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Differential Effect of Morphine and Morphine-6-glucuronide on the Control of Breathing in the Anesthetized Cat

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Background: **Morphine's metabolite, morphine-6-glucuronide (M6G), activates the μ-opioid receptor. Previous data suggest that M6G activates a unique M6G receptor that is selectively antagonized by 3-methoxynaltrexome (3mNTX). The authors compared the effects of M6G and morphine on breathing in the anesthetized cat and assessed whether 3mNTX reversal was selective for M6G.**

Methods: **Step changes in end-tidal carbon dioxide concentration were applied in cats anesthetized with α-chloralose-urethane. In study 1, the effect of the 0.15 mg/kg morphine followed by 0.2 mg/kg 3mNTX and next 0.8 mg/kg M6G was assessed in six cats. In study 2, the effect of 0.8 mg/kg M6G followed by 0.2 mg/kg 3mNTX and 0.15 mg/kg morphine was tested in another six cats. The ventilatory carbon dioxide responses were analyzed with a two-compartment model of the ventilatory controller, which consists of a fast peripheral and a slow central component.**

Results: **Both opioids shifted the ventilatory carbon dioxide responses to higher end-tidal carbon dioxide levels. Morphine had a preferential depressant effect within the central chemoreflex loop. In contrast, M6G had a preferential depressant effect within the peripheral chemoreflex loop. Irrespective of the opioid, 3mNTX caused full reversal of and prevented respiratory depression.**

Conclusions: **In anesthetized cats, the μ-opioids morphine and M6G induce respiratory depression at different sites within the ventilatory control system. Because 3mNTX caused full reversal of the respiratory depressant effects of both opioids, it is unlikely that a 3mNTX-sensitive unique M6G receptor is the cause of the differential respiratory behavior of morphine and M6G.**

IN animals and humans, morphine's metabolite, morphine-6-glucuronide (M6G), activates the μ-opioid receptor causing typical opioid behavior.[1–3] This includes analgesia/antinociception, miosis, respiratory depression, and nausea/vomiting. M6G is present in the blood of patients after just a single morphine dose, but its contribution to morphine analgesia and toxicity (*e.g.*, sedation and respiratory depression) becomes significant only after long-term morphine treatment and in patients with renal impairment (the primary route of M6G clearance is *via* the kidneys).[4–7] Several studies from Pasternak *et al.* indicate the existence of a unique M6G receptor responsible for its analgesic activity: (1) In morphine-insensitive CBX mice, M6G analgesia is uncompromised.[8] (2) M6G shows no analgesic cross-tolerance in mice made tolerant to morphine.[8] (3) Labeled M6G binding to bovine brain tissue indicates the existence of a high- and a low-affinity component. The low-affinity component corresponds to labeling of traditional μ-opioid receptors, whereas the high-affinity component shows selectivity to M6G.[9,10] (4) 3-Methoxynaltrexone (3mNTX) is an opioid receptor antagonist selective for the M6G binding site. In CD1 mice and rats, 3mNTX displaces the M6G dose–response (analgesia) curve without affecting the curve for morphine.[10,11] (5) Rats treated with antisense probes against exon 1 of the μ-opioid receptor gene (*Oprm1*) display reduced morphine analgesia but normal M6G analgesia. Similar observations were made for probes targeting specific G protein α subunits.[8,12,13] (6) Finally, exon 1 μ-opioid receptor (*Oprm*) gene knockout mice show the persistence of M6G but not morphine analgesia.[14] The evidence from items 5 and 6 is less compelling because exon 1 μ-opioid receptor knockout mice do not display any G-protein activation.[15] Furthermore, the effect of M6G analgesia in this mouse strain was not reproduced by others.[16] Interestingly, the M6G opioid receptor seems equally sensitive to heroin.[8–14]

There are indications from animal and human studies that M6G produces less respiratory depression than morphine at equiantinociceptive/equianalgesic doses.[17–19] This is an important feature of a potent opioid analgesic because respiratory depression is a potentially lethal side effect of acute opioid administration.[20] Possibly, the differential effect of M6G and morphine on respiration reflects activation of distinct μ-opioid receptors with differential effects on the ventilatory control system. The current study was designed to quantify the respiratory effects of M6G *versus* morphine and assess whether the effect of M6G is related to the previously classified unique M6G receptor. We initially measured the effects of morphine and M6G on the dynamic ventilatory response to carbon dioxide in anesthetized cats and next investigated the effect of the M6G receptor–selective antagonist 3mNTX on morphine- and M6G-induced respiratory depression. The ventilatory responses were analyzed using a two-compartment model of the respiratory controller, reflecting the peripheral and central chemoreflex pathways.[21–24] These studies provide infor-

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mation about the sites of action of M6G, morphine, and 3mNTX with respect to their dynamic and steady state effects on the ventilatory carbon dioxide response curves.

Materials and Methods

The experiments were performed after approval of the protocol by the local Ethical Committee for Animal Experiments (UDEC, Leiden, The Netherlands). Eighteen purebred (European shorthair) cats (8 males and 10 females; mean (± SD) body weight, 3.3 ± 1.0 kg) were sedated with 10 mg/kg intramuscular ketamine hydrochloride. Next, the animals were anesthetized with a gas containing 0.7–1.4% sevoflurane and 30% oxygen in nitrogen. The right femoral vein and artery were cannulated, after which 20 mg/kg α-chloralose and 100 mg/kg urethane were slowly administered intravenously. Subsequently, the volatile anesthetic was withdrawn. Approximately 1 h later, an infusion of an α-chloralose–urethane solution was started at a rate of 1.0–1.5 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} α-chloralose and 5.0–7.5 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} urethane. This regimen leads to conditions in which the level of anesthesia is sufficient to suppress pain withdrawal reflexes but light enough to preserve the corneal reflex. The stability of the ventilatory parameters was studied previously, and they were found to be similar than those in awake animals, and stable over a period of at least 6 h.[24–26] We use a feline experimental model because (1) it allows the application of the dynamic end-tidal forcing (DEF) technique, which is an important requirement for studying ventilatory control in a reliable fashion; and (2) cat data are often well comparable to human data.

To measure inspiratory and expiratory flow, the trachea of the animals was cannulated and connected to a Fleisch No. 0 flow transducer (Fleisch, Lausanne, Switzerland), which was attached to differential pressure transducer (Statham PM197; Los Angeles, CA). The flow transducer was connected to a T-piece of which one arm received a continuous fresh gas flow of 5 l/min. Three computer-controlled mass flow controllers (High-Tec, Veenendaal, The Netherlands) composed desired inspiratory gas mixtures of oxygen, carbon dioxide, and nitrogen. The inspiratory and expiratory fractions of oxygen and carbon dioxide were measured with a Datex Multicap monitor (Datex-Engstrom, Helsinki, Finland). The temperature of the animals was controlled within 1°C and ranged among cats between 38° and 39°C. All signals were recorded digitally (sample frequency, 100 Hz) and stored on a breath-to-breath basis on a computer for further analysis.

Study Design

The dynamic ventilatory response to carbon dioxide was studied with the DEF technique.[21–24,27] Stepwise changes in end-tidal partial pressure of carbon dioxide (P_{ETCO_2}) at a constant end-tidal partial pressure of oxygen (P_{ETO_2}; 110 mmHg) were applied. Each DEF run started with a steady state period of 2 min, during which P_{ETCO_2} was maintained at 4 mmHg above resting values. Thereafter, the P_{ETCO_2} was increased by 7.5 mmHg for 7 min and then decreased to the initial value and kept constant for another 7 min. To avoid irregular breathing at partial pressure of carbon dioxide values close to the apneic threshold, we adjusted clamped baseline P_{ETCO_2} at a level approximately 3–4 mmHg higher than the apneic threshold during any given experimental condition (*i.e.*, in control and after each drug infusion).

Initially, in two cats, the effects of four cumulative M6G doses (0.15, 0.3, 0.6, and 0.9 mg/kg) followed by two cumulative 3mNTX doses (0.1 and 0.2 mg/kg) on ventilation were tested (with P_{ETCO_2} clamped 4 mmHg above resting). This was done to determine the M6G and 3mNTX doses to be used. Next, we performed three separate studies. In study 1, the effect of the intravenous infusion of morphine (0.15 mg/kg) followed by 3mNTX (0.2 mg/kg intravenous) and subsequently M6G (0.8 mg/kg intravenous) on the dynamic ventilatory response to carbon dioxide was assessed. In study 2, ventilatory carbon dioxide responses were obtained after the intravenous infusion of M6G (0.8 mg/kg), followed by 3mNTX (0.2 mg/kg intravenous) and lastly morphine (0.15 mg/kg intravenous). Finally, the effect of just 3mNTX (0.2 mg/kg intravenous) was assessed in four cats. In all studies, three or four control DEF runs were obtained before any drug infusion (control runs); after each drug infusion and a pause of approximately 20–30 min, two to four DEF runs were performed. M6G was obtained from CeNeS Ltd. (Cambridge, United Kingdom), morphine was obtained from Pharmachemie BV (Haarlem, The Netherlands), and 3mNTX was obtained from Sigma BV (Zwijndrecht, The Netherlands).

Data and Statistical Analysis

The steady state relation between inspired minute ventilation ($\dot{V}_I$) and P_{ETCO_2} is linear down to apnea and described by $\dot{V}_I = [Gc + Gp] \cdot [P_{ETCO_2} - B]$,[21–24,27] where Gc and Gp are the ventilatory carbon dioxide sensitivities of the central and peripheral chemoreflex loops, and B represents the apneic threshold or extrapolated P_{ETCO_2} at zero $\dot{V}_I$. When applying rapid changes in P_{ETCO_2} at constant P_{ETO_2}, it is possible to quantify the contributions of the peripheral and central chemoreflex loops to total ventilation. This is based on the difference in response times and dynamics of the two chemoreflexes in response to a change in P_{ETCO_2}.[21–24] The central chemoreflex loop displays a relative large time delay (average response time in cats is 8 s) with slow dynamics (average time constant in cats is 100 s); the response time of the peripheral chemoreflex loop is on average 4 s, with a time constant of approximately 10 s.[21,22,24] To

estimate Gc, Gp, and B, we fitted the ventilatory responses to a two-compartmental model using a least-squares fitting routine as described previously.[21–24] In the fitting procedure, parameters Gp and B were not restricted to values of zero or greater. Occasionally, a negative optimal value for Gp was obtained, which then was set to zero in the statistical analysis.

Initially, the data were tested for normality using the Kolmogorov–Smirnov test. Because all of the data were normally distributed, next, to determine the level of significance of the treatment effects, we performed an analysis of variance on the group data. A separate analysis was performed on the data from studies 1, 2, and 3. *Post hoc* comparisons were made with the Bonferroni test. To correct for multiple comparisons, P values less than 0.05 were considered significant. The analysis was performed using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL). Values reported are mean of the cat means ± SD.

Results

The M6G and 3mNTX doses used in the study were based on the effects of incrementing doses of the two drugs on resting ventilation as observed in two cats (see fig. 1 for the results in one animal). M6G produced a dose-dependent depression of resting ventilation. The M6G dose causing a depression similar to that observed with 0.15 mg/kg morphine (see Lötsch *et al.*,[20] Berkenbosch *et al.*,[21] and Berkenbosch *et al.*[22]) was 0.8 mg/kg. We therefore used an M6G dose of 0.8 mg/kg in the subsequent studies. 3mNTX produced no effect at 0.1 mg/kg but displayed full reversal of the depressed resting ventilation at a dose of 0.2 mg/kg.

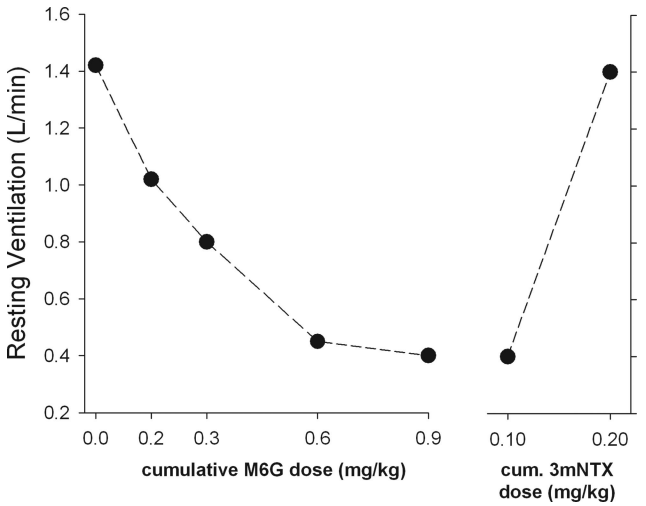

Fig. 1. Effect of four cumulative morphine-6-glucuronide (M6G) doses flowed by two cumulative 3-methoxynaltrexone (3mNTX) doses on resting ventilation in one cat. The data are obtained at a clamped end-tidal partial pressure of carbon dioxide of 45 mmHg. Only at M6G doses of 0.6 mg/kg and greater was a reduced response to carbon dioxide observed (data not shown).

To get an appreciation of the quality of the DEF experiments and data fits, we plotted four examples obtained in one cat from study 2 in figure 2. The top diagrams show the applied steps into and out of P_{ETCO_2}. In the bottom graphs, each dot represents one breath. The slow central components and a fast peripheral component are shown together with the least-squares model fits (the thick lines through the data points). As may be observed by the eye, the model adequately described the data. In these examples, M6G increased the apneic threshold and reduced the ventilatory carbon dioxide sensitivity of the peripheral chemoreflex loop without affecting the ventilatory carbon dioxide sensitivity of the central chemoreflex loop. The subsequent infusion of 3mNTX caused the return of apneic threshold to control values and increased peripheral carbon dioxide sensitivity to values greater than control. Finally, the infusion of morphine after 3mNTX did not further influence any of the model parameters.

Study 1

In the six cats of study 1, we performed 22 control experiments, 20 after morphine, 19 after 3mNTX, and 16 after M6G. Treatment effects were observed on the apneic threshold and central and total carbon dioxide sensitivities, with no effects on peripheral carbon dioxide sensitivity and the ratio of peripheral to central carbon dioxide sensitivity (fig. 3). Morphine caused a significant increase of the apneic threshold from 27.5 ± 3.6 to 31.5 ± 2.2 mmHg, and reduced the central and total carbon dioxide sensitivities from 0.13 ± 0.06 to 0.07 ± 0.04 and from 0.16 ± 0.07 to 0.08 ± 0.04 $l \cdot min^{-1} \cdot mmHg^{-1}$, respectively ($P < 0.01$). After the infusion of the opioid antagonist 3mNTX, the apneic threshold decreased to values below baseline (24.8 ± 2.5 mmHg), central carbon dioxide sensitivity increased to a value between morphine and M6G (0.11 ± 0.04 $l \cdot min^{-1} \cdot mmHg^{-1}$), and total carbon dioxide sensitivity returned to control values (0.11 ± 0.05 $l \cdot min^{-1} \cdot mmHg^{-1}$). Infusion of M6G after 3mNTX had no further effect on any of the model parameters.

Study 2

In the six cats of study 2, we performed 27 control experiments, 17 after M6G, 18 after 3mNTX, and 17 after morphine. Treatment effects were observed for all model parameters except central carbon dioxide sensitivity (fig. 4). M6G caused a significant increase of the apneic threshold from 26.3 ± 5.7 to 34.2 ± 25.0 mmHg, and reduced the peripheral and total carbon dioxide sensitivities from 0.031 ± 0.013 to 0.013 ± 0.017 and from 0.16 ± 0.02 to 0.13 ± 0.05 $l \cdot min^{-1} \cdot mmHg^{-1}$, respectively ($P < 0.01$). The ratio of peripheral to central carbon dioxide sensitivity was reduced from 0.26 ± 0.13 to 0.09 ± 0.13. Infusion of the opioid antagonist after M6G caused full return to baseline levels of the

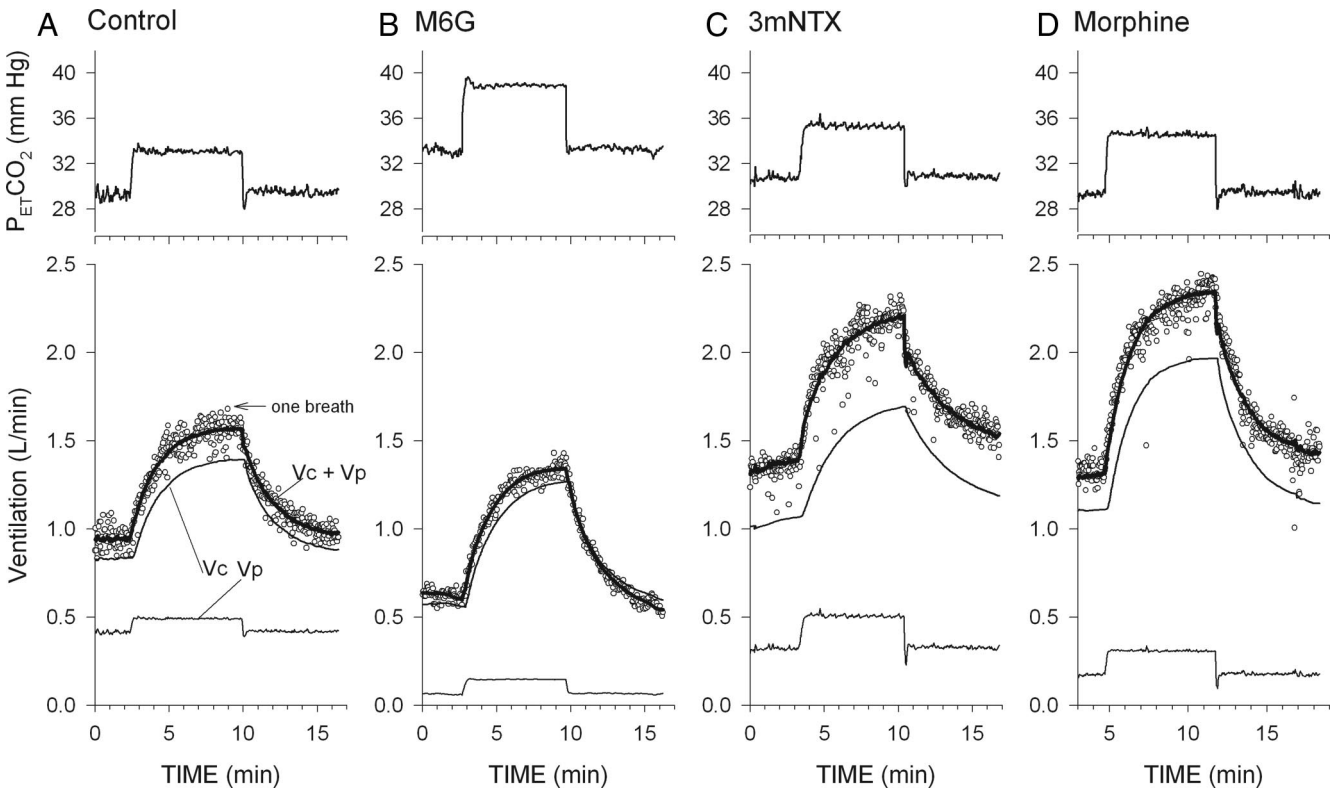

Fig. 2. Example of the dynamic ventilatory responses to end-tidal partial pressure of carbon dioxide (P_{ETCO_2}) in one cat and data fits. One control response (*A*), one response after 0.8 mg/kg morphine-6-glucuronide (M6G; *B*), one response after a subsequent dose of 3-methoxynaltrexone (3mNTX; *C*), and one response after a subsequent dose of 0.15 mg/kg morphine (*D*) are shown. The *top panels* show the input function to the system (*i.e.*, P_{ETCO_2}). Each *circle* represents one breath. The *line* with the fast dynamics is the estimated output of the peripheral chemoreflex loop (Vp); the *thin line* with the slow dynamics is the estimated output of the central chemoreflex loop (Vc). The sum of Vp and Vc is the *thick line* through the data points. Parameter values for the shown data fits are as follows: (*A*) apneic threshold = 23.6 mmHg, central carbon dioxide sensitivity = 0.13 l · min^{-1} · mmHg^{-1}, peripheral carbon dioxide sensitivity = 0.020 l · min^{-1} · mmHg^{-1}; (*B*) apneic threshold = 28.7 mmHg, central carbon dioxide sensitivity = 0.13 l · min^{-1} · mmHg^{-1}, peripheral carbon dioxide sensitivity = 0.011 l · min^{-1} · mmHg^{-1}; (*C*) apneic threshold = 22.8 mmHg, central carbon dioxide sensitivity = 0.13 l · min^{-1} · mmHg^{-1}, peripheral carbon dioxide sensitivity = 0.04 l · min^{-1} · mmHg^{-1}; (*D*) apneic threshold = 22.5 mmHg, central carbon dioxide sensitivity = 0.16 l · min^{-1} · mmHg^{-1}, peripheral carbon dioxide sensitivity = 0.025 l · min^{-1} · mmHg^{-1}.

apneic threshold (26.5 ± 5.1 mmHg), the peripheral and total carbon dioxide sensitivities (0.024 ± 0.017 and 0.17 ± 0.05 l · min^{-1} · mmHg^{-1}, respectively), and the ratio of peripheral to central carbon dioxide sensitivity (0.18 ± 0.11). Infusion of morphine after 3mNTX had no further effect on any of the model parameters.

Study 3

In the four cats of study 3, we performed 30 experiments (15 control and 15 after 3mNTX). Infusion of 0.2 mg/kg 3mNTX had no systematic effect on any of the estimated model parameters (fig. 5).

Discussion

The main findings of our study are as follows: (1) Morphine (0.15 mg/kg) affects the control of breathing by increasing the apneic threshold and by reducing central ventilatory carbon dioxide sensitivity. (2) The effect of morphine is fully antagonized by 3mNTX, and subsequent infusions of M6G are without further effects. (3) M6G (0.8 mg/kg) caused an increase of the apneic threshold together with a reduction of the peripheral carbon dioxide sensitivity without affecting central carbon dioxide sensitivity. This indicated a preferential effect of M6G within the peripheral chemoreflex loop. (4) The effect of M6G is fully antagonized by 3mNTX, and subsequent infusions of morphine are without further effects. (5) 3mNTX (0.2 mg/kg) has no effect on the apneic threshold and the peripheral and central carbon dioxide sensitivities when given without previous opioid infusion.

We used an M6G dose that was 5.3 times greater than the morphine dose. The M6G dosing was based on our pilot experiments in two cats showing that at 0.8 mg/kg, M6G causes a reduction in resting ventilation of similar magnitude as 0.15 mg/kg morphine. This observation was later confirmed: The morphine and M6G ventilatory carbon dioxide response curves intersect at 38 mmHg (just above the metabolic hyperbola), a value close to the mean clamped P_{ETCO_2} value in our study (fig. 6). In contrast to our observation of greater morphine po-

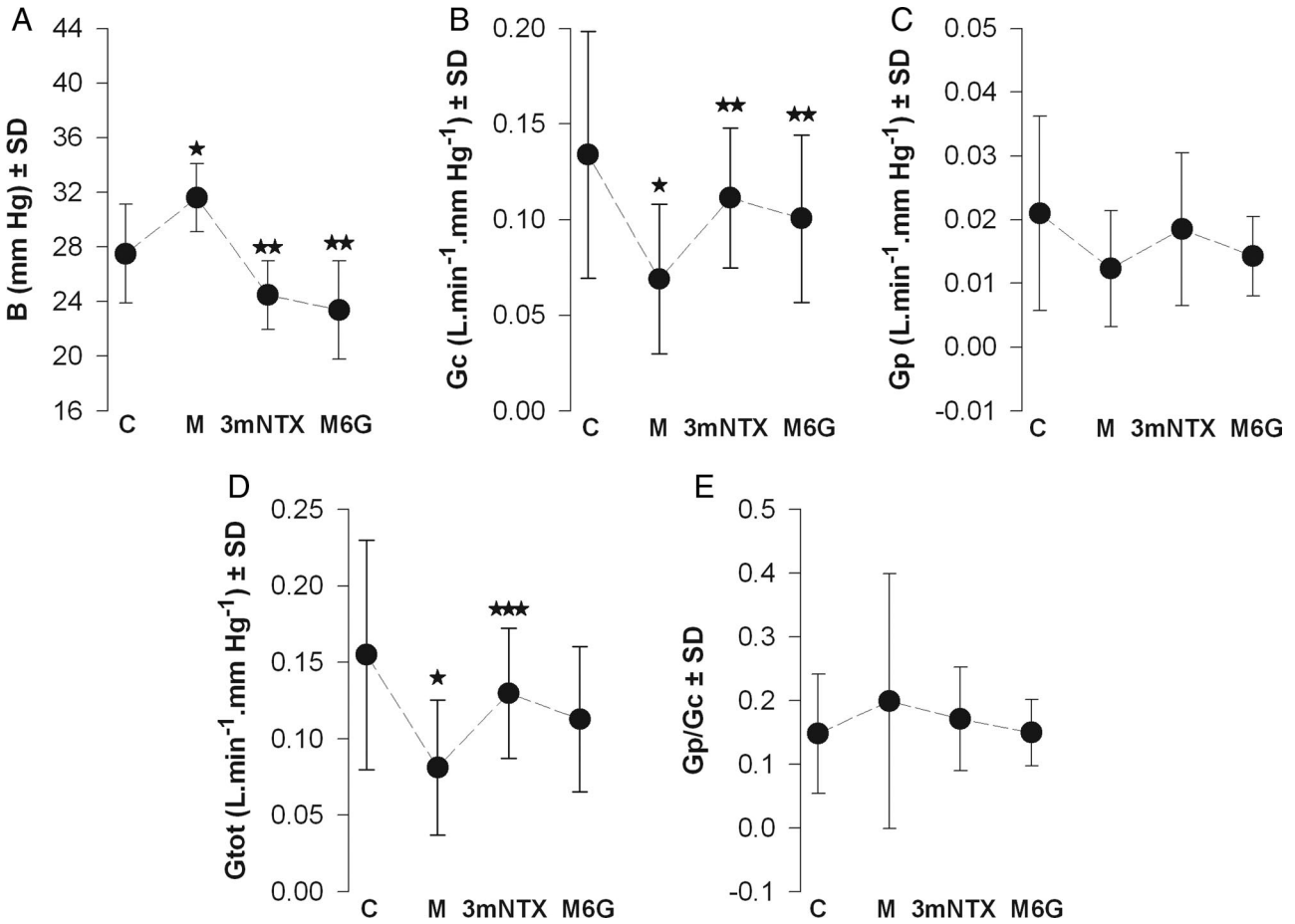

Fig. 3. Study 1: Effect of morphine (M) followed by 3-methoxynaltrexone (3mNTX) and morphine-6-glucuronide (M6G) on apneic threshold (B; *A*), central carbon dioxide sensitivity (Gc; *B*), peripheral carbon dioxide sensitivity (Gp; *C*), total carbon dioxide sensitivity (sum of peripheral and central carbon dioxide sensitivity; *D*), and ratio of peripheral to central carbon dioxide sensitivity (*E*). Data were obtained in six cats. Values are the mean of the cat means ± SD. Treatment effects were obtained for apneic threshold ($P < 0.001$), central carbon dioxide sensitivity ($P < 0.001$), and total carbon dioxide sensitivity ($P < 0.001$). * $P < 0.01$ *versus* control. ** $P < 0.01$ *versus* morphine and control. *** $P < 0.01$ *versus* morphine. C = control.

tency, animal studies usually show that M6G is the more potent drug with respect to antinociception (see Kilpatrick and Smith[3] and references cited therein) and respiratory depression. For example, in mice, rats, dogs, and neonatal guinea pigs, morphine:M6G potency ratios for respiratory depression vary from 1:4 after intraperitoneal or intravenous injections to 1:10 after intracerebroventricular injection.[28–31] Apparently, cats form an exception to this rule, which may be related to the absence of an effect on the carbon dioxide sensitivity of the central chemoreflex loop, the major component of total chemical drive.

In the current study, morphine had no effect on the peripheral carbon dioxide sensitivity (fig. 3). This contrasts with previous studies on morphine using a similar cat model,[21,22,27] as well as with our observation that morphine did not affect the ratio of peripheral to central carbon dioxide sensitivity (fig. 3). This latter observation, together with the reduction of central carbon dioxide sensitivity, suggests an effect of morphine on neuronal structures common to both the peripheral and central chemoreflex pathway (such as the respiratory centers in the ventrolateral medulla). Some effect of morphine on the peripheral chemoreflex is expected. There are indications for the presence of opioid receptors in cat carotid bodies: 98% of type I carotid body cells exhibit enkephalin immunoreactivity,[32] and naloxone enhances the response to hypoxia as measured from single or paucifiber preparations of carotid body afferents.[33] Taking into account the above information, we believe that our current study may have been underpowered to observe a morphine effect on the peripheral carbon dioxide sensitivity ($P = 0.07$ *vs.* control). However, we cannot exclude that study differences in the effect of morphine on the peripheral chemoreflex loop are also partly related to differences in the genetic background of the cats we used in our studies: mongrel cats in our previous studies *versus* inbred animals in the current study.[21,22,27]

Compared with morphine, M6G showed important differences in its effect on ventilatory control. At the relatively high dose tested, M6G increased the apneic

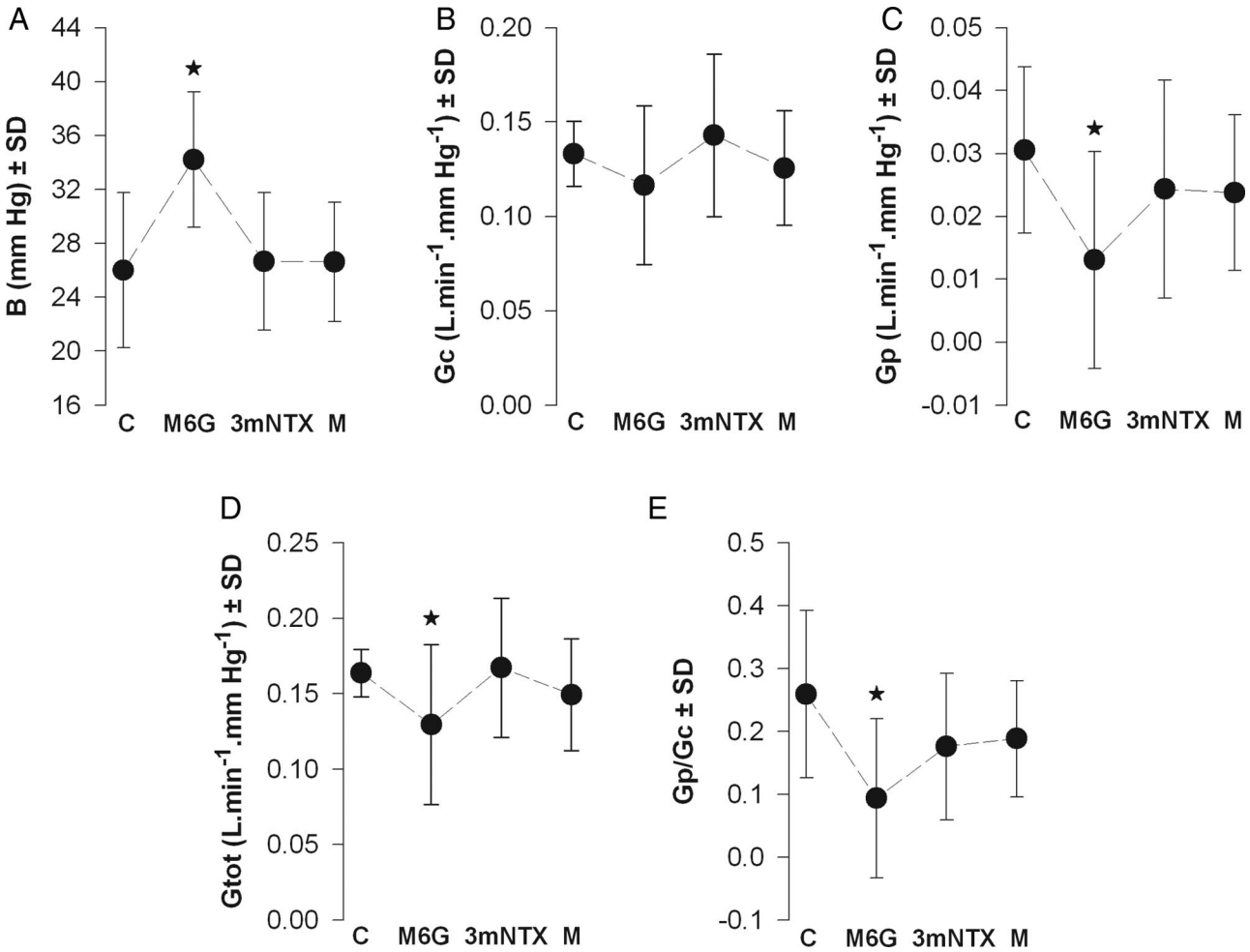

Fig. 4. Study 2: Effect of morphine-6-glucuronide (M6G) followed by 3-methoxynaltrexone (3mNTX) and morphine (M) on apneic threshold (B; *A*), central carbon dioxide sensitivity (Gc; *B*), peripheral carbon dioxide sensitivity (Gp; *C*), total carbon dioxide sensitivity (Gtot; sum of peripheral and central carbon dioxide sensitivity; *D*), and ratio of peripheral to central carbon dioxide sensitivity (Gp/Gc; *E*). Data were obtained in six cats. Values are the mean of the cat means ± SD. Treatment effects were observed for apneic threshold ($P < 0.001$), central carbon dioxide sensitivity ($P = 0.01$), peripheral carbon dioxide sensitivity ($P = 0.001$), total carbon dioxide sensitivity ($P < 0.001$), and ratio of peripheral to central carbon dioxide sensitivity ($P = 0.001$). *$P < 0.01$ *versus* control and 3mNTX. C = control.

threshold by 8 mmHg, whereas the peripheral carbon dioxide sensitivity decreased by more than 60% without any effect on central carbon dioxide sensitivity (morphine reduced the central carbon dioxide sensitivity by approximately 50%; see also fig. 6). There are several possible explanations for the difference in respiratory behavior between the two opioids. In contrast to morphine, M6G may not have crossed the blood–brain bar-

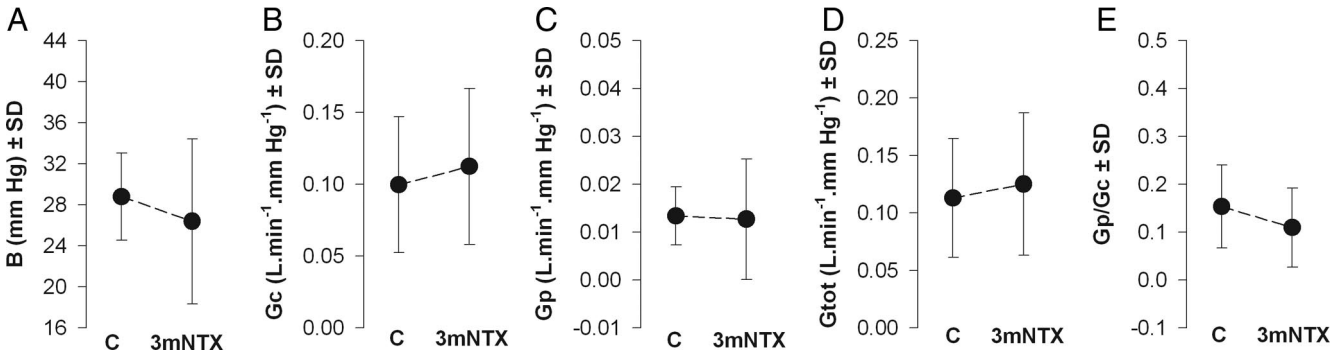

Fig. 5. Study 3: Effect of 3-methoxynaltrexone (3mNTX) on apneic threshold (B; *A*), central carbon dioxide sensitivity (Gc; *B*), peripheral carbon dioxide sensitivity (Gp; *C*), total carbon dioxide sensitivity (Gtot; sum of peripheral and central carbon dioxide sensitivity; *D*), and ratio of peripheral to central carbon dioxide sensitivity (Gp/Gc; *E*). Data were obtained in four cats. Data are the mean of the cat means ± SD. C = control.

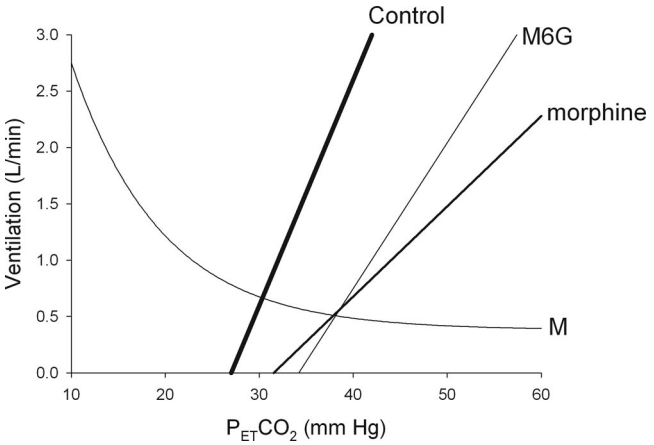

Fig. 6. Effect of 0.15 mg/kg morphine and 0.8 mg/kg morphine-6-glucuronide (M6G) on the ventilatory response to carbon dioxide. The control response is also given. During anesthesia, resting ventilation occurs at the intersection of the ventilatory carbon dioxide response curve and the metabolic hyperbola (M). While resting ventilation did not differ between the two drugs, the end-tidal partial pressure of carbon dioxide (P_{ETCO_2}) to reach a ventilation level of 2 l/min was 7 mmHg (approximately 1 vol%) greater for morphine than for M6G (50 *vs.* 57 mmHg). This is an indication that M6G produces less respiratory depression than morphine at the drug doses used.

rier in sufficient amounts but had its effect at the carotid bodies. M6G is much more polar than morphine[34] and consequently passes the blood–brain barrier much slower than morphine does.[35] However, at the dose used by us and the relatively low volume of distribution and clearance, there is a large M6G concentration gradient across the blood–brain barrier.[36] This results in sufficient passage of M6G into the brain to cause a central effect. Furthermore, although opioid receptors are assumed to exist in the carotid body (see previous paragraph), there are no studies that directly demonstrate the existence of μ-opioid receptors in the carotid chemoreceptors. Of interest to our discussion is the observation that in our anesthetized cat model, we were unable to observe an effect of morphine (0.15 mg/kg) on the steady state ventilatory response to hypoxia.[22] In humans, an experimental opioid that does not cross the blood–brain barrier has no effect on the ventilatory response to acute hypoxia,[37] whereas intrathecal morphine has a profound and long-lasting effect on this response.[38] In summary, we suggest that an appreciable amount of the M6G that we infused did cross the blood–brain barrier and consequently may have affected the ventilatory control system for a large part at central sites (*i.e.*, within the central nervous system).

Another possibility for the observed differences between morphine and M6G is that whereas morphine acts at the classic μ-opioid receptor, ubiquitously present on the neuronal substrates of the ventilatory control system, M6G acts at the proposed unique M6G receptor,[8–14] which is then present in the peripheral chemoreflex pathways and/or brain stem neurons that control the apneic threshold but not within the central chemoreflex pathway. An important feature of this opioid receptor system is its selective antagonism by 3mNTX.[10,11] However, we were unable to demonstrate 3mNTX selectivity for M6G-induced respiratory depression: 3mNTX antagonized both morphine and M6G-induced respiratory changes, and administration of either opioid after 3mNTX was without effect. One can contend that we missed a distinctive effect of 3mNTX between morphine and M6G because we did not perform dose–response studies. There are strong arguments to dismiss this suggestion. In mice, intracerebroventricular infusion of 2.5 ng 3mNTX significantly decreased the analgesic actions of M6G without affecting morphine analgesia (see fig. 2 of Brown *et al.* [10]). Five to six times higher doses of 3mNTX were required to reduce morphine analgesia to the same effect.[10] We observed that at the lowest dose at which 3mNTX caused full reversal of M6G respiratory effect (0.2 mg/kg), full reversal of morphine respiratory effect already occurred. Because the dose–response of 3mNTX seemed to be very steep (no effect at 0.1 mg/kg; fig. 1) we decided not to test the effect of 3-mNTX on M6G or morphine at doses less than 0.2 mg/kg. Hence, our data permit the conclusion that in contrast to the data obtained in mice and rats on analgesia,[8–14] our data do not suggest the presence of a unique 3mNTX-sensitive M6G receptor in the ventilatory control system of cats. In agreement with our findings, in rhesus monkeys, 3mNTX was able to antagonize the antinociceptive effects of heroin as well as morphine.[39] However, our design is unable to exclude the existence of a separate (3mNTX-insensitive) M6G binding site. It may be that such a binding site may need to be pursued in less complex systems than the ventilatory control system.

There are several alternative explanations for our observations. (1) Morphine and M6G interact with distinct subpopulations of the μ-opioid receptor, which are differentially expressed on the various neuronal substrates of the ventilatory control system. These subpopulations may be splice variants of the μ-opioid receptor gene. In mice, at least 15 of such variants arising from alternative splicing have been identified.[40] (2) Morphine and M6G acting at the same opioid receptor may activate different G proteins, causing differences in signaling events and consequently divergence in behavioral responses.[41] (3) Differences in distribution of morphine and M6G exist within the brain compartment.[42] (4) Finally, morphine and M6G may differentially activate excitatory pathways within the ventilatory control system. This may be similar to the hyperalgesic responses observed after M6G infusion but not morphine in mice lacking the μ-opioid receptor.[16,28]

A final point of criticism may be that in the current study, we found larger predrug (*i.e.*, baseline) values for the peripheral and central carbon dioxide sensitivities compared with some of our previous studies (*e.g.*, see Berkenbosch *et al.*[21]). In (awake and anesthetized) animals and humans, the variability in ventilatory carbon dioxide and hypoxic sensitivities is considerable (20–30%),[43,44] and this applies particularly to the relative contributions of the peripheral and central chemoreceptors to the total ventilatory carbon dioxide response.[45] By itself, the ratio of peripheral to central carbon dioxide is insensitive to the depth of anesthesia.[46] This does not exclude, however, that the depth of anesthesia in our current animals may have been somewhat less because, compared with our previous studies, we adapted premedication (reducing the ketamine dose) and the inhalational and intravenous anesthesia (using sevoflurane rather than halothane for maintenance and reducing the chloralose–urethane dose). This then may have resulted in larger baseline ventilatory carbon dioxide sensitivities than in some of our previous studies. Other causes for the observed differences may be biologic variability related to genetic components (*e.g.*, the use of inbred animals in our current study). It is important to note, however, that irrespective of the baseline parameter values, the chosen anesthetic regimen results in a stable preparation and steady experimental conditions over several hours (> 6 h).[25]

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