animal studies

In **chapter 2** we describe the effects of ACET on the ventilatory CO_2 response curve in normoxic cats using the DEF technique The aims of this study were firstly, to find a dose sufficient to cause ventilatory stimulation and not causing a complete inhibition of erythrocytic carbonic anhydrase Secondly, we studied the effects of the drug on the peripheral and central chemoreflex loops

In **chapter 3** the results of administration of ACET in hypoxic cats are presented

In **chapter 4** the effects of MPA in combination with ACET in cats are presented In this study we hypothesized that a combination therapy with ACET and MPA could possibly result in a more than additive effect on the control of breathing, via both the chemoreceptor and a hormonal route

Part II consists of two human studies

In **chapter 5** the results of a double blind cross over placebo controlled study in a group of hypercapnic and hypoxic COPD patients are presented The aim of this study was to evaluate the effects of ACET and MPA as monotherapy on ventilation and gas exchanges during day and night-time

Finally, in **chapter 6** we investigated the effect of combined treatment with ACET and MPA in order to answer the question, whether there is an interaction between chemoreceptor and hormonal ventilatory drives in this group of COPD-patients

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CHAPTER 2

THE EFFECT OF LOW-DOSE ACETAZOLAMIDE ON THE VENTILATORY CO_2 RESPONSE CURVE IN THE ANAESTHETIZED CAT

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2.1. SUMMARY

1 The effect of 4 mg kg^{-1} acetazolamide (i.v.) on the slope (S) and intercept on the $PaCO_2$ axis (B) of the ventilatory CO_2 response curve of anaesthetized cats with intact or denervated carotid bodies was studied using the technique of dynamic end-tidal forcing (DEF)

2 This dose did not induce an arterial-to-end-tidal PCO_2 ($P(a\text{-}_{ET})CO_2$) gradient, indicating that erythrocytic carbonic anhydrase was not completely inhibited. Within the first 2 h after administration, the small dose caused only a slight decrease in mean standard bicarbonate of 1.8 and 1.7 mmol L^{-1} in intact (n=7) and denervated animals (n=7), respectively. Doses of acetazolamide larger than 4 mg kg^{-1} (up to 32 mg kg^{-1}) caused a significant increase in $P(a\text{-}_{ET})CO_2$ gradient

3 In carotid body denervated cats, 4 mg kg^{-1} acetazolamide caused a decrease in the CO_2 sensitivity of the central chemoreflex loop (Sc) from 1.52 ± 0.42 to 0.96 ± 0.32 L min^{-1} kPa^{-1} (mean ± S.D.), while the intercept on the $PaCO_2$ axis (B) decreased from 4.5 ± 0.5 to 4.2 ± 0.7 kPa

4 In carotid body intact animals, 4 mg kg^{-1} acetazolamide caused a decrease in the CO_2 sensitivity of the peripheral chemoreflex loop (Sp) from 0.28 ± 0.18 to 0.19 ± 0.12 L min^{-1} kPa^{-1}. Sc and B decreased from 1.52 ± 0.55 to 0.84 ± 0.21 L min^{-1} kPa^{-1} and from 4.0 ± 0.5 to 3.0 ± 0.6 kPa, respectively, not significantly different from changes encountered in the denervated animals

7 It is argued that the effects of acetazolamide on the CO_2 sensitivity of the peripheral chemoreflex loop in intact cats may be caused by a direct effect on the carotid bodies. Both in the intact and denervated animals the effect of the drug on Sc and B may not be due to a direct action on the central nervous system, but rather to an effect on cerebral vessels resulting in an altered relationship between brain blood flow and brain tissue PCO_2

2.2. INTRODUCTION

Carbonic anhydrase is present in several tissues directly or indirectly involved in the control of breathing, for example in renal tubular cells, erythrocytes, lung and brain capillary endothelium, in peripheral and possibly also central chemoreceptors [1-6] The most widely used inhibitor of the enzyme is acetazolamide [4] At doses sufficient to cause more than 99% inhibition of erythrocytic carbonic anhydrase, this drug effects a large gradient between arterial PCO_2 (determined from *in vitro* samples) and end-tidal PCO_2, and an increase in ventilation *e g* [7-9]

Clinically, acetazolamide is mostly used in too small a dose to inhibit erythrocytic carbonic anhydrase completely Usually, however, ventilation increases at these low doses, which is probably caused by an ensuing metabolic acidosis *e g* [10-13]

Several authors have studied the effect of acetazolamide on the ventilatory response curve to CO_2 by applying the Read rebreathing technique *e g* [11, 12] It has been shown, however, that applying this rebreathing technique in conditions of metabolic acidosis may result in a considerable overestimation of the CO_2 response slope [14, 15] Furthermore, neither the Read technique nor conventional steady state techniques are able to assess possible effects of drugs on the sensitivities of the peripheral and central chemoreflex loops separately from each other

In this study in anaesthetized cats we investigated the effect on the peripheral and central chemoreflex loop of a low dose of acetazolamide (4 mg kg^{-1}) not causing complete inhibition of erythrocytic carbonic anhydrase, indicated by the absence of an arterial-to-end-tidal PCO_2 ($P(a\text{-}ET)CO_2$) gradient To this aim, we applied the dynamic end-tidal forcing (DEF) technique in animals with intact carotid bodies, to assess the CO_2 sensitivity of the peripheral and central chemoreflex loops, as well as the intercept on the $PaCO_2$ axis of CO_2 response curve [16] In this study we also determined the effect of acetazolamide on the CO_2 response curve in carotid body-denervated cats The aim was to see whether applying the data thus obtained on a steady-state CO_2 mass balance of the brain [15, 17] could give us more insight into a possible mechanism of action of this drug on the central chemoreflex loop

2.3. METHODS

Animals and surgery

Fourteen adult cats (weight 4.0-5.6 kg) were sedated with 15 $mg.kg^{-1}$ ketamine hydrochloride (i.m.). Atropine sulphate (0.5 mg s.c) was given. The animals were anaesthetized by inhalation of a gas mixture containing 0.5-1% halothane and 30% O_2 in N_2 while the femoral arteries and veins were cannulated Subsequently a dose of 20 $mg\,kg^{-1}$ α-chloralose and 100 $mg.kg^{-1}$ urethane was slowly infused intravenously and the addition of halothane to the inspirate was discontinued. Anaesthesia was maintained with a continuous infusion of 1 $mg.kg^{-1}.h^{1}$ α-chloralose and 5 $mg.kg^{-1}.h^{-1}$ urethane. Rectal temperature was monitored with a thermistor and maintained within 0.5°C in the range from 36.3 to 39.3°C by a heating blanket and an infrared lamp.

The trachea was cannulated at midcervical level and connected to a respiratory circuit. In seven cats both carotid sinus nerves were identified at their junctions with the glossopharyngeal nerves and were cut. To check the effectiveness of carotid nerve section, these animals were exposed to a short hypoxic challenge. All seven cats responded with a decrease in ventilation, indicating that the peripheral chemoreceptors were functionally eliminated.

The animals were connected to an extracorporeal circuit (ECC) for continuous blood gas measurement Using the ECC, blood from the left femoral artery was pumped back via the right femoral vein with a flow of 6 $ml.min^{-1}$

Measurements

Respiratory airflow was measured with a Fleisch No 0 flow transducer (Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Statham PM197, Los Angeles, California, USA), and was electrically integrated to yield tidal volume. The CO_2 and O_2 concentrations in the tracheal gas were measured with an infrared analyser (Gould Godart MK2 Capnograph, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger O_2-test, Würzburg, Germany), respectively. The inspiratory gas concentrations were

made with mass flow controllers (type AFC 260, Advanced Semiconductor Materials, De Bilt, The Netherlands) Arterial pH and PCO_2 in the blood passing the extracorporeal circuit were measured continuously with a pH electrode (Radiometer E-5037-0, Copenhagen, Denmark), calibrated with phosphate buffers and a CO_2 electrode (General Electric A312AB, Milwaukee, Wisonscin, USA), calibrated with water equilibrated with CO_2-O_2-N_2 gas mixtures delivered by a gas mixing pump (Wosthoff, Bochum, Germany) The transport delay from the lungs to this CO_2 electrode was approximately 50 seconds The CO_2 electrode was recalibrated about every 2 hours and corrections were made for drift when necessary Arterial blood pressure was measured using a Statham pressure transducer (P23ac, Los Angeles, California, USA)

All signals were recorded on polygraphs, digitized (sample frequency 100 Hz), processed by a PDP 11/23 computer (Digital Equipment Corp , Maynard, Massachusetts, USA) and stored on disc Steady-state values of ventilation, blood pressure, end-tidal and arterial blood gas tensions were averaged over twenty breaths

Experimental protocol

Each DEF-run started with a steady-state period of ventilation of about 2 min Thereafter the $P_{ET}CO_2$ was elevated by about 1-1 5 kPa within one or two breaths, maintained at a constant level for a period of about 7 min and then lowered stepwise to the previous value and kept constant for a further 7 min (see Fig 3) The $P_{ET}O_2$ was held constant at about 15 kPa throughout all runs In this way three to five control DEF-runs were performed in each cat Subsequently in ten animals (five of seven intact and five of seven carotid body denervated cats) the effects on the $P(a\text{-}_{ET})CO_2$ gradient of low cumulative doses of acetazolamide (Diamox, Lederle) up to 4 mg kg^{-1} (i v) were determined The drug was dissolved in saline The doses, which were infused at constant end-tidal PCO_2, were 0 5, 0 5, 1 and 2 mg kg^{-1} respectively (i e 4 mg kg^{-1} in total) There was at least a 20 min pause after each dose in order to let all parameters stabilize Four animals (two of seven intact cats and two of seven carotid body denervated cats) received a bolus infusion of 4 mg kg^{-1} About 45-60 min after the animals had received 4 mg kg^{-1} of the agent, another 3-5 DEF runs (acetazolamide runs) were performed Thereafter, to three of the (seven) carotid body

denervated animals a single subsequent intravenous dose of respectively 8, 17 and 34 mg of bovine carbonic anhydrase C (Sigma, dialysed and lyophilized from bovine erythrocytes, approx 5500 Wilbur-Anderson units mg^{-1}) in saline was administered and again 3 DEF-runs were performed After completion of the acetazolamide DEF-runs the respiratory effects of additional doses (respectively 2, 2, 8, 16 and 16 mg kg^{-1}) acetazolamide were studied In five intact cats and in three animals with denervated carotid bodies we determined minute ventilation at three different steady state levels of end-tidal PCO_2 after they had received a total dose of 32 mg kg^{-1}

Data analysis

The steady state relation of ventilation (V'_I) to $P_{ET}CO_2$ at constant $P_{ET}O_2$ in the cat is linear down to the $P_{ET}CO_2$ axis as well described by DeGoede *et al* [18]

$$V'_I = (Sp+Sc)(P_{ET}CO_2 - B) \qquad (1)$$

The parameters Sc and Sp are the central and peripheral ventilatory CO_2 sensitivities and the offset B represents the apnoeic threshold or extrapolated $P_{ET}CO_2$ of the steady-state ventilatory response to CO_2 at zero ventilation

For the analysis of the breath-to-breath data obtained in the DEF-runs we used a two-compartment model [16]

$$\tau_c V'_c/dt + V'_c = Sc(P_{ET}CO_2\,(t-T_c)-Bc) \qquad (2)$$

$$\tau_p V'_p/dt + V'_p = Sp(P_{ET}CO_2\,(t-T_p)-Bp) \qquad (3)$$

$$\tau_c = \tau_{on}\,x + (1-x)\tau_{off} \qquad (4)$$

$$V'_I(t) = V'_c(t) + V'_p(t) + C\,t \qquad (5)$$

In the equations V'_c and V'_p denote the contributions of the central and peripheral chemoreceptors to the ventilation V'_I Bc and Bp are the offsets of the central and peripheral ventilatory response The time constants of the central end peripheral ventilatory responses are denoted by τ_c and τ_p T_c and T_p are the delay times needed to transport the CO_2 disturbance from the lungs to the central and peripheral chemoreceptors, respectively To model the central time constant of the ventilatory on-transient to be different from that of the off-transient, τ_c is written according to eqn (4) in which x=1 when $P_{ET}CO_2$ is high and x=0 when $P_{ET}CO_2$ is low In some experiments a small drift in ventilation was present Therefore

we included a drift term C t in the model (eqn (5)) However, the trend was usually small and in multiple DEF studies in the same cat it was positive as well as negative

We emphasize that the DEF technique can only separate the change in ventilation following a change in end-tidal CO_2 into parts belonging to the central and peripheral chemoreflex loops This is reflected in the fact that the offset parameters Bc and Bp in eqns (2) and (3), respectively, cannot be estimated individually since they are not identifiable We therefore reduce the number of parameters in the model To this end it is customary to choose the same offset parameters for both loops, viz Bc=Bp=B [19] The offset B is then equal to the extrapolated $P_{ET}CO_2$ of the steady state ventilatory response curve to zero ventilation (apnoeic threshold) As a consequence when a drug causes a change in apnoeic threshold it cannot be determined, using the DEF technique, whether the change has a central or peripheral origin Although it is not correct to call V'_c and V'_p the central and peripheral part of the ventilation due to the arbitrary choice of Bc=Bp, we usually do so for the sake of simplicity of the presentation For the steady state the two compartment model reduces to eqn (1) as it should

All the parameters of the model were estimated simultaneously using the actual $P_{ET}CO_2$ as input and by fitting the data of each DEF study with a least-squares method To obtain optimal time delays a "grid search" was applied and all combinations of Tc and Tp with increments of 1 s and with Tc> Tp were tried until a minimum in the residual sum of squares was found The minimal time delays were somewhat arbitrarily chosen to be 1 s and τ_p was constrained to be at least 0 3 s [16] For the analysis of the response of the carotid body denervated cats Sp and τ_p were set at zero, since no fast component was present

Statistical analysis

To compare the values obtained from the analysis of the DEF runs in the control situation with those after acetazolamide infusion, a two-way analysis of variance was performed, using a fixed model The level of significance was set at 0 05 Results are given as means ± S D

The design of this study and the use of cats were approved by the Ethical Committee for Animal Experiments of the Leiden University

2.4. RESULTS

In figure 1 cumulative dose-response curves of acetazolamide are shown for intact as well as denervated animals It appeared that, in a (total) dose smaller than or equal to 4 mg kg^{-1}, the drug did not induce a systematic $P(a\text{-}ET)CO_2$ gradient, indicating incomplete inhibition of erythrocytic carbonic anhydrase [4] Maximal widening of the arterial-to-end-tidal PCO_2 difference (approximately 2 5-3 kPa) was reached at a total dose of approximately 30 mg kg^{-1} in intact cats, similar to the gradient reached after a bolus infusion of 50 mg kg^{-1} [9]

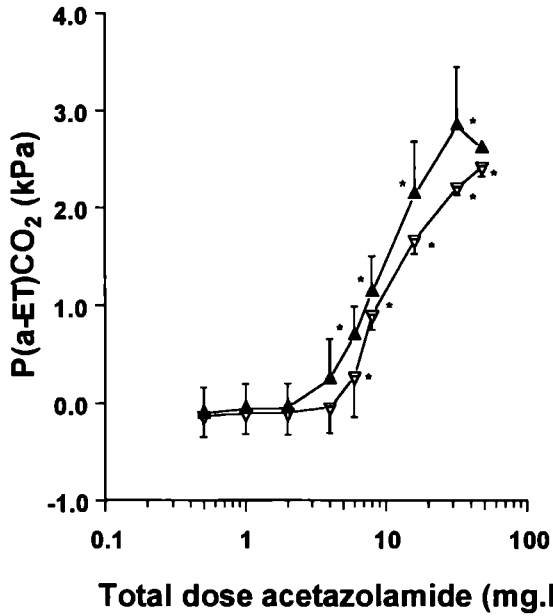

Figure 1 Dose response curve of acetazolamide
The effect of acetazolamide on the arterial-to-end-tidal PCO_2 difference ($P(a\text{-}ET)CO_2$) Mean values ± SD ▲ intact cats, n=5 at 0 5, 1, 2, 4, 6, 8, 16, and 32 mg kg^{-1}, n =1 at 48 mg kg^{-1} Δ carotid body denervated cats, n=5 at 0 5, 1, 2, an 4 mg kg^{-1}, n=3 at 6, 8, 16, 32 and 48 mg kg^{-1} * significantly different from control

Figure 2 shows a recording of the respiratory effects of 4 mg kg^{-1} acetazolamide in one of the two carotid body intact animals to which a bolus infusion of this dose was given The infusion was performed at constant end-tidal PCO_2 It shows that no arterial $P(a\text{-}ET)CO_2$ gradient was induced since arterial PCO_2 remained also virtually constant Shortly after the injection ventilation decreased and then slowly increased to a level above that existing

before drug administration. The second intact animal showed a similar response However, the two denervated animals receiving a bolus of 4 mg.kg^{-1} did not show the slow ventilatory decrease

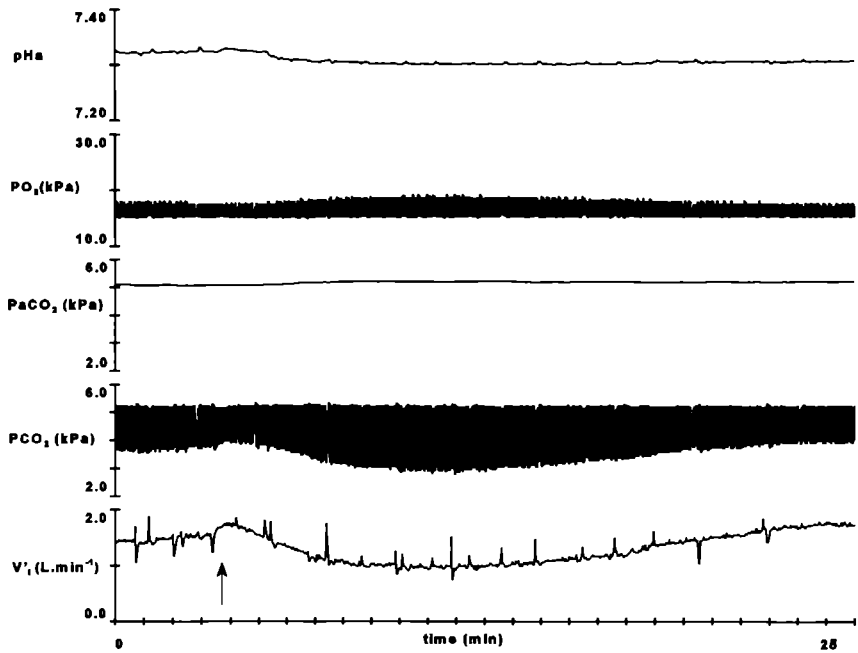

Figure 2 Respiratory effects of 4 mg kg^{-1} acetazolamide (i v.).
Intravenous infusion of 4 mg.kg^{-1} acetazolamide (arrow) at constant end-tidal PCO_2 in a cat with intact carotid bodies results in a rapid initial increase in ventilation V'_I. This initial increase is followed by a slow decrease, for which an effect of the drug on the peripheral chemoreceptors may be responsible and a gradual increase in ventilation to a level above control. Note that the arterial PCO_2 ($PaCO_2$) remains virtually constant indicating ineffective inhibition of erythrocytic carbonic anhydrase PO_2 and PCO_2 denote gas tensions in tracheal gas; pH_a is pH in arterial blood.

In Fig 3 two examples of a computer analysis of DEF runs from an intact animal are shown, performed before and after infusion of 4 mg.kg^{-1} acetazolamide. It shows that after acetazolamide administration Sp, Sc and B were decreased. A total of fifty-one DEF runs (31 control and 20 acetazolamide runs) were analysed

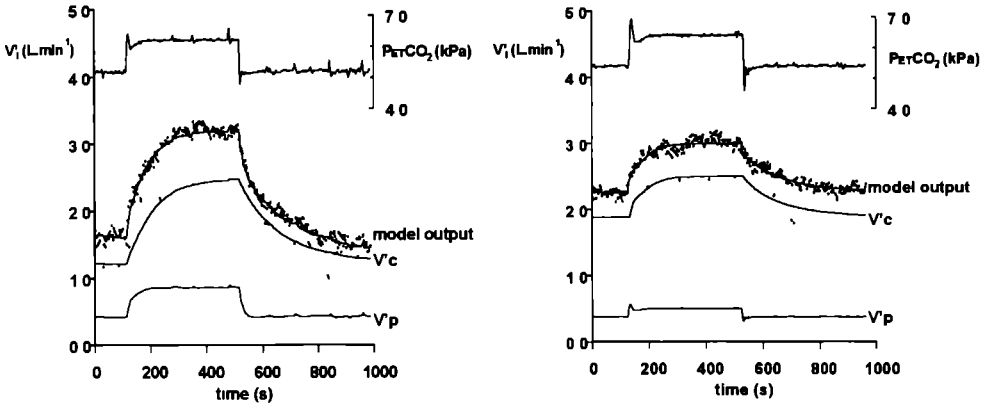

Figure 3 DEF runs before and after 4 mg kg^{-1} acetazolamide
Examples of two representative DEF runs in a carotid body intact animal before (left panel) and after (right panel) acetazolamide administration The upper part of each panel shows the $P_{ET}CO_2$ The dots represent breath-to-breath ventilation The curve through these data points is the model fit, the sum of the central component V'_c and the peripheral component V'_p and the drift (not shown separately) The values in the control run of the intercept on the $P_{ET}CO_2$ axis (B), the sensitivity of the central and peripheral chemoreflex loops Sc and Sp are 4 21 kPa, 1 24 and 0 43 L min^{-1} kPa^{-1}, respectively After acetazolamide these values decreased to levels of B, Sc and Sp of 2 27 kPa, 0 61 and 0 12 L min^{-1} kPa^{-1}, respectively

In table 1 the effects of 4 mg kg^{-1} acetazolamide on the DEF parameters in intact animals, together with those on standard bicarbonate and on the $P(a\text{-}_{ET})CO_2$ gradient are summarized The decrease in Sc, Sp and B were highly significant A small but significant effect on T_c and τ_{off} was found The significant but slight decrease in standard bicarbonate indicates that the acute renal effect of acetazolamide was mild An arterial-to-end-tidal PCO_2 difference was not detectable, indicating incomplete inhibition of erythrocytic carbonic anhydrase at this dose [4]

In table 2 the mean data obtained from twenty-six control and twenty-four acetazolamide runs of 4 mg kg^{-1} acetazolamide in the seven carotid body-denervated animals are summarized In these animals we also found a decrease in Sc and B As in the intact animals, no arterial-to-end-tidal PCO_2 gradient was found, and only a slight (insignificant) metabolic acidosis The change in mean B in the denervated animals was not significantly different from the mean change found in those with intact carotid bodies (two-tailed t-test for

independent samples, p=0 06) The same was true for the effect of acetazolamide on mean Sc in both groups (p=0 5) Infusion of respectively 8, 17 and 34 mg bovine carbonic anhydrase C (approximately 5500 Wilbur-Anderson units per mg) in three denervated animals did not change the values of Sc and B obtained after 4 mg kg^{-1} acetazolamide From data reported by Travis *et al* [8] and Maren [4] we assume that about 2 h after the intravenous infusion of 4 mg kg^{-1} acetazolamide the concentration of free, unbound, acetazolamide in plasma will be too low to completely inhibit these large quantities of infused carbonic anhydrase

Finally, in five of seven intact cats and three of seven carotid body denervated cats we determined ventilatory CO_2 sensitivity after a total dose of 32 mg kg^{-1} of the inhibitor In the denervated animals, the slope of the V'_I-$PaCO_2$ response curve decreased further to a mean value of 20 ± 7% of the control value, i e that existing before any acetazolamide infusion In the intact animals the slope decreased to a mean value of 32 ± 7% of the control value In all animals a large decrease in B was seen, corresponding to that encountered during complete inhibition of erythrocytic carbonic anhydrase induced by an infusion of 70 mg kg^{-1} benzolamide [20]

	control	acetazolamide	p
Sc (L min^{-1} kPa^{-1})	1 52 ± 0 55	0 84 ± 0 21	0001
Sp (L min^{-1} kPa^{-1})	0 28 ± 0 18	0 19 ± 0 12	0001
B (kPa)	4 0 ± 0 5	3 0 ± 0 6	0001
$P(a-ET)CO_2$ (kPa)	0 04 ± 0 31	0 17 ± 0 56	45
St bicarbonate (mmol L^{-1})	20 6 ± 0 9	18 8 ± 0 6	0003
T_p (s)	5 ± 2	5 ± 3	31
τ_p (s)	4 ± 3	6 ± 4	11
T_c (s)	8 ± 3	10 ± 4	03
τ_{on} (s)	89 ± 11	107 ± 28	24
τ_{off} (s)	141 ± 31	115 ± 20	01

Table 1 Effects of 4 mg kg^{-1} acetazolamide in cats with intact carotid bodies
Sp and Sc are the CO_2 sensitivities of the peripheral and central chemoreflex loops, with delay times T_p and T_c τ_p is the peripheral time constant and τ_{on} and τ_{off} the central on and off-transient time constants and B is the intercept on the $PaCO_2$ axis of the CO_2 ventilatory response curve Values are presented as mean of the means per cat ± S D p-values are obtained from the ANOVA on the individual data

	control	acetazolamide	p
Sc (L min^{-1} kPa^{-1})	1 52 ± 0 42	0 96 ± 0 32	0001
B (kPa)	4 5 ± 0 5	4 2 ± 0 7	0021
P(a-ET)CO_2 (kPa)	0 02 ± 0 20	-0 02 ± 0 18	60
St bicarbonate (mmol L^{-1})	19 9 ± 2 1	18 2 ± 2 5	18
T_c(s)	4 ± 1	6 ± 3	06
τ_{on}(s)	85 ± 25	116 ± 83	03
τ_{off}(s)	132 ± 31	105 ± 21	005

Table 2 Effects of 4 mg kg^{-1} acetazolamide in carotid body denervated cats
Sc is the sensitivity of the central chemoreflex loop with time constants τ_{on} and τ_{off} and delay time T_c
B is the intercept on the $PaCO_2$ axis of the ventilatory CO_2 response curve Values are presented as mean of the means per cat ± S D p-values are obtained from the ANOVA on the individual data

2.5. DISCUSSION

This study shows that acetazolamide, at doses of 4 mg kg^{-1} and below, induced no significant P(a-ET)CO_2 gradient, indicating absence of effective inhibition of erythrocytic carbonic anhydrase In dogs, such a dose causes an appreciable gradient [21], and the maximal widening of the P(a-ET)CO_2 difference is reached at 20 mg kg^{-1} [4] We found that the maximal effect in the intact cat is reached at about 30 mg kg^{-1} Red cell enzyme activity in cat is about three times higher than in dog [22] This may explain the higher doses needed to achieve inhibition in the cat

The main findings of this study are that in carotid body intact cats Sp and Sc as well as B decreased after 4 mg kg^{-1} acetazolamide In carotid body denervated cats Sc and B were decreased to about the same extent

For the interpretation of these results we start with the denervated cats in which we consider ventilation a function of brain tissue PCO_2 ($PtCO_2$) Since in these animals 4 mg kg^{-1} acetazolamide induced neither a significant decrease in standard bicarbonate nor a P(a-ET)CO_2 gradient (table 2), we conclude that the observed changes in the slope and intercept of

the CO_2 response curve (relating ventilation to $PaCO_2$) were not due to renal or erythrocytic carbonic anhydrase inhibition of the drug The infused low dose of acetazolamide, if evenly distributed, would yield a brain concentration of $1\ 8 \times 10^{-5}$ M which is insufficient to give full inhibition of local carbonic anhydrase [4] Furthermore and importantly, acetazolamide is not evenly distributed at all, and is relatively excluded from the brain, even when administered in large doses [4, 23, 24] Consequently the brain concentration reached 1-2 hours after infusion of 4 mg kg^{-1} will be very much smaller than that needed to achieve effective inhibition of CNS carbonic anhydrase We therefore reason that the observed decreases in Sc and B were not due to a direct effect on central chemoreceptors or other CNS nerve cells and ascribe these effects to a change in the relation between brain tissue PCO_2 and arterial PCO_2 To express this relationship we have previously used a steady-state mass balance equation for CO_2 for a brain compartment [20] which was originally proposed by Read & Leigh [17] and modified by Berkenbosch *et al* [15] (see eqn (A1)) Our model yields a linear relationship between $PtCO_2$ and $PaCO_2$ (see also Fig 5) The slope and intercept of this relationship depend, among other factors, on brain metabolism, on the slope of the blood CO_2 dissociation curve and on the relationship between cerebral blood flow density (Q') and $PtCO_2$ (see eqn (A3)) As reasoned above brain metabolism will not be changed Apparently the slope of the blood CO_2 dissociation curve remained also constant, since the dose of 4 mg kg^{-1} did not result in a $P(a\text{-}ET)CO_2$ gradient and since subsequent intravenous infusion of carbonic anhydrase did not further influence the effects of acetazolamide on the CO_2 response curve The effects of the drug on S and B should thus be caused by an alteration of the relationship between Q' and $PtCO_2$, resulting in a change in the $PtCO_2$-$PaCO_2$ relationship

Figure 4 shows the relationship between brain blood flow density and arterial and brain tissue PCO_2, the dashed curves show the calculated course of both hyperbolas after acetazolamide infusion if the effects of the agent on the CO_2 response curve were entirely due to an action on cerebral vessels We calculate from the slope ratio of the CO_2 response curve after and before drug infusion (0 63) that a decrease of about 50% in the "shape factor" of the hyperbola (parameter *a* in eqns (A2) and (A3)) accounts for the observed decrease in S The change in B would result from this decrease in *a*, combined with a left shift of the asymptotes of the hyperbolas (parameter *b* in the equations in the Appendix) This effect of acetazolamide on cerebral blood flow leads to a change in the $PtCO_2$-$PaCO_2$ relationship

shown in Fig 5 Figure 4 implies that the effects of acetazolamide on Q', in a dose which does not completely inhibit the erythrocytic enzyme, depends on the $PaCO_2$ at which it is given In many studies a considerable increase in Q' was reported, but in most of these large doses were used cf [25-27] This appreciable effect on Q' is probably mediated via an increase in brain tissue PCO_2 (see also Vorstrup *et al* [26]) resulting from a decrease in slope of the blood CO_2 dissociation curve [20] However, at a usual clinical dose, when given at normocapnic or slightly hypocapnic $PaCO_2$ values, acetazolamide did not increase cerebral blood flow in man (cf [28] As illustrated in Fig 4 our calculations show that in normocapnic $PaCO_2$ range, a low dose acetazolamide will have an effect on Q' which is hard to detect, thus explaining the findings of Huang *et al* [28] The effect of a low dose acetazolamide on the Q'-$PaCO_2$ and Q'-$PtCO_2$ relationship may be due to a direct action on cerebral vessels There are indeed experimental data indicating that vascular carbonic anhydrase may play an important role in the control of vascular tone by endothelial cells (for references see [29]) We also mentioned that acetazolamide has been shown to induce vasoconstriction in an *in vitro* choroid plexus preparation from the rat [30] We suggest that acetazolamide may act on a carbonic anhydrase isoenzyme located intracellularly in brain arteriolar and/or capillary endothelial cells, to which, due to its physical and chemical properties, benzolamide has no access [31] These properties also deny benzolamide access to the brain [4, 8]

An alternative explanation for the observed effects of acetazolamide on Sc and B might be that despite incomplete red cell inhibition the slope of the *in vitro* CO_2 dissociation curve was reduced by inhibition of a membrane-bound carbonic anhydrase at the luminal side of brain capillaries cf [24] Ridderstråle and Hanson [5] showed that the structure of the cat brain which was most intensely stained for carbonic anhydrase is the capillary endothelium It is reasonable to assume that this easily accessible luminal carbonic anhydrase will be inhibited by the low dose of acetazolamide This may tend to affect the CO_2 transport capacity of the capillary blood while perfusing the brain, albeit to a much lesser degree than during complete red cell inhibition However, under these circumstances, assuming an unchanged tissue PCO_2, one would expect an increase in the contribution of carboxyhaemoglobin to total CO_2 transport cf [32], thus compensating for the lesser contribution of rapidly formed bicarbonate

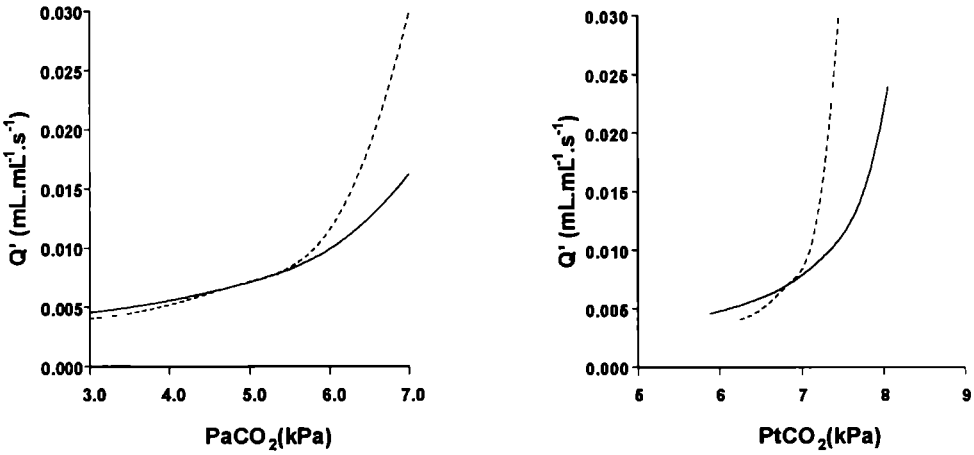

Figure 4 Cerebral blood flow density (Q') as a function of arterial or brain tissue PCO_2
Cerebral blood flow density (Q') calculated from the hyperbolic relation

$$Q' = \frac{a}{b - PCO_2}$$

in which either $PaCO_2$ (left panel) or $PtCO_2$ (right panel) can be taken as independent variable The continuous curves represent the control situation, the dashed curves that after 4 mg kg^{-1} acetazolamide For calculation of parameters *a* and *b* in both conditions see Appendix

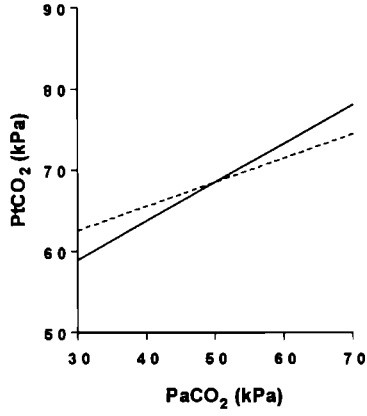

Figure 5 Relation between $PtCO_2$ and $PaCO_2$
Applying the mass balance equation for CO_2 for a brain compartment yields a linear relationship between $PtCO_2$ and $PaCO_2$ (eqn (A3)) The continuous line represents the control situation and the dashed line the one calculated after administration of 4 mg kg^{-1} acetazolamide The difference in slope and intercept between both conditions is caused by an effect on the coupling between brain blood flow and brain tissue PCO_2

This would tend to restore the slope of the *in vivo* CO_2 dissociation curve of the capillary blood towards normal, thus masking a physiological effect of inhibition of the luminal carbonic anhydrase

In our opinion, the facts that after infusion of acetazolamide a $P(a\text{-}ET)CO_2$ gradient was absent and that large quantities of intravenously infused carbonic anhydrase (probably sufficient to restore the normal contribution of rapidly formed bicarbonate) failed to reverse the decrease in Sc and B, indicate that this indeed might have been the case This led us to suggest the effect of acetazolamide on the $Q'\text{-}PaCO_2$ and $Q'\text{-}PtCO_2$ relationships as mechanism by which acetazolamide, at a low dose, may change slope and intercept of the CO_2 response curve It is obvious, however, that our explanation awaits further experimental verification

Our model predicts that if the only additional effect of a supplemental dose of about 30 mg kg^{-1} acetazolamide is to decrease the slope of the *in vivo* blood CO_2 dissociation curve to the same value as with 70 mg kg^{-1} benzolamide [20], the slope of the ventilatory CO_2 response curve should decrease to 23% of the control value (see Appendix) This corresponds closely to the observed value of 20% in the four denervated animals in which the effect of 32 mg kg^{-1} acetazolamide was studied

We cannot exclude the possibility that the effects of acetazolamide on Sc and B are mediated by an action on the central nervous system, however, for reasons mentioned above we consider this less likely Furthermore, the decrease in Sc and B developed within 30-50 min, a period too short for this effect to be mediated centrally (see [23]) We are unaware of central actions of acetazolamide occurring independently of inhibition of carbonic anhydrase

In carotid body intact cats the decrease in Sc was of about the same magnitude as in denervated animals (45 vs 38 %) as expected The decrease in mean B, in the intact animals also did not differ significantly from that observed in the denervated animals, although the effect tended to be more pronounced in the former Note that the numeric values for the parameters *a* and *b* after acetazolamide administration used in Fig 4 were calculated for denervated animals only In the intact animals, these values could have been somewhat different, thus resulting in different values for B Furthermore, in intact cats the intercept on the $PaCO_2$ axis (B) of the CO_2 response curve is also dependent on the peripheral chemoreflex loop [18] Finally, as remarked earlier, the DEF technique is not able to

separate the effect on B into a peripheral and a central part but can only separate the change in ventilation following a change in end-tidal CO_2 into parts belonging to the peripheral and central chemoreflex loops cf. [18].

If the effect of acetazolamide is mediated by a direct action on the central nervous system, the effect on the peripheral chemoreflex loop (decrease in Sp) in the intact animals is probably caused by a local action on the carotid bodies Several studies have indeed reported a decrease in baseline carotid body activity and/or sensitivity to $PaCO_2$ changes e g [33, 34] In these studies, however, high inhibitor doses were used. We believe we have indirect evidence that carotid body output may be decreased by a dose of acetazolamide as small as 4 $mg.kg^{-1}$ Figure 2 shows that, shortly after a bolus infusion of the drug, ventilation decreased and then underwent a secondary gradual increase. This was also found in the other cat (carotid body intact) receiving the bolus but not in the two denervated animals receiving the drug in this way During hypoxia, when the contribution of the carotid bodies to ventilation is relatively large, we consistently observe a considerable initial decrease in ventilation (authors' unpublished observations). When larger doses are infused during hypoxia, an initial period of apnoea ensues [9].

Acetazolamide may also act on respiratory muscles. The role of muscle carbonic anhydrase may be complicated, since isoenzymes have been identified at various muscular sites We mention cytosolic CA III in muscle cells (mainly type I), cytosolic CA I and II in muscle cells as well as in capillary endothelium, and a membrane-bound form in sarcolemma, sarcoplasmatic reticulum and capillary endothelium [35].

Scheid and Siffert [36] showed that concentrations > 10^{-4} M acetazolamide were necessary to inhibit maximal isometric force of frog skeletal muscle by 50% Barclay [37] found that exposing mouse soleus muscle to 10^{-5} M acetazolamide for 25 min did not affect isometric tension From these and other data no conclusions can be drawn as to a possible effect of low doses acetazolamide on respiratory muscles *in vivo* in the anaesthetized cat. Given the low permeability of acetazolamide and the small dose that we used, we think, however, that the observed decreases in S and B are unlikely to be mediated at muscular level Further studies are necessary to investigate this.

The present observation of a decrease in slope of the CO_2 response curve may seem to conflict with known studies in humans reporting either an increase (cf. [12, 13]) or no change in slope, but only an upward shift of the response curve (cf. [10, 11]).

Several of these studies used the Read rebreathing technique or a modification to determine CO_2 sensitivity However, chronic use of acetazolamide (as applied in these studies) by humans usually leads to a considerable metabolic acidosis, an increase in ventilation and a substantial decrease in arterial PCO_2 However, during metabolic acidosis, using conditions formulated by Read, the rebreathing technique results in a considerable overestimation of the response slope (see [14, 15]) In most studies in which a conventional steady state technique was used, end-tidal PCO_2 was taken as independent variable However, in patients suffering from lung disease a relatively large $P(a\text{-}ET)CO_2$ gradient may be present which, in addition, may be altered if lung carbonic anhydrase is inhibited [38] If sufficiently large oral doses of the drug are used to cause partial inhibition of erythrocytic carbonic anhydrase, a gradient may also be present in healthy subjects (cf [12, 13]) One should therefore preferably use arterial PCO_2 as the independent variable, combined with end-tidal data it can then be judged if an unusual arterial-to-end-tidal gradient exists It may be illustrative that in re-analysing the data of Lerche *et al* [10] by means of linear regression, using their arterial PCO_2 values (see their table 2), we found a decrease of 30% in the slope of their CO_2 response curve by acetazolamide Finally, Swenson and Hughes [13] showed that chronic and acute treatments with the drug led to different effects on the CO_2 response curve Although baseline ventilation was increased, they concluded that acute (intravenous) treatment has an inhibitory effect on the control of breathing Obviously further studies are needed to document the effect of clinical doses of acetazolamide on the control of breathing in humans, taking into consideration these methodological problems

In conclusion, the effects of low dose acetazolamide on Sc and B are probably due to an effect on cerebral vessels resulting in an altered relationship between cerebral blood flow and brain tissue PCO_2 The effect on the peripheral chemoreflex loop may be caused by a direct action on the carotid bodies

2.6. Appendix

The mass balance for CO_2 of a brain compartment in steady state can be written as [15, 17].

$$PtCO_2 = \frac{(1 - \gamma)(M' - h)}{lQ'} + PaCO_2 \qquad (A1)$$

where $PaCO_2$ and $PtCO_2$ denote the arterial PCO_2 and brain tissue PCO_2 respectively. Q' and M' are the brain blood flow density and brain metabolism density, respectively, *l* the slope of the linearized blood CO_2 dissociation curves, *h* the Haldane parameter and γ a parameter which locates $PtCO_2$ between $PaCO_2$ and the cerebral venous PCO_2 ($PvCO_2$). The cerebral blood flow density is assumed to be coupled to $PtCO_2$ in a hyperbolic fashion [20].

$$Q' = \frac{a}{(b - PtCO_2)} \qquad (A2)$$

with "shape factor" *a* and PCO_2-asymptote *b*. Substituting eqn (A2) in eqn (A1) yields a linear relation between $PtCO_2$ and $PaCO_2$

$$PtCO_2 = \frac{1}{1 + \frac{(1 - \gamma)(M' - h)}{al}} PaCO_2 + \frac{b}{1 + \frac{al}{(1 - \gamma)(M' - h)}} \qquad (A3)$$

A linear relation between $PtCO_2$ and $PaCO_2$ was indeed found experimentally [39] according to

$$PtCO_2 = \alpha PaCO_2 + \beta \qquad (A4)$$

with slope α and intercept β. Using eqns (A3) and (A4) it follows that:

$$b = \frac{\beta}{1 - \alpha} \qquad (A5)$$

We assume that in carotid body denervated animals ventilation (V'_I) is linearly related to $PtCO_2$ so that:

$$V'_I = St(PtCO_2 - Bt) \qquad (A6)$$

in which St is the CO_2 sensitivity at the site of the central chemoreceptors and Bt an offset The ventilation as function of the $PaCO_2$ is

$$V'_I = S\,(PaCO_2 - B) \qquad (A7)$$

with the slope S and the intercept B on the $PaCO_2$ axis
From eqns (A1), (A4) and (A6) it follows that the slope S is

$$S = \alpha St = \frac{1}{1 + \frac{(1 - \gamma)(M' - h)}{aI}}\, St \qquad (A8)$$

Using eqns (A4), (A5), (A6), (A7) and (A8) the intercept on the $PaCO_2$ axis can be written as

$$B = \frac{Bt - b}{\alpha} + b \qquad (A9)$$

From eqn (A8) it follows that

$$a = \frac{\alpha(1 - \gamma)(M' - h)}{I(1 - \alpha)} \qquad (A10)$$

In the control situation, were α = 0 48, γ = 0 5, M' = 8 17 x 10^{-4} ml ml^{-1} s^{-1}, h = 1 83 x 10^{-4} and I = 2 4 x 10^{-2} ml ml^{-1} kPa^{-1} [20] we calculate a value for a of 1 23 x 10^{-2} ml ml^{-1} s^{-1} kPa Since acetazolamide crosses the blood-brain barrier only very slowly we assume that under the present experimental conditions the parameters γ, M', h, and St remained constant after administration of the drug Since infusion of 4 mg kg^{-1} was not followed by a $P(a\text{-}\varepsilon T)CO_2$ gradient and since the decreases in S and B were not affected by a subsequent intravenous infusion of carbonic anhydrase, we conclude that parameter I also remained constant In this way the change in S by acetazolamide can be entirely attributed to a change in a, (cf eqn (A8)) i e to a change in the shape of the hyperbola relating Q' to $PtCO_2$ (eqn (A2)) Introducing the subscripts d for the parameters after drug administration and 'n' for the control situation, the ratio a_d/a_n can be written as

$$\frac{a_d}{a_n} = \frac{\frac{S_d}{S_n}(1 - \alpha_n)}{1 - \frac{S_d}{S_n}\alpha_n} \qquad (A11)$$

where we have used the relation:

$$\alpha_d = S_d \frac{\alpha_n}{S_n} \qquad (A12)$$

In the carotid body denervated cats we found for the ratio S_d/S_n a value of 0 63 Taking a value of 1.23 x 10^{-2} ml.ml^{-1}.s^{-1} kPa for a_n (see above) it can now be calculated that a_d= 0.057 ml.ml^{-1} s^{-1} kPa. It also follows from eqn (A8) that if α_n = 0.48, the value of α_d should be 0 30. Using eqn (A5) and (A9), taking a value of 4.45 for β_n (see Teppema *et al.* 1995 [20]) and assuming that B_t remained constant, it can be calculated that the observed change in the intercept on the $PaCO_2$ axis (B) from 4.5 to 4.2 kPa can be explained by a shift in the value of *b* (i.e. the asymptote of the Q'-$PtCO_2$ relation) from 8 6 to 7.6 kPa, together with the change in α from 0.48 to 0.30. It is thus possible to attribute the effect of 4 mg.kg^{-1} acetazolamide on the CO_2 response curve entirely to an effect of this agent on cerebral vessels.

Since acetazolamide is relatively excluded from the brain [4, 23], even an additional dose of about 30 mg.kg^{-1} will not result in effective inhibition of CNS carbonic anhydrase within a time span as short as 30 min (see also [24]). We thus assume that the only additional short-term effect of this dose will consist of a decrease in the slope *I* of the *in vivo* blood CO_2 dissociation curve, due to inhibition of erythrocytic carbonic anhydrase. In a previous study [20] we have calculated that complete inhibition of the red cell enzyme leads to a decrease in *I* from 2 4 x 10^{-2} to 6.9 x 10^{-3} ml.ml^{-1} kPa^{-1} Using eqn (A8) and taking a value for *a* of 0 057 ml.ml^{-1}.s^{-1}.kPa already existing after 4 mg.kg^{-1} acetazolamide (see above), we calculate that infusion of this large dose of the drug should result in a 77% decrease in slope of the CO_2 response curve. This corresponds closely to the observed mean decrease of 80% in slope in the four carotid body denervated animals tested.

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EFFECT OF LOW-DOSE ACETAZOLAMIDE ON THE VENTILATORY CO_2 RESPONSE DURING HYPOXIA IN THE ANAESTHETIZED CAT

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3.1. Summary

Acetazolamide, a carbonic anhydrase inhibitor, is used in patients with chronic obstructive pulmonary diseases and central sleep apnoea syndrome and in the prevention and treatment of the symptoms of acute mountain sickness. In these patients, the drug increases minute ventilation, resulting in an improvement in arterial oxygen saturation. However, the mechanism by which it stimulates ventilation is still under debate.

Since hypoxaemia is a frequently observed phenomenon in these patients, the effect of 4 $mg.kg^{-1}$ acetazolamide (i.v.) on the ventilatory response to hypercapnia during hypoxaemia (arterial oxygen tension (PaO_2) = 6.8 ± 0.8 kPa, mean ± SD) was investigated in seven anaesthetized cats The dynamic end-tidal forcing (DEF) technique was used enabling the relative contributions of the peripheral and central chemoreflex loops to the ventilatory response to a step change in end-tidal carbon dioxide tension, ($P_{ET}CO_2$) to be separated.

Acetazolamide reduced the CO_2 sensitivities of the peripheral (Sp) and central (Sc) chemoreflex loops from 0.22 ± 0.08 to 0.11 ± 0.03 $L.min^{-1}.kPa^{-1}$ (mean±SD) ($p<0.01$) and from 0.74 ± 0.32 to 0.40 ± 0.10 $L.min^{-1}.kPa^{-1}$ ($p<0.01$), respectively. The apnoeic threshold B (x-intercept of the ventilatory CO_2 response curve) decreased from 2.88 ± 0.97 to 0.95 ± 0.92 kPa ($p<0.01$) The net-result was a stimulation of ventilation at $P_{ET}CO_2$ <5 kPa.

The effect of acetazolamide is possibly due to a direct effect on the peripheral chemoreceptors as well as to an effect on the cerebral blood flow regulation. Possible clinical implications of these results are discussed.

3.2. INTRODUCTION

The carbonic anhydrase inhibitor acetazolamide stimulates ventilation, resulting in an improvement in arterial oxygen tension ($PaCO_2$) in patients with chronic obstructive pulmonary disease (COPD) or central sleep apnoea syndrome and in those suffering from acute mountain sickness [1-10] The ventilatory effect with the drug is believed to be mediated by a metabolic acidosis, induced by inhibition of renal carbonic anhydrase [11-14] However, other local effects of acetazolamide could also contribute to the observed ventilatory effects, since carbonic anhydrase is present in several tissues of the pathways involved in the control of breathing For example, the enzyme is present in the peripheral and possibly also the central chemoreceptors [15-18], erythrocytes [16] and muscles [19] and in lung- as well as brain capillary endothelium [20-22] Usually, acetazolamide is administered in doses which do not completely inhibit red cell carbonic anhydrase Complete inhibition of erythrocytic carbonic anhydrase occurs at a fractional inhibition > 99 8%, for which doses >10 mg kg^{-1} acetazolamide are required [16,23] In COPD patients, this situation would result in impeded washout of CO_2 from the lungs, leading to CO_2 accumulation in the tissues Such an undesired complication can be avoided by administering small doses, preventing an increase in the arterial to end-tidal carbon dioxide tension ($P(a_{ET})CO_2$) gradient

In a previous study in anaesthetized cats, it was found that doses up to 4 mg kg^{-1} acetazolamide (i v) did not cause a $P(a_{ET})CO_2$ gradient [24] In the same study the effect of 4 mg kg^{-1} acetazolamide on the ventilatory response to CO_2 during normoxaemia was also investigated utilizing the technique of dynamic end-tidal forcing (DEF) [25], decreases in the CO_2 sensitivities of the peripheral (Sp) and central (Sc) chemoreflex loops and in the apnoeic threshold (extrapolated carbon dioxide tension (PCO_2) at zero ventilation) were found These effects were attributed to a possible direct action of acetazolamide on the peripheral chemoreceptors and to a change in the relation between brain tissue PCO_2 ($PbtCO_2$) and arterial PCO_2 ($PaCO_2$), due to a possible effect of the drug on cerebral blood flow regulation

Since this previous study was performed during normoxia, its results may not be directly relevant to a situation of hypoxaemia, such as frequently occurs in patients with COPD During hypoxaemia, both cerebral blood flow and the relative contribution of the peripheral chemoreceptors to total ventilation are different from that in normoxaemia Therefore, the

aim of this study, in anaesthetized cats, was to investigate the acute ventilatory effects of 4 mg kg^{-1} acetazolamide on the peripheral and central chemoreflex loops on a background of moderate hypoxaemia (PaO_2 6 8 kPa)

Owing to different pharmacokinetics, the ventilatory effect of oral acetazolamide in patients with CO_2 retention may differ from that after an acute i v infusion of a low dose, as performed in the present study However, both situations are the same to the extent that the effects of the drug will be mediated independently of erythrocytic carbonic anhydrase inhibition, since in both cases the red cell enzyme will not be inhibited effectively So despite different pharmacokinetics to those after chronic oral administration, it was decided to study the effect of a low dose of acetazolamide in an acute animal preparation which was made moderately hypoxaemic, a condition which frequently occurs in the clinical situations in which the drug is used

3.3. METHODS

Animals, surgery and measurements

Seven adult cats (body weight 4 0-5 6 kg) were premedicated with 15 mg kg^{-1} ketamine hydrochloride (i m) and atropine sulphate (0 5 mg s c) Anaesthesia was induced via inhalation of a gas mixture containing 0 5-1% halothane and 30% O_2 in N_2 After cannulation of the femoral veins and arteries, an initial dose of 20 mg kg^{-1} α-chloralose and 100 mg kg^{-1} urethane was slowly infused intravenously and the addition of halothane to the inspirate was discontinued Anaesthesia was maintained with a continuous infusion of 1-1 5 mg kg^{-1} h^{-1} α-chloralose and 5 0-7 5 mg kg^{-1} h^{-1} urethane This anaesthetic regimen provides a constant level of ventilatory control [26] Rectal temperature was monitored with a thermistor, kept within 1 °C in each cat and ranged from 36 3 to 38 2 °C among the animals The trachea was cannulated and connected to a respiratory circuit One femoral artery and vein were connected to an extracorporeal circuit (ECC, flow 6 ml min^{-1}) for continuous blood gas measurement

The ventilatory responses to CO_2 were studied before and 1 hour after i v administration of 4 mg kg^{-1} acetazolamide, using the DEF technique (see below)

The end-tidal PCO_2 ($P_{ET}CO_2$) was forced stepwise, while the end tidal oxygen tension ($P_{ET}O_2$) was kept constant This was achieved by manipulating the inspired CO_2 and O_2 concentrations by feedback control with a computer. Respiratory airflow was measured with a Fleisch No. 0 flow transducer (Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Statham PM197, Statham, Los Angeles, CA, USA), and was electronically integrated to yield tidal volume. The composition of the inspirate was regulated by computer-controlled mass flow controllers (type AFC 260, Advanced Semi-conductor Materials, De Bilt, The Netherlands), using pure O_2, CO_2 and N_2 The CO_2 and O_2 concentrations in the tracheal gas were continuously measured with an infrared analyser (Gould Godart MK2 Capnograph, Gould Godart, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger O_2-test, Jaeger, Würzburg, Germany), respectively.

Arterial pH, PCO_2 and PO_2 in the blood passing through the ECC were measured continuously with a pH electrode (Radiometer E-5037-0, Radiometer, Copenhagen, Denmark), calibrated with phosphate buffers, a PCO_2 electrode (General Electric A312AB, General Electric, Milwaukee, WI, USA) and a home made Clark-type oxygen tension PO_2 electrode. The PCO_2 and PO_2-electrodes were calibrated with water equilibrated with CO_2-O_2-N_2 gas mixtures delivered by a gas mixing pump (Wösthoff, Bochum, Germany) The PCO_2 electrode was recalibrated approximately every 2 hours and corrections were made for drift when necessary. Arterial blood pressure was measured using a pressure transducer (Statham P23ac)

All signals were recorded on polygraphs, digitized (sample frequency 100 Hz), processed by a PDP 11/23 computer (Digital Equipment Corp., Maynard, MA, USA) and stored on disk. Values for ventilation, tidal volume, respiratory frequency, arterial blood pressure, end-tidal and arterial blood gas tensions ($P_{ET}CO_2$, $P_{ET}O_2$, $PaCO_2$, PaO_2) were stored on breath by breath basis.

Expenmental protocol and data analysis

Each DEF-run was started after a steady-state period of ventilation of about 2 min. Next, the $P_{ET}CO_2$ was elevated by about 1-1.5 kPa within one or two breaths, maintained constant for a period of 6 - 7 min, and then lowered stepwise to the previous value and kept constant

for a further 6 - 7 min (fig 1) The PaO_2 was kept constant at 6 8±0 8 kPa throughout all runs In each cat three to five control DEF-runs were performed After the control runs, all cats received an i v injection of 4 mg kg^{-1} acetazolamide (Diamox, AHP-Pharma, Hoofddorp, The Netherlands), dissolved in saline (2 mg ml^{-1}) $P_{ET}CO_2$ and $P_{ET}O_2$ were kept constant during infusion Sixty minutes after infusion, another three to five DEF-runs (acetazolamide runs) were performed

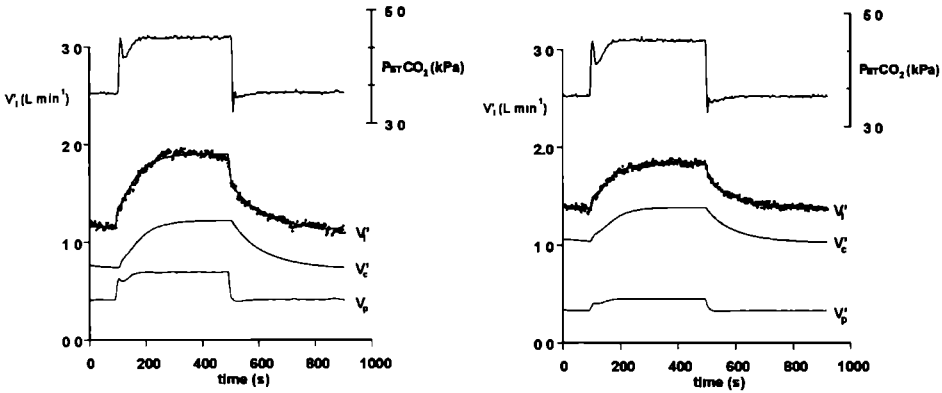

Figure 1 Dynamic End-tidal Forcing runs before (left panel)and after 4 mg kg^{-1} acetazolamide (right panel) Examples of two DEF runs and the model fits of the ventilatory responses The upper trace of each panel shows the input function of end-tidal carbon dioxide tension $P_{ET}CO_2$ The curve through the breath to breath ventilatory data points (dots) is the model fit The two lower traces show the contributions to ventilation of the central (V'c) and peripheral (V'p) chemoreflex loops, respectively, to the model output Insets Sc and Sp are the CO_2 sensitivities of the central and peripheral chemoreflex loops (L min^{-1} kPa^{-1}), respectively, and B is the extrapolated X-intercept of the ventilatory CO_2 response curve (kPa)

For the analysis of the breath-to-breath data obtained in the DEF-runs a two-compartment model was used [25]

$$\tau_c \frac{d}{dt} V'_c(t) + V'_c = Sc[P_{ET}CO_2(t-T_c)-B] \quad (1)$$

$$\tau_p \frac{d}{dt} V'_p(t) + V'_p = Sp[P_{ET}CO_2(t-T_p)-B] \quad (2)$$

$$\tau_c = \tau_{on}\, x + (1-x)\tau_{off} \quad (3)$$

$$V'_I(t) = V'_c(t) + V'_p(t) + C\,t \quad (4)$$

Equation (1) describes the ventilatory dynamics of the slow central chemoreflex loop with the contribution of the central chemoreceptors to the ventilation (V'_c), CO_2 sensitivity (Sc),

time constant (τ_c) and transport delay time (T_c) of the CO_2 change from lungs to central chemoreceptors, similarly, equation (2) describes the ventilatory dynamics of the fast peripheral chemoreflex loop with the contribution to the ventilation (V'_p), CO_2 sensitivity (Sp), time constant (τ_p) and transport delay time (T_p) The offset B (equations (1) and (2)) represents the apnoeic threshold, *i e* the extrapolated ventilatory response to $P_{ET}CO_2$ at zero ventilation Equation (3) was used to model the difference in the central time constant of the on-transient (τ_{on}) versus the off-transient (τ_{off}) When $P_{ET}CO_2$ is raised (on-transient) we use x = 1 and when $P_{ET}CO_2$ is lowered (off-transient) we use x = 0 In some experiments a small drift in the ventilation (V'_I) was present Therefore, we a drift term C t was included (equation (4)) The parameters of the model were estimated by fitting the model to the data with a least squares method A grid search was performed to obtain optimal time delays All combinations of T_c and T_p (increments of 1s and $T_c \geq T_p$) were tried until a minimum in the residual sum of squares was found The minimal and maximal time delays were, somewhat arbitrarily, chosen to be 1 s and 15 s, respectively and τ_p was constrained to be at least 0 3s

Statistical analysis

To compare the means of the values obtained from the analysis of the DEF runs in the control situation with those after acetazolamide infusion, a two-way analysis of variance (ANOVA) was performed, using a fixed model The level of significance was set at 0 05 Results are given as mean of the means ± S D

The design of this study and the use of cats were approved by the Ethical Committee for Animal Experiments of the Leiden University

3.4. RESULTS

After an initial transient decrease in minute ventilation, all cats responded with a slow increase in ventilation to an i v infusion of 4 mg.kg^{-1} acetazolamide at the prevailing $P_{ET}CO_2$ level. An example is shown in figure 2.

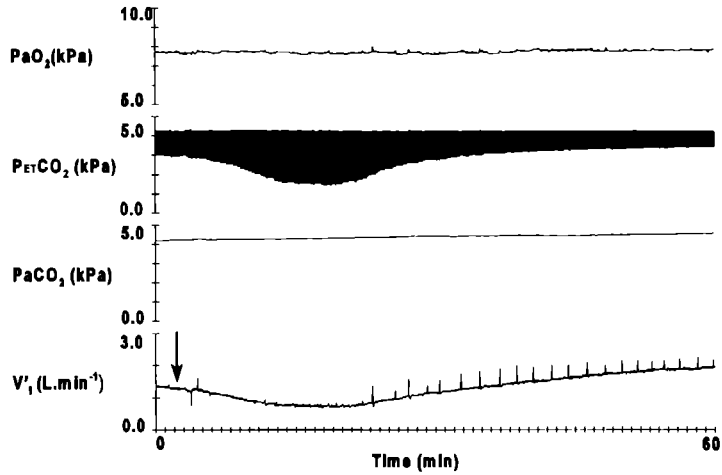

Figure 2. Acute effect of an i.v. infusion of 4 mg.kg^{-1} acetazolamide in a hypoxaemic cat (arrow). An initial decrease in ventilation (V'i) when a constant end-tidal PCO_2 was maintained, was followed by a slow secondary increase. PaO_2: arterial oxygen tension; PCO_2: carbon dioxide tension in the respiratory air; $PaCO_2$ arterial carbon dioxide tension

Thirty-one DEF-runs were performed during the control situation and 31 runs after infusion of 4 mg.kg^{-1} acetazolamide. Two examples of DEF-runs in the same cat are shown in figure 1 together with the computer analysis. one run before and one after administration of the drug. This figure illustrates that Sc and Sp were decreased after acetazolamide infusion. The effects of acetazolamide on Sc and Sp and on the apnoeic threshold of all individual cats are shown in the scatter diagrams of figure 3. As shown, in all individual cats each parameter decreased after infusion of 4 mg.kg^{-1} acetazolamide. Table 1 summarizes all of the parameters obtained by the analysis of the DEF responses before and after infusion of 4 mg.kg^{1} acetazolamide as well as the effect on standard bicarbonate (PCO_2 = 5 32 kPa, pH=7 4) and on the $P(a\text{-}_{ET})CO_2$ difference.

The apnoeic threshold B diminished significantly by about 2 kPa (from 2.88±0.97 to 0.95 ± 0.92 kPa). Sp and Sc decreased significantly to about half their control values (from 0.22 ± 0.08 to 0.11 ± 0.03 L $min^{-1}.kPa^{-1}$ and from 0.74 ± 0.32 to 0.40 ± 0.10 L.min^{-1}. kPa^{-1}, respectively). Of all remaining DEF-parameters only T_p and τ_{on} changed significantly. A small, but significant, increase in the $P(a\text{-}ET)CO_2$ gradient and a slight decrease in standard bicarbonate was observed.

As shown in figure 4, the decrease in total CO_2 sensitivity (Sp+Sc) combined with a diminished apnoeic threshold imply that the ventilatory response curves to CO_2 intersect at a $P_{ET}CO_2$ of about 5 kPa. At a $P_{ET}CO_2$ below this value acetazolamide stimulates ventilation during moderate hypoxaemia in anaesthetized cats.

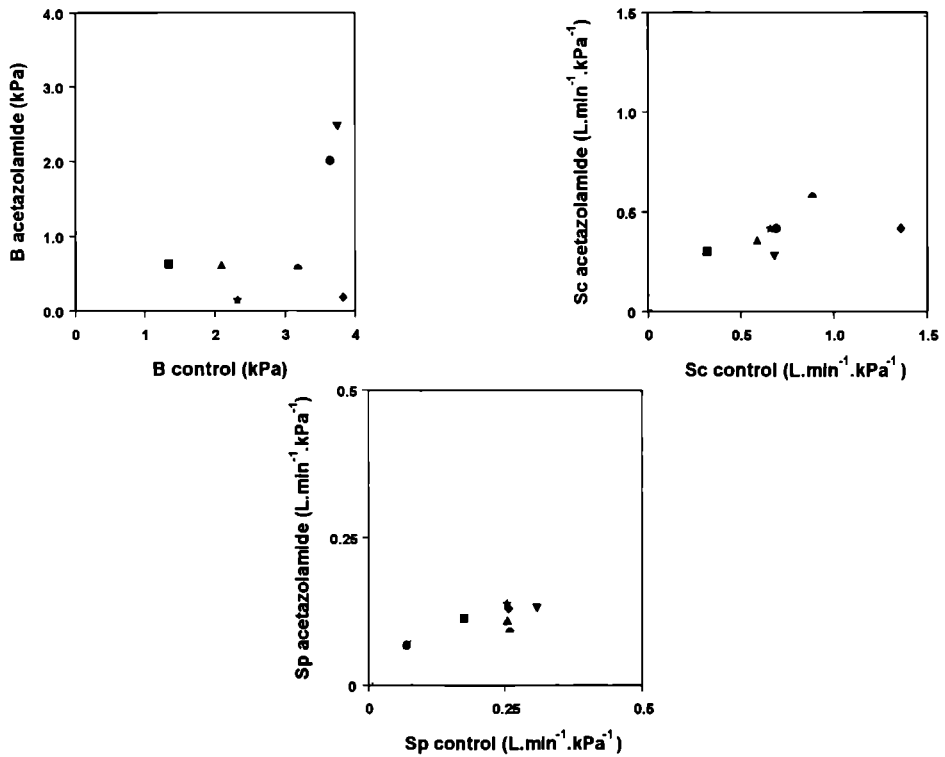

Figure 3. Scatter diagrams of the effect of 4 $mg.kg^{-1}$ acetazolamide on Sc, Sp and B.
Intravenous infusion of 4 $mg.kg^{-1}$ acetazolamide results in a decrease in the values of all parameters shown. Each individual cat is represented by a separate symbol.

PARAMETERS	CONTROL	ACETAZOLAMIDE
B (kPa)	2.88 ± 0.97	0.95 ± 0.92*
Sc ($L.min^{-1}.kPa^{-1}$)	0.74 ± 0.32	0.40 ± 0.10*
Sp ($L.min^{-1}.kPa^{-1}$)	0.22 ± 0.08	0.11 ± 0.03*
T_p (s)	4.2 ±1.1	5.6 ± 0.9*
τ_p (s)	2.6 ±1.9	2.9 ± 2.0
T_c (s)	9.9 ±2.4	9.3 ± 2.8
τ_{on} (s)	56.3 ±21.5	73.8 ± 30.9*
τ_{off} (s)	108.7 ±24.2	119.1 ± 22.4
St. Bicarbonate ($mmol.L^{-1}$)	21.89 ± 1.42	19.94 ± 1.00*
$P(a\text{-}ET)CO_2$ (kPa)	0.30 ± 0.20	0.44 ± 0.25*

Table 1. Effects of 4 $mg.kg^{-1}$ acetazolamide on the ventilatory CO_2 response curve in seven cats during hypoxaemia (PaO_2=6.8 ± 0.8 kPa). B is the X-intercept of the ventilatory CO_2 response curve Sp and Sc are the gains of the peripheral and central chemoreflex loops, with time constants τ_p and τ_{on} or τ_{off}, respectively and delay times T_p and T_c. Values are presented as mean of the means per animal ± S.D. *: significantly different from control.

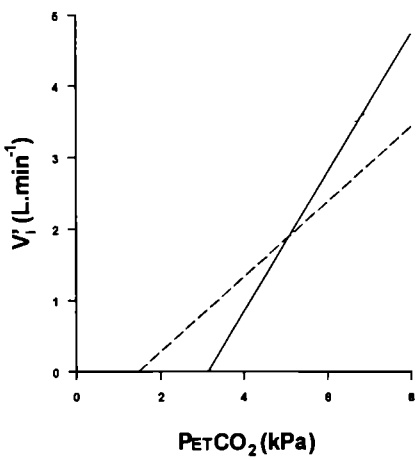

Figure 4. Effect of acetazolamide on the ventilatory CO_2 response curve.
Effect of 4 $mg.kg^{-1}$ acetazolamide on the ventilatory CO_2 response curve, calculated from the mean data in eight cats The continuous line represents the control situation and the dashed line the situation after 4 $mg.kg^{-1}$ acetazolamide. The dotted line is a hypothetical line representing the CO_2 response curve when an additional metabolic acidosis is allowed to develop as in chronic administration in humans. Note that a metabolic acidosis causes an appreciable left shift of the curve resulting in a widening of the therapeutic range in which acetazolamide induces an increase in ventilation.

3.5. DISCUSSION

In this study the effects of acetazolamide on the ventilatory CO_2 response curve were investigated in hypoxaemic cats A dose of 4 $mg.kg^{-1}$ caused an increase in the $P(a\text{-}ET)CO_2$ difference as small as 0.14 kPa, indicating marginal inhibition of erythrocytic carbonic anhydrase [11,25]. Based on this finding it was concluded that the effects of acetazolamide could be studied without the complication of significant tissue CO_2 retention.

The main results of this study are that, in hypoxaemic cats, 4 $mg.kg^{-1}$ acetazolamide causes a decrease in both the Sp and Sc and a decrease in the value of the apnoeic threshold B, resulting in a ventilatory stimulation at $P_{ET}CO_2$ levels below 5 kPa.

The decrease in Sp from 0.22 ± 0.08 to 0.11 ± 0.03 $L.min^{-1}.kPa^{-1}$ could possibly be explained by a direct effect of the drug on the carotid bodies, since they contain the enzyme carbonic anhydrase [15]. The decrease in Sp found in the present study is in agreement with our previous observation in normoxaemic cats [24]. The absolute values of Sp and Sc in this study are lower than the ones in the normoxaemic study [24]. For unknown reasons, the cats in the present study needed more supplemental anaesthesia than the previous group This made both Sp and Sc decline by the same percentage. This means that the ratio Sp/Sc is unaffected by anaesthesia as shown by van Dissel *et al.* [27].

After a bolus infusion of 4 $mg.kg^{-1}$ acetazolamide a transient initial fall in ventilation was observed (fig. 2). This may indicate that acetazolamide acts directly on the carotid bodies since, during hypoxaemia, the contribution of the peripheral chemoreceptors to the ventilation is larger than in normoxaemia. This is illustrated by the fact that in the present study the ratio Sp/Sc was 0.30, while in the previous study in normoxaemic cats - where the initial fall in ventilation induced by acetazolamide was smaller - this ratio was 0.18 [24]. In hypoxaemic cats, 50 $mg.kg^{-1}$ acetazolamide causes a short initial period of apnoea, while the ventilatory response to hypoxia is virtually abolished at this dose [28,29]. Several other studies have shown that acetazolamide at doses of 25-100 $mg.kg^{-1}$ (i.v.) causes a decrease in chemosensitivity of the carotid bodies [30,31].

The central nervous system, particularly glial cells, and possibly also the central chemoreceptors contain carbonic anhydrase [16,17,21]. Consequently, the decrease in Sc could be due to a direct effect on the central chemoreceptors, affecting the central chemoreflex loop

This is unlikely for the following reasons 1 because of its physicochemical properties, acetazolamide passes the blood brain barrier very slowly [16,18,32] and 2 to achieve the full physiological effect, more than 99% inhibition of the carbonic anhydrase is needed [16,18] Therefore, 1 hour after administration of 4 mg kg^{1} acetazolamide insufficient inhibitor will have reached the central chemoreceptors to cause effective inhibition of local carbonic anhydrase Inhibition of central nervous system carbonic anhydrase by the more lipophilic drug methazolamide results in an *increase* in Sc [33], the opposite effect to that observed in the present study

It was previously suggested that the effect of acetazolamide on Sc is probably due to an indirect effect on the central chemoreflex loop, namely by a direct effect on cerebral vessels and consequently on cerebral blood flow regulation This results in a change in the relationship between $PbtCO_2$ (which is considered as the direct stimulus to the central chemoreceptors) and $PaCO_2$ [24] This relationship was previously described by Berkenbosch *et al* [34], Read *et al* [35] and Teppema *et al* [33] and is simplified to

$$PbtCO_2 = \alpha PaCO_2 + \beta$$

Among other factors, the slope α depends on the cerebral blood flow response to changes in $PaCO_2$ The intercept β includes brain metabolism density and the slope of the linearized blood CO_2 dissociation curve, which we assumed to be constant [24]

During hypoxaemia, cerebral blood flow is increased resulting in a parallel shift of this linear relation between $PbtCO_2$ and $PaCO_2$ to lower levels of $PbtCO_2$ without a change in slope (i e no change in parameter α in the above formula) [36] So, the present finding that during hypoxaemia the decrease in Sc by acetazolamide was about equal to that observed in normoxaemic cats is not unexpected, and indicates that the effect of low-dose acetazolamide on the slope of the relation between $PbtCO_2$ and $PaCO_2$ is not influenced by the level of PaO_2 and the concomitant change in cerebral blood flow

There is no clear explanation for the fact that the decrease in the apnoeic threshold in the hypoxaemic cats of the present study was larger than in normoxaemic animals (2 vs 1 kPa) [24] The DEF technique is unable to separate the apnoeic threshold B into a peripheral and a central part As shown previously, the value of B depends on the relation between $PbtCO_2$ and $PaCO_2$ (i e parameter α in the above formula) [24], but also on Sp [37]

Comparison with human studies

In a previous study in the authors' laboratory [4] in which hypercapnic and hypoxaemic COPD patients were examined, an increase in the slope of the expiratory minute ventilation (V'_E) - $P_{ET}CO_2$ response curve was observed after administration of acetazolamide This was also found in healthy volunteers [13, 38] These results differ from the present data obtained in anaesthetized animals, since a *decrease* was found in both slope and apnoeic threshold

Apart from the use of anaesthesia [39] and from species differences [16] there are several other possible explanations for these differences

The use of $P_{ET}CO_2$ as independent variable In most human studies, ventilatory CO_2 response curves are measured using the $P_{ET}CO_2$ as the independent variable In COPD patients, however, $P_{ET}CO_2$ will not be representative of $PaCO_2$ because of the presence of a $P_{(a-ET)}CO_2$ gradient Furthermore, after acetazolamide infusion the relationship between $PaCO_2$ and $P_{ET}CO_2$ may change, due to both a higher level of ventilation and a change in ventilation/perfusion ratio's So, to find out whether the sensitivities of the chemoreceptors to changes in PCO_2 are altered, $P_{ET}CO_2$ seems to be an inappropriate independent variable Illustrative in this context may be the study of Lerche *et al* [12] who reported a parallel shift of the CO_2 response curve in healthy subjects Re-analysing their data by means of linear regression, using $PaCO_2$ as the independent variable, reveals that acetazolamide reduced the slope of the V'_E-$PaCO_2$ relationship by approximately 30%

The use of the rebreathing method for the CO_2 response In several human studies the Read rebreathing method was used to estimate ventilatory CO_2 sensitivity [1,9,13] During chronic use of acetazolamide, however, a considerable metabolic acidosis ensues, resulting in a decrease in $PaCO_2$ (and $P_{ET}CO_2$) As argued by Linton *et al* [40] and Berkenbosch *et al* [34] this may lead to a considerable overestimation of the CO_2 response slope in this situation

Acute versus chronic use of acetazolamide Acetazolamide is used in COPD-patients in a chronic setting and administered orally Swenson and Hughes [38] showed that chronic and

acute administration of acetazolamide affected the slope of the V'_E-$P_{ET}CO_2$ response curve differently chronic use resulted in an increase in slope of the hypoxaemic V'_E-$P_{ET}CO_2$ response curve, whereas acute administration of the drug had the opposite effect Therefore, acute and chronic administration of acetazolamide may have different effects on the CO_2 response slope and this may explain why acute administration in cats - as in humans - results in a decrease in slope of the hypoxaemic V'_E-$P_{ET}CO_2$ response curve

Another difference in the effect of chronic and acute administration of acetazolamide is that in the former case a metabolic acidosis ensues, while this is absent in the latter [38] This agrees with the present study in which acute administration of acetazolamide only caused a very limited metabolic acidosis

Clinically, the chronic use of acetazolamide can be extra advantageous since even in the case of a decrease in slope of the V'_E-$PaCO_2$ response curve the point of iso-ventilation will be shifted to a higher PCO_2 level Consequently, the $PaCO_2$ range in which acetazolamide increases the level of ventilation will be extended considerably (see fig 4) This is caused by a shift of the ventilatory CO_2 response curve to lower PCO_2 values induced by the ensuing metabolic acidosis

Further clinical studies are needed to document the effects of chronic use of acetazolamide in patients with chronic obstructive pulmonary disease, using arterial carbon dioxide tension as independent variable

Acknowledgement

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CHAPTER 4

MEDROXYPROGESTERONE ACETATE WITH ACETAZOLAMIDE STIMULATES BREATHING IN CATS

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4.1. SUMMARY

Both medroxyprogesterone acetate (MPA) and acetazolamide (ACET) increase ventilation Combined administration of these agents could result in an additional improvement of blood gases, for example in patients with chronic obstructive pulmonary diseases The aim of this study in anaesthetized female (ovariohysterectomized, pre-treated with 17-β-estradiol) cats was to compare the effects on the CO_2 response curve of MPA alone (4 μg kg^{-1}, i v) with those after MPA followed by ACET (4 mg kg^{-1} ,i v) We performed dynamic end-tidal CO_2 forcing and analysed the data with a two-compartment model comprising a fast peripheral and slow central compartment, characterized by CO_2 sensitivities (Sp and Sc, respectively) and a single offset (the apnoeic threshold B) MPA reduced Sp from 0 22 ± 0 09 (mean ± S D) to 0 13 ± 0 06 L min^{-1} kPa^{-1} ($p<0$ 01) and Sc from 1 01 ± 0 38 to 0 88 ± 0 32 L min^{-1} kPa^{-1} ($p<0$ 01) B decreased from 4 02 ± 0 27 to 3 64 ± 0 42 kPa (P<0 01) Subsequent administration of ACET reduced Sp and Sc further to 0 09 ± 0 06 and to 0 70 ± 0 49 L min^{-1} kPa^{-1} ($p<0$ 01), respectively The apnoeic threshold decreased further to 2 46 ± 1 50 kPa ($p<0$ 01) Because both treatments reduced ventilatory CO_2 sensitivity, we conclude that a simulating effect on ventilation is due to a decrease in the apnoeic threshold Combined administration of MPA and ACET may lead to larger increases in ventilation than treatment with either drugs alone

4.2. INTRODUCTION

During human pregnancy and the luteal phase of the menstrual cycle ventilation is increased, for which the high plasma level of progesterone may be responsible (for review see Dempsey *et al*,1986 [1]) Progesterone, or the synthetic progesterones medroxyprogesterone acetate (MPA) and chlormadinone acetate are sometimes used in hypoxic and hypercapnic patients with severe chronic obstructive pulmonary disease to stimulate ventilation and improve blood gas values [2-4] In man, progesterone may increase ventilation by an effect on the hypothalamus and/or structures in the medulla oblongata *e g* [1, 2, 5-7] A possible action on the peripheral chemoreceptors is indicated by the finding that during human pregnancy the hypoxic ventilatory response is increased [8] Progesterone may also influence the tone of upper airway muscles in patients with obstructive sleep apnea, MPA was found to reduce the frequency of upper airway obstructions [9, 10] Data from animal studies also indicate that progesterone may stimulate breathing by an effect on peripheral and/or central sites [11-13] Guinea pigs, dogs and cats but not rats, goats, ponies and cows respond with an increased hypercapnic ventilatory response to progesterone administration [1] Failure to show this response may be related to species or gender, or may be due to specific experimental circumstances such as level of arousal, pre-treatment with estradiol and dose [1, 14, 15] An increase in hypoxic sensitivity by progesterone was reported in cat and rat [13, 16] A second agent used to improve blood gases in some hypercapnic and hypoxic patients with chronic obstructive pulmonary disease is the carbonic anhydrase inhibitor acetazolamide (ACET) *e g* [3, 4] It is generally believed that this beneficial effect of ACET is due to a metabolic acidosis - induced increase in ventilatory drive However, since carbonic anhydrase is present in many tissues and cells involved in the control of breathing, the respiratory effects of ACET may be much more complicated For example, in a previous study in anaesthetized cats we showed that low-dose ACET (4 mg kg^{-1}) causes a decrease in both the slope and X-intercept of the CO_2 response curve [17] A large dose of the agent (50 mg kg^{-1}) totally abolishes the hypoxic ventilatory response [18, 19] Although MPA and ACET may act via different mechanisms, it is possible that (part of) their respiratory effects are due to an action on common structures, for example carotid bodies It would be interesting, therefore, to compare the effects of a combined application with those of single treatments, and this was the aim of this study In ovariohysterectomized, lightly anaesthetized female cats pre-treated with estradiol, we

measured the effects of MPA and those of a subsequent administration of acetazolamide on the slope and intercept of the CO_2 response curve. This enabled us to compare the effects of combined MPA + ACET administration with those of a single treatment with ACET as documented in a previous study [17]. To be able to separate the effects of MPA and ACET on the peripheral and central chemoreflex loops, we applied the dynamic end-tidal forcing technique and analysed the ventilatory data with a two-compartment model, comprising a fast peripheral and slow central component [20].

4.3. METHODS

Animals, surgery and measurements

The present experiments were performed in eight female cats (body weight 3.4–4.1 kg). The use of the animals was approved by the Ethical Committee for Animal Experiments of the Leiden University Medical Center. An ovariohysterectomy was performed at least 1 month prior to the experiments The animals were pre-medicated with 10 $\mu g.kg^{-1}$ 17-β-estradiol (E2) (Sigma-Aldrich, Bornem, Belgium), dissolved in sesame oil (100 $\mu g.ml^{-1}$), twice daily subcutaneously during 3 days immediately prior to the study [11]. Plasma concentration of 17-β-estradiol in seven cats was estimated by a radioimmunoassay (RIA) method with extraction using anti-estradiol antibody-coated tubes (Coat-A-Count TKE; Diagnostic Products, Los Angeles, CA, USA) [21] For the RIA of 17-β-estradiol, the limit of quantitation was 2 $pg.ml^{-1}$ and the intra- and interassay coefficients of variation were 8 and 10%, respectively. On the day of the experiment the animals were pre-medicated with 15 $mg\ kg^{-1}$ ketamine hydrochloride (i.m.). Anaesthesia was induced by inhalation of a gas mixture containing 0.5–1% halothane and 30% O_2 in N_2 . After cannulation of the femoral veins and arteries, an initial dose of 20 $mg.kg^{-1}$ α-chloralose and 100 $mg.kg^{-1}$ urethane was slowly infused intravenously and the addition of halothane to the inspirate was discontinued. Anaesthesia was maintained with a continuous infusion of 1–1.5 $mg.kg^{-1}\ h^{-1}$ α-chloralose and 5.0–7.5 $mg.kg^{-1}.h^{-1}$ urethane. Comparison of our studies with those of others in awake cats shows that this anaesthetic regimen has little effect on the ventilatory response to CO_2,

and does not yield systemic changes of the ventilatory parameters in time [22] Rectal temperature was monitored with a thermistor and ranged from 36 1 to 38 3°C among animals The trachea was cannulated and connected to a respiratory circuit One femoral artery and vein were connected to an extracorporeal circuit (ECC, flow 6 ml min^{-1}) for continuous blood gas measurements Respiratory airflow was measured with a Fleisch No 0 flow transducer (Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Statham PM197, Los Angeles, CA USA), and was electronically integrated to yield tidal volume The composition of the inspirate was regulated by computer-steered mass flow controllers (type AFC 260, Advanced Semi-conductor Materials, De Bilt, The Netherlands), using pure O_2, CO_2 and N_2 The CO_2 and O_2 concentrations in the tracheal gas were continuously measured with an infrared analyser (Gould Godart MK2 Capnograph, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger O_2-test, Wurzburg, Germany), respectively Arterial pH, PCO_2 and PO_2 in the blood passing through the ECC were measured continuously with a pH electrode (Radiometer E-5037-0, Copenhagen, Denmark), calibrated with phosphate buffers, a PCO_2 electrode (General Electric A312AB, Milwaukee, Wisonscin, USA) and a home made Clark-type PO_2 electrode The PCO_2 and PO_2 electrodes were calibrated with water equilibrated with CO_2 – O_2 – N_2 gas mixtures delivered by a gas-mixing pump (Wosthoff Bochum, Germany) The PCO_2 electrode was recalibrated approximately every 2 h and corrections were made for drift when necessary Arterial blood pressure was measured using a pressure transducer (Statham P23ac, Los Angeles, CA, USA) All signals were recorded on polygraphs, digitized (sample frequency 100 Hz), processed by a PDP 11/23 computer (Digital Equipment Corp , Maynard, MA, USA) and stored on disc Values of ventilation, tidal volume, breathing frequency, arterial blood pressure, end-tidal and arterial blood gas tensions ($P_{ET}CO_2$, $P_{ET}O_2$, $PaCO_2$ PaO_2) were stored on a breath-by-breath basis

Experimental protocol

The ventilatory responses to CO_2 were studied using the dynamic end-tidal forcing technique (DEF) [20] This technique was developed to force the end-tidal PCO_2 ($P_{ET}CO_2$) and PO_2 ($P_{ET}O_2$) to follow a specific dynamic pattern by manipulating the inspired CO_2 and O_2 concentrations, performed automatically by feedback control with a computer

The $P_{ET}CO_2$ and $P_{ET}O_2$ can be adjusted to desired values independent of the ventilatory response and of the gas tensions in the mixed venous return [20]. Each DEF-run started with a steady-state period of ventilation of about 2 min. Next, the $P_{ET}CO_2$ was elevated stepwise by about 1–1.5 kPa within one or two breaths, maintained constant for a period of 6–7 min, and then lowered stepwise to the previous value and kept constant for a further 6–7 min (Fig. 1). The $P_{ET}O_2$ was kept constant at about 15 kPa throughout all runs. In each cat three to five control DEF-runs were performed. After the control runs, 4 $\mu g.kg^{-1}$ medroxyprogesterone acetate (MPA) (Sigma-Aldrich, Bornem, Belgium), dissolved in 9.6% ethanol (0.4 $ml.kg^{-1}$) was administered intravenously. About 15 min after administration of the drug, another three to five DEF runs were performed. Thereafter, 4 $mg.kg^{-1}$ acetazolamide (ACET; Diamox, AHP Pharma, Hoofddorp, The Netherlands) in saline (2 $mg.ml^{-1}$) was given intravenously. After a stabilisation period of about 45 min, three to five final DEF runs were performed.

Data analysis

The steady-state relation of ventilation (V'_I) to $P_{ET}CO_2$ at constant $P_{ET}O_2$ in the cat is linear down to the $P_{ET}CO_2$ axis:

$$V'_I = (Sp+Sc)(P_{ET}CO_2 - B) \quad (1)$$

The parameters Sc and Sp are the CO_2 sensitivities of the peripheral and central chemoreflex loops, respectively and the offset B represents the apnoeic threshold or extrapolated $P_{ET}CO_2$ at zero ventilation.

For the analysis of the dynamic ventilatory response, we used a two-compartment model [20]

$$\tau_c V'_c/dt + V'_c = Sc(P_{ET}CO_2\,(t-T_c)-Bc) \quad (2)$$

$$\tau_p V'_p/dt + V'_p = Sp(P_{ET}CO_2\,(t-T_p)-Bp) \quad (3)$$

$$\tau_c = \tau_{on}.x + (1-x)\tau_{off} \quad (4)$$

$$V'_I(t) = V'_c(t) + V'_p(t) + C.t \quad (5)$$

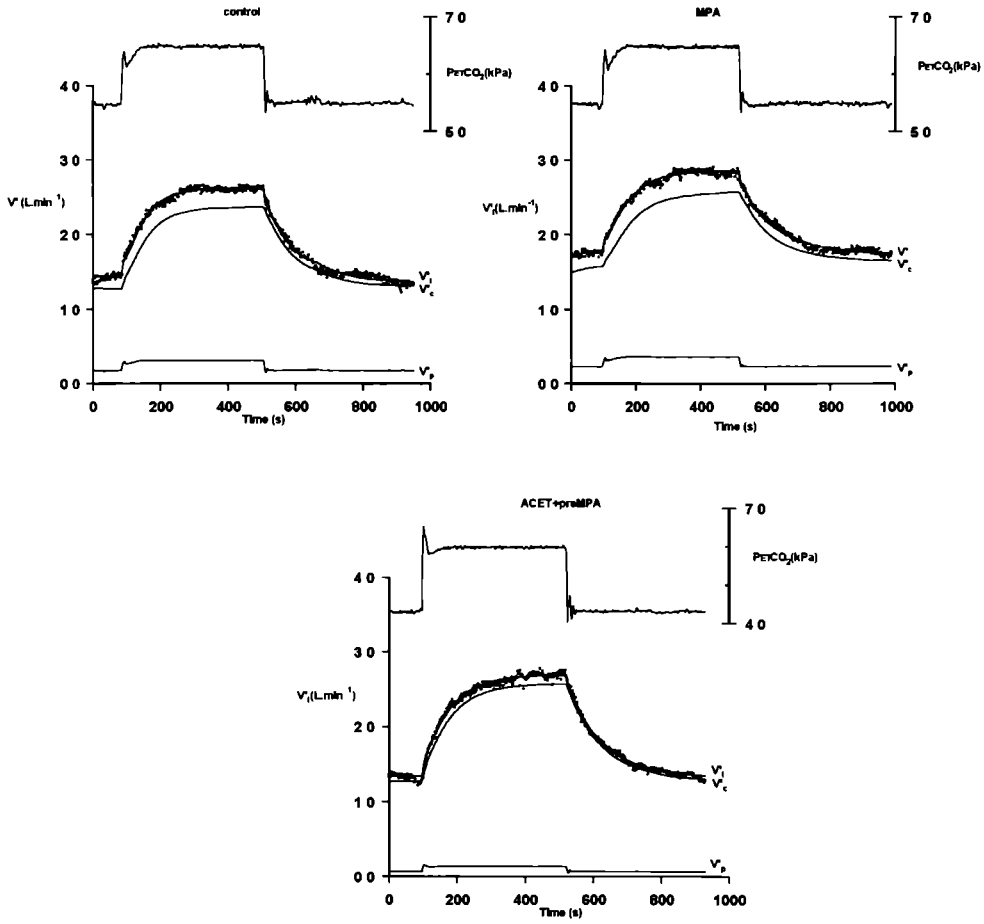

Fig 1 Examples of three DEF runs and the model fits of the ventilatory responses control situation, after MPA and after additional ACET administration

The upper trace shows the input function of $P_{ET}CO_2$ The curve through the breath-by-breath ventilatory data points (dots) is the model fit The two lower traces show the contributions of the central (V'_c) and peripheral (V'_p) chemoreflex loops, respectively, to the model output (V'_I)

Eq (2) describes the dynamics of the slow central chemoreflex loop with the central CO_2 sensitivity Sc, time constant τ_c and transport delay time to the central chemoreceptors Tc , similarly Eq (3) describes the dynamics of the fast peripheral chemoreflex loop with the peripheral CO_2 sensitivity Sp , time constant τ_p and delay time Tp The offset B represents the apnoeic threshold or extrapolated $P_{ET}CO_2$ at zero ventilation To model the central time constant of the on-transient to be different from the off-transient, we used Eq (4) When $P_{ET}CO_2$ is high (on-transient) we take x=1 and when $P_{ET}CO_2$ is low (off-transient) we take x=0 In most experiments a small drift in the ventilation was present Therefore, we included a drift term C t, as can be seen in Eq (5) The parameters of the model were estimated simultaneously using the actual $P_{ET}CO_2$ as input and by fitting the data with a least squares method To obtain optimal time delays, we performed a "grid search" All combinations of Tc and Tp (increments of 1 sec and $Tc \geq Tp$) were tried until a minimum in the residual sum of squares was found The minimal time delays were, somewhat arbitrarily, chosen to be 1 sec and τ_p was constrained to be at least 0 3s [20]

Statistical analysis

To compare the values of the parameters obtained in the three different experimental conditions with each other, ANOVA was performed, using a fixed model The level of significance was set at 0 02 (with Bonferroni correction) To compare the combined therapy (MPA+ACET) with the results of single ACET administration from our previous study [17], the Rayn–Einot–Gabriel–Welsh multiple range test was performed (significance at $p<0\ 05$) Unless otherwise indicated, results are given as means ± S D

4.4. RESULTS

One month after ovariohysterectomy in seven of eight cats, the mean plasma concentration of (E2) was 6 92 ± 1 7 pg ml^{-1} (see also [16]) After E2 -priming, the E2 concentration in these cats increased to a mean of 567 2 ± 396 4 pg ml^{-1} (in one animal, E2 concentrations were not determined) Thirty-seven DEF runs were performed during the control situation, 33 after MPA administration and 24 after additional infusion of ACET In Fig 1 examples of three DEF-runs in one animal are shown, each performed under these three different experimental conditions In Table 1 the effects of 4 µg kg^{-1} MPA and 4 mg kg^{-1} ACET on all relevant parameters are summarized After treatment with MPA, the mean CO_2-sensitivity of the peripheral chemoreflex loop (Sp) in eight cats decreased significantly from 0 22 ± 0 09 to 0 13 ± 0 06 L min^{-1} kPa^{-1} , while that of the central chemoreflex loop (Sc) was reduced from 1 01 ± 0 30 to 0 88 ± 0 32 L min^{-1} kPa^{-1} The mean apnoeic threshold B decreased from 4 02 ± 0 27 to 3 64 ± 0 42 kPa

Parameter	control (a)	MPA (b)	MPA+ACET (c)	p-value (a-b)	(b-c)	(a-c)
B (kPa)	4 02 ± 0 27	3 64 ± 0 42	2 46 ± 1 50	0 0001	0 0001	0 0001
Stot (L min^{-1} kPa^{-1})	1 23 ± 0 39	1 02 ± 0 33	0 78 ± 0 51	0 0001	0 0001	0 0001
Sc (L min^{-1} kPa^{-1})	1 01 ± 0 38	0 88 ± 0 32	0 70 ± 0 49	0 0001	0 0001	0 0001
Sp (L min^{-1} kPa^{-1})	0 22 ± 0 09	0 13 ± 0 06	0 09 ± 0 06	0 0001	0 0248	0 0001
St bicarbonate (mmol L^{-1})	20 61 ± 2 98	21 11 ± 2 55	20 44 ± 3 91	0 2000	0 0003	0 0056

Table 1 Effects of 4 µg kg^{-1} MPA and additional infusion of 4 mg kg^{-1} acetazolamide on the ventilatory CO_2 response curve in eight cats
Sc and Sp are the CO_2-sensitivities of the central and peripheral chemoreflex loops B is the intercept on the $P_{ET}CO_2$ -axis of the CO_2 ventilatory response curve Values are presented as mean of the means per cat ± S D The p-values are obtained from the ANOVA on all individual data

The effects of MPA in each individual animal are shown in the scatter diagrams of Fig 2 After ACET administration, the mean Sp and Sc were further reduced to 0 09 ± 0 06 and to 0 70 ± 0 49 L min^{-1} kPa^{-1}, respectively The mean apnoeic threshold B decreased further to 2 46 ± 1 50 kPa

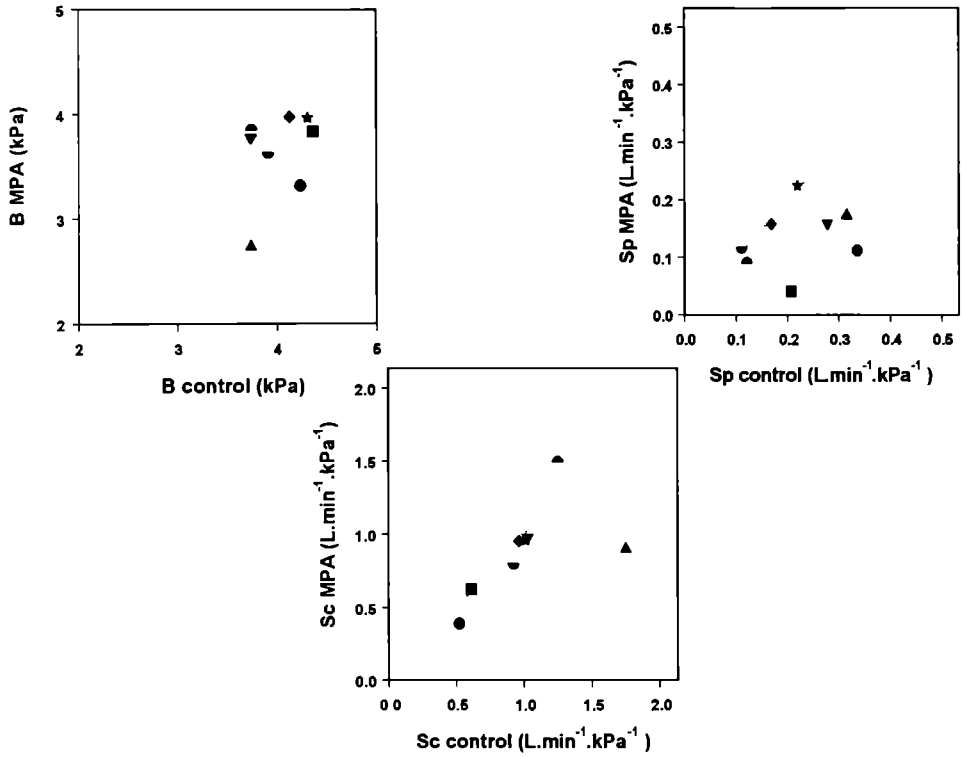

Fig. 2. Scatter diagrams of the effect on 4 µg.kg^{-1} MPA on the ventilatory parameters
Intravenous infusion of 4 µg.kg^{-1} MPA results in a decrease in the values of the parameters shown. Each individual cat is represented by a separate symbol. Sc and Sp are the CO_2 sensitivities of the central and peripheral chemoreflex loops (L.min^{-1}. kPa^{-1}), respectively, and B is the x-intercept of the ventilatory $P_{ET}CO_2$ response curve (kPa).

Fig. 3 shows the effects of ACET in each individual animal. For comparison, in Table 2 we also show the effects of a treatment with 4 mg.kg^{-1} ACET alone as reported previously in anaesthetized cats [17]. Since both MPA and ACET cause a reduction in both slope and X-intercept of the CO_2 response curve, the qualitative effect on ventilation (stimulation or inhibition) will depend on the arterial PCO_2 level at which the infusions are performed. Any stimulatory effect on ventilation must result from the reduction in the apnoeic threshold. Conversely, any inhibiting effect would result from the decrease in CO_2 sensitivity, despite the reduction in B. The mean values of the slope (Stot) and intercept (B) of the CO_2 response curve shown in Tables 1 and 2 can be used to calculate the level of ventilation at any constant end-tidal PCO_2 value after the different drug treatments. The overall result of a combined administration of MPA and ACET was a reduction in slope by 37%.

A slightly larger reduction (42%) was observed after ACET alone (Table 2, from Wagenaar *et al.*, 1996 [17] comparison with the Rayn–Einot–Gabriel–Welsh multiple range test yielded no significant difference between these decreases in slope). MPA alone caused a slope reduction by 17% only (Table 1). Given the much larger decrease in mean apnoeic threshold after the combined treatment (1.56 kPa; Table 1) than after MPA (0.38 kPa; Table 1) or ACET (1.00 kPa; Table 2) alone, it can be calculated that the stimulatory effect on ventilation of MPA+ACET, when administered at normo- and hypocapnic PCO_2 values, is considerably larger than that of a single treatment with MPA or ACET.

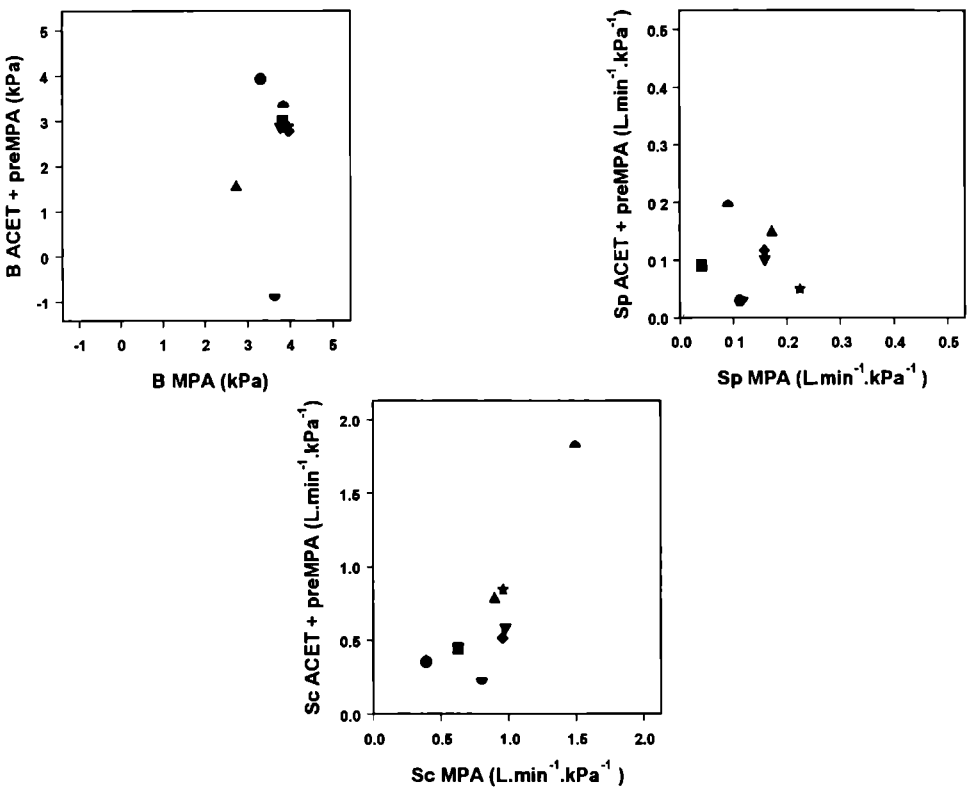

Fig. 3. Scatter diagrams of the effect on 4 mg.kg^{-1} ACET after pre-treatment with MPA on the ventilatory parameters.
Intravenous infusion of 4 mg.kg^{-1} ACET after pre-treatment with MPA results in a decrease in the values of the parameters B, Sc and Sp. Each individual cat is represented by a separate symbol. Sc and Sp are the CO_2 sensitivities of the central and peripheral chemoreflex loops (L.min^{-1}.kPa^{-1}), respectively, and B is the x-intercept of the ventilatory $P_{ET}CO_2$ response curve (kPa).

Parameter	Control	ACET	p-value
B (kPa)	4 0 ± 0 5	3 0 ± 0 6	0 0001
Stot (L min^{-1} kPa^{-1})	1 80 ± 0 69	1 03 ± 0 26	0 0001
Sc (L min^{-1} kPa^{-1})	1 52 ± 0 55	0 84 ± 0 21	0 0001
Sp (L min^{-1} kPa^{-1})	0 28 ± 0 18	0 19 ± 0 12	0 0001

Table 2 Effects of a single ACET treatment (4mg kg^{-1}) on the ventilatory CO_2 response curve in eight cats as determined in a previous study [17]
Sc and Sp are the CO_2-sensitivities of the central and peripheral chemoreflex loops B is the intercept on the $P_{ET}CO_2$ -axis of the CO_2 ventilatory response curve Values are presented as mean of the means per cat ± S D The p-values are obtained from the ANOVA on all individual data

4.5. DISCUSSION

In this study we found that in ovariohysterectomized cats pre-treated with 17-β-estradiol, 4 μg kg^{-1} MPA decreased the CO_2 -sensitivities of the peripheral (Sp) and central chemoreflex (Sc) loops with 41 and 13%, respectively A subsequent administration of 4 mg kg^{-1} ACET caused a further reduction in carbon dioxide sensitivities (Sp 31% and Sc 20%) Both agents lowered the mean apneic threshold by 0 38 and 1 18 kPa, respectively

Effect of 17-β-*estradiol*

We used 17-β-estradiol for premedication Bayliss *et al* [11] showed that priming with E2 is required to achieve a sustained facilitation of phrenic nerve activity by progesterone They showed progesterone receptors to be up-regulated by estradiol as was earlier described by MacLusky and McEwen [23] and Brodeur *et al* [14]

Effects of MPA

In man, progesterone increases the isocapnic acute hypoxic ventilatory response (AHR)[5, 8, 24] Tatsumi *et al* [25] showed that chlormadinone acetate increased the AHR, provided it was measured at pre-drug $P_{ET}CO_2$ level Ovariohysterectomized cats possess smaller

isocapnic acute hypoxic ventilatory and carotid sinus nerve (CSN) responses to hypoxia than intact cats [26] Hannhart *et al* [27] showed that CSN responses to isocapnic hypoxia increased after chronic exogenous or endogenous elevations in progesterone. Male rats treated with female hormones show a larger hypoxic ventilatory response than untreated males [13] The above studies clearly suggest a stimulatory action of female hormones on the peripheral chemoreceptors. Favier *et al* [13] showed that a direct effect may consist of a reduction in carotid body catecholamine content and -turnover. It seems unlikely, therefore, that the decrease in carbon dioxide sensitivity of the peripheral chemoreflex loop by MPA that we observed, is due to a direct effect on the carotid bodies. Apart from the peripheral chemoreceptors, the peripheral chemoreflex loop contains all afferent projection sites of chemosensory CSN fibers, respiratory integrating centres and the neuro mechanical link between the brain stem respiratory network and respiratory muscles. In caudal regions of the nucleus tractus solitarius (NTS) where chemosensory CSN afferents terminate, progesterone inhibits noradrenergic activity in the A_2 catecholaminergic cell group [13] This may have a modulating effect on local afferent impulse transmission resulting in an alteration of the peripheral chemoreflex gain An important observation in the present study was that MPA not only reduced the peripheral, but also the central carbon dioxide sensitivity This decrease in chemoreflex gain is reminiscent of the decrease in baroreflex gain observed in pregnant rats and animals treated with 3α-OH dihydroprogesterone (3α-OHDHP), an important metabolite of progesterone [28]. This effect is probably due to a potentiation by 3α-OHDHP of $GABA_A$ inhibitory influences in the NTS and in the rostro ventrolateral medulla, in the region were baro- as well as chemoreceptor inputs are integrated [28]. MPA, or its metabolites, may have reduced the CO_2 sensitivity in our animals via a GABA-related mechanism: an increase in GABA-ergic tonus in brain stem has an inhibitory effect on ventilation [29]. A similar mechanism could play a role in the hypothalamus, some regions of which contain progesterone receptors [11]. CO_2 sensitive neurons in the caudal hypothalamus modulate the respiratory response to increases in PCO_2 and are exposed a tonic GABA-ergic inhibition [30]

Metabolites of progesterone increase anaesthetic depth and lessen the need for anaesthetics for surgery [31, 32]. This effect could be due to an increase in GABA-induced chloride conductance, and in this respect 3α-OHDHP may also be an important modulator [28]. It is possible that both factors, an increase in GABA-ergic tonus in the brain stem and/or hypothalamus and an increase in anaesthetic depth contributed to the observed

decrease in carbon dioxide sensitivity by MPA or its metabolites Apart from different routes of administration and experimental techniques, an effect related to anaesthetic depth may explain why no progesterone-induced decrease in ventilatory CO_2 sensitivity was reported in awake animals [15, 33, 34] or in humans [4, 5, 24, 35]

The finding that the synthetic progestagenes MPA and chlormadinone acetate, despite a much larger progestational activity, do not have larger effects on ventilation than progesterone [2, 36], may indicate that progesterone's metabolites are responsible for its respiratory effects, rather than the hormone itself

The data in Table 1 show that the effect of MPA on ventilation is a mixture of excitatory (decrease in apnoeic threshold) and inhibitory (decrease in S) effects In other words, the net effect on ventilation depends on the PCO_2 level at which the agent is administered The rise in phrenic activity by progesterone in cats pre-treated with estrogen is probably mediated via hypothalamic structures [12] Stimulation of hypothalamic progesterone receptors may result in an increased excitatory input into the respiratory integrating centres explaining the decrease in apnoeic threshold that we observed in our cats An alternative explanation for the decrease in B is a possible excitatory action of MPA on the carotid bodies (despite the decrease in Sp) A fall in the value of B with approximately 0 4 kPa is within the range reported in the literature From data reported in humans and animals, we calculate that it varies between 0 2–1 7 kPa [16, 24, 35-37]

Effect of ACET after MPA treatment

After pre-treatment with MPA, ACET administration caused an additional decrease in CO_2 sensitivity of both the peripheral and central chemoreflex loops and a decrease in B The latter effect, a fall in the mean apnoeic threshold by 1 18 kPa, was equal to that found without MPA pre-treatment (1 0 kPa, see Table 2, unpaired t-test yields p=0 34) The relative reduction in carbon dioxide sensitivity of the peripheral chemoreflex loop by ACET seems also independent of the pre-treatment with MPA in both studies we found a decrease by 32% ACET may cause this reduction in Sp by a direct action on the peripheral chemoreceptors [17] The relative decreases in Sc, with and without MPA pre-treatment, however, were 20 and 45%, respectively The relative changes in Stot were 37 and 42%, respectively Although unpaired t-tests yielded no significant difference between these changes in Sc and Stot by ACET, we cannot exclude that the respiratory effects of both

agents are not quite independent from each other. Previously, we ascribed the reduction in central carbon dioxide sensitivity by ACET to a possible action of the agent on cerebral vessels [17]. The fact that - to our knowledge - no direct effects of MPA on cerebral vessels have been described, could argue against an influence of MPA on the acetazolamide - induced decrease in central CO_2 sensitivity The additional finding that MPA apparently does not influence the ACET-induced considerable decrease in B, means that after pretreatment with MPA, ACET will cause a much larger net ventilatory stimulation than without pretreatment with the steroid. From the data in Tables 1 and 2, we calculate that a combined treatment (i.e. MPA followed by ACET), when applied at a constant end-tidal PCO_2 of 4.5 kPa, would augment ventilation by 116%. Without MPA pre-treatment, ACET would cause an increase by 66%. Single treatment with MPA would result in a rise of 35% only Note, however, that these calculations apply to a situation in which the end-tidal PCO_2 is clamped, and that these differences in ventilatory effects depend on the prevailing PCO_2 level. An extra ventilatory drive induced by a combined treatment of MPA with ACET could be efficient in (severe) hypercapnic COPD patients. Before treatment, resting ventilation and PCO_2 in these patients may be on the flat portion of the metabolic hyperbola. Independent decreases by both agents of the apnoeic threshold B (as shown in this study in cats), would then result in a relatively small increase in ventilatory drive (shift of the CO_2 response curve to the left), but to a large fall in arterial PCO_2 and, depending on the occurrence of lung regions with very low ventilation perfusion ratios, possibly also to a large rise in arterial PO_2. So in this respect, a combined treatment of MPA and ACET may be clearly more beneficial than single treatments. The present data were obtained after acute infusion of both agents. In clinical practice, however, ventilatory stimulants for patients with COPD are usually applied chronically, and this may lead to different pharmacodynamic effects. We have no animal data on effects of a combined treatment in which the agents were administered in the reverse order, i.e. ACET followed by MPA. In this study we did not exclude that MPA might modulate the effects of ACET on central CO_2 sensitivity. If this would be indeed the case, the respiratory effects of ACET followed by MPA could differ from the effects reported in this study. From what is known about the mechanisms of action of both agents, however, we consider it more likely that MPA and ACET act independently on the control of breathing. Our data do not conflict with this scenario.

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CHAPTER 5

COMPARISON OF ACETAZOLAMIDE AND MEDROXYPROGESTERONE AS RESPIRATORY STIMULANTS IN HYPERCAPNIC COPD PATIENTS.

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Keywords: Medroxyprogesterone acetate (MPA), acetazolamide (ACET), hypercapnia, Chronic obstructive pulmonary disease (COPD).

5.1. SUMMARY

Background. Acetazolamide (ACET) and medroxyprogesterone acetate (MPA) are two respiratory stimulants that can be used in patients with stable hypercapnic chronic obstructive pulmonary disease (COPD).

Design and methods. The effects of ACET (250 mg b.i.d.) and MPA (30 mg b.i.d.) on day- and nighttime blood gas values and the influences on the hypercapnic and hypoxic ventilatory and $P_{0.1}$ response were studied in a cross-over design in 12 hypercapnic patients with stable COPD (mean FEV_1 33 ± 4 % Pred, mean ±sem).

Results. The daytime $PaCO_2$ decreased from 6.3 ± 0.1 (placebo) to 5.6 ± 0.2 kPa during ACET treatment ($p<0.05$) and to 5.7 ± 0.2 kPa during MPA ($p<0.05$). The daytime PaO_2 improved with ACET from 8.7 ± 0.3 to 10.0 ± 0.4 kPa ($p<0.05$), whereas no significant changes were seen with MPA. Mean nocturnal $P_{ET}CO_2$ decreased with both treatments; from 5.6 ± 0.3 to 4.7 ± 0.3 kPa with ACET ($p<0.05$) and to 4.6 ± 0.1 kPa with MPA ($p<0.05$). The percentage of time that the nocturnal SaO_2 was lower than 90%, was reduced significantly with ACET from 34.9 ± 10.7 to 16.3 ± 7.5% ($p<0.05$). Mean nocturnal saturation did not change with MPA.

Resting minute ventilation increased significantly only with MPA from 9.6 ± 0.7 to 10.8 ± 0.8 $L.min^{-1}$ ($p<0.05$). The slope of the hypercapnic ventilatory response did not change during ACET and MPA therapy. The hypoxic ventilatory response increased from -0.2 ± 0.05 to -0.4 ± 0.1 $L.min^{-1}.\%^{-1}$ during ACET ($p<0.05$) and to -0.3 ± 0.1 $L.min^{-1}.\%^{-1}$ during MPA ($p<0.05$). The hypoxic $P_{0.1}$ response improved with ACET treatment from -0.007 ± 0.002 to -0.02 ± 0.003 $kPa.\%^{-1}$($p<0.05$).

Conclusions. This study shows that ACET and MPA both have favourable effects on day- and nighttime blood gas parameters in ventilatory limited patients with stable COPD. However, the use of ACET is preferred because of its extra effect on nocturnal saturation.

5.2. INTRODUCTION

Some patients with severe chronic obstructive pulmonary disease (COPD) become chronically hypoxic and/or hypercapnic as the disease progresses In general, their prognosis is poor as shown by Cooper *et al* [1], who analyzed COPD patients retrospectively from the moment of death and concluded that hypoxia as well as hypercapnia were signs of imminent death within three years Moreover, Foucher *et al* [2] found that the rate of death in COPD patients on long term oxygen therapy (LTOT) was higher in the hypercapnic group ($PaCO_2$ > 5 7 kPa) as compared to the normocapnic group ($PaCO_2$ < 5 7 kPa) Hence, hypercapnia itself is probably an independent poor prognostic factor, although this is not supported by all investigators [1-3] Treatment of hypoxia with supplemental oxygen improves survival [4, 5] However, the degree of hypercapnia worsens in many patients during oxygen therapy, which may influence the prognosis This hypercapnia is probably due to a combination of a slightly worsened V'/Q' mismatch, initial CO_2 retention with O_2 mediated blunting of the peripheral chemoreceptor drive, Haldane-effect related changes in CO_2 and H^+ buffering by hemoglobin [6] During sleep, hypoxia and hypercapnia increase due to hypoventilation caused by a diminished intercostal and accessory muscle function during REM sleep stage, superimposed on a dysfunctional diaphragm [7] Also, low hypoxic and hypercapnic ventilatory responses may contribute to the nocturnal hypoventilation

The respiratory stimulants medroxyprogesterone acetate (MPA) and acetazolamide (ACET) may improve day- and nighttime blood gas values in COPD patients, although not all studies are consistent [8-13]

ACET stimulates ventilation by inducing a metabolic acidosis [8, 14] In a previous study, we found an increase in the slope of the hypercapnic ventilatory response (HCVR) with ACET in COPD patients [9], in contrast to other studies in which only changes in intercept are shown [15, 16] The effect of ACET on the hypoxic ventilatory response (HVR) is equivocal in various studies ranging from no change [9, 15, 17] to an improvement [16]

Progesterone increases ventilation via progesterone receptors in the hypothalamus [18] Most human studies show an increase in HCVR during (synthetic) progestagens [9, 13, 19] The results, however, on HVR are conflicting [9, 13, 19-21]

The aim of the present study is to compare the effects of ACET and of MPA on day- and nighttime blood gas values as well as hypercapnic and hypoxic ventilatory responses in chronic hypercapnic patients with COPD

5.3. METHODS

Patients

Nineteen hypercapnic patients with severe COPD (age 68 ± 2 years), as defined by the American Thoracic Society [22], were enrolled in this study The patient's characteristics are shown in table 1 Inclusion criteria were optimal bronchodilator treatment, daytime $PaCO_2 \geq$ 6 0 kPa, no long term oxygen treatment and the ability to lower the $P_{ET}CO_2$ at least 1 kPa, in a voluntary hyperventilation test, to establish the ventilatory pump reserve Exclusion criteria were exacerbation of COPD within the last three months, abnormal renal and liver functions, use of respiratory stimulating drugs and obstructive sleep apnea/hypopnea syndrome Seven patients dropped out of the study, after inclusion, because of an exacerbation, evenly distributed over both treatment modalities

Study design

In a double blind, randomized, cross-over study, the effects of ACET and MPA on daytime and nocturnal blood gas and ventilatory parameters were investigated The design is shown in figure 1 The patients were studied four times in six weeks, at the start of the study and before a change in medication (study points T1-4 fig 1) All patients received placebo during the first two weeks to assess the intra-individual variability After the second and third measurements patients received either ACET (250 mg) + placebo or MPA (30 mg) + placebo twice a day (b i d), all in identical capsules Written informed consent was obtained from all patients and the study was approved by the Hospital Ethics Committee

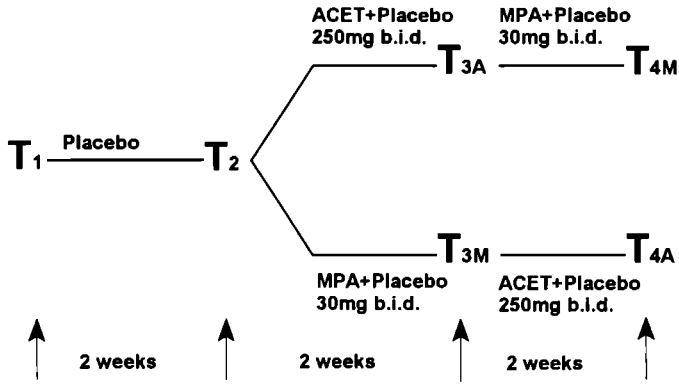

Fig 1. Study protocol double blind cross over study.

Tx,↑=measurement. ACET= acetazolamide, MPA=medroxyprogesterone acetate.

Daytime measurements

Laboratory tests

After each study night (at 9 a.m.) an arterial blood sample was taken after 15 minutes of rest (Ciba-Corning 278 blood gas analyzer, Houten, The Netherlands). Liver and renal functions were tested.

Pulmonary function tests

-Hypercapnic ventilatory response (HCVR) and hypoxic ventilatory response (HVR)-

The HCVR was assessed by the steady-state method [23]. The patient was connected to a closed spirometric circuit (Pulmotest, Godart, Bilthoven, The Netherlands) via a mouthpiece. A Rudolph valve 2700 (Kansas City, Miss, USA) was placed in the system, to maintain a

one way circuit. The end-tidal carbon dioxide tension ($P_{ET}CO_2$) level was measured by a side stream capnograph (Dräger, Lübeck, Germany). Inspiratory PCO_2 could be gradually raised by adjusting a three-way valve, partly short-circuiting the CO_2 absorber in the inspiratory limb of the circuit. Two different levels of $P_{ET}CO_2$ were studied ($P_{ET}CO_2$ at zero P_ICO_2, and $P_{ET}CO_2$ 1-1.5 kPa above resting value). Both levels of $P_{ET}CO_2$ were maintained for at least 7 minutes. Oxygen was added to the system to keep SaO_2 level >90%. SaO_2 was measured with a pulse oximeter (Oxyshuttle; Sensor Medics, Anaheim, CA, USA). At the end of each steady state period of the HCVR ("high" PCO_2 level) an arterialized capillary blood sample was taken.

The steady state relation of ventilation (V'_E) to $P_{ET}CO_2$ at constant $P_{ET}O_2$ was considered to be linear down to the $P_{ET}CO_2$ axis:

$V'_E = S (P_{ET}CO_2 - B)$,

in which the parameter S is the CO_2 sensitivity of the peripheral and central chemoreflex loops and the offset B the apnoeic threshold or extrapolated $P_{ET}CO_2$ at zero ventilation. The mouth occlusion pressure ($P_{0.1}$) [24] was measured during the final two minutes of each steady state period at the two different levels of $P_{ET}CO_2$ using a solenoid valve, in the inspiratory part of the circuit.

The HVR was performed by inducing progressive hypoxia. $P_{ET}CO_2$ was maintained at the initial pre-drug level (placebo, T_2) during all HVR tests, by adding CO_2 to the inspirate, when necessary. All patients started the test at a SaO_2 level of 98% by adding supplemental oxygen. Then, the oxygen supplementation was stopped and the HVR test was performed until SaO_2 reached 80%, which took less than seven minutes.

-Spirometry-

Forced expiratory volume in 1 second (FEV_1), inspiratory vital capacity (IVC), total lung capacity (TLC) and residual volume (RV) were performed with a wet spirometer and He dilution technique (Pulmonet III, SensorMedics, Anaheim, CA, USA), respectively. Reference spirometric values were derived from ERS standards [25].

Subjective parameters

After each study period the dyspnea sensation was scored with the modified Medical Research Council (MRC)-scale [26]. Side effects of the drugs were noted.

Nocturnal measurements

The nocturnal arterial oxygen saturation (SaO_2) was measured with a pulse oximeter The baseline SaO_2 'awake' was defined as the mean SaO_2 during the first 15 minutes, when the patient was awake and in supine position [27]. The $P_{ET}CO_2$ was measured by sampling air through a naso-pharyngeal cannula (Mijnhardt capnolyser, Bilthoven, The Netherlands) [9] The mean $P_{ET}CO_2$ and SaO_2 were defined as the mean $P_{ET}CO_2$ and SaO_2 of the total recording time. Oxygen saturation, and $P_{ET}CO_2$ signals during the night were stored on a computer (Compaq 4/66, Houston, TX, USA).

Statistical analysis

Data are presented as mean values ± sem. Carryover effects were analyzed according to Pocock [28] by comparing the treatment with ACET as well as MPA in the different study arms (Mann-Whitney-U-test) (*i.e.* for ACET: T_{3A} minus T_2 and T_{4A} minus T_2, for MPA: T_{4M} minus T_2 and T_{3M} minus T_2 ;(fig 1.)). After determining that there were no significant differences in mean values of the group between control (T_1) and placebo (T_2), and that carryover effects could not be measured two weeks after stopping the previous medication, the data obtained in the "baseline situation" (mean of T_1 and T_2) were compared with those after ACET and MPA treatment (Wilcoxon signed rank tests). Statistical significance was defined as a p-value <0.05. For all analyzes SPSS (version 6.1.3, SPSS inc, Chicago, ILL, 1995) was used.

5.4. RESULTS

No significant differences were observed in ventilatory or in blood gas variables after placebo treatment compared to control Hence, the mean data of the control and placebo measurements were used as baseline values in the analysis of the effects of ACET and MPA treatment Baseline characteristics are shown in table 1 No carryover effects could be demonstrated with either drug regimen as shown in table 2 Therefore the analysis of the drug treatment could be simplified to baseline *vs* ACET treatment and baseline *vs* MPA treatment, irrespective of the sequence of the drugs

general	
sex (M/F)	8/4
age (years)	68 ± 2
weight (kg)	68 ± 3
height (cm)	170 ± 3
BMI (kg m^{-2})	23 ± 1
lung function	
TLC (%pred)	105 ± 5
RV (%pred)	168 ± 14
FEV_1 (L)	0 9 ± 0 1
(%pred)	33 ± 4
FEV_1/IVC (%pred)	46 ± 3
arterial blood gases	
pH	7 40 ± 0 01
PaO_2 (kPa)	8 8 ± 0 3
$PaCO_2$ (kPa)	6 3 ± 0 2
BE (mmol L^{-1})	3 9 ± 0 5
SaO_2 (%)	92 4 ± 0 9

Table 1 Baseline characteristics (T_1) (n=12)
Data are presented as mean ± sem BMI=body mass index, TLC=total lung capacity, RV=residual volume, FEV_1 =forced expiratory volume in 1 second, IVC=inspiratory vital capacity, $PaCO_2$ = arterial carbon dioxide tension, PaO_2 = arterial oxygen tension, BE = base excess, SaO_2 = arterial oxygen saturation,%pred [25].

	ACET			MPA		
PARAMETER	**T_{4A} minus T_2**	**T_{3A} minus T_2**	**p**	**T_{4M} minus T_2**	**T_{3M} minus T_2**	**p**
$PaCO_2$ (kPa)	0.5 ± 0.1	0.9 ± 0.2	0.24	0.6 ± 0.2	0 7 ± 0 1	0.94
PaO_2 (kPa)	0.5 ± 0.3	0.6 ± 0.5	0.94	1.1 ± 0.3	1 7 ± 0.7	0.49
V'_E ($L.min^{-1}$)	0 7 ± 0.9	1.6 ± 0.7	0.59	0.0 ± 0.8	0.9 ± 0 6	0.7
$P_{0.1}$ (kPa)	0 1 ± 0.2	0.1 ± 0.0	0.43	0.1 ± 0.1	0.2 ± 0.1	1
S_{HCVR} ($L.min^{-1}.kPa^{-1}$)	4.6 ± 1.3	5.8 ± 1.3	0.59	4.5 ± 1 1	3 7 ± 0 4	0.59
S_{HVR} ($L.min^{-1}.\%^{-1}$)	-0.1 ± 0.1	-0.2 ± 0.1	0 49	-0.3 ± 0.1	-0.3 ± 0.1	0.31

Table 2 Carryover effects.
Differences in ventilatory and blood gas parameters between the various stages in the study, indicating that at T_4, no carryover effects from the previous medication could be shown Data are presented as mean ± sem. $PaCO_2$ = arterial carbon dioxide tension; PaO_2 = arterial oxygen tension, V'_E =minute ventilation; $P_{0.1}$ = mouth occlusion pressure at 100 msec; S_{HCVR} ($\Delta V'_E/\Delta PaCO_2$) = the slope of the ventilatory CO_2 response curve; S_{HVR} ($\Delta V'_E/\Delta Sa_{O_2}$) = the slope of the hypoxic ventilatory response

Daytime laboratory measurements

The effects of ACET and MPA on daytime blood gas parameters are shown in table 3. The mean PaO_2 improved significantly during ACET treatment by 1.3 ± 0.4 kPa ($p<0.05$), whereas the mean PaO_2 did not significantly increase with MPA. The mean $PaCO_2$ decreased significantly after treatment with ACET (0.7 ± 0.1 kPa) and MPA (0.6 ± 0.1 kPa) (both $p<0.05$). The mean decrease of $PaCO_2$ with ACET was significantly larger than that obtained with MPA treatment ($p<0.05$) (table 3, fig 2.). The base excess (BE) decreased significantly after ACET (~6.0 $mmol.L^{-1}$) and after MPA treatment (~2 $mmol.L^{-1}$).

PARAMETER	BASELINE (T_1 and T_2)	ACET (T_{3A} and T_{4A})	MPA (T_{3M} and T_{4M})
pH	7 40 ± 0 01	7 35 ± 0 01*	7 41 ± 0 01
PaO_2 (kPa)	8 8 ± 0 3	10 0 ± 0 4*	9 3 ± 0 4
$PaCO_2$ (kPa)	6 3 ± 0 2	5 6 ± 0 2*	5 7 ± 0 2*
$\Delta PaCO_2$ (kPa)	-	-0 72 ±0 13†	-0 ò1 ± 0 10
BE (mmol L^{-1})	3 9 ± 0 5	-2 0 ± 0 7*	2 1 ± 0 4*
$P_{(a-ET)}CO_2$ (kPa)	0 8 ± 0 2	0 7 ± 0 2	0 6 ± 0 2
SaO_2 (%)	92 4 ± 0 9	93 8 ± 0 7	93 4 ± 0 8

Table 3 Effect of ACET and MPA on daytime blood gas parameters
Data are presented as mean ± sem $P_{(a-ET)}CO_2$ = arterial-to-end-tidal PCO_2 gradient $\Delta PaCO_2$ = mean change in $PaCO_2$ (drug versus baseline) (*p<0 05 between treatment and baseline, † p<0 05 between ACET and MPA treatment)

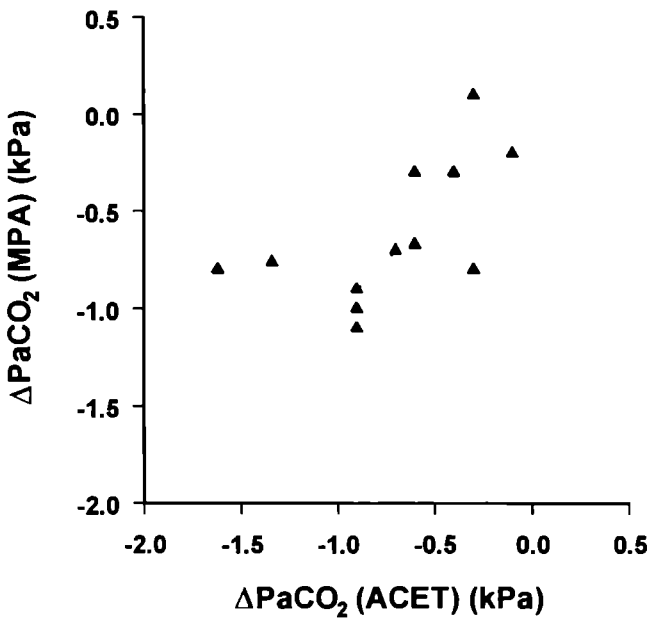

Fig 2 Effect of ACET and MPA on Δ $PaCO_2$
The effect of Δ $PaCO_2$ (ACET) versus Δ $PaCO_2$ (MPA) in twelve COPD patients All data points are the individual data of each patient Dotted line identity

Ventilation and hypercapnic/hypoxic responses (table 4)

Minute ventilation did not change significantly with ACET but increased significantly with MPA treatment (1.2 L.min^{-1}). The slope of the HCVR-curve did not differ significantly during ACET nor during MPA, as compared to baseline (fig 3). The ventilatory CO_2 response curve showed a non significant shift to the left as indicated by a small decrease in apnoeic threshold B_{HCVR} (x-intercept) both with ACET (from 3.2 to 2.1 kPa) and with MPA (from 3.2 to 2 6 kPa).

$P_{0.1}$ increased significantly with MPA, however, the slope and x-intercept of the response of $P_{0.1}$ to $PaCO_2$ did not change significantly with ACET nor with MPA treatment

The slope of the HVR significantly increased during ACET as well as during MPA therapy (fig 4) Only during ACET, the slope of the relationship between $\Delta P_{0.1}$ and ΔSaO_2 was significantly increased (fig 5).

PARAMETER	BASELINE (T_1 and T_2)	ACET (T_{3A} and T_{4A})	MPA (T_{3M} and T_{4M})
V'_E (L min^{-1})	9.7 ± 0.8	10.0 ± 0.6	10.8 ± 0.8*
$P_{0.1}$ at rest (kPa)	0 4 ± 0.04	0.4 ± 0.03	0 5 ± 0.04*
S_{HCVR} (L.min^{-1}.kPa^{-1})	5.0 ± 0.8	4.1 ± 0.6	5.2 ± 0.9
B_{HCVR} (kPa)	3.6 ± 0.6	2.1 ± 0.7	2.6 ± 0.7
$S_{HCR_{0.1}}$ (kPa.kPa^{-1})	0.20 ± 0.02	0.3 ± 0.05	0.3 ± 0.04
$B_{HCR_{0.1}}$ (kPa)	4.0 ± 0.3	3.6 ± 0.3	3.4 ± 0.4
S_{HVR} (L.min^{-1}.%$^{-1}$)	-0 16 ± 0.04	-0.4 ± 0.1*	-0.3 ± 0.1*
$S_{HR_{0.1}}$ (kPa.%$^{-1}$)	-0.007 ± 0.001	-0.02 ± 0.003*	-0.01 ± 0.003

Table 4. Effect of ACET and MPA on ventilatory variables
Data are presented as: mean ± sem. V'_E = minute ventilation; V_T = tidal volume; f_{resp} = respiratory frequency; $P_{0.1}$ at rest = mouth occlusion pressure at rest; S_{HCVR} ($\Delta V'_E/\Delta PaCO_2$) = slope of the ventilatory CO_2 response curve; B_{HCVR} (x-intercept) = apnoeic threshold or extrapolated $P_{ET}CO_2$ at zero ventilation; $S_{HCR_{0.1}}$ ($\Delta P_{0.1}/\Delta PaCO_2$) = slope of the mouth occlusion response to CO_2; $B_{HCR_{0.1}}$ = x-intercept of the mouth occlusion pressure response to CO_2; S_{HVR} ($\Delta V'_E/\Delta SaO_2$) = slope of the hypoxic ventilatory response. $S_{HR_{0.1}}$ ($\Delta P_{0.1}/\Delta SaO_2$) = slope of the mouth occlusion response to O_2. (*p<0.05 between baseline and treatment)

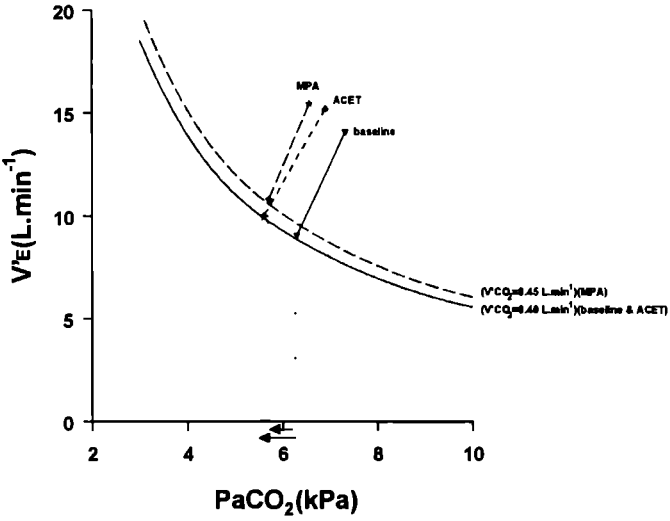

Fig 3 Effect of ACET and MPA on the ventilatory CO_2 response curve (HCVR).
The effect of treatment with 250 mg b.i.d. ACET and with 30 mg b.i.d. MPA on the ventilatory CO_2 response curve, calculated from the mean data in twelve severe COPD patients.
The continuous line reprsents the baseline situation and the dashed lines the situations during ACET and MPA therapy. The continuous line of the metabolic hyperbola represents the situation with ACET treatment, the dashed metabolic hyperbola the one during MPA administration (calculated lines; Standard temperature and pressure (STPD)).

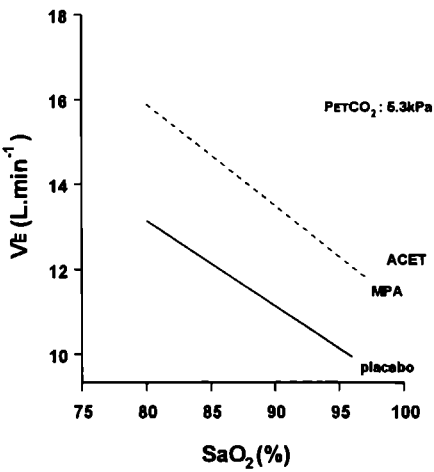

Fig 4. Effect of ACET and MPA on the relation Hypoxic Ventilatory Response (HVR).
The effect of treatment with 250 mg b.i.d. ACET and with 30 mg b.i.d. MPA on the ventilatory response to O_2, calculated from the mean data in twelve severe COPD patients.
The continuous line represents the baseline situation and the dashed lines the situations during ACET and MPA therapy.

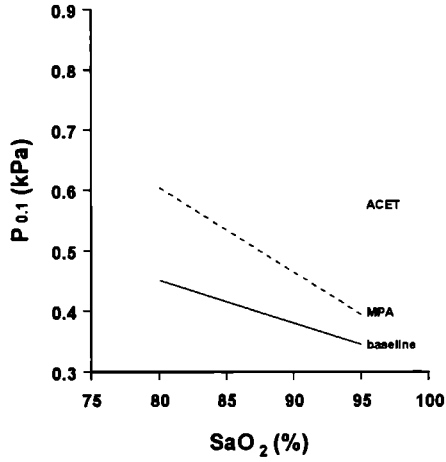

Fig 5. Effect of ACET and MPA on the relation between $P_{0.1}$ and SaO_2.
The effect of treatment with 250 mg b.i.d. ACET and with 30 mg b.i.d MPA on the relation between $P_{0.1}$ and SaO_2 calculated from the mean data in twelve severe COPD patients.
The continuous line represents the baseline situation and the dashed lines the situations after 250 mg b.i.d. ACET and after 30 mg b.i.d. MPA, respectively.

Subjective parameters

In the ACET group three patients complained of paraesthesias and three patients with gastro-intestinal discomfort. Seven patients reported side effects during MPA therapy (gastro-intestinal discomfort (n=5) and fatigue (n=2)). Dyspnea sensation (MRC-scale) did not change significantly after introduction of the drugs. 1.83 ± 0.17 at baseline to 1.83 ± 0.24 (n.s.) during ACET therapy and to 2.33 ± 0.31 (n.s.) during MPA therapy.

Nocturnal parameters

Mean $P_{ET}CO_2$ decreased significantly by both ACET and MPA, with 0.9 and 1.0 kPa, respectively (table 5). The mean SaO_2 did not improve significantly with ACET. However,

the percentage of nocturnal recording time with saturation below 90% did improve significantly with ACET therapy from 34.9 ± 10.7 to 16 3 ± 7 5%. MPA did not significantly change nocturnal saturation.

	BASELINE (T_1 and T_2)	ACET (T_{3A} and T_{4A})	MPA (T_{3M} and T_{4M})
Mean $P_{ET}CO_2$ (kPa)	5.6 ± 0.3	4.7 ± 0.3*	4.6 ± 0.1*
Mean SaO_2 (%)	90.6 ± 1.0	92.0 ± 0.9	91.6 ± 1.0
Lowest SaO_2 (%)	84.0 ± 1.8	83.7 ± 2.7	83.8 ± 2.1
% time SaO_2 <90%	33.3 ± 10 5	16.3 ± 7.5*	23.8 ± 11 5

Table 5. Nocturnal parameters (n=12)
Data are presented as: mean ± sem.(*p<0.05 treatment and baseline)

5.5. DISCUSSION

The present study showed that, in hypercapnic COPD patients, ACET and MPA both have favourable effects on several day- and nighttime blood gas parameters as well as on ventilatory responses; ACET showed more and larger effects than MPA.

Daytime laboratory tests

The present study showed that ACET improved daytime $PaCO_2$ and PaO_2. This is in agreement with the literature [8, 9, 15]. The mechanism by which ACET improves daytime blood gas values is not yet clear. However, it is generally believed that the metabolic acidosis induced by ACET is responsible for the improvements. We observed an increase in daytime PaO_2 of 1.3 kPa only with ACET. If one calculates A-aPO_2 differences, it appears that (assuming a respiratory exchange ratio of 0 8) acetazolamide has reduced the A-aPO_2 difference by 0 5 kPa. Therefore, the improvement in PaO_2 cannot be caused by an increase in alveolar ventilation alone, but it may also be due to a slight improvement of V'/Q' matching and/or a small diuretic effect resulting in a decrease in interstitial lung water [6, 29].

In our previous animal studies [30, 31], we discussed that the effect of ACET on the HCVR may be caused by a direct effect on the carotid bodies as well as on the cerebral vessels, which may be responsible for the decrease in $PaCO_2$ and increase in PaO_2.
MPA improved daytime $PaCO_2$ as was shown by others [11, 32]. Skatrud *et al.* [33] divided their COPD patients in "correctors" (10/15), in whom tidal volume increased and $PaCO_2$ decreased ($\geq$ 0.67 kPa) during both MPA and ACET administration, versus "non-correctors"(5/15), showing no fall in $PaCO_2$ ($\leq$ 0.67 kPa). This is in agreement with our study where minute ventilation increased by MPA treatment. In 7 out of 12 patients $PaCO_2$ decreased >0.67 kPa and they should thus be considered "correctors". Our figure 2 suggests that there is a wide inter individual range of responses, but we found no suggestion of two distinct groups of responders and non responders.

Ventilation and hypercapnic/hypoxic responses

The present data showed that neither ACET nor MPA therapy changed the slope and x-intercept of the hypercapnic ventilatory response (HCVR). This is in contrast with animal studies. In *anaesthetized* cats it was shown that the slope of the HCVR decreased by an acute low intravenous dose of ACET, which was attributed to a direct effect of ACET on the peripheral chemoreflex loop as well as an effect on cerebral blood flow regulation [30]. In humans, several authors studied the effect of acetazolamide on the ventilatory CO_2-response using the Read rebreathing technique [17, 34]. It has been shown however, that during metabolic acidosis the Read rebreathing technique results in a considerable overestimation of the response slope [35] Swenson *et al.* [15] showed that in healthy volunteers acute intravenous infusion of the drug (1 intravenous dose of 500 mg ACET) resulted in a parallel shift of the HCVR to the left, whereas chronic administration (3 oral doses of 500 mg over 24 hours ACET) resulted in an increase in the slope of the HCVR. An explanation for the contradiction with our study could be the large chronic dose of ACET used by Swenson [15], the time sequence the drug was administered (2 vs 3 oral doses), as well as the different study group (hypercapnic patients vs healthy volunteers).

We found that MPA did not significantly influence the slope and x-intercept of the HCVR This was also found by Morikawa [21]

$P_{0.1}$ is considered to be useful in the assessment of central inspiratory neuromuscular drive, especially in COPD patients, since $P_{0.1}$ is less affected by airway resistance [24] Therefore, mouth occlusion pressure ($P_{0.1}$) was measured at rest and the $P_{0.1}$ response to CO_2 was calculated However, $P_{0.1}$ may underestimate the respiratory drive in COPD patients because it reflects the force output of respiratory muscles, which are sometimes weak, and reflects the neuro-mechanical coupling which is changed [36] In our patients the $P_{0.1}$ values prior to drug administration were higher and the relation between $\Delta P_{0.1}$ and $\Delta PaCO_2$ were lower than in healthy subjects, which is in agreement with literature [8, 37] During ACET treatment no significant change in $P_{0.1}$ and in the relationship between $\Delta P_{0.1}$ and $\Delta PaCO_2$ was observed, whereas $P_{0.1}$ increased significantly by MPA This is consistent with the observed increase in ventilation with MPA therapy The relation $\Delta P_{0.1}$ and $\Delta PaCO_2$ did not change with MPA treatment These findings are in agreement with Skatrud *et al* and Morikawa *et al*, who also did not find any change in this relationship [8, 21] When considering the CO_2-response curves in COPD, the ventilation of the patients will increase during hypercapnia This will lead to increased hyperinflation, taking the lungs and thoracic wall to a stiffer part of their pressure-volume curve Furthermore, the process of shortening of the diaphragm due to hyperinflation will bring it on a disadvantageous part of the length-tension curve This might explain the lack of response in $P_{0.1}$ in the CO_2-response curves [37]

The ventilatory control system can be subdivided into a controller or controlling system, and a controlled system The controller has an input ($PaCO_2$) and an output (minute ventilation) The characteristic of the controller is the ventilatory response to CO_2 The controlled system is the gas exchanging process in the lungs, its input is minute ventilation and its output is the $PaCO_2$ The characteristic of the controlled system depends on the metabolic CO_2-production, and is called the metabolic hyperbola In our model, due to a higher metabolic rate in COPD patients [38], we calculated a resting CO_2-production of 400 ml min^{-1} by using the steady state resting $PaCO_2$ and V'_E values as points of the hyperbola Metabolism will even be a little higher during MPA Therefore, CO_2-production was calculated at 450 ml min^{-1} (steady state resting $PaCO_2$ and V_E values during MPA therapy) [19, 32] During spontaneously breathing, the output of the controlled system ($PaCO_2$) is at the same time

input of the controller Alternatively, the output of the controller (minute ventilation) is the input of the controlled system Thus the system settles at the point where the CO_2-response curve crosses the metabolic hyperbola This is called the 'closed loop' situation

As shown in figure 3, for both ACET and MPA, this model will predict a decrease in $PaCO_2$ of 0 8 and 0 5 kPa, respectively This is in close agreement with the actual decrease of 0 7 and 0 6 kPa that we found in our patients Since the point of intersection of the ventilatory CO_2 response curve with the metabolic hyperbola is in the relatively flat part of the hyperbola, it is not surprising that with ACET treatment, a decrease in $PaCO_2$ was observed without a significant increase in ventilation This also means that ACET seems to be able to improve blood gas values without adding a substantial load to the ventilatory muscles As shown in figure 3 the (non-significant) ventilatory increase of 0 4 L min^{-1} ($\Delta V'_E$) would result in a decrease in $PaCO_2$ of only ± 0 3 kPa as indicated by the metabolic hyperbola Since we observed a larger decrease in $PaCO_2$ (0 8 kPa) other factors in the gas exchange must be responsible for this "extra" decrease in $PaCO_2$ A decrease in V'_{CO_2} as observed by Hirahara [39] could possibly explain this extra decrease This is due to the fact that the point of intersection of the ventilatory CO_2 response curve with the metabolic hyperbola is in the relatively flat part of the hyperbola Another explanation could be the appearance of tissue CO_2-retention, since the carbonic anhydrase is inhibited, leading to a relative tissue hypercapnia, with either no change or a relative arterial hypocapnia [15]

Taken all together, the concern of Swenson *et al* [10] about prescribing ACET to severe COPD patients with ventilatory pump failure seem to be of great clinical importance as can also be concluded from the present study ACET should not be used in patients with very severe obstruction and hypercapnia (FEV_1 <25% and $PaCO_2$ >8 kPa) or in those who have not the mechanical ability to lower their $PaCO_2$ by voluntary hyperventilation

In the current study as well as in others, the HVR was augmented by ACET and MPA, which indicates that both respiratory stimulants have an effect on the peripheral chemoreflex loop [19, 20] The HVR was not increased in all ACET and MPA studies [9, 12, 17] A possible explanation could be a different technique measurement of the HVR at pre-drug PCO_2 level resulted in an increased HVR [12, 15, 19] instead of a decrease under lower post-drug PCO_2 levels [9, 12, 17]

The slope of $\Delta P_{0.1}$ versus ΔSa_{O_2} was significantly increased by ACET (fig 5), but did not reach any significance with MPA For MPA this is in agreement with the observations of Schoene *et al* [40], although this is inconsistent with the observed increase in HVR This might be due to the large inter and intra individual variability as mentioned by Whitelaw [24]

Nocturnal parameters

ACET significantly decreased nocturnal desaturation time (% time Sa_{O_2} <90%) It is known that the respiratory stimulant may contribute to improvement of desaturation time by diminishing central sleep apnea and periodic breathing [41] This supports the opinion that ACET augments the chemical drive as was shown in those patients This may also explain the decrease in $P_{ET}CO_2$, although occasional nocturnal hypoventilation may still be present MPA has a beneficial effect on $P_{ET}CO_2$ as shown in table 5 This findings corroborates that of others [42]

Clinical implications

The current study showed that short-term ACET treatment is more beneficial compared to short term MPA treatment as can be seen from a larger change in daytime $PaCO_2$ in hypercapnic COPD patients However, the long-term clinical benefit of ACET and MPA treatment on severe COPD patients remains to be investigated The possible effect on survival of a modest increase in PaO_2 and a decrease in $PaCO_2$ is not yet clear Hypoxia and hypercapnia are both considered to be independent poor prognostic factors for survival [2, 4, 43], yet others question the prognostic role of hypercapnia [3] If the role of $PaCO_2$ as an independent predictor for a prognostic poor prognosis will be further established, the use of ACET might be preferred because of its extra effect on nocturnal saturation

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COMBINED TREATMENT WITH ACETAZOLAMIDE AND MEDROXYPROGESTERONE IN COPD PATIENTS

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6.1. SUMMARY

Medroxyprogesterone acetate (MPA) and Acetazolamide (ACET) are two ventilatory stimulants which are used in hypoxic and hypercapnic patients with chronic obstructive pulmonary diseases

In a double blind, randomized study, the effects of a two week treatment with MPA (30 mg bid) or ACET (250 mg bid), followed by a two week treatment with a combination of both drugs (MPA-ACET), were investigated on daytime and nocturnal ventilatory and blood gas parameters in 17 stable hypercapnic COPD patients

ACET, MPA and MPA-ACET treatment decreased mean daytime $PaCO_2$ by 0 4, 0 7 and 1 2 kPa (all $p<0$ 02), respectively V'_E was improved only with combined therapy from 9 3 to 11 2 L min^{-1} ($p<0$ 02)

The hypercapnic and hypoxic ventilatory responses were significantly increased by MPA-ACET therapy from 3 7 to 5 8 L min^{-1} kPa^{-1} and from -0 13 to -0 40 L min^{-1} $\%^{-1}$, respectively

The $P_{0.1}$ response to hypoxia increased during the combination therapy from -0 01 to -0 03 kPa $\%^{-1}$ Nocturnal $P_{ET}CO_2$ decreased with MPA and MPA-ACET treatment by 0 9 and 1 4 kPa ($p<0$ 02), respectively MPA-ACET significantly increased mean nocturnal SaO_2 values from 85 5 to 90 2%

We concluded that combined treatment with MPA-ACET has a more favourable effect on day and night-time blood gas values and chemical drive, than single drug treatment

6.2. INTRODUCTION

A sub-group of patients with severe chronic obstructive disease (COPD) are not able to maintain their blood gas values at normal levels The life expectancy of these patients depends on both the severity of hypoxaemia and hypercapnia, although the latter is under debate [1] Long term oxygen treatment improves survival rate in these patients [2,3], however, in some COPD patients, this will aggravate the carbon dioxide retention [1] It was suggested that the hypercapnic ventilatory failure was associated with early death in patients using long-term oxygen therapy

The goal of the treatment in this patient group is to avoid total respiratory failure In the model of Karpel *et al* [4] respiratory failure is the result of a decrease in central respiratory drive, an excessive respiratory work load, inadequate respiratory muscle endurance and malnutrition Therefore, non invasive ventilation, supplemental oxygen, respiratory stimulants, inspiratory muscle training and nutritional support may be beneficial, although variable degrees of success were reported [5-7]

The current study focuses on the effect of the respiratory stimulants acetazolamide (ACET) and medroxyprogesterone acetate (MPA), which can be used to increase the ventilatory drive and so avoid respiratory failure

The carbonic anhydrase inhibitor ACET has successfully been used in the treatment of hypoventilation in COPD patients and nocturnal hypoventilation [7-9] By inhibition of renal carbonic anhydrase, ACET causes a metabolic acidosis which induces an increase in ventilatory drive However, since ACET is present in many tissues and cells that are involved in the regulation of breathing, the effect of ACET on the control of breathing may be much more complicated Our previous animal studies showed a decrease in the slope and X-intercept of the ventilatory CO_2 response curve, which suggests that acetazolamide may also act on the peripheral chemoreceptors and on the cerebral blood flow [10-12]

The other respiratory stimulant, the synthetic progestagene MPA, increases arterial oxygen tension (PaO_2) and reduces arterial carbon dioxide tension ($PaCO_2$) in COPD patients [7, 13, 14] The ventilatory effect of MPA is controlled by receptors in the hypothalamus [15] Ventilatory effects via the central and peripheral chemoreflex loops have also been described, *e g* [12, 16] Verbraecken *et al* [17] described a persistent ventilatory stimulation

with ACET in patients with central hypoventilation, that lasted 1-6 months. The possible sustained effects of MPA and ACET are not known

Simultaneous administration of both respiratory stimulants, aiming for a more efficient ventilatory stimulation in COPD patients has not yet been described. We hypothesised that stimulating both afferent systems (chemoreceptor and hypothalamus) to the ventilatory centers in the brainstem, will yield a better ventilatory stimulation.

The aim of this study was to determine to what extent combined treatment with MPA-ACET ameliorates hypoxia and hypercapnia, and to compare the results with a single drug treatment, in a group of hypercapnic COPD patients.

6.3. METHODS

Study design and patients

Twenty-three hypercapnic out-patients with stable severe COPD, as defined by the European Respiratory Society [18], were enrolled in this study. Inclusion criteria were: daytime arterial PCO_2 ($PaCO_2$) ≥ 6.0 kPa and ventilatory pump reserve. The latter was measured prior to supplying the respiratory stimulants, by a voluntary hyperventilation test with capnography. Exclusion criteria were: non-stable COPD, i.e. exacerbation in the last three months, long term oxygen treatment, abnormal renal and liver functions, use of respiratory stimulating drugs and obstructive sleep apnea/hypopnea syndrome. Six patients were withdrawn because of an exacerbation during the study. The remaining seventeen patients received optimal medication, consisting of bronchodilators and in some cases loop diuretics and inhaled corticosteroids. The medication remained unchanged during the study period

Study protocol

In a double blind, double dummy, randomized study the effects of a two week treatment of ACET (250 mg b.i.d.) or MPA (30 mg b.i.d.), followed by a combination of MPA-ACET on

daytime and nocturnal ventilatory and blood gas parameters were studied The study design is shown in figure 1 The patients were studied four times over a period of six weeks at the beginning of the study and before a change of medication, after introduction of the placebo, single or combined drug treatment, at intervals of two weeks (study points T1-4 fig 1) The measurements consisted of nocturnal measurements followed by daytime tests All patients received a placebo during the first two weeks to determine the intra-individual variability in the outcome parameters After the second measurements (T2) patients received either MPA (30 mg) + placebo (arm I) or ACET (250 mg) + placebo (arm II) twice a day (b i d) Over the last study period all patients received combined treatment with MPA (30 mg bid) and ACET (250 mg bid), all in identical capsules

At one and three months after the end of the last treatment, hypercapnic ventilatory response and arterial blood gas values were measured in nine and seven patients respectively, to observe possible sustained effects of MPA-ACET Written informed consent was obtained from all patients and the study was approved by the Hospital Ethics Committee

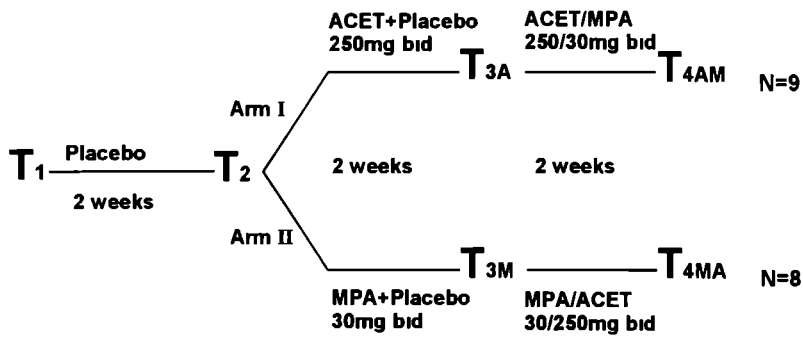

Fig 1 Study design
Tx = measurement Interval between every measurement was two weeks MPA = medroxyprogesterone acetate, ACET= acetazolamide T1= baseline measurements, T2 = measurements after 2 weeks placebo, T3A = measurements after 2 weeks acetazolamide + placebo, T3M = measurements after 2 weeks medroxyprogesterone acetate + placebo , T4AM = measurements after 2 weeks acetazolamide + placebo followed by 2 week treatment with acetazolamide and medroxyprogesterone acetate and T4MA = measurements after 2 weeks medroxyprogesterone acetate + placebo followed by 2 week treatment with and medroxyprogesterone acetate and acetazolamide

Daytime measurements

All daytime measurements were performed on the morning after the nocturnal measurement

-Arterial blood gas values

After each study night at 9 a m an arterial blood sample was taken after 15 minutes of rest (Ciba-Corning 278 blood gas analyser, Houten, The Netherlands)

-Pulmonary function tests

Spirometric tests, including inspiratory vital capacity (IVC), total lung capacity (TLC), residual volume (RV) and forced expiratory volume in 1 second (FEV_1) were performed with a wet spirometer and He dilution technique (Pulmonet III, SensorMedics, Anaheim, CA, USA) Reference values were derived from ERS standards [19]

-Pressure measurements

Maximal inspiratory and expiratory mouth pressure (PI_{max} and PE_{max}) were measured as described by Wilson *et al* [20]

-Hypercapnic ventilatory response (HCVR) and Hypoxic ventilatory response (HVR)

The HCVR was assessed by the steady-state method [21] The patient was connected to a closed spirometric circuit (Pulmotest, Godart, Bilthoven, The Netherlands) via a mouthpiece A Rudolph valve 2700 (Kansas City, USA) was placed in the system, to maintain a one way circuit The end-tidal carbon dioxide tension ($P_{ET}CO_2$) level was measured by a side stream capnograph (Drager, Lubeck, Germany) Inspiratory PCO_2 could be raised by adjusting a

three-way valve, partly short-circuiting the CO_2 absorber in the inspiratory limb of the circuit Two different levels of $P_{ET}CO_2$ were determined ($P_{ET}CO_2$ at zero P_ICO_2, and $P_{ET}CO_2$ at 1-1 5 kPa above resting value) and the slope ($\Delta V'/\Delta P_{ET}CO_2$) and x-intercept B were calculated, according to the equation of the steady state relation of ventilation and $P_{ET}CO_2$ at constant $P_{ET}O_2$

$$V'_E = S(P_{ET}CO_2 - B)$$

Each level of $P_{ET}CO_2$ was maintained for at least 7 minutes Oxygen was added to the system to keep arterial oxygen saturation (SaO_2) constant at levels >90% SaO_2 was measured with a pulse oximeter (Oxyshuttle, SensorMedics, Anaheim, California, USA) At the end of the steady state period of the HCVR an arterialized capillary blood sample was taken Mouth occlusion pressure ($P_{0.1}$) was measured during the final 2 minutes of each steady state period at the two different levels of PCO_2 using a solenoid valve, in the inspiratory part of the circuit

The HVR was performed by inducing progressive isocapnic hypoxia $P_{ET}CO_2$ was maintained at the pre-drug level (T2, placebo treatment) during all HVR tests, by adding CO_2 to the inspirate, when necessary All patients started the test at normoxia SaO_2 level of >95% by adding an adequate amount of oxygen Next, inspiratory O_2 was decreased by stopping the oxygen supplementation and the HVR test was performed until SaO_2 reached 80%

During the recording of both the HCVR and HVR, the $P_{ET}CO_2$, breathing frequency (f), tidal volume (V_T), ventilation (V'_E), SaO_2 and $P_{0.1}$ were measured and stored on an analogue chart recorder (BD101, Kipp & zonen, Delft, The Netherlands)

Nocturnal measurements

The arterial oxygen saturation (SaO_2) was measured by a pulse oximeter The baseline SaO_2 awake was defined as the mean SaO_2 during the first 15 minutes of the recording, when the patient was awake and in supine position The $P_{ET}CO_2$ was measured by sampling air through a naso-pharyngeal cannula inserted via the nose and connected to the sampling capnograph (Mijnhardt capnolyser, Bilthoven, The Netherlands) Oxygen saturation, and $P_{ET}CO_2$ signals during the night were stored on a computer (Compaq 4/66, Houston, TX, USA)

Subjective parameters

After each study period patients were asked for side effects of the drugs The dyspnea sensation was analysed with the modified Medical Research Council (MRC) scale [22]

Statistical analysis

In this study four repeated measurements were made, at the beginning and at the end of each period (T_1 to T_4) Data are presented as mean ± sem In order to test whether the effect of the combined treatment was different in both arms of the study (arm-effects), we compared the differences ($T_{1\text{-}4AM}$- T_2 arm I) and ($T_{1\text{-}4MA}$ - T_2, arm II) using the Mann-Whitney-U-test After showing that there was no significant intra-individual variability by comparing T_1 and T_2 and that there were no arm effects at T_4 the data obtained in the placebo situation were compared with those after combined treatment with MPA-ACET Combination therapy was compared with single drug treatment (Wilcoxon signed rank tests) A Bonferoni correction was used because of multiple comparisons, a $p<0.025$ was considered statistically significant For all analysis SPSS version 6 1 3 was used (SPSS inc, Chicago, 1995)

6.4. RESULTS

The anthropometric characteristics, baseline respiratory function data, acid base status and nocturnal parameters of the patients in both arms are summarized in table 1 After placebo, no significant differences were observed in ventilatory and blood gas parameters as well as nocturnal measurements compared to baseline (T_2 -T_1) Hence, there was no significant intra- individual variability, and the data of the placebo measurements were used as reference values in the analysis of the effects of ACET, MPA and the combined drug treatment No arm-effects were found with MPA-ACET (table 2), therefore the data of the combined treatment of both arms were analysed as one group Furthermore, placebo *vs* single treatments with either MPA (n=8) or ACET (n=9) were compared to placebo *vs*

combined treatment with MPA/ACET (n=17) and combined treatment was compared to single treatment

	arm I (n=9)	arm II (n=8)
age (years)	66 ± 2	69 ± 2
weight (kg)	83 ± 8	70 ± 5
height (m)	1 72 ± 0 04	1 65 ± 0 03
Body Mass Index (kg m^{-2})	28 ± 2	26 ± 2
FEV_1 (%pred)	37 ± 6	30 ± 4
FEV_1/VC (%pred)	46 ± 7	48 ± 6
TLC (%pred)	100 ± 6	92 ± 7
IVC (%pred)	80 ± 4	68 ± 1
RV (%pred)	145 ± 20	137 ± 10
pH	7 39 ± 0 01	7 41 ± 0 01
PaO_2(kPa)	8 2 ± 0 4	8 4 ± 0 5
$PaCO_2$(kPa)	6 6 ± 0 1	6 3 ± 0 2
BE (mmol L^{-1})	4 3 ± 1 0	4 6 ± 0 9
SaO_2 (%)	90 0 ± 1 0	91 1 ± 1 6
nocturnal parameters		
mean SaO_2(%)	84 8 ± 2 0	86 7 ± 2 6
% time SaO_2<90% (%)	72 2 ± 12 8	48 9 ± 15 3
Lowest SaO_2 (%)	70 5 ± 3 2	72 1 ± 4 2
Mean $P_{ET}CO_2$(kPa)	6 1 ± 0 3	5 0 ± 0.3

Table 1 Baseline characteristics (T_1)
Data are presented as mean ± sem %pred = percentage of predicted value [19]

parameter	$X_{1\text{-}4AM} - X_2$	$X_{1\text{-}4MA} - X_2$	P
$\Delta PaCO_2$ (kPa)	-1 0 ± 0 2	-1 4 ± 0 2	0 09
ΔPaO_2 (kPa)	1 4 ± 0 2	2 2 ± 0 4	0 06
$\Delta V'_E$ (L min^{-1})	0 5 ± 0 3	0 6 ± 0 4	0 82
$\Delta P_{0.1}$ (kPa)	0 3 ± 0 1	-0 2 ± 0 1	0 41

Table 2 Analysis of arm-effects
Data are presented as mean ± sem

Daytime blood gas parameters

Single treatment

Compared to the placebo, as shown in table 3, ACET significantly decreased $PaCO_2$, pH and Base Excess (BE) and improved PaO_2 and SaO_2 values MPA increased PaO_2 and SaO_2 and decreased $PaCO_2$ and BE

Combined treatment

During combined treatment, patients became normocapnic and almost normoxic compared to the placebo treatment (table 3)
Analysis of differences between combined and single treatment resulted in a significant difference in pH (combined vs MPA), PaO_2 and BE (combined vs both single drug treatments) and $PaCO_2$ (combined vs ACET) (table 3)

	PLACEBO (T2) (n=17)	ACET (T_{3A}) (n=9)	MPA (T_{3M}) (n=8)	MPA-ACET (T4) (n=17)
pH	7 39 ± 0 01	7 34 ± 0 01*	7 40 ± 0 01*	7 36 ± 0 01*‡
PaO_2 (kPa)	7 9 ± 0 3	8 5 ± 0 2*	8 9 ± 0 6*	9 6 ± 0 4*†‡
$PaCO_2$(kPa)	6 5 ± 0 2	6 1 ± 0 2*	5 8 ± 0 3*	5 3 ± 0 2*†
BE (mmol L^{-1})	4 5 ± 0 7	0 8 ± 1 3*	2 6 ± 1 0*	-2 4 ± 0 8*†‡
$P(a\text{-}_{ET})CO_2$ (kPa)	0 7 ± 0 2	0 6 ± 0 2	0 7 ± 0 2	0 7 ± 0 2
SaO_2(%)	89 0 ± 1 3	90 6 ± 0 8*	92 0 ± 1 7*	92 3 ± 1 0*
nocturnal parameters				
Mean $P_{ET}CO_2$(kPa)	5 5 ± 0 2	5 2 ± 0 3	4 6 ± 0 3	4 1 ± 0 2*†‡
Mean SaO_2 (%)	85 5 ± 1 6	87 2 ± 1 3	88 6 ± 2 3	90 2 ± 1 0*†‡
Lowest SaO_2(%)	70 9 ± 2 9	74 9 ± 3 1*	80 1 ± 3 3	78 6 ± 1 8*
% time SaO_2<90%	61 8 ± 9 9	63 4 ± 13 4	46 8 ± 16 7	42 0± 10 4*

Table 3 Effect of Medroxyprogesterone acetate and Acetazolamide on daytime and nocturnal parameters
Data are presented as mean ± sem $P(a\text{-}_{ET})CO_2$ = arterial-to-end-tidal PCO_2 gradient *p < 0 025 any treatment versus placebo, †p< 0 025 combined treatment vs ACET ‡p< 0 025 combined treatment vs MPA

Ventilatory parameters

The main results of the ventilatory data including HCVR and HVR are shown in table 4 Ventilation did not increase after single treatment but did significantly increase after combined therapy compared to the placebo

The slope of the ventilatory CO_2 response curve significantly increased with combination therapy compared to the placebo (fig 2) The slope of the hypoxic ventilatory response ($S_{HVR}=\Delta V'_E/\Delta SaO_2$) and the $P_{0.1}$ response to hypoxia, expressed as function of SaO_2 ($S_{HP0.1}=\Delta P_{0.1}/\Delta SaO_2$) increased with combined treatment, both compared to placebo No significant changes were found with the single drug therapy $S_{HP0.1}$ expressed as function of V'_E resulted in a correlation coefficient of r=0 98

A significant increase in maximal inspiratory mouth pressure was found with combination therapy compared to placebo

	PLACEBO (T_2) (n=17)	ACET (T_{3A}) (n=9)	MPA (T_{3M}) (n=8)	MPA-ACET (T_4) (n=17)
V'_E ($L.min^{-1}$)	9.3 ± 0.5	9.5 ± 0.9	10.2 ± 0.6	11.2 ± 0.8*†
V_T (L)	0.6 ± 0.06	0.8 ± 0.2	0.6 ± 0.04	0.7 ± 0.1*
$P_{0.1}$ at rest (kPa)	0.5 ± 0.04	0.5 ± 0.03	0.6 ± 0.05	0.5 ± 0.04
S_{HCVR} ($L.min^{-1}.kPa^{-1}$)	3.7 ± 0.6	5.5 ± 1.4	4.8 ± 1.4	5.8 ± 1.0*
B_{HCVR} (kPa)	2.9 ± 0.5	3.6 ± 0.4	2.8 ± 0.5	2.9 ± 0.3
$S_{HCP0.1}$ ($kPa.kPa^{-1}$)	0.3 ± 0.04	0.2 ± 0.04	0.4 ± 0.08	0.4 ± 0.04
$B_{HCP0.1}$ (kPa)	4.2 ± 0.3	3.7 ± 0.3	3.6 ± 0.4	2.9 ± 0.3
S_{HVR} ($L.min^{-1}/\%$)	-0 13 ± 0.03	-0.20 ± 0.05	-0.40 ± 0.11	-0.40 ± 0.07*
$S_{HP0.1}$ (kPa/%)	-0.01 ± 0.0	-0.01 ± 0.0	-0.02 ± 0.01	-0.03 ± 0.01*
$P_{I_{max}}$ (kPa)	5.7 ± 0.5	6.3 ± 0.8	5.4 ± 0.5	6.7 ± 0.7*
$P_{E_{max}}$ (kPa)	9.3 ± 0.7	10.0 ± 1.1	8.6 ± 0.8	9.6 ± 0.7

Table 4. Effect of Medroxyprogesterone acetate and Acetazolamide on ventilatory parameters and respiratory muscle strength.
$P_{0.1}$ at rest (kPa) = mouth occlusion pressure at rest; S_{HCVR} ($\Delta V'_E/\Delta PaCO_2$) = slope of the ventilatory CO_2 response curve; B_{HCVR} (x-intercept) = apnoeic threshold or extrapolated $P_{ET}CO_2$ at zero ventilation; $S_{HCP0.1}$ ($\Delta P_{0.1}/\Delta PaCO_2$) = slope of the mouth occlusion response to CO_2; $B_{HCP0.1}$ = x-intercept of the mouth occlusion pressure response to CO_2. S_{HVR} ($\Delta V'_E/\Delta SaO_2$) = slope of the hypoxic ventilatory response. $S_{HP0.1}$ ($\Delta P_{0.1}/\Delta SaO_2$) = slope of the mouth occlusion response to hypoxia. $P_{I_{max}}$= inspiratory mouth occlusion pressure, $P_{E_{max}}$= expiratory mouth occlusion pressure.

Nocturnal measurements

Single treatment

As shown in table 3, the lowest nocturnal SaO_2 increased significantly with single drug therapies

Combined treatment

In comparing the combined treatment with the single drug and the placebo treatments, there was a significant decrease in $P_{ET}CO_2$ and a significant improvement of SaO_2 values. In comparing only the placebo with the combined treatment, the lowest SaO_2 values were improved and the percentage of time SaO_2 <90% was significantly reduced (table 3)

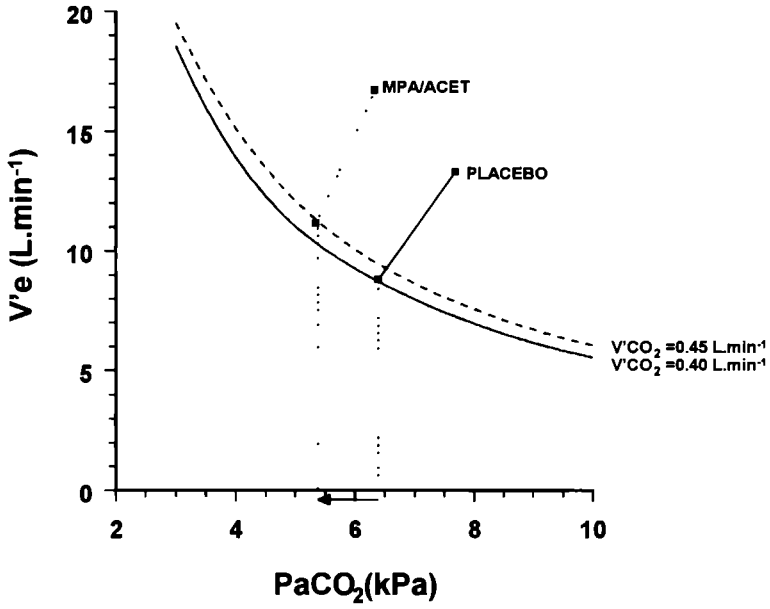

Fig 2 Effect of combined therapy with Medroxyprogesterone acetate and Acetazolamide on the ventilatory control system. The continuous straight line of the ventilatory CO_2 response curve represents the placebo situation and the dashed lines the situations during combination therapy (MPA/ACET). The continuous line of the metabolic hyperbola represents the situation with placebo, the dashed metabolic hyperbola the one after combination therapy (MPA/ACET).

Subjective Parameters

Single treatment

During single MPA therapy 4 patients reported gastro-intestinal discomfort and 4 patients fatigue. In the single ACET-group two patients complained of gastro-intestinal discomfort, one patient of headaches and three patients complained of fatigue. Dyspnea sensation (MRC-scale) remained unchanged: 2.06 ± 0.18 at placebo to 2.00 ± 0 33 with ACET therapy and to 2.88 ± 0.23 during MPA therapy.

Combined therapy

During combined treatment gastro-intestinal complaints (n=3), headache (n=1), fatigue (n=3) and paraesthesia (n=6) were reported. The dyspnea score did not change significantly (2.47 ± 0.19).

Off-treatment

At one month and three months after discontinuing the combined treatment, no significant differences were found between placebo and the off-treatment data (table 5).

PARAMETER	PLACEBO	1 MONTH AI	3 MONTHS AI
		n=9	n=7
PaO_2(kPa)	7 9 ± 0.3	9.0 ± 0.4	7.8 ± 0.5
$PaCO_2$(kPa)	6.5 ± 0.2	5.9 ± 0.1	6.6 ± 0.4
V'_E (L min^{-1})	9.3 ± 0.5	9.3 ± 1.4	11.2 ± 1.7
S_{HCVR} (L.min^{-1}.kPa^{-1})	3 7 ± 0.6	6.7 ± 1 6	4 1 ± 1 9

Table 5. Off-treatment effects
Data are presented as mean ± sem AI = after interruption ACET/MPA treatment

6.5. DISCUSSION

The present study showed that in hypercapnic patients with COPD, combination therapy with MPA-ACET normalized daytime blood gas values and improved night-time and ventilatory parameters. The improvement was greater with the combined drug treatment than with either of the single drug treatments.

Daytime blood gas parameters

ACET and MPA given as a single drug, decreased the daytime $PaCO_2$ (0.4 kPa (6%) and 0.7 kPa (11%), respectively) and increased PaO_2(0.6 kPa (9%) and 1.0 kPa (13%), respectively). The effect of combined treatment was additive; $PaCO_2$ decreased 1.2 kPa (19%) and PaO_2 increased 1.7 kPa (23%), reaching normocapnic and almost normoxic values. The findings on monotherapy are not new and in agreement with the literature [7, 14, 23, 24], in contrast to the results of combination therapy, which has not been shown before. The reason for the improvement of combination therapy might be that MPA and ACET both slightly increased V'_E, whereas a combination of both drugs significantly increased V'_E by 1 9 $L.min^{-1}$, which was more than the sum of the increase in V'_E with single drug treatment.

Hypercapnic ventilatory response (HCVR)

The slope of the HCVR increased significantly with MPA-ACET therapy, whereas with the single drug treatments no significant change were observed The latter agrees with some existing data [14], although an increase in HCVR on monotherapy was found by others [7, 23]. The fact that combined treatment with MPA-ACET on the HCVR and on the blood gas parameters was slightly better than the single drug treatment suggests that combination therapy was additive. This also suggests that there is no interaction between the ventilatory drive from the chemoreceptors and the drive mediated by the hypothalamus.

The ventilatory control system as graphically shown in figure 2 consists of two parts: (a) the linear ventilatory response to CO_2, which represents the controller or controlling system with $PaCO_2$ as input parameter and minute ventilation as output parameter, and (b) the metabolic hyperbola which represents the controlled system and depends on the metabolic CO_2-production. The controlled system comprises of the gas exchanging process in the lungs, with input parameter minute ventilation and output parameter the $PaCO_2$. In a spontaneously breathing man, in the so-called "closed loop" situation, the working-point is represented by the intersection of both curves: the output of the controlled system ($PaCO_2$)

is the input of the controller and the output of the controller (minute ventilation) is the input of the controlled system In our model we calculated a resting CO_2-production of 400 ml min ^{1}, which increased during MPA to a calculated 450 ml min ^{1}, based on the actual values of V'_E and $PaCO_2$ of our patient groups

It is important to recognize whether the chronic hypercapnia is due to neuromuscular weakness, the excessive work of breathing ("Can't Breathe") or due to inadequate drive from the ventilatory centers in the CNS ("Won't breathe") According to the metabolic hyperbola, a substantial decrease in $PaCO_2$ of 1 2 kPa can be generated by a very small increase in ventilation of 1 9 L min ^{1} during chronic hypercapnia

Therefore, respiratory stimulants like ACET and MPA can be considered for hypercapnic patients with severe COPD, as also discussed by Teppema [25], because they are able to improve blood gas values considerably with only minimal changes in ventilation and without an increase in dyspnea sensation

Hypoxic ventilatory response (HVR)

We found that the hypoxic ventilatory response was not augmented by single drug treatment, whereas combination therapy increased HVR In the current literature, the effects of MPA and ACET on the HVR are confounded by the fact that the same pre- and post-drug levels of $P_{ET}CO_2$ were not always maintained [7, 26] HVR is not only determined by PO_2 but also by the prevailing PCO_2 This is well illustrated by the study of Tojima *et al* [26], who showed that the effect on HVR measured during acetazolamide-induced hypocapnia did not significantly increase, whereas responses to hypoxia measured under pre-drug $P_{ET}CO_2$ levels did increase significantly

Vos *et al* [7] showed no significant increase in HVR with chlormadinone acetate (CMA), which was measured at hypocapnic post-drug level We used pre-drug $P_{ET}CO_2$ levels in this study Both ACET and MPA probably have an effect on the peripheral chemoreceptors, whereas progesterone induces an increase in metabolic rate, which in turn may be responsible for the increase in HVR [27] The lower BE (metabolic acidosis) will also contribute to the increased HVR during the combined treatment

Whitelaw [28] introduced $P_{0.1}$ measurement as an output parameter of the controlling part of the respiratory control system, because in COPD patients ventilation as an output parameter can be influenced by chronic airway obstruction $P_{0.1}$ is a measure of neuromuscular drive, minimizing the influences of airway obstruction The $P_{0.1}$ values at rest, measured in our COPD patients, were comparable with those described in other studies [14,28]

The $P_{0.1}$-SaO_2 response slope was augmented by combination therapy, whereas single drug treatment only caused a slight, non-significant, increase Apparently, in our COPD patients the measurement of $P_{0.1}$ as an output parameter of the respiratory controlling system, is comparable to V'_E as shown by the high correlation coefficient

The combination of MPA and ACET showed a significant increase in inspiratory mouth pressure A possible mechanism could be an increase in central and voluntary drive, although this could not be confirmed with the rare data in the literature [29] Single drug treatment did not improve respiratory muscle function and for MPA this was shown by Contreras [30]

Nocturnal measurements

In COPD patients MPA and ACET are proven to have a beneficial effect on central sleep apnea and oxygenation, respectively [8, 9, 13, 17] In the present study we found an improvement of mean nocturnal $P_{ET}CO_2$, SaO_2 and lowest SaO_2 and a decrease of the percentage of time SaO_2 <90% It remains to be established whether nocturnal desaturation needs to be treated at all [31]

One month after discontinuation of the combined treatment with MPA-ACET, all parameters which were found to have changed significantly had returned to baseline values Verbraecken *et al* [17] showed in five out of eight patients with central sleep apneas who were six months after cessation of ACET, that the number of central apneas were still reduced, whereas the number of obstructive apneas and hypopneas did not significantly differ from the selection night They argued that ACET induces a resetting of the central chemoreceptor drive, which may continue after stopping the drug therapy We could not confirm this finding, as our patients have only been treated for a period of four weeks,

whereas theirs were treated for five months Furthermore, we studied hypercapnic COPD patients instead of patients with non-hypercapnic central sleep apnea syndrome

In conclusion, the present study shows that combined treatment with MPA-ACET, in hypercapnic COPD patients, normalizes arterial blood gas values and improves nocturnal saturation and the chemical drive The combination of MPA-ACET is more favourable than either single drug treatments

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Chapter 7. Summary and conclusions.

7.1. Introduction.

Some patients with severe chronic obstructive pulmonary disease (COPD) are hypercapnic and hypoxic, due to respiratory pump failure Hypoxia influences life expectancy Several studies have shown a beneficial effect of long term oxygen therapy (LTOT) on survival However, LTOT is an expensive and cumbersome treatment Furthermore, LTOT may lead to an increase in hypercapnia due to increased ventilation-perfusion mismatching and suppression of the hypoxic ventilatory drive Hypercapnia by itself may independently influence survival adversely Therefore, other therapeutic options than LTOT or in addition to LTOT such as respiratory stimulants, could be considered

In this thesis the short-term effects on the ventilation of the respiratory stimulants acetazolamide (ACET), a carbonic anhydrase inhibitor and medroxyprogesterone acetate (MPA), a synthetic progesterone, were studied as monotherapy and in a combined treatment

In Chapter 1 the physiology and measurements of the control of breathing are described The major respiratory stimulants used in COPD patients and the objectives of the current thesis are outlined

The thesis can be divided into two parts

Part I consists of three animal studies The specific questions in these studies were

- What dose of ACET does not impede CO_2-transport by inhibition of the erythrocytic carbonic anhydrase?
- What is the effect of low dose ACET on the control of breathing during normoxia? (Chapter 2)
- What is the effect of low dose ACET on the control of breathing under hypoxic conditions? (Chapter 3)
- Is there an interaction (supra-additive effect) between the ventilatory effects of ACET and MPA? (Chapter 4)

Part II consists of two human studies On the basis of the animal studies the specific questions were

- What is the effect of 250 mg twice daily (b i d) ACET(equivalent to the optimal dose found in chapter 2) and 30 mg b i d MPA as monotherapy in hypercapnic COPD patients? (Chapter 5)
- Does a combined therapy with ACET and MPA (thus influencing the ventilation via chemoreceptors as well as via hypothalamic pathways) provide a better ventilatory stimulation and better treatment of hypoventilation in patients with severe COPD, than single drug therapy? (Chapter 6)

7.2 Transition from animal model to human studies

Acetazolamide (ACET)

- In cats a low dose of 4 mg kg^{-1} ACET does not cause an arterial-to-end-tidal PCO_2 gradient, indicating no complete inhibition of erythrocytic carbonic anhydrase The acute effects of ACET on ventilation in the animal studies were performed with this dose Extrapolation to human doses is in fact precarious, since (1) in humans we have studied the "chronic effects" of ACET (2 weeks therapy) It is known from literature data that the effects of acute vs chronic administration of ACET on the ventilation may be different (2) We are not precisely informed about the distribution of the drug to intra and extracellular compartments of the human body compared to the situation in animals In the literature no dose response curves are available leading to the answer what dose one should use in a chronic situation in humans In the human studies in the present thesis a dose of 250 mg twice daily (b i d) ACET was administered based on extrapolation of the dose found in the animal study and on literature data (3) The animal studies were performed on anaesthetized cats, whereas the human studies were evidently in unanaesthetized condition Anaesthesia influences ventilation and may likewise influences the ventilatory responses to drugs

- The findings of the animal and human studies seem not consistent In the animal studies the sensitivities of the peripheral and central chemoreflex loops and the apnoeic threshold decreased after administration of ACET (open loop situation), whereas in human studies a tendency of increase of the sensitivity of the ventilatory CO_2 response curve was found Extrapolation to the ventilation using the equation, $V'_I = S(PaCO_2-B)$ would imply that in cats ventilation only increases in relative hypocapnia after administration of the drug in contrast to the human studies However, all studies were performed in an open loop situation Considering the closed loop situation, the working point of the ventilatory control system changes along the metabolic hyperbola (the representation of the controlled system) Considering this, the acute effect of 4 mg kg^{-1} ACET, both intravenously and orally administered is the same a decrease in $PaCO_2$ with a small increase in ventilation

- A possible explanation for the decrease in slope of the ventilatory CO_2 response curve in the animal studies after ACET administration may be an effect of anaesthesia

- In the animal studies it is argued that the effect of 4 mg kg^{-1} ACET is caused by a direct effect on the peripheral chemoreceptors (carotid bodies), because of an inhibiting effect of the drug on the CO_2 sensitivity of the peripheral chemoreflex loop The effect of the drug on the CO_2 sensitivity of the central chemoreflex loop may not be due to a direct effect on the central nervous system, but rather to an effect on cerebral vessels resulting in an altered relationship between $PaCO_2$ and brain tissue PCO_2 as discussed in chapter 2

- Under hypoxic condition in cats the effect of 4 mg kg^{-1} ACET on the ventilation was confirmed, being a larger effect on the peripheral chemoreceptors

- Starting from the findings in the animal studies, two studies with ACET were performed in hypercapnic COPD patients A dose of 250 mg b i d ACET improved blood gas values to normocapnia and almost normoxia This is important, since literature data suggest that hypercapnia may be an independent determinant of life

expectancy in COPD patients. Whether chronic administration of ACET (>2 weeks treatment) will yield a better life expectancy has to be established in further studies.

Medroxyprogesterone acetate (MPA)

- In cats and in COPD patients MPA leads to a decrease in $PaCO_2$ and an increase in ventilation.

- The mechanisms by which MPA act on the ventilatory control system is probably via two pathways: on one hand the female hormone has a stimulatory action on the peripheral chemoreceptors. On the other hand a central pathway may be active, since data in the literature suggest that the hypothalamus contains progesterone receptors, which may be an input to the ventilatory control mechanism. Whether MPA alone or its metabolites are responsible for the effects, has to be studied further. From the results in this thesis no clear distinction can be made between action via chemical and humoral ventilatory drives.

MPA +ACET

- In cats, combined treatment of (4 $\mu g.kg^{-1}$) MPA and (4 $mg.kg^{-1}$) ACET causes a larger net ventilatory stimulation than either drug alone. The same was found in hypercapnic COPD patients; combined treatment with MPA and ACET improved the day-time and night-time arterial blood gas values and the chemical drive more than compared with single drug treatment. Combination of both drugs works favourably on the nocturnal SaO_2 as compared with single drug treatment.

- Whether MPA modulates the effect of ACET on the central CO_2 sensitivity could not be excluded nor confirmed. However, from the findings in the present thesis as well as what is known from literature data it seems more likely that both agents independently affect the ventilatory control system.

7.3. The effect of low-dose acetazolamide on the ventilatory CO_2 response curve in the anaesthetized cat during normoxia.

Chapter 2 describes a dose response study of intravenous acetazolamide (ACET) in intact and in carotid body denervated cats on the arterial-to-end-tidal PCO_2 ($P(a\text{-}ET)CO_2$) gradient
A low dose of ACET (4 mg kg^{-1}) caused an increase in ventilation of approximately 65% at a feline normocapnic value of a $PaCO_2$ of 4 5 kPa without inducing an $P(a\text{-}ET)CO_2$ gradient, indicating that CO_2-transport was not impeded by complete blockade of erythrocytic carbonic anhydrase Within the first two hours after administration, this small dose caused only a slight decrease in mean standard bicarbonate of 1 8 and 1 7 mmol L^{-1} in intact (n=7) ($p<0$ 05) and in carotid body denervated animals (n=7) ($p>0$ 05), respectively Doses of ACET larger than 4 mg kg^{-1} caused a significant increase in $P(a\text{-}ET)CO_2$ gradient
In addition, in both carotid body intact and in denervated cats the effect of 4 mg kg^{-1} ACET on the central and both central and peripheral chemoreflex loops were studied
In this chapter, as well as in the other animal studies, the dynamic end-tidal forcing technique was used By applying stepwise changes in $PETCO_2$ and by analysing the ventilatory responses with a two compartment model, we were able to separate the contributions from the peripheral and central chemoreflex loops (chapter 1)
As shown in table 1 in carotid body-denervated cats, 4 mg kg^{-1} ACET caused an increase in ventilation of 64% at a $PaCO_2$ of 4 5 kPa A decrease in the CO_2 sensitivity of the central chemoreflex loop (Sc) of 0 56 L min^{-1} kPa^{-1} was observed, whereas the intercept on the $PaCO_2$ axis or apnoeic threshold (B) decreased by 0 3 kPa In carotid body-intact animals, the same dose caused an increase in ventilation of 66% at a $PaCO_2$ of 4 5 kPa The CO_2 sensitivity of the peripheral chemoreflex loop (Sp) decreased by 0 09 L min^{-1} kPa^{-1} Sc and B decreased by 0 68 L min^{-1} kPa^{-1}, and by 1 0 kPa, respectively The latter findings were not significantly different from the changes found in the denervated animals
The effect of ACET on the CO_2 sensitivity of the peripheral chemoreflex loop may be caused by a direct effect on the carotid bodies The effects of the drug on Sc and B may not be due to a direct action on the central nervous system, since from the literature it is known that the drug hardly penetrates into the brain It is argued that the effect of ACET on Sc and B can be due to an effect of the drug on cerebral vessels, which results in an altered relationship between arterial PCO_2 and brain tissue PCO_2

7.4. Effect of low dose acetazolamide on the ventilatory CO_2 response during hypoxia in the anaesthetized cat.

Hypoxia is a frequently observed phenomenon in patients with severe COPD Therefore we have studied the acute effect of acetazolamide (ACET) (4 mg kg^{-1}) on the ventilatory response to CO_2 during hypoxaemia in seven anaesthetized cats (chapter 3) After a bolus of 4 mg kg^{-1} ACET a transient initial fall in ventilation was observed, suggesting a direct action of ACET on the peripheral chemoreceptors, since during hypoxia the contribution of the peripheral chemoreceptors to the ventilation is larger than during normoxia In the subsequent steady state, ACET caused an increase in ventilation As shown in table 1 the drug reduced the CO_2 sensitivities of the peripheral (Sp) and central (Sc) chemoreflex loops with 0 11 and 0 34 L min^{-1} kPa^{-1}, respectively The apnoeic threshold B (x-intercept of the ventilatory CO_2 response curve) decreased by 1 93 kPa (all parameters $p<0.01$) The net result was a stimulation of ventilation of about 50% at a fixed $PaCO_2$ of 4 kPa It is argued that the steady state effect of ACET is probably due to a direct effect on the peripheral chemoreceptors as well as an indirect central effect on the cerebral blood flow regulation as discussed in chapter 2

7.5. Medroxyprogesterone acetate with acetazolamide stimulates breathing in cats.

Chapter 4 describes the effect of medroxyprogesterone acetate (MPA), and MPA followed by acetazolamide (ACET), on the ventilatory CO_2 response curve in eight anaesthetized female cats, which underwent an ovariohysterectomy 1 month prior the study The animals were pre-treated with 17 β-estradiol(E_2), since priming with a standard dose of 10 μg kg^{-1} E_2 is required to achieve a sustained facilitation of phrenic activity by progesterone The effects of 0 4 μg kg^{-1} MPA and 4 mg kg^{-1} ACET on the ventilatory parameters are summarized in table 1 After infusion of 0 4 μg kg^{-1} MPA the ventilation increased by 35% at a fixed $PaCO_2$ of 4 5 kPa Sp was reduced with 0 09 L min^{-1} kPa^{-1} and Sc with 0 13 L min^{-1} kPa^{-1} A decrease of the apnoeic threshold was observed (a decrease in B by 0 38 kPa (all parameters $p < 0.01$)

Subsequent administration of ACET increased ventilation up to a total of 116%(MPA+ACET) at a fixed $PaCO_2$ of 4.5 kPa. Sp and Sc further decreased by 0.04 and 0.18 $L.min^{-1}.kPa^{-1}$, respectively The apnoeic threshold further decreased by 1.18 kPa (all parameters $p < 0.01$). These results imply a more than twofold increase of ventilation at resting PCO_2 (=4.5 kPa) as compared to separate treatment with either one of the drugs. Because both treatments reduced ventilatory CO_2 sensitivity, it is concluded that a stimulating effect on ventilation is due to a decrease in the apnoeic threshold. Combined administration of MPA and ACET will lead to larger increases in ventilation than treatment with either drugs alone.

animal studies (chapter)		V'_I *) (% control)	Sp ($L.min^{-1}.kPa^{-1}$)	Sc ($L.min^{-1}.kPa^{-1}$)	B (kPa)
normoxia (7 3.)					
intact cats	control	100	0.28 ± 0.18	1.52 ± 0.55	4.0 ± 0.5
	ACET	166	0.19 ± 0.12	0.84 ± 0.21	3.0 ± 0.6
CBD cats	control	100	-	1.52 ± 0.42	4.5 ± 0.5
	ACET	164	-	0.96 ± 0.32	4.2 ± 0.7
hypoxia (7.4.)	control	100	0.22 ± 0.08	0.74 ± 0.32	2.88 ± 0.97
	ACET	150**)	0.11 ± 0.03	0.40 ± 0.10	0.95 ± 0.92
normoxia(7.5.)	control	100	0.22 ± 0.09	1.01 ± 0.38	4.02 ± 0.27
	MPA	135	0.13 ± 0.06	0.88 ± 0.32	3.64 ± 0.42
	MPA+ACET	216	0.09 ± 0.06	0.70 ± 0.49	2.46 ± 1.50

Table 1. Summary of the data obtained by analysis of the ventilatory responses to CO_2 from the different animal studies
*) at a fixed $PaCO_2$ =4.5 (kPa), **) at a fixed $PaCO_2$ =4.0 (kPa). Sp, Sc and B data are presented as mean ± SD. CBD cats = carotid body denervated cats.

7.6. Comparison of acetazolamide and medroxyprogesterone as respiratory stimulants in hypercapnic COPD patients.

In Chapter 5 the effects of acetazolamide (ACET) (250 mg b i.d.) and medroxyprogesterone acetate (MPA) (30 mg b.i.d.) on day- and nighttime blood gas values and the influences on the hypercapnic and hypoxic ventilatory and $P_{0.1}$ response were studied in a cross-over design in 12 hypercapnic patients with stable COPD (mean FEV_1 33 ± 4 % pred (mean ± sem)).

As shown in table 2., the daytime $PaCO_2$ was decreased by 0.7 kPa during ACET treatment and by 0.6 kPa during MPA. The daytime PaO_2 improved with ACET by 1.3 kPa, whereas no significant changes were seen with MPA. Mean nocturnal $P_{ET}CO_2$ decreased by both treatments. with 0 9 kPa during ACET and with 1 0 kPa during MPA treatment. The percentage of time that the nocturnal SaO_2 was lower than <90%, was reduced with ACET by almost 50%. Nocturnal saturation parameters did not change with MPA.

Resting minute ventilation increased only with MPA by 1.2 $L.min^{-1}$. The slope of the hypercapnic ventilatory response did not change during ACET and MPA therapy. The hypoxic ventilatory response increased by 0.2 $L.min^{-1}.\%^{-1}$ during ACET and with 0.1 $L.min^{-1}.\%^{-1}$ during MPA. The hypoxic $P_{0.1}$ response improved with ACET treatment by 0.013 $kPa.\%^{-1}$, (all parameters $p<0.05$).

This study shows that ACET and MPA both have favourable effects on day- and nighttime blood gas parameters in hypercapnic COPD patients, however, the effects of ACET are more evident, especially on nocturnal saturation.

7.7. Combined treatment with acetazolamide and medroxyprogesterone in COPD patients

In chapter 6 we describe the second human study, in which the effects of a two week treatment with medroxyprogesterone acetate (MPA) (30 mg b.i.d.) or acetazolamide (ACET) (250 mg b.i.d.) followed by a two week treatment with a combination of both drugs (MPA-ACET) was investigated. The effect on daytime and nocturnal ventilatory and blood gas

parameters was studied in 17 severe stable COPD patients (FEV_1 34 ± 4% pred) in a double blind controlled fashion

Single treatment with ACET and MPA decreased mean daytime $PaCO_2$ by 0 4 and 0 7 kPa, respectively Mean daytime PaO_2 improved with both drugs with 0 6 and 1 0 kPa, respectively

As shown in table 2 , MPA-ACET treatment significantly decreased mean daytime $PaCO_2$ by 1 2 kPa V'_E increased with combined therapy by 1 9 L min^{-1}

The hypercapnic and hypoxic ventilatory responses were significantly increased by MPA-ACET therapy with 2 1 L min^{-1} kPa^{-1} and 0 27 L min^{-1} $\%^{-1}$, respectively The $P_{0.1}$ response to hypoxia increased during the combination therapy by 0 02 kPa $\%^{-1}$ Nocturnal $PETCO_2$ significantly decreased with MPA-ACET treatment by 1 4 kPa MPA-ACET significantly increased mean nocturnal SaO_2 values by 4 7%

We concluded that under hypercapnic conditions combined treatment with ACET and MPA induced a larger improvement in day- and night-time $PaCO_2$ than separate treatments with either drug The increase in ventilation that is necessary to 'blow-off' an excess of CO_2, in the hypercapnic situation, is very limited (approx 2 L min^{-1} in our patients) The combination of MPA-ACET treatment in stable hypercapnic COPD patients is concluded to be effective in a short period Whether chronic administration of both drugs alone or in a combined fashion may alter life expectancy and/or quality needs further to be investigated

human studies (chapter)		single treatment (7.6.)		combined treatment (7.7.)	
	placebo	ACET	MPA	placebo	MPA+ACET
V'_E (L min^{-1})	9.6 ± 0.7	*10.0 ± 0.6*	10.8 ± 0.8	9.3 ± 0.5	11.2 ± 0.8
$PaCO_2$(KPa)	6.3 ± 0.1	5.6 ± 0.2	5.7 ± 0.2	6.5 ± 0.2	5.3 ± 0.2
PaO_2(KPa)	8.7 ± 0.3	10.0 ± 0.4	*9.3 ± 0.4*	7.9 ± 0.3	9.6 ± 0.4
noct. $PETCO_2$(KPa)	5.6 ± 0.3	4.7 ± 0.3	4.6 ± 0.1	5.5 ± 0.2	4.1 ± 0.2
% time noct. SaO_2 <90% (%)	34.9 ± 10.7	16.3 ± 7.5	*23.8 ±11.5*	61.8 ± 9.9	42.0 ± 10.4
S_{HCVR} (L min^{-1}.kPa^{-1})	*4.3 ± 0.6*	*4.1 ± 0.6*	*5.2 ± 0.9*	3.7 ± 0.6	5.8 ± 1.0
S_{HVR} (L.min^{-1}.$\%^{-1}$)	-0.2 ± 0.05	-0.4 ± 0.1	-0.3 ± 0.1	-0.13 ± 0.03	-0.40 ± 0.07
S_{HPO1} (kPa/%)	-0.007 ± 0.002	-0.02 ± 0.003	*-0.01 ± 0.003*	-0.01 ± 0.00	-0.03 ± 0.01

Table 2. Selection of data obtained from the human studies described in chapter 7.5 and 7.6 which changed significantly (Data denoted in *italics* did not significantly change from placebo) Data are presented as mean ± sem.

Chapter 8. Samenvatting en conclusies.

8.1. Inleiding

Sommige patienten met een ernstige chronisch obstructieve longziekte (COPD) zijn hypercapnisch en hypoxisch ten gevolge van het falen van de adempomp Hypoxie beinvloedt de levensverwachting Uit een aantal studies is gebleken, dat de langdurige toediening van zuurstof (LTOT) een gunstig effect heeft op de overleving Echter, LTOT is een dure en omslachtige behandeling Daarnaast kan het gebruik van LTOT leiden tot een toename van de hypercapnie ten gevolge van een toegenomen ongelijkmatigheid van de ventilatie-perfusie verhoudingen en onderdrukking van de hypoxische ademstimulus

Hypercapnie heeft zelf, onafhankelijk van andere factoren, een slechte invloed op de levensverwachting Daarom zouden andere en additionele therapeutische opties naast LTOT, zoals het gebruik van ademhalingsstimulatia, overwogen kunnen worden

In dit proefschrift zijn de korte termijn effecten van de respiratoire stimulantia acetazolamide (ACET), een carbo anhydrase remmer, en medroxyprogesteron acetaat (MPA), een synthetisch progestageen, op de ventilatie beschreven Deze behandelingen zijn als monotherapie en in combinatie gegeven

In hoofdstuk 1 worden de fysiologie en de metingen van de regeling van de ademhaling beschreven De meest gebruikte respiratoire stimulantia die gebruikt worden bij COPD patienten en de doelstellingen van dit proefschrift worden toegelicht

Het proefschrift kan verdeeld worden in twee delen

Deel I bevat drie dierexperimentele studies De specifieke vragen in deze studies waren

- Welke dosis van ACET geeft geen vermindering van het CO_2- transport door remming van het carbo anhydrase in de erythrocyt?
- Wat is het effect van lage dosis ACET op de regeling van de ademhaling tijdens normoxie? (Hoofdstuk 2)
- Wat is het effect van lage dosis ACET op de regeling van de ademhaling tijdens hypoxie? (Hoofdstuk 3)
- Is er een interactie (supra-additief effect) tussen de ventilatoire effecten van ACET en MPA? (Hoofdstuk 4)

Deel II bevat 2 humane studies Op basis van de gegevens uit de dierexperimentele studies waren de vragen

- Wat zijn de effecten van 2 dd 250 mg ACET (equivalent aan de optimale dosis welke gevonden is als beschreven is in hoofdstuk 2) en 2 dd 30 mg MPA als monotherapie bij hypercapnische COPD patienten? (Hoofdstuk 5)
- Geeft gecombineerde behandeling van ACET en MPA (dus het stimuleren van de ventilatie via chemoreceptoren en via de hypothalame weg) een betere stimulatie van de ademhaling en zo een betere behandeling van hypoventilatie bij ernstige COPD patienten dan enkelvoudige behandeling? (Hoofdstuk 6)

8.2. Herleiden van diermodel naar humane studies

Acetazolamide (ACET)

- Een lage dosis ACET (4 mg kg^{-1}) veroorzaakt geen gradient tussen de arteriéle en eind expiratoire PCO2, wat inhoudt dat er geen complete remming is van het carbo anhydrase in de erythrocyt De acute effecten van ACET op de ventilatie werden met deze dosis uitgevoerd Extrapolatie naar doses die gebruikt worden bij mensen is hachelijk, (1) omdat we de "chronische effecten" van ACET (2 weken durende therapie) hebben bestudeerd Uit de literatuur is bekend dat, de effecten van acute vs chronische toediening van ACET op de ventilatie verschillend zijn (2) We weten niet precies hoe de verdeling van het geneesmiddel over de intra- en extra cellulaire compartimenten in het menselijk lichaam is, vergeleken met de situatie in dier modellen Er zijn in de literatuur geen dosis-respons studies beschikbaar die een antwoord kunnen geven op de vraag welke dosis in de chronische situatie gebruikt moet worden Gebaseerd op extrapolatie van de dosis die werd gevonden in de dierexperimentele studies en de gegevens uit de literatuur werd In de humane studies in dit proefschrift een dosis van 2 dd 250 mg ACET gebruikt (3) De dierexperimentele studies werden uitgevoerd op genarcotiseerde proefdieren Narcose veroorzaakt altijd enige ventilatoire depressie, en zal dus mogelijk ook de ventilatoire responsen op de medicatie beinvloeden

- De bevindingen van de dierexperimentele studies en de humane studies lijken niet eenduidig. In de dierexperimentele studies werd een daling gevonden van de gevoeligheden van de perifere en centrale chemoreflex lussen en van het apneupunt na toediening van ACET. Daarentegen werd er in de humane studies een stijgende tendens werd gezien van de gevoeligheden van de ventilatoire CO_2 responscurve. Extrapolatie naar de ventilatie, gebruik makend van de vergelijking $V'_I=S(PaCO_2-B)$ zou betekenen dat de ventilatie in katten alleen toeneemt tijdens relatieve hypocapnie na toediening van het geneesmiddel in tegenstelling tot de humane studies. Echter, alle studies werden verricht in de "open systeem" situatie. Gaan we uit van de "gesloten systeem" situatie, dan verschuift het "werkpunt" langs de metabole hyperbool (het geregelde systeem). Als we hiervan uitgaan is het netto-effect van acute en chronische toediening van 4 $mg.kg^{-1}$ ACET, zowel i.v. als oraal hetzelfde: een daling in $PaCO_2$ en een kleine toename van de ventilatie.

- Een potentiële verklaring voor de daling in helling van de ventilatoire CO_2 respons curve in de dierexperimentele studies na toediening van ACET is mogelijk een anaesthesie effect.

- In de dierexperimentele studies wordt bediscussieerd dat het effect van 4 mg kg^{-1} ACET wordt veroorzaakt door een direct effect op de perifere chemoreceptoren ("glomuslichaampjes"), vanwege een inhiberend effect van het geneesmiddel op de CO_2 gevoeligheid van de perifere chemoreflex lus. Het effect van het geneesmiddel op de CO_2 gevoeligheid van de centrale chemoreflex lus is mogelijk niet het gevolg van een direct effect op het centraal zenuwstelsel, maar een effect op de cerebrale vaten resulterend in een veranderde relatie tussen de $PaCO_2$ en de PCO_2 van het hersenweefsel hetgeen bediscussieerd wordt in hoofdstuk 2.

- Het effect van 4 $mg.kg^{-1}$ ACET op de ventilatie onder hypoxische condities in de kat werd bevestigd, zijnde een groter effect op de perifere chemoreceptoren, die verhoogd actief zijn in hypoxie.

- Uitgaande van de bevindingen uit de dierexperimentele studies werden twee humane studies met ACET uitgevoerd in COPD patienten met een totale respiratoire insufficientie Een dosis van 2 dd 250 mg ACET gaf een verbetering van de bloedgas waarden tot normocapnie en bijna normoxie Dit is een belangrijke bevinding, omdat uit de literatuur blijkt dat hypercapnie een onafhankelijke factor is voor de levensverwachting van COPD patienten Of chronische toediening van ACET (>2 weken behandeling) zal leiden tot een betere levensverwachting zal moeten worden bevestigd in toekomstige studies

Medroxyprogesteron acetaat (MPA)

- Zowel bij katten als bij COPD patienten leidt de toediening van MPA tot een verlaging in $PaCO_2$ en tot een toename van de ventilatie

- Het mechanisme waardoor MPA zijn effect heeft op de ventilatie is mogelijk via twee wegen enerzijds heeft het vrouwelijke geslachtshormoon zijn werking via de perifere chemoreceptoren Anderzijds is een centrale weg beschikbaar, omdat uit literatuur gegevens blijkt dat de hypothalamus progesteronreceptoren bevat, die de ademhaling stimuleren Of MPA of de metabolieten van MPA verantwoordelijk zijn voor de gevonden effecten moet nog verder worden bestudeerd Uitgaande van de gevonden effecten uit dit proefschrift kan geen onderscheid gemaakt worden tussen een effect via de chemische of humorale ademstimulus

MPA +ACET

- Gecombineerde behandeling met (4 µg kg^{-1}) MPA en (4 mg kg^{-1}) ACET in katten veroorzaakt een grotere netto-ventilatoire stimulatie dan separate toediening van beide geneesmiddelen Dit werd ook gevonden bij hypercapnische COPD patienten, gecombineerde behandeling met MPA en ACET gaf een grotere verbetering van de arteriele bloedgas waarden overdag en 's nachts als ook van de chemische "drive"

(gevoeligheid van de centrale en perifere chemoreceptoren) in vergelijking met separate toediening van beide geneesmiddelen. Gecombineerde behandeling met beide geneesmiddelen gaf een verbetering van de nachtelijke SaO_2 in vergelijk met enkelvoudige behandeling met beide geneesmiddelen.

- Of MPA het effect van ACET op de centrale CO_2 gevoeligheid moduleert kon noch ontkend noch bevestigd worden Echter, uitgaande van de bevindingen welke in dit proefschrift beschreven zijn, gecombineerd met de gegevens uit de literatuur is het aannemelijk dat de beide geneesmiddelen onafhankelijk van elkaar werken op de regeling van de ademhaling.

8.3. De effecten van lage dosis acetazolamide op de ventilatoire CO_2 respons curve in de genarcotiseerde kat.

In hoofdstuk 2 wordt een dosis respons studie van intraveneuze toediening van ACET beschreven, in intacte katten en in katten met gedenerveerde perifere chemoreceptoren, op de gradiënt tussen de arteriële en eind expiratoire PCO_2
Een lage dosering ACET (4 $mg.kg^{-1}$) veroorzaakt een toename van de ventilatie van ongeveer 65% bij een $PaCO_2$ van 4.5 kPa, zonder een gradiënt te veroorzaken tussen de arteriële en eind-expiratoire PCO_2 ($P(a\text{-}_{ET})CO_2$. Dit betekent dat het CO_2 transport
nog niet belemmerd wordt door het compleet remmen van het erytrocyten carbo anhydrase. In de eerste twee uur na toediening veroorzaakte deze lage dosering een lichte daling van het standaard bicarbonaat van respectievelijk 1.8 en 1.7 $mmol.L^{-1}$ in respectievelijk intacte katten ($n=7$) ($p<0.05$) en in katten met gedenerveerde perifere chemoreceptoren ($n=7$) ($p>0.05$). Doseringen hoger dan 4 $mg.kg^{-1}$ veroorzaakten een significante $P(a\text{-}_{ET})CO_2$ gradiënt.
Aansluitend werd in zowel katten met gedenerveerde perifere chemoreceptoren als in intacte katten het effect van 4 $mg.kg^{-1}$ ACET op de centrale en zowel centrale als perifere chemoreflexen bestudeerd.
In dit hoofdstuk, als ook in de andere dierexperimentele studies, werd de "dynamische eind-expiratoire koolzuur sturings" techniek (DEF-techniek) gebruikt. Deze techniek maakt het mogelijk een scheiding aan te brengen tussen de bijdrage van de perifere en centrale

chemoreflex lussen door ventilatoire responsen te analyseren die verkregen worden door stapvormige veranderingen aan te brengen in de $P_{ET}CO_2$

Zoals te zien is in tabel 1 veroorzaakte een dosis van 4 mg kg^{-1} ACET in katten met gedenerveerde perifere chemoreceptoren een stijging in ventilatie van 64% bij een normocapnische waarde voor katten met een $PaCO_2$ = 4 5 kPa Er werd een daling van de CO_2 gevoeligheid van de centrale chemoreflex (Sc) waargenomen van 0 56 L min^{-1} kPa^{-1} Het intercept op de $PaCO_2$-as, ofwel het apneupunt (B) daalde met 0 3 kPa In intacte katten veroorzaakte dezelfde dosis een stijging in ventilatie van 66% bij een $PaCO_2$=4 5 kPa De CO_2 gevoeligheid van de perifere chemoreflex (Sp) daalde met 0 09 L min^{-1} kPa^{-1} Sc en B namen af met respectievelijk 0 68 L min^{-1} kPa^{-1} en 1 0 kPa De laatste bevindingen waren niet significant verschillend van de bevindingen die werden gevonden in katten met gedenerveerde perifere chemoreceptoren Het effect van ACET op de perifere chemoreflex wordt waarschijnlijk veroorzaakt door een direct effect op de perifere chemoreceptoren ("glomuslichaampjes") De effecten van het geneesmiddel op Sc en B worden waarschijnlijk niet veroorzaakt door een direct effect op het centrale zenuwstelsel, omdat uit literatuur gegevens blijkt dat ACET de bloed-liquor barriere vrijwel niet passeert Er wordt bediscussieerd dat het effect van ACET op Sc en B mogelijk berust op een effect van dit geneesmiddel op de cerebrale bloedvaten Dit resulteert in een veranderde relatie tussen de arteriele PCO_2 en de PCO_2 van het hersenweefsel

8.4. Het effect van lage dosis acetazolamide op de ventilatoire CO_2 respons tijdens hypoxie in de genarcotiseerde kat

Hypoxie komt vaak voor bij patienten met een ernstig COPD Dit was de reden om het acute effect van ACET (4 mg kg^{-1}) op de ventilatoire CO_2 respons te bestuderen in zeven genarcotiseerde katten onder hypoxische omstandigheden (hoofdstuk 3) Na toediening van een bolus van 4 mg kg^{-1} ACET werd een transiente initiele daling van de ventilatie waargenomen Dit wijst op een direct effect op de perifere chemoreflex, omdat tijdens hypoxie de bijdrage van de perifere chemoreflex aan de ventilatie groter is dan tijdens normoxie In de daaropvolgende stabiele situatie veroorzaakte ACET een stijging van de ventilatie Zoals te zien is in tabel 1 verlaagde het geneesmiddel de CO_2 gevoeligheid van de perifere (Sp) en

centrale (Sc) chemoreflex met respectievelijk 0.11 en 0.34 $L.min^{-1}.kPa^{-1}$. Het apneupunt B (x-intercept van de ventilatoire CO_2 respons curve) daalde met 1.93 kPa (alle parameters $p<0.01$). Het netto resultaat was een stimulatie van de ventilatie met ongeveer 50% bij een $P_{ET}CO_2$ van 4 kPa. Er wordt bediscussieerd dat de effecten van ACET tijdens de stabiele situatie waarschijnlijk worden veroorzaakt door een direct effect op de perifere chemoreceptoren als ook een indirect centraal effect op de regeling van de cerebrale blood doorstroming, zoals werd beschreven in hoofdstuk 2.

8.5. Medroxyprogesteron acetaat in combinatie met acetazolamide stimuleert de ademhaling in katten.

In hoofdstuk 4 worden de effecten beschreven op de ventilatoire CO_2 respons curve van MPA, en MPA gevolgd door ACET, in acht genarcotiseerde vrouwtjeskatten, die 1 maand voor de studie een ovariohysterectomie hadden ondergaan. De katten werden voorbehandeld met 17 β-estradiol (E2) om een stabiele en vergelijkbare endocriene status in alle proefdieren te verkrijgen. Een standaard dosis van 10 $\mu g.kg^{-1}$ E2 is nodig om een facilitatie van de ventilatie door progesteron bij proefdieren te bewerkstelligen.

In tabel 1 zijn de effecten van 0.4 $\mu g.kg^{-1}$ MPA en van 4 $mg.kg^{-1}$ ACET op de ventilatoire parameters samengevat. Na infusie van 0.4 $\mu g.kg^{-1}$ MPA nam de ventilatie toe met 35% bij een $PaCO_2$ van 4.5 kPa. Sp daalde met 0.09 $L.min^{-1}.kPa^{-1}$ en Sc daalde met 0.13 $L.min^{-1}.kPa^{-1}$. Er werd een linksverschuiving van het apneupunt waargenomen (een daling in B met 0 38 kPa (alle parameters $p < 0.01$)).

Daaropvolgende toediening van ACET liet een toename in de ventilatie zien van in totaal 116% (MPA+ACET) bij een $PaCO_2$ van 4.5 kPa. Sp en Sc daalden verder met respectievelijk 0.04 en 0.18 $L.min^{-1}.kPa^{-1}$. Het apneupunt daalde verder met 1.18 kPa (alle parameters $p < 0.01$). De bovenstaande resultaten impliceren een meer dan verdubbeling van de ventilatie bij een $PaCO_2$ van 4.5 kPa, vergeleken met monotherapie met een van beide geneesmiddelen Omdat met beide behandelingen de ventilatoire CO_2 gevoeligheid daalde wordt geconcludeerd, dat het stimulerende effect op de ventilatie veroorzaakt wordt door een daling van het apneupunt. Gecombineerde toediening van MPA en ACET leidt tot een grotere toename in ventilatie dan separate behandeling met een van beide geneesmiddelen.

dierexperimentele studies (Hoofdstuk)		V'_E *) (% control)	Sp (L min^{-1} kPa^{-1})	Sc (L min^{-1} kPa^{-1})	B (kPa)
normoxie (8 3)					
intacte katten	controle	100	0 28 ± 0 18	1 52 ± 0 55	4 0 ± 0 5
	ACET	166	0 19 ± 0 12	0 84 ± 0 21	3 0 ± 0 6
katten met gedenerveerde	controle	100	-	1 52 ± 0 42	4 5 ± 0 5
perifere chemoreceptoren	ACET	164	-	0 96 ± 0 32	4 2 ± 0 7
hypoxie (8 4)	controle	100	0 22 ± 0 08	0 74 ± 0 32	2 88 ± 0 97
	ACET	150**)	0 11 ± 0 03	0 40 ± 0 10	0 95 ± 0 92
normoxie (8 5)	controle	100	0 22 ± 0 09	1 01 ± 0 38	4 02 ± 0 27
	MPA	135	0 13 ± 0 06	0 88 ± 0 32	3 64 ± 0 42
	MPA+ACET	216	0 09 ± 0 06	0 70 ± 0 49	2 46 ± 1 50

Tabel 1 Samenvatting van de data verkregen door analyse van de ventilatoire CO_2 responsen uit de verschillende dierexperimentele studies *) $PaCO_2$=4 5 (kPa), **) $PaCO_2$=4 0 (kPa) Sp, Sc and B data zijn weergegeven als gemiddelde ± SD

8.6. Vergelijking van acetazolamide met medroxyprogesteron als respiratoire stimulantia in hypercapnische COPD patiënten.

In hoofdstuk 5 werden de effecten van ACET (2 dd 250 mg) en MPA (2 dd 30 mg) bestudeerd bij 12 hypercapnische patienten met een stabiel COPD (gemiddelde FEV_1 33 ± 4% voorspeld (gemiddelde ± sem)) op de dag en nachtelijke bloedgas waarden en het effect op de hypercapnische en hypoxische ventilatoire en $P_{0.1}$ responsen in een "cross-over" studie Zoals te zien is in tabel 2 daalde de $PaCO_2$ overdag met 0 7 kPa tijdens ACET behandeling en met 0 6 kPa tijdens MPA behandeling De PaO_2 overdag verbeterde met ACET met 1 3 kPa, terwijl geen significante verandering werd waargenomen met MPA De gemiddelde nachtelijke $P_{ET}CO_2$ daalde met beide behandelingen met 0 9 kPa gedurende ACET en met 1 0 kPa gedurende MPA behandeling Het percentage van de tijd dat de nachtelijke SaO_2 lager was dan 90%, werd met ACET gereduceerd met 50%, (alle parameters $p<0.05$) Gedurende MPA behandeling werden er geen veranderingen gevonden op de nachtelijke parameters

De toename in rust ventilatie was alleen significant met MPA behandeling 1 2 L min^{-1} De helling van de hypercapnische ventilatoire respons veranderde niet gedurende ACET en MPA behandeling De hypoxische ventilatoire respons nam toe met 0 2 L min^{-1} %$^{-1}$ tijdens ACET behandeling en met 0 1 L min^{-1} %$^{-1}$ gedurende MPA behandeling De hypoxische $P_{0.1}$ respons verbeterde met ACET behandeling met 0 013 kPa %$^{-1}$, (alle parameters $p<0.05$)
Deze studie laat zien dat ACET en MPA beiden een gunstig effect hebben op de bloedgas waarden overdag en 's nachts in hypercapnische COPD patienten Echter, de effecten van ACET zijn evidenter, vooral op de nachtelijke saturatie

8.7. Gecombineerde behandeling van acetazolamide met medroxyprogesteron bij COPD patiënten.

In hoofdstuk 6 wordt de laatste humane studie beschreven, waarin de effecten zijn bestudeerd van een twee weken durende behandeling met MPA (2 dd 30 mg) of ACET (2 dd 250 mg) gevolgd door een twee weken durende behandeling met beide geneesmiddelen (MPA-ACET)
In 17 stabiele patienten met een ernstig COPD (FEV_1 34 ± 4% voorspeld) zijn de effecten bestudeerd op de ventilatoire en bloedgas parameters overdag en 's nachts in een dubbelblinde gecontroleerde vorm
Met de enkelvoudige behandeling van ACET, MPA daalde de gemiddelde $PaCO_2$ overdag significant met respectievelijk 0 4 en 0 7 kPa De gemiddelde PaO_2 overdag verbeterde met beide geneesmiddelen met respectievelijk 0 6 en 1 0 kPa
Zoals te zien is in tabel 2 daalde de gemiddelde $PaCO_2$ overdag met gecombineerde therapie met MPA-ACET met 1 2 kPa De ventilatie nam toe met gecombineerde behandeling met 1 9 L min^{-1}
De hypercapnische en hypoxische ventilatoire responsen namen significant toe met MPA-ACET behandeling met respectievelijk 2 1 L min^{-1} kPa^{-1} en met 0 27 L min^{-1} %$^{-1}$
De $P_{0.1}$ respons op hypoxie nam toe bij de combinatie therapie met 0 02 kPa %$^{-1}$ De nachtelijke $P_{ET}CO_2$ daalde significant MPA-ACET behandeling met 1 4 kPa Met de combinatie van MPA-ACET nam de gemiddelde nachtelijke SaO_2 toe met 4 7%
We concludeerden dat, onder hypercapnische condities, gecombineerde behandeling met ACET en MPA een grotere verbetering in bloedgas waarden overdag en 's nachts gaf dan

separate behandeling met een van beide geneesmiddelen. De toename in ventilatie die nodig is om een overschot in CO_2 af te blazen gedurende hypercapnie is gering (ongeveer 2 L.min^{-1} bij onze patiënten). De combinatie van MPA-ACET behandeling in hypercapnische COPD patiënten lijkt effectief te zijn gedurende een korte periode. Of chronische behandeling met beide geneesmiddelen als monotherapie of in combinatie met elkaar de levensverwachting en/of kwaliteit verbetert moet verder bestudeerd worden.

	behandeling				
humane studies (Hoofdstuk)	enkelvoudige (8.6.)			gecombineerd (8.7.)	
	placebo	ACET	MPA	placebo	MPA+ACET
V'_E (L min^{-1})	9.6 ± 0.7	*10.0 ± 0.6*	10.8 ± 0.8	9.3 ± 0.5	11.2 ± 0.8
$PaCO_2$ (KPa)	6.3 ± 0.1	5.6 ± 0.2	5.7 ± 0.2	6.5 ± 0.2	5.3 ± 0.2
PaO_2 (KPa)	8.7 ± 0.3	10.0 ± 0.4	*9.3 ± 0.4*	7.9 ± 0.3	9.6 ± 0.4
nacht $PETCO_2$ (KPa)	5.6 ± 0.3	4.7 ± 0.3	4.6 ± 0.1	5.5 ± 0.2	4.1 ± 0.2
% tijd nacht. SaO_2<90% (%)	34.9 ± 10.7	16.3 ± 7.5	*23.8 ± 11.5*	61.8 ± 9.9	42.0 ± 10.4
S_{HCVR} (L min^{-1} kPa^{-1})	*4.3 ± 0.6*	*4.1 ± 0.6*	*5.2 ± 0.9*	3.7 ± 0.6	5.8 ± 1.0
S_{HVR} (L.min^{-1} %$^{-1}$)	-0.2 ± 0.05	-0.4 ± 0.1	-0.3 ± 0.1	-0.13 ± 0.03	-0.40 ± 0.07
$S_{HP0.1}$ (kPa.%$^{-1}$)	-0.007 ± 0.002	-0.02 ± 0.003	*-0.01 ± 0.003*	-0.01 ± 0.00	-0.03 ± 0.01

Tabel 2. Selectie van data uit de humane studies, beschreven in hoofdstuk 8.6 en 8.7 welke significant veranderden. (Data genoteerd in *schuinschrift* veranderden niet significant t.o.v. placebo). Data zijn weergegeven als gemiddelde ± sem.

Dankwoord

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Curriculum vitae

Michiel Wagenaar werd geboren op 31 maart 1964 te Leiden In 1985 behaalde hij zijn VWO diploma aan het Willem de Zwijger college te Bussum De studie geneeskunde werd gevolgd aan de Rijksuniversiteit te Leiden, waar hij in 1991 zijn doctoraal examen en in 1993 zijn artsexamen haalde Aansluitend werkte hij gedurende een half jaar als arts-assistent niet-in-opleiding op de afdeling longziekten van het Academisch Ziekenhuis Utrecht (Hoofd Prof Dr J-W Lammers) In 1994 werd gestart met het wetenschappelijk onderzoek, dat in dit proefschrift is beschreven Het werd ten dele uitgevoerd op de afdeling cardiopulmonaal gastransport en ademregulatie van de vakgroep fysiologie, medische faculteit, Universiteit van Leiden (hoofd Prof Dr Ph H Quanjer) en ten dele op de afdeling longfunctie van het Universitair Longcentrum Dekkerswald (hoofd Prof Dr H Th M Folgering) Van 1999 tot 2001 volgde hij de vooropleiding longziekten op de afdeling interne geneeskunde in het Rijnstate ziekenhuis te Arnhem (hoofd Dr L Verschoor, Dr R Van Leusen) Thans is hij werkzaam als longarts in opleiding in het Universitair Longcentrum Dekkerswald/Universitair Medisch Centrum St Radboud (hoofd Prof Dr C L A van Herwaarden)

Michiel is gehuwd met Marjan Pos Samen hebben zij drie kinderen Casper (1997), Thijs (1999) en Florine (2001)

Stellingen

behorende bij het proefschrift

Ventilatory stimulation bij acetazolamide and medroxyprogesterone acetate
A study in cats and COPD patients

M. Wagenaar
15 januari 2003

1 Intraveneuze toediening van 4 $mg.kg^{-1}$ acetazolamide geeft nog geen significante gradiënt tussen de arteriële en de end-tidal PCO_2. *(dit proefschrift)*

2 Het gebruik van bepaalde respiratoire stimulantia bij COPD patienten met een hypercapnie kan op korte termijn leiden tot een normocapnie *(dit proefschrift)*

3 Of het gebruik van carbo-anhydrase remmers en progesteron een positief effect heeft op de lange termijn overleving bij COPD patiënten zal nog bewezen moeten worden

4 Het is maar goed dat er patienten zijn Anders zou dit proefschrift niet tot stand zijn gekomen.

5 Het uitvoeren van een hypoxische ventilatoire respons manoeuvre kan ook bij gezonde proefpersonen een adembenemende inspanning zijn *(eigen waarneming)*

6 Een slecht-nieuwsgesprek kan zeer aangrijpend zijn voor de patient èn voor de arts.

7 Het routinematig maken van een röntgenopname van de thorax in expiratiestand bij het vermoeden van een pneumothorax is naast het maken van een inspiratieopname niet gerechtvaardigd. (Schramel F, Wagenaar M, Sutedja T, Golding R, Postmus P. Ned Tijdschr Geneeskd, 1995)

8 Bij patiénten met een achalasie van de oesophagus is periodiek endoscopisch onderzoek geïndiceerd

9 Indien binnen een politieke partij het dualisme ontaardt in voortdurende incompatibilité d'humeur, is deze ten dode opgeschreven.

10 Een medisch wetenschappelijk proefschrift is vaak onleesbaar voor de niet-medisch wetenschapper.