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# Medroxyprogesterone acetate with acetazolamide stimulates breathing in cats

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**Abstract**

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 ($S_p$ and $S_c$, respectively) and a single offset (the apnoeic threshold B). MPA reduced $S_p$ from $0.22 \pm 0.09$ (mean ± S.D.) to $0.13 \pm 0.06$ L $min^{-1}$ $kPa^{-1}$ ($P < 0.01$) and $S_c$ from $1.01 \pm 0.38$ to $0.88 \pm 0.32$ L $min^{-1}$ $kPa^{-1}$ ($P < 0.01$). B decreased from $4.02 \pm 0.27$ to $3.64 \pm 0.42$ kPa ($P < 0.01$). Subsequent administration of ACET reduced $S_p$ and $S_c$ further to $0.09 \pm 0.06$ and to $0.70 \pm 0.49$ L $min^{-1}$ $kPa^{-1}$ ($P < 0.01$), respectively. The apnoeic threshold decreased further to $2.46 \pm 1.50$ 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.


*Keywords:* Control of breathing, $CO_2$ response; Mammals, cat; Pharmacological agents, acetazolamide; Pharmacological agents, medroxyprogestron

## 1. 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., 1988). Progesterone, or the synthetic proges-

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terones 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 (Skatrud et al., 1978; Skatrud and Dempsey, 1983; Vos et al., 1994).

In man, progesterone may increase ventilation by an effect on the hypothalamus and/or structures in the medulla oblongata (e.g. Skatrud et al., 1978; Zwillich et al., 1978; Dolly and Block, 1983; Pfaff and McEwen, 1983; Dempsey et al., 1988). A possible action on the peripheral chemoreceptors is indicated by the finding that during human pregnancy the hypoxic ventilatory response is increased (Moore et al., 1987). 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 (Hensley et al., 1980; Popovic and White, 1998).

Data from animal studies also indicate that progesterone may stimulate breathing by an effect on peripheral and/or central sites (Bayliss et al., 1990, 1991; Favier et al., 1997). Guinea pigs, dogs and cats but not rats, goats, ponies and cows respond with an increased hypercapnic ventilatory response to progesterone administration (see Dempsey et al., 1988). 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 (Brodeur et al., 1986; Dempsey et al., 1988; Tatsumi et al., 1991). An increase in hypoxic sensitivity by progesterone was reported in cat and rat (Hannhart et al., 1989; Favier et al., 1997).

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. Skatrud and Dempsey, 1983; Vos et al., 1994). 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 (Wagenaar et al., 1996). A large dose of the agent (50 mg $kg^{-1}$) totally abolishes the hypoxic ventilatory response (Teppema et al., 1988, 1992).

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 (Wagenaar et al., 1996). 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 (De Goede et al., 1985).

## 2. Methods

### 2.1. 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 μg $kg^{-1}$ 17-β-estradiol ($E_2$) (Sigma-Aldrich, Bornem, Belgium), dissolved in sesame oil (100 μg $ml^{-1}$), twice daily subcutaneously during 3 days immediately prior to the study (Bayliss et al., 1990). Plasma concentration of 17-β-estradiol in seven cats was esti-

mated by a radioimmunoassay (RIA) method with extraction using anti-estradiol antibody-coated tubes (Coat-A-Count TKE; Diagnostic Products; Los Angeles, CA, USA) (Dieleman and Bevers, 1987). 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 (Teppema et al., 1997). 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 Godard MK2 Capnograph, Bilthoven, The Netherlands) and a fast-responding zirconium oxide cell (Jaeger $O_2$-test, Würzburg, Germany), respectively.

Arterial pH, $P_{CO_2}$ and $P_{O_2}$ 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 $P_{CO_2}$ electrode (General Electric A312AB, Milwaukee, Wisonscin, USA) and a home made Clark-type $P_{O_2}$ electrode. The $P_{CO_2}$ and $P_{O_2}$ 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 $P_{CO_2}$ 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 ($P_{ET_{CO_2}}$, $P_{ET_{O_2}}$, $Pa_{CO_2}$, $Pa_{O_2}$) were stored on a breath-by-breath basis.

### 2.2. Experimental protocol

The ventilatory responses to $CO_2$ were studied using the dynamic end-tidal forcing technique (DEF) (De Goede et al., 1985). This technique was developed to force the end-tidal $P_{CO_2}$ ($P_{ET_{CO_2}}$) and $P_{O_2}$ ($P_{ET_{O_2}}$) 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 (De Goede et al., 1985).

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 μg $kg^{-1}$ 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.

### 2.3. Data analysis

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

$$\dot{V}_I = (S_p + S_c)(P_{ET_{CO_2}} - B) \quad (1)$$

The parameters $S_p$ and $S_c$ are the $CO_2$ sensitivities of the peripheral and central chemoreflex loops, respectively, and the off-set 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 (De Goede et al., 1985):

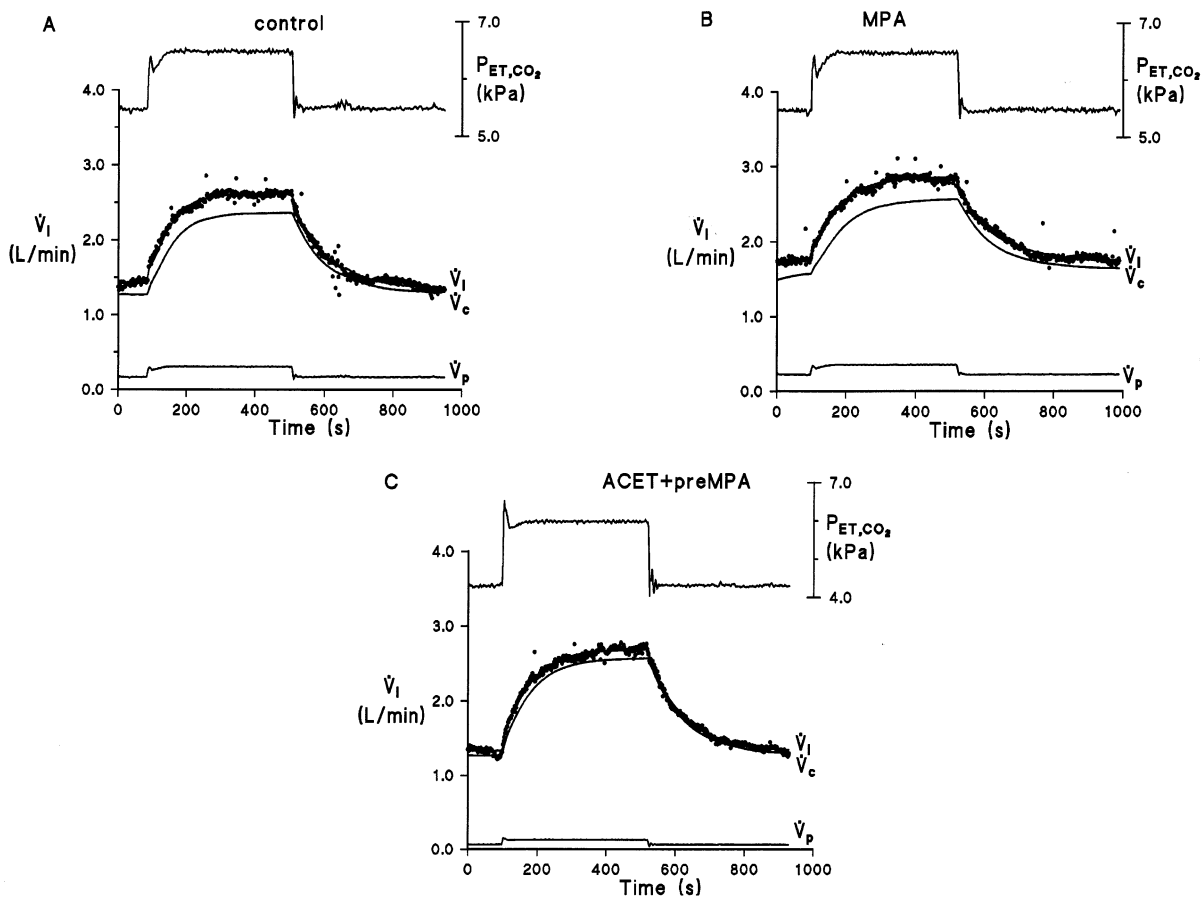


Fig. 1. Examples of three DEF runs and the model fits of the ventilatory responses: control situation, after MPA and after additional ACET administration. The upper trace shows the input function of $P_{ET_{CO_2}}$. The curve through the breath-by-breath ventilatory data ($\dot{V}_I$) points (dots) is the model fit. The two lower traces show the contributions of the central ($\dot{V}_c$) and peripheral ($\dot{V}_p$) chemoreflex loops to the model output, respectively.

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[a]

| Parameter | Control | MPA | MPA + ACET | *P*-value | | |
|---|---|---|---|---|---|---|
| | (a) | (b) | (c) | (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 |
| $S_{tot}$ (L $min^{-1}$ $kPa^{-1}$) | 1.23 ± 0.39 | 1.02 ± 0.33 | 0.78 ± 0.51 | 0.0001 | 0.0001 | 0.0001 |
| $S_c$ (L $min^{-1}$ $kPa^{-1}$) | 1.01 ± 0.38 | 0.88 ± 0.32 | 0.70 ± 0.49 | 0.0001 | 0.0001 | 0.0001 |
| $S_p$ (L $min^{-1}$ $kPa^{-1}$) | 0.22 ± 0.09 | 0.13 ± 0.06 | 0.09 ± 0.06 | 0.0001 | 0.0248 | 0.0001 |
| Standard bicarbonate (meq $L^{-1}$) | 20.61 ± 2.98 | 21.11 ± 2.55 | 20.44 ± 3.91 | 0.2000 | 0.0003 | 0.0056 |

[a] $S_c$ and $S_p$ 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 means ± S.D. The *P*-values are obtained from the ANOVA on all individual data.

$$\tau_c \dot{V}_c/dt + \dot{V}_c = S_c[P_{ET_{CO_2}}(t - T_c) - B] \quad (2)$$

$$\tau_p \dot{V}_p/dt + \dot{V}_p = S_p[P_{ET_{CO_2}}(t - T_p) - B] \quad (3)$$

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

$$\dot{V}_I(t) = \dot{V}_c(t) + \dot{V}_p(t) + C.t \quad (5)$$

Eq. (2) describes the dynamics of the slow central chemoreflex loop with the central $CO_2$ sensitivity $S_c$, time constant $\tau_c$ and transport delay time to the central chemoreceptors $T_c$; similarly Eq. (3) describes the dynamics of the fast peripheral chemoreflex loop with the peripheral $CO_2$ sensitivity $S_p$, time constant $\tau_p$ and delay time $T_p$. 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 $T_c$ and $T_p$ (increments of 1 sec and $T_c \geq T_p$) 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 $\tau_p$ was constrained to be at least 0.3 sec (De Goede et al., 1985).

### 2.4. 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 (Wagenaar et al., 1996), 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.

## 3. Results

One month after ovariohysterectomy in seven of eight cats, the mean plasma concentration of ($E_2$) was 6.92 ± 1.7 pg $ml^{-1}$ (see also Hannhart et al., 1989). After $E_2$-priming, the $E_2$ concentration in these cats increased to a mean of 567.2 ± 396.4 pg $ml^{-1}$ (in one animal, $E_2$ 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 $0.22 \pm 0.09$ to $0.13 \pm 0.06$ L $min^{-1}$ $kPa^{-1}$, while that of the central chemoreflex loop ($S_c$) was reduced from $1.01 \pm 0.30$ to $0.88 \pm 0.32$ L $min^{-1}$ $kPa^{-1}$. The mean apnoeic threshold B decreased from $4.02 \pm 0.27$ to $3.64 \pm 0.42$ kPa. The effects of MPA in each individual animal are shown in the scatter diagrams of Fig. 2. After ACET administration, the mean $S_p$ and $S_c$ were further reduced to $0.09 \pm 0.06$ and to $0.70 \pm 0.49$ L $min^{-1}$ $kPa^{-1}$, respectively. The mean apnoeic threshold B decreased further to $2.46 \pm 1.50$ 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. (Wagenaar et al., 1996). 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 $P_{CO_2}$ 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 ($S_{tot}$) 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

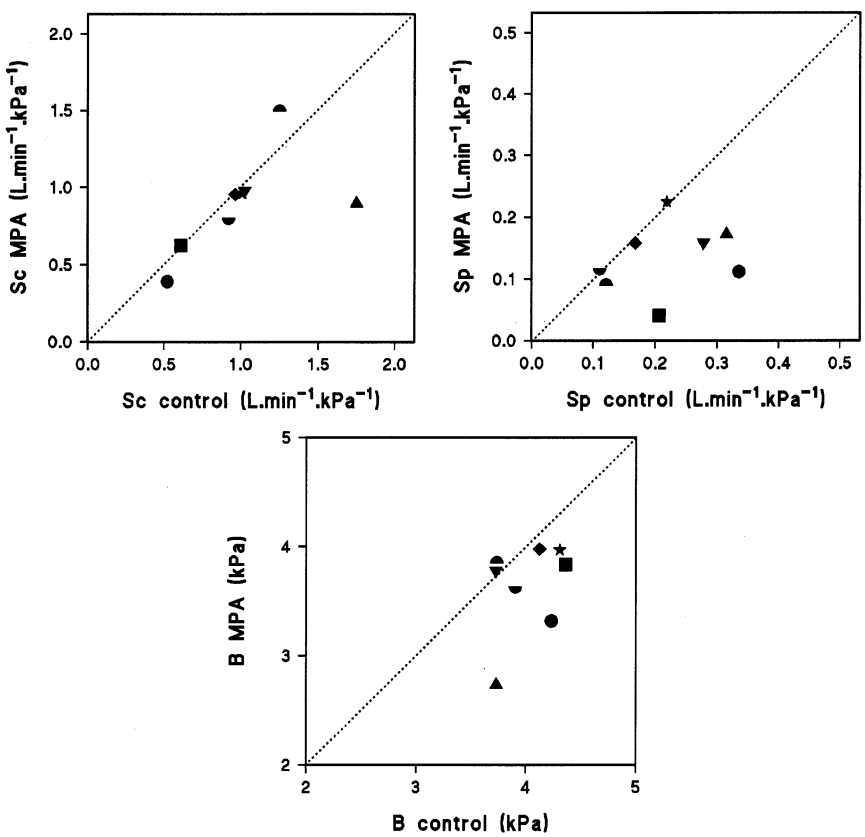


Fig. 2. Scatter diagrams of the effect on 4 μg $kg^{-1}$ MPA on the ventilatory parameters. Intravenous infusion of 4 μg $kg^{-1}$ MPA results in a decrease in the values of the parameters shown. Each individual cat is represented by a separate symbol. $S_c$ and $S_p$ are the $CO_2$ sensitivities of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

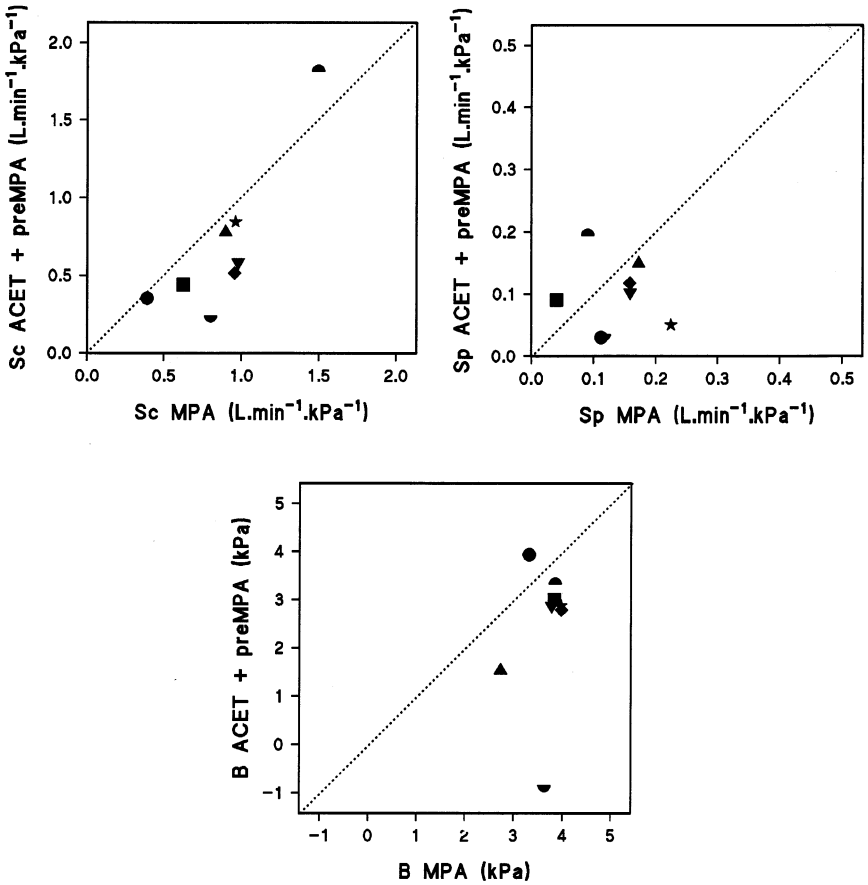


Fig. 3. Scatter diagrams of the effect on 4 mg kg$^{-1}$ ACET after pre-treatment with MPA on the ventilatory parameters. Intravenous infusion of 4 mg kg$^{-1}$ ACET after pre-treatment with MPA results in a decrease in the values of the parameters B, $S_c$ and $S_p$. Each individual cat is represented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

$P_{CO_2}$ 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 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 $P_{CO_2}$ values, is considerably larger than that of a single treatment with MPA or ACET.

## 4. 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 ($S_p$) and central chemoreflex ($S_c$) loops with 41 and 13%, respectively. A subsequent administration of 4 mg kg$^{-1}$ ACET caused a further reduction in carbon dioxide sensitivities ($S_p$ 31%

and $S_c$ 20%). Both agents lowered the mean apneic threshold by 0.38 and 1.18 kPa, respectively.

### 4.1. Effect of 17-β-estradiol

We used 17-β-estradiol for premedication. Bayliss et al. (1990) showed that priming with $E_2$ 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 (1978) and Brodeur et al. (1986).

### 4.2. Effects of MPA

In man, progesterone increases the isocapnic acute hypoxic ventilatory response (AHR; Zwillich et al., 1978; Moore et al., 1987; Regensteiner et al., 1989). Tatsumi et al. (1986) 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 (Tatsumi et al., 1997). Hannhart et al. (1989, 1990) 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 (Favier et al., 1997).

Table 2
Effects of a single ACET treatment (4 mg $kg^{-1}$) on the ventilatory $CO_2$ response curve in eight cats as determined in a previous study (Wagenaar et al., 1996)[a]

| Parameter | Control | ACET | *P*-value |
|---|---|---|---|
| B (kPa) | $4.0 \pm 0.5$ | $3.0 \pm 0.6$ | 0.0001 |
| $S_{tot}$ (L $min^{-1}$ $kPa^{-1}$) | $1.80 \pm 0.69$ | $1.03 \pm 0.26$ | 0.0001 |
| $S_c$ (L $min^{-1}$ $kPa^{-1}$) | $1.52 \pm 0.55$ | $0.84 \pm 0.21$ | 0.0001 |
| $S_p$ (L $min^{-1}$ $kPa^{-1}$) | $0.28 \pm 0.18$ | $0.19 \pm 0.12$ | 0.0001 |

[a] $S_c$ and $S_p$ 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 means ± S.D. The *P*-values are obtained from the ANOVA on all individual data.

The above studies clearly suggest a stimulatory action of female hormones on the peripheral chemoreceptors. Favier et al. (1997) 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 neuromechanical 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 (Favier et al., 1997). 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 (Heesch and Rogers, 1995). This effect is probably due to a potentiation by 3α-OHDHP of $GABA_A$ inhibitory influences in the NTS and in the rostroventrolateral medulla, in the region were baro- as well as chemoreceptor inputs are integrated (Heesch and Rogers, 1995). 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 (Yamada et al., 1981). A similar mechanism could play a role in the hypothalamus, some regions of which contain progesterone receptors (Bayliss et al., 1990). $CO_2$ sensitive neurons in the caudal hypothalamus modulate the respiratory response to increases in $P_{CO_2}$ and are exposed a tonic GABA-ergic inhibition (Waldrop, 1991). Metabolites of progesterone increase anaesthetic depth and lessen the need for anaesthetics

for surgery (Bukusoglu et al., 1993; Hirabayashi et al., 1995). This effect could be due to an increase in GABA-induced chloride conductance, and in this respect 3 α-OHDHP may also be an important modulator (references see Heesch and Rogers, 1995). 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 (Smith and Kellog, 1980; Keith et al., 1982; Tatsumi et al., 1991) or in humans (Zwillich et al., 1978; Schoene et al., 1980; Regensteiner et al., 1989; Vos et al., 1994). The finding that the synthetic progestagenes MPA and chlormadinone actetate, despite a much larger progestational activity, do not have larger effects on ventilation than progesterone (Skatrud et al., 1978; Kimura et al., 1984), 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 $P_{CO_2}$ level at which the agent is administered. The rise in prenic activity by progesterone in cats pre-treated with estrogen is probably mediated via hypothalamic structures (Bayliss et al., 1991). 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 (Schoene et al., 1980; Kimura et al., 1984; Morikawa et al., 1987; Hannhart et al., 1989; Regensteiner et al., 1989).

### 4.3. 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 pretreatment (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 (Wagenaar et al., 1996). The relative decreases in $S_c$, with and without MPA pre-treatment, however, were 20 and 45%, respectively. The relative changes in $S_{TOT}$ were 37 and 42%, respectively. Although unpaired $t$-tests yielded no significant difference between these changes in $S_c$ and $S_{TOT}$ by ACET, we can not 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 (Wagenaar et al., 1996). 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 $P_{CO_2}$ 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 $P_{CO_2}$ is clamped, and that these differences in ventilatory effects depend on the prevailing $P_{CO_2}$ 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 $P_{CO_2}$ 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 $P_{CO_2}$ and, depending on the occurrence of lung regions with very low ventilation perfusion ratios, possibly also to a large rise in arterial $P_{O_2}$. 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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