LABORATORY INVESTIGATIONS

Anesthesiology
80:1303–1310, 1994

J. B. Lippincott Company, Philadelphia

Effects of Morphine and Physostigmine on the Ventilatory Response to Carbon Dioxide

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Background: **It has been reported that physostigmine antagonizes morphine-induced respiratory depression, but it is not known whether this is due to a central chemoreceptor effect, an effect on the peripheral chemoreflex loop, or both. We therefore assessed the effect of morphine and physostigmine on the normoxic hypercapnic ventilatory response mediated by the central and peripheral chemoreceptors in ten α-chloralose–urethan–anesthetized cats.**

Methods: **The breath-by-breath ventilatory responses to stepwise changes in end-tidal CO_2 tension were determined before (control), after administration of morphine hydrochloride (0.15 $mg \cdot kg^{-1}$) and during intravenous infusion of physostigmine salicylate (bolus of 0.05 $mg \cdot kg^{-1}$ followed by 0.025 $mg \cdot kg^{-1} \cdot h^{-1}$). Each response was separated into a central and a peripheral chemoreflex characterized by CO_2 sensitivity (S_c and S_p), time constant, time delay, and apneic threshold (a single off-set B).**

Results: **Morphine increased B and decreased S_c and S_p ($P < 0.01$), but not the ratio S_p/S_c. Subsequent infusion of physostigmine decreased B ($P < 0.01$), without further change of S_p and S_c. Premedication with physostigmine decreased B, S_p and S_c ($P < 0.01$) *vs.* control, but not S_p/S_c. Subsequent administration of morphine decreased S_p and S_c further but increased B ($P < 0.01$), while S_p/S_c remained constant.**

Conclusions: **Because morphine diminishes the S_c and S_p of the chemoreflex loop to the same extent this depressant effect is presumably due to an action on the respiratory integrating centers rather than on the peripheral and central chemoreceptors as such and is not antagonized by physostigmine. We argue that the increase in B may be due to changes in the amount of acetylcholine available in the brain and can be antagonized by physostigmine. (Key words: Analgesics, opioid: morphine. Measurement techniques: carbon dioxide ventilatory response. Parasympathetic nervous system, cholinergic agonists: physostigmine. Receptors, chemoreceptors: central; peripheral.)**

IT is well known that opiates are potent depressants of breathing. The site of opioid-induced respiratory depression may be on the central chemosensitive structures located in the medulla oblongata,[1] the peripheral chemoreceptors of the carotid bodies[2] and the integrating centers in the brain stem where the information from both groups of chemoreceptors is processed. From a survey of the literature it is not clear whether the depressant action on the ventilatory response to CO_2 is on the apneic threshold (B) (*i.e.*, a parallel shift of the ventilation–CO_2 tension line), on the CO_2 sensitivities of the central and peripheral chemoreflex loops (S_c and S_p, respectively) (*i.e.*, the slope), or on both.[3,4] This uncertainty may be due to species differences or to differences between the anesthetized and nonanesthetized states, or to differences in methods used (rebreathing *vs.* steady-state measurements)[5] to study this effect.

It has been reported that morphine reduces the release of acetylcholine in the brain[6] and there are numerous indications that acetylcholine is involved in the regulation of breathing. An increase in acetylcholine stimulates the central chemosensitive structures of the ventral medulla oblongata and the peripheral chemoreceptors.[7,8] In clinical practice physostigmine, an anticholinesterase agent is sometimes used in the postoperative phase to improve breathing, because it has been reported that physostigmine antagonizes morphine-induced respiratory depression.[9–11] However, the reports on the effect of physostigmine on the opioid-induced respiratory depression are not unanimous; e.g. Bourke and coworkers[12] conclude that physostigmine is ineffective as an antagonist.

The aim of the present study is to investigate in the anesthetized cat the effects of morphine on the central and peripheral component of the ventilatory response

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Received from the University of Leiden and Erasmus University Rotterdam, The Netherlands. Accepted for publication February 1, 1994.

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to CO_2 and the action of physostigmine on these effects. A method which is very well suited to investigate this is the dynamic end-tidal forcing (DEF) technique. This technique, uses the difference in time to transport a CO_2 change in the lung to the peripheral and central chemoreceptors and the difference in speed of their response, to separate the contributions of the peripheral and central chemoreceptors to the ventilatory response to CO_2.[13,14]

Materials and Methods

Experiments were performed on ten anesthetized adult cats of either sex (body weight 2.2–3.0 kg). The use of the animals was approved by the Ethical Committee for Animal experiments of the University of Leiden. Anesthesia was induced with 15 mg kg^{-1} ketamine hydrochloride intramuscularly, followed by halothane inhalation. The right femoral vein was cannulated and 20 mg kg^{-1} α-chloralose and 100 mg kg^{-1} urethan were slowly administered intravenously and the volatile anesthetic was withdrawn. About 1 h later an infusion of an α-chloralose–urethan solution was started at a rate of 1.0–1.5 mg kg^{-1} h^{-1} α-chloralose and 5.0–7.5 mg kg^{-1} h^{-1} urethan. This regime leads to conditions in which the level of anesthesia is sufficient to suppress the pain-withdrawal reflex but low enough to preserve the corneal reflex. Ventilatory depression is minimal because resting end-tidal CO_2 tension ($P_{ET_{CO_2}}$) and the slope and intercept of the ventilatory response to CO_2 are not different from those reported in awake cats.[15,16] Furthermore, the ventilatory response to CO_2 did not show systematic changes for several hours.[17]

All cats were studied with the DEF method before and after the administration of drugs. Because the DEF technique has been described previously we restrict ourselves to a brief description.[17]

In the DEF technique the $P_{ET_{CO_2}}$ is forced to follow a specific pattern in time while the $P_{ET_{O_2}}$ is kept constant. This is performed by manipulating the inspired CO_2 and O_2 concentrations by feedback control with a computer. The ventilatory response after a prescribed change in $P_{ET_{CO_2}}$ is assessed on a breath-by-breath basis.

To measure inspiratory and expiratory flow the trachea was cannulated and connected via a Fleisch no. 0 flow transducer head to a T piece of which one arm was receiving a continuous gas flow of 5 1 min^{-1}. With the aid of three computer-steered mass flow controllers, a prescribed composition of the inspirate from pure O_2, CO_2, and N_2 could be obtained. The respiratory fractions of O_2 and CO_2 were continuously measured with a fast-responding zirconium oxide cell (O_2 Test, Jaeger, Germany) and an infrared analyzer (MK-2 capnograph, Gould Godart, The Netherlands). Temperature was controlled within 1°C in each cat and ranged between cats from 37.0 to 39.9°C. Femoral arterial pressure was measured with a strain gauge transducer. An extracorporeal circuit was connected between the cannulated left femoral artery and the femoral vein for the measurement of arterial blood gas tensions. Blood was pumped at a rate of 6–7 ml · min^{-1}.

All signals were recorded on polygraphs and processed by a PDP 11/23 minicomputer (sample frequency 100 Hz). Tidal volume, breathing frequency, ventilation, $P_{ET_{CO_2}}$, and $P_{ET_{O_2}}$ were determined by the minicomputer and stored on a breath-by-breath basis. For monitoring purposes during the experiment averages over 20 breaths of ventilation, blood gas tensions and arterial pressure were calculated, displayed on a monitor and stored on disk.

Experimental Design

Experiments consisted of changes in $P_{ET_{CO_2}}$ of about 7–10 mmHg during normoxia ($P_{ET_{O_2}}$ controlled at 110 mmHg). After a period of steady-state ventilation during which $P_{ET_{CO_2}}$ was slightly raised above resting values, $P_{ET_{CO_2}}$ was increased in a stepwise fashion and kept constant for about 7 min. Thereafter the $P_{ET_{CO_2}}$ was decreased to its original value and kept constant for another 7 min. In each cat at least 3 of such control studies were performed.

Protocol I. In 5 cats after assessment of the control studies 0.15 mg/kg morphine hydrochloride was infused iv. After about 0.5 h, when ventilation had stabilized, at least 3 DEF studies were performed. After the morphine studies 0.05 mg/kg physostigmine was given iv in a period of 3 min and this was followed by a continuous infusion of 0.025 mg/kg^{-1}/h^{-1}. After about 15 min, data collection started again and at least 3 DEF studies were assessed. In 4 of the 5 cats 0.1 mg/kg naloxone was iv administered and after about 15 min a few DEF studies were performed. The infusion of physostigmine was continued till the end of the experiment.

Protocol II. This protocol was the same as protocol I except that the order in which morphine and physostigmine was administered, was reversed. It was performed in 5 cats. DEF studies after administration of naloxone were performed in 4 cats.

Data Analysis

The steady-state relation of ventilation to $P_{ET_{CO_2}}$ at constant $P_{ET_{O_2}}$ in the cat is linear down to the $P_{ET_{CO_2}}$-axis and well described by[18,19]

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

where $\dot{V}_I$ is ventilation, and the off-set B represents the apneic threshold, or extrapolated $P_{ET_{CO_2}}$ of the steady-state ventilatory response to CO_2 at zero ventilation.

For the analysis of the dynamic response of the ventilation, we used a two-compartment model,[17] *viz.*:

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

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

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

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

where τ_c and τ_p are the time constants of the central and peripheral ventilatory responses; $\dot{V}_c$ and $\dot{V}_p$ are the contributions of the central and peripheral chemoreceptors to ventilation; T_c and T_p are the time delays needed to transport the CO_2 change from the lungs to the central and peripheral chemoreceptors; B_c and B_p are the off-sets of the central and peripheral ventilatory response; τ_{on} and τ_{off} are the central time constants of the ventilatory on-transient and off-transient, respectively. To model τ_{on} to be different from τ_{off}, τ_c is written according to equation 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 (eq. 5). However, the trend C 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 after a change in $P_{ET_{CO_2}}$ into parts belonging to the central and peripheral chemoreflex loops. This is reflected in the fact that the off-set parameters B_c and B_p in equations 2 and 3 cannot be estimated individually because they are not identifiable. We therefore reduce the number of parameters in the model. To this end it is customary to choose the same off-set parameter for both loops, viz. $B_c = B_p = B$.[20] This off-set B is then equal to the extrapolated $P_{ET_{CO_2}}$ of the steady-state ventilatory response curve to zero ventilation (apneic threshold). As a consequence, when a drug causes a change in apneic 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 $\dot{V}_c$ and $\dot{V}_p$ in equations 2, 3, and 5 the central and peripheral parts of the ventilation due to the arbitrary choice of $B_c = B_p$, we usually do so for the sake of simplicity of the presentation. For the steady state the two-compartment model reduces to equation 1 as it should.

All the parameters of the model were estimated simultaneously 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 T_c and T_p in increments of 1 s and with $T_c \geq T_p$ were tried until a minimum in the residual sum of squares was found. The minimal T_c and T_p were somewhat arbitrarily chosen to be 1 s, and τ_p was constrained to be at least 0.3 s.[17]

Statistical Analysis

To detect the significance of differences between the control, morphine and physostigmine groups, analysis of variance with a two-way layout was performed on the estimated parameters of all the individual DEF studies. Differences between treatments were tested with the Student-Newman-Keuls test. For protection against type I errors a probability level of 0.01 was chosen for differences to be significant. Group values are mean ± SEM of the means per cat unless otherwise stated.

Results

Protocol I

An actual recording of a DEF study is shown in fig. 1. A stepwise increase in $P_{ET_{CO_2}}$ was initiated by giving the animal one or two breaths of a gas mixture containing about 6% CO_2 to inhale. The inspired CO_2 was then regulated to keep $P_{ET_{CO_2}}$ constant. Visual inspection already shows that there is a fast change in ventilation followed by a slower one. The increase in ventilation is mainly due to an increase in tidal volume with little change in breathing frequency. The model fit to the data obtained from the study depicted in figure 1 is shown in figure 2 together with a model fit from a study obtained in the same cat after administration of morphine. The points are the breath-by-breath ventilation data. The curve through the data is the least squares model fit using the actual $P_{ET_{CO_2}}$ as input. The total ventilation is broken up into a slow central and a

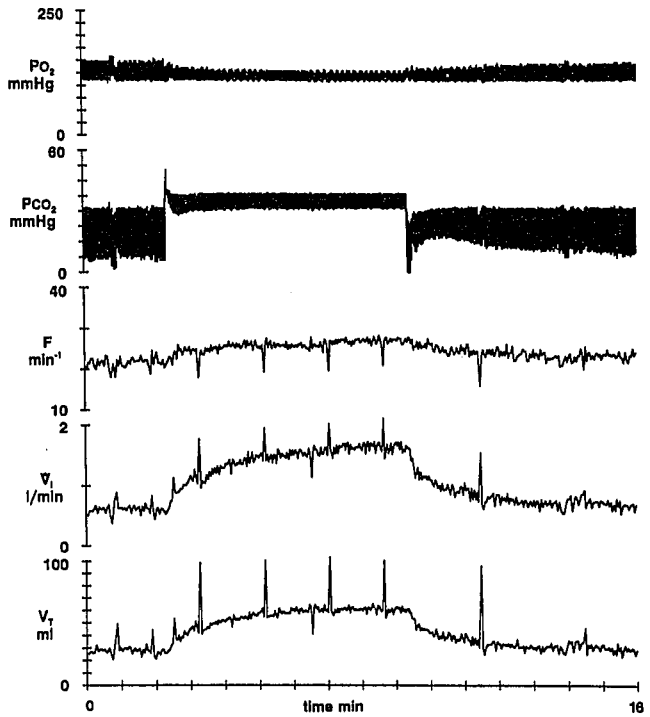

Fig. 1. Recording of a dynamic end-tidal forcing study. Plotted against time are tidal volume (V_T), breath-by-breath ventilation ($\dot{V}_I$), breathing frequency (F), and CO_2 and O_2 tensions (P_{CO_2} and P_{O_2}, respectively) in tracheal gas.

fast peripheral component. Figure 2 illustrates the finding that both the central and the peripheral component are depressed.

To illustrate the effects of the interventions on the overall ventilatory response to CO_2 a representative example of the curves of one cat are shown in the left panel of fig. 3. The points were obtained by averaging over 20 breaths ventilation and end-tidal CO_2 tension just before a change in CO_2 tension was applied. Administration of morphine shifted the CO_2 response curve to higher CO_2 tension values and decreased the slope. Subsequent infusion of physostigmine caused a decrease in B. However, the slope of the response curve remained depressed with respect to the control curve. The slope increased after the administration of naloxone, but B remained decreased. Fig. 4 summarizes the effects of the administration of morphine followed by physostigmine on the S_p and S_c of the chemoreflex loop and B. S_p and S_c were significantly depressed by morphine ($P < 0.01$ *vs.* control) but were not further changed by physostigmine. The B value was increased significantly by morphine ($P < 0.01$ *vs.* control) and was decreased significantly by physostigmine ($P < 0.01$ *vs.* morphine) to values even lower than the control values ($P < 0.01$ *vs.* control). There were no significant changes in the ratio S_p/S_c as in the other parameters of the model. Mean values of the trend parameter (C) were -3.6 ± 4.1 for control, 1.3 ± 1.7 for morphine and -1.5 ± 2.4 ml · min^{-2} for physostigmine.

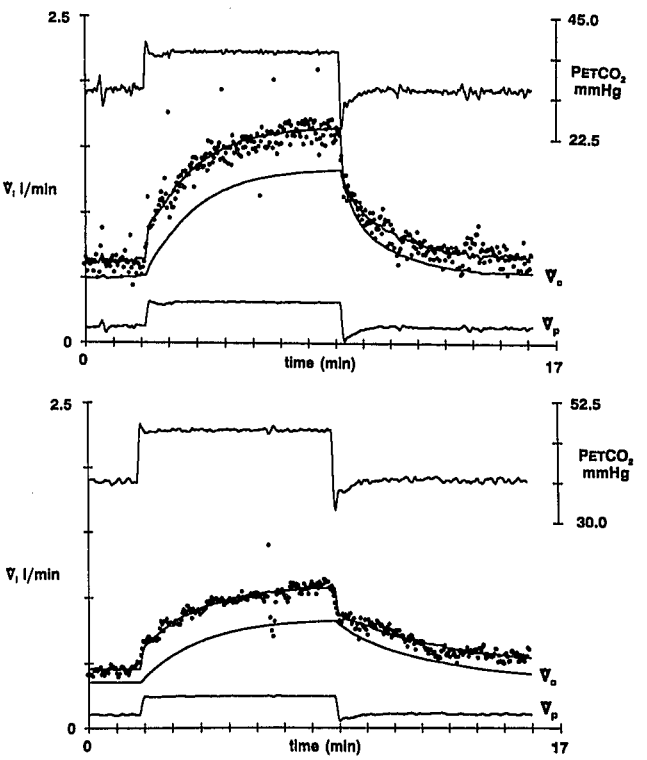

Fig. 2. (*Top*) Response of ventilation and model fit of the control dynamic end-tidal forcing study shown in figure 1. End-tidal CO_2 ($P_{ET_{CO_2}}$) stimulus (millimeters mercury). Smooth curve running in between points is model-fit. The slow central ($\dot{V}_c$) and the fast peripheral ($\dot{V}_p$) components are also shown. The estimated parameters are central time constant of the ventilatory on-transient (τ_{on}) = 104 s, central time constant of the ventilatory off-transient (τ_{off}) = 89 s, time constant of the peripheral ventilatory response (τ_p) = 4.1 s, apneic threshold (B) = 26.7 mmHg, CO_2 sensitivity of the central chemoreflex loop (S_c) = 0.1133 l · min^{-1} · mmHg^{-1}, CO_2 sensitivity of the peripheral chemoreflex loop (S_p) = 0.0265 l · min^{-1} · mmHg^{-1}, time delay needed to transport the CO_2 change from the lungs to the central chemoreceptors (T_c) = 4 s, time delay needed to transport the CO_2 change from the lungs to the peripheral chemoreceptors (T_p) = 3 s, and trend (C) = 0.0002 l · min^{-2}. (*Bottom*) Response of ventilation and model fit of a dynamic end-tidal forcing study after morphine administration. The estimated parameters are τ_{on} = 122 s, τ_{off} = 209 s, τ_p = 3.5 s, B = 31.0 mmHg, S_c = 0.0511 l · min^{-1} · mmHg^{-1}, S_p = 0.0148 l · min^{-1} · mmHg^{-1}, T_c = 5 s, T_p = 4 s, and C = 0.0014 l · min^{-2}.

Mean arterial pressure of the 5 cats decreased from 105 ± 5 to 98 ± 4 mmHg after morphine ($P < 0.01$) and decreased further to 92 ± 5 mmHg after physostigmine ($P < 0.01$ *vs.* control).

Protocol II

In the lower panel of fig. 4 the results of the experiments of protocol II are shown. Physostigmine caused a significant decrease of the S_c and S_p and of B ($P < 0.01$ *vs.* control). Subsequent administration of morphine caused a further decrease in S_c and an increase in B value ($P < 0.01$ *vs.* physostigmine). The decrease in S_p was not significant. The ratio S_p/S_c was not significantly different between the 3 treatments as were the other parameters. Mean values of the trend parameter were 6.0 ± 2.7 for control, 0.4 ± 2.6 for physostigmine and −3.6 ± 1.2 ml · min^{-2} for morphine. The ventilatory response curves of a representative cat are shown in the right panel of fig. 3.

Mean arterial pressure increased from 104 ± 9 to 113 ± 10 mmHg after physostigmine ($P < 0.01$) and decreased to 95 ± 10 mmHg after morphine ($P < 0.01$ *vs.* control).

Effects of Naloxone

The scatter diagrams of fig. 5 show the results for the administration of naloxone in the experiments of protocol I and II. After naloxone S_p and S_c returned to the control values. B, however, remained significantly decreased ($P < 0.01$ *vs.* control).

Mean arterial pressure of the 8 cats of protocol I and II after naloxone (105 ± 23 mmHg) was not significant different from their corresponding control values (102 ± 14 mmHg).

Discussion

The effects of morphine and physostigmine on the control of breathing were evaluated with the DEF technique. As shown previously[17] the DEF technique together with our two-compartment model and least squares parameter estimation, can be used to assess the S_c and S_p and B.

Our study shows that in the anesthetized cat, morphine depresses ventilation by an effect on S_p, S_c and B. However, the ratio S_p/S_c did not change. Recently, Sato *et al.*[21] showed, using identification of medullary CO_2 chemoreceptors by c-*fos* immunochemistry, that the effect of morphine on ventilation is not mediated by blockade of CO_2 receptors in the rostral and caudal chemosensitive areas of the ventral medulla of rats. Furthermore, local application of opiates to the intermediate area, but not to the caudal area, depresses ventilation.[1] It is worth noting that the intermediate area itself is not chemosensitive but is thought to be involved in the transmission of chemoreceptor signals to the respiratory centers.[22] With respect to the effect of morphine on the peripheral chemoreceptors, it is reported that the spontaneous chemoreceptor discharge of the carotid bodies is slightly increased at low doses (intracarotid injection of 0.1 μg) and only at doses larger than 100 μg a decrease in discharge is observed which lasts longer than 45 s.[2] We therefore believe that the change in B we observed is not due to an effect of morphine on the chemoreceptors themselves although we can not entirely exclude such an effect. The finding that the S_p and S_c are depressed to the same extent, because the ratio S_p/S_c was constant, supports the idea that the depressant effect of morphine is mainly on the neuronal structures common to both the pe-

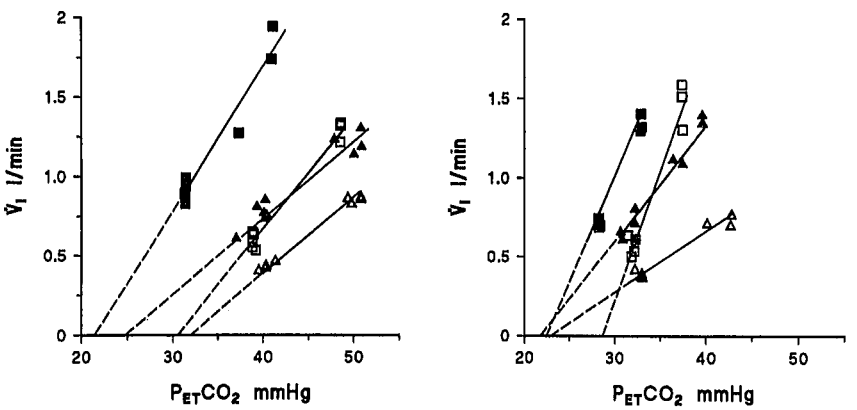

Fig. 3. Ventilatory CO_2 response curves during control conditions (open squares), after administration of morphine (open triangles), during infusion of physostigmine (filled triangles), and after further administration of naloxone (filled squares). (*Left*) Response curves for one cat in protocol I. (*Right*) Curves for one cat in protocol II.

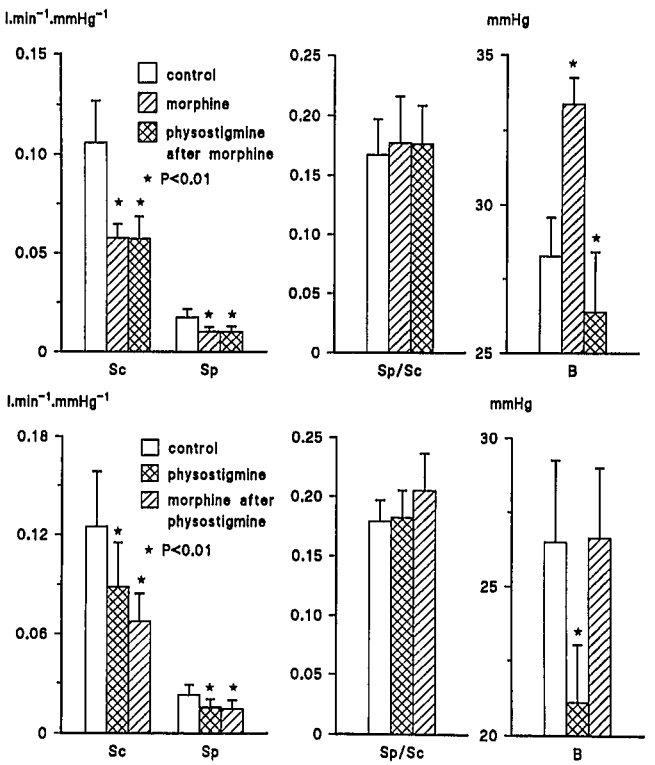

Fig. 4. CO_2 sensitivities of the central (S_c) and the peripheral (S_p) chemoreflex loop, their ratio, and the apneic threshold (B). Values are means and SEM of the means per cat (n = 5). *Significantly different from control values. (*Top*) Results of experiments of protocol I. Morphine and physostigmine data were not significantly different, except for the B value. (*Bottom*) Results of experiments of protocol II. The S_c and B of the morphine experiments were significantly different from those of the physostigmine experiments.

ripheral and central chemoreflex pathway, *i.e.*, the respiratory centers, rather than on the peripheral and central chemoreceptors as such.

Several reports describe the effectiveness of physostigmine against opioid-induced respiratory depression.[9-11] When we infused physostigmine after the administration of morphine, a significant decrease in B value was observed toward or even lower than control values. However, the S_p and S_c of the chemoreflex loop remained depressed. This implies that, in agreement with the above mentioned reports, we too observed that at resting values of $P_{ET_{CO_2}}$ the ventilatory depression is significantly reduced or even completely reversed. However, at higher $P_{ET_{CO_2}}$ levels ventilation is still lower than control values (see fig. 3). Our findings are in contrast with those of Bourke *et al.*, who did not observe an effect of physostigmine on the morphine-induced depressed ventilatory response to CO_2 in humans.[12] On the other hand our findings are partly in agreement with the observations of Snir-Mor *et al.*[10] These authors found that physostigmine not only restored the position but also the slope of the ventilatory response to CO_2 to premorphine values.

It has long been known that cholinergic agents stimulate respiration. Acetylcholine stimulates the peripheral chemoreceptors.[8] However, there is some controversy about effects of physostigmine on the peripheral chemoreceptors. McQueen[23] reported no effect of physostigmine on the spontaneous discharge of the carotid bodies in contrast to other investigators[24,25] who used an *in vitro* preparation and observed a potentiated response to CO_2 and remarkably not to pH. Application of physostigmine and the muscarinic agonist carbachol to the chemosensitive zones of the ventral medulla enhanced ventilation leaving the slope of the CO_2 response the same or slightly decreased.[26,27] However, local application may have different effects than intravenous administration. Physostigmine is rapidly metabolized to eseroline and two other metabolites, and it has been shown that eseroline possesses, besides anticholinesterase activity, opioidlike antinociceptive

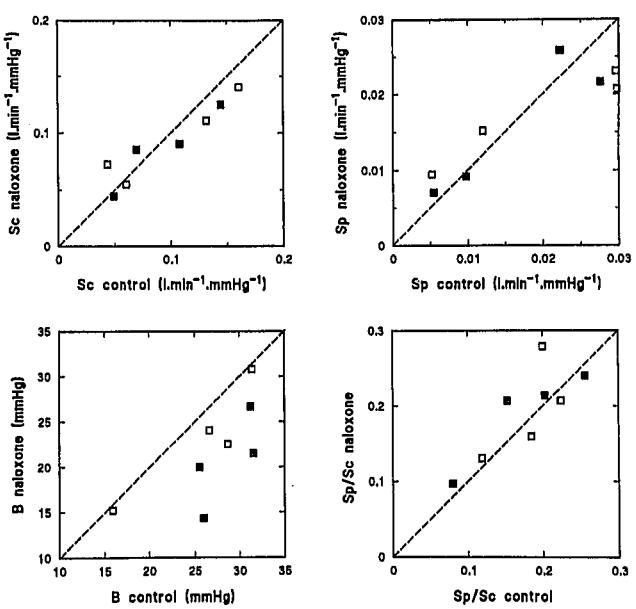

Fig. 5. Scatter diagrams for each cat of the means of CO_2 sensitivities of the central and peripheral chemoreflex loops (S_c and S_p, respectively), the S_p/S_c ratio, and apneic threshold (B) of control experiments and experiments in which naloxone was given after morphine and physostigmine. Filled squares = protocol I; open squares = protocol II.

activity.[28–30] We recently investigated the effects of eseroline on the ventilatory response to CO_2 and found that it decreases the slope of the response probably due to its opioidlike activity because this effect could be antagonized by naloxone.[31] It may well be that the decreased CO_2 sensitivities we found when physostigmine is infused before the administration of morphine (protocol II) is due to the opioidlike effect of the metabolite eseroline. In that case it is unlikely that infusion of physostigmine after the administration of morphine will lead to CO_2 sensitivities of premorphine values as found by Snir-Mor *et al.*[10] The finding that naloxone restored the S_p and S_c of the chemoreflex loops to control values strengthens this idea.

Upon administration of naloxone after the administration of morphine and physostigmine, B decreased to values about 5 mmHg lower than control, a decrease similar as found when physostigmine was infused alone. This strongly suggests that the changes in B observed in this study are mainly due to an action of acetylcholine on its receptors. It has been shown that morphine reduces the release of acetylcholine in the brain.[6] By blocking acetylcholine esterase with physostigmine the amount of acetylcholine available increases.

In summary, our results are consistent with the idea that the depressant effect of morphine on B is caused by a decrease in the release of acetylcholine in the medulla oblongata and this effect can be antagonized by the acetylcholine esterase inhibitor physostigmine. The diminished ventilatory CO_2 sensitivity is presumably due to an effect on opiate receptors in the respiratory integrating centers rather than on the peripheral and central chemoreceptors as such.

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