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Morphine has latent deleterious effects on the ventilatory responses to a hypoxic challenge

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Abstract

The aim of this study was to determine whether morphine depresses the ventilatory responses elicited by a hypoxic challenge (10% O_2, 90% N_2) in conscious rats at a time when the effects of morphine on arterial blood gas (ABG) chemistry, Alveolar-arterial (A-a) gradient and minute ventilation (V_M) had completely subsided. In vehicle-treated rats, each episode of hypoxia stimulated ventilatory function and the responses generally subsided during each normoxic period. Morphine (5 mg/kg, i.v.) induced an array of depressant effects on ABG chemistry, A-a gradient and V_M (via decreases in tidal volume). Despite resolution of these morphine-induced effects, the first episode of hypoxia elicited substantially smaller increases in V_M than in vehicle-treated rats, due mainly to smaller increases in frequency of breathing. The pattern of ventilatory responses during subsequent episodes of hypoxia and normoxia changed substantially in morphine-treated rats. It is evident that morphine has latent deleterious effects on ventilatory responses elicited by hypoxic challenge.

Keywords

morphine; hypoxia; minute ventilation; arterial blood gas chemistry; conscious rats

1. INTRODUCTION

Opioids depress minute ventilation (V_M) and disturb arterial blood gas (ABG) chemistry and Alveolar-arterial (A-a) gradients in humans via central and peripheral effects [1–4]. The ability of morphine to depress the ventilatory responses of humans to hypoxic [5–8] and hypercapnic [8] challenges, strongly suggests that opioids depress carotid body mechanisms responsive to these challenges [9,10] and central mechanisms responsive to hypercapnia [7, 8]. Evidence that the metabolite, morphine-6-glucurodide (M6G), blunts the ventilatory response to hypercapnic challenge in humans without affecting resting ventilation [5] poses

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Conflicts of Interest
None

the unaddressed question as to whether analgesic doses of morphine have latent deleterious effects on ventilatory systems responding to hypoxic/hypercapnic challenges. This is an important question since patients are deemed to have recovered from the acute ventilatory depressant effects of morphine when respiratory rate and ABG chemistry are normal. However, if morphine was still able to depress ventilatory responses to hypoxic challenge, patients may be prone to respiratory collapse because chemoafferent systems fail to trigger appropriate ventilatory responses.

Animal studies have provided extensive insight into the mechanisms by which opioids such as morphine depress ventilation and ABG chemistry [11–15]. The mechanisms include (1) centrally-mediated depression of ventilatory drive [16], (2) skeletal muscle rigidity in the chest-wall [17], (3) increases in airways resistance [18,19], and (4) an increase pulmonary vascular resistance, which suggests decreased perfusion of alveoli [20]. Opioids also blunt the hypoxic ventilatory response in rats [21] and inhibit carotid body chemoafferent responses to hypoxia and hypercapnia [22–24]. As with humans, it is not known whether morphine has latent deleterious effects on ventilatory control systems in the rat. As such, our aim was to determine whether morphine depressed the ventilatory responses of conscious rats elicited by hypoxic challenges given when the effects of morphine on ABG chemistry, A-a gradient and V_M had completely subsided.

2. METHODS

2.1. Rats and surgeries

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the Animal Care and Use Committee of the University of Virginia. Adult male Sprague-Dawley rats (Harlan, Madison, WI, USA) were implanted with jugular vein and femoral arterial catheters (ABG studies) or jugular vein catheters only (ventilation studies) under 2% isoflurane anesthesia. The rats were allowed 4 days to recover from surgery before use. All catheters were flushed with saline at least 4h before commencement of experiments. All studies were performed in a quiet room with relative humidity of 52 ± 4% and room temperature of 21.3 ± 0.2 °C.

2.2. Analgesia assay

Analgesia was assayed using Hargreaves thermal sensitivity method [25,26]. The rats were placed into plexiglass animal enclosures surrounding a 30°C heated glass platform and were allowed to acclimate for at least 30 min. A radiant heat source was activated with a timer and focused onto the animal hindpaw. Paw-withdraw latency was determined by a detector that halted both lamp and timer upon paw withdrawal. Lamp intensity was adjusted to obtain average baseline withdrawal latencies of 20 sec, to allow for detection of possible hyperalgesia. A cutoff of 40 sec was used to prevent tissue damage.

2.3. Blood gas measurements and determination of Arterial-alveolar gradient

pH, pCO_2, pO_2 and sO_2 of arterial blood samples (100 μL) were measured by a Radiometer Blood-gas Machine (ABL800 FLEX). The A-a gradient measures the difference between

alveolar arterial blood pO_2 and is an index of ventilation-perfusion in the lung [27,28]. An increased gradient reflects an abnormally low pO_2 in lung blood versus alveoli. A decrease in PaO_2, without a change in A-a gradient is caused by hypoventilation whereas a decrease in PaO_2 with an increase in A-a gradient indicates ventilation-perfusion mismatch or shunting [27,28]. The formulas used to derive A-a gradient are shown in the legend to Table 1.

2.4. Ventilatory parameters

Ventilatory parameters were continuously recorded in conscious unrestrained rats using a whole-body plethysmography system (PLY 3223; BUXCO Incorporated, Wilmington, NC, USA) [29,30]. The parameters were (1) frequency of breathing (f_R), (2) tidal volume (V_T), (3) minute volume (V_M), (4) inspiratory time (T_I), (5) expiratory time (T_E), (6) end inspiratory pause (EIP), time between end of inspiration and start of expiration, (7) peak inspiratory flow (PIF), and (8) peak expiratory flow (PEF). V_T/T_I, an index of Respiratory Drive [31], was calculated from the recorded V_T and T_I, data. The provided software constantly corrected digitized values for changes in chamber temperature and humidity and a rejection algorithm was included in the breath-by-breath analysis to exclude motion-induced artifacts. Due to the closeness of body weights in the experimental groups, ventilatory data are presented without corrections.

2.5. Body Temperature measurements

Body temperatures (B_T) of male Sprague Dawley rats were measured via telemetry [32]. Rats were anesthetized (2% isoflurane) and a telemetry probe (ETA-F20, Data Sciences International, MN) was implanted into the peritoneal cavity. A jugular vein catheter was also implanted. After 4 days, the rats were placed in plethysmography chambers, under each of which was placed a telemetry receiver connected via a data exchange matrix to a computer. The B_T signals were sampled once a minute using internal hardware and Dataquest ART Gold 3.1 software. Digitized signals were transferred to MATLAB (MathWorks, Natick, MA) for analysis.

2.6. Protocols for analgesia studies

Paw-withdraw latencies were determined in a group of 4 rats (290 ± 4 grams) before and 20, 40, 60 and 90 min after a bolus injection of morphine (5 mg/kg, i.v.). All studies used an injectable form of (+)-morphine sulfate (10 mg/ml) from Baxter Healthcare (Deerfield, IL, USA).

2.7. Protocols for arterial blood gas and Alveolar-arterial gradient studies

Arterial blood sample (100 μL) were taken before (2–3 samples and values averaged) and 3, 6, 9, 12, 15 and 30 min after bolus injections of vehicle (saline, n=8 rats; 293 ± 3 grams) or morphine (5 mg/kg, i.v., n=8 rats; 291 ± 3 grams).

2.8. Protocols for ventilation studies

The rats were placed in the plethysmography chambers and allowed 45–60 min to acclimatize. Data was continuously recorded (i.e., breath by breath) throughout the

acclimatization period (the last 15 min was used for analysis and graphing) and subsequent experimentation. The data were collected into 1 min bins for analysis and graphing. Two groups of rats were used in these studies. One group of rats (n=12, 281 ± 1 grams) received an injection of vehicle (saline, i.v.) whereas another group (n=12, 284 ± 3 grams) received an injection of morphine (10 mg/kg, i.v.). After 15 min, the rats were exposed to 3 consecutive 15 min episodes of hypoxia (10% O_2, 90% N_2), each of which was separated by a 15 min exposure to room air.

2.9. Protocols for body temperature studies

Rats with jugular vein catheters and telemetry devices were placed in plethysmography chambers and allowed 45–60 min to acclimatize. One group (n=6, 303 ± 2 g) received an i.v. injection of vehicle (saline) whereas another group (n=6, 304 ± 3 g) received morphine (5 mg/kg, i.v.). After 15 min, the rats underwent the hypoxia-normoxia challenges as described above.

2.10. Statistics

Data recorded during the ventilatory studies (1 min bins) and derived parameters, namely, TV/Ti and cumulative response (cumulative arithmetic changes from pre-values), were taken for analyses. All data are presented as mean ± SEM and were analyzed by one-way or two-way ANOVA followed by Student's modified *t* test with Bonferroni corrections for multiple comparisons between means [33]. $P < 0.05$ denoted significance.

3. RESULTS

3.1. Analgesia

Paw-withdraw latencies in rats prior to injection of morphine (5 mg/kg, i.v.) were 18.9 ± 0.5 sec. The paw withdrawal latencies at 20, 40, 60 and 90 min after morphine injection were 40 ± 0, 40 ± 0, 39.4 ± 0.6 and 38.9 ± 1.1 sec, respectively ($P < 0.05$, for all comparisons to pre-injection values). Remembering that the cutoff-latency was 40 seconds, it was evident that of morphine elicited a sustained analgesia in these rats.

3.2. Arterial blood gas and Alveolar-arterial gradient studies

The effects of vehicle or morphine (5 mg/kg, i.v.) on ABG chemistry and A-a gradient are summarized in Fig. 1 and Table 1. Vehicle did not affect these parameters whereas morphine elicited prompt decreases in arterial blood pH, pO_2 and sO_2 and increases in pCO_2 and A-a gradient. All responses were resolved by 15 min and no changes were observed at 30 min.

3.3. Ventilatory responses elicited by morphine

Morphine (5 mg/kg, i.v.) elicited transient increases in f_R which were associated with decreases in V_T and V_M of 9–10 min in duration (Fig. 2, Table 2). Despite the transient nature of the morphine-induced increase in f_R, morphine elicited sustained increases in T_I and EIP but a sustained decrease in T_E (Fig. 3, Table 2). In addition, morphine elicited sustained decreases in V_T/T_I and PIF but relatively transient changes in PEF (Fig. 4, Table

2). Vehicle elicited minor responses (Figs. 2–4, Table 2). Taken together, vehicle elicited minor cumulative responses whereas morphine elicited significant cumulative responses in all parameters except f_R and PEF (Table 3).

3.4. Ventilatory responses during hypoxic challenges and upon return to room-air

f_R, V_T and V_M: Episode 1 of hypoxia (H1) elicited rapid and sustained increases in f_R, V_T and V_M in vehicle-treated (VEH) rats (Fig. 2, Table 4). The increases in f_R and V_M developed more slowly during H2 and H3. In morphine-treated (MOR) rats, H1–H3 transiently increased f_R (Fig. 2, Table 4). The increases in V_T during H1 were similar to those in VEH rats whereas the increases during H2 and H3 were greater. The increases in V_M during H1 in MOR rats were smaller than in VEH rats (due to lesser increases in f_R) whereas the increases in V_M during H2 and H3 were similar in both groups. In VEH rats, f_R returned to pre-levels during episode 1 of normoxia (N1) but fell below pre-values for several min during N2 and N3. V_T and V_M dipped below pre-levels during N1–N3 but normalized within 5–10 min. In MOR rats, f_R changed minimally during N1 whereas it rose and remained elevated during N2 and N3. V_T tended to return to pre-levels during N1–N3 in MOR rats. V_M returned to pre-levels during N1–N3 in MOR rats but remained above pre-levels during N2 and N3. f_R and V_M values in MOR rats were higher than in VEH rats at the end of N3 (Fig. 2).

T_I, T_E and EIP: H1–H3 elicited sustained decreases in T_I, T_E and EIP in VEH rats (Fig. 3, Table 4). H1–H3 elicited sustained decreases in T_I in MOR rats (Fig. 3, Table 4) but due to higher baseline values, nadir values did reach those in VEH rats. In contrast, T_E (depressed by morphine) tended to recover to pre-levels during H-H3. Moreover, EIP (elevated by morphine) rapidly returned to pre-levels during H1–H3. In VEH rats, T_I and EIP returned to baseline during N1–N3 (Fig. 3, Table 4). T_E rapidly returned to and exceeded pre-values during N1–N3 but returned to pre-levels before each episode of hypoxia (Fig. 3, Table 4). In MOR rats, T_I rose rapidly upon return to room-air during N1, less so during N2 and minimally during N3. T_E fell back to (morphine-induced) nadir levels during N1–N3. EIP returned to the elevated levels elicited by morphine during N1–N3. T_I and EIP values in MOR rats were similar to VEH rats at the end of N3 whereas T_E was decreased (Fig. 3).

V_T/T_I, PIF and PEF: H1–H3 elicited sustained increases in V_T/T_I in VEH and MOR rats (Fig. 4, Table 4). V_T/T_I values in MOR rats did not reach those of VEH rats during H1 but approached them in H2 and equaled them in H3. In VEH rats, V_T/T_I rapidly returned to baseline levels during N1–N3. In MOR rats, V_T/T_I returned to the lower morphine-induced baseline during N1. The decreases in V_T/T_I during N2 and N3 were similar in both groups. H1–H3 elicited sustained increases in PIF in VEH and in MOR rats. The responses during H1 and H2 in MOR rats did not reach the maxima in VEH rats whereas the responses during H3 were equal to VEH rats. In VEH rats, PIF returned to pre-levels during N1 whereas it fell below pre-levels during N2 and N3. In MOR rats, PIF returned to post-morphine levels during N1 but returned to pre-morphine levels during N2 and N3. H1–H3 elicited sustained increases in PEF in VEH and MOR rats (Fig. 4, Table 4). The responses during H1 in MOR rats were smaller than in VEH rats. The responses during H2 and H3 were similar in both groups although higher values were reached in MOR rats during H3 because of higher pre-

hypoxia values. In VEH rats, PEF returned to pre-levels during N1–N3. In contrast, PEF in MOR rats remained elevated during N2 and N3. V_T/T_I values were similar in VEH and MOR rats at the end of N3, whereas PIF was lower in VEH rats because of the sustained post-hypoxic depression (Fig. 4). PEF was higher in MOR rats at the end of N3 because of the sustained effects of morphine (Fig. 4).

Cumulative responses: In VEH rats. H1–H3 elicited similar cumulative responses in V_T, T_I, T_E, and EIP whereas those in f_R, V_M, V_T/T_I, PIF, and PEF gradually diminished with each episode (Table 5). H1–H3 elicited markedly different cumulative responses in MOR rats than in VEH rats, and the magnitude of these differences changed with each hypoxic episode (Table 5). N1–N3 elicited substantial cumulative responses in f_R, V_M, T_I, EIP, PIF, and PEF but minimal responses in V_T, T_I, and V_T/T_I. Moreover, the magnitude and direction of these cumulative responses changed with each episode of normoxia. For example, N1 was associated with positive cumulative response for f_R and V_M, whereas N2 and N3 were associated with negative responses. N1–N3 also elicited substantial cumulative responses in MOR rats that changed markedly with each successive challenge (Table 5). Although the cumulative responses during N1 for f_R, V_T, and V_M were similar in VEH and MOR rats, the cumulative responses in MOR rats greatly exceeded those in VEH rats during N2 and N3.

3.5. Body Temperature

H1–H3 elicited minor but sustained decreases in B_T in VEH rats (e.g., H1 at +15 min, −0.39 ± 0.04 °C, $P < 0.05$) (Fig. 5). B_T returned to pre-hypoxia levels upon return to room-air (N1–N3). Morphine elicited a minor increase in B_T, which was still evident at the time of exposure to H1 (+0.44 ± 0.08 °C, $P < 0.05$). H1–H3 elicited minor decreases in B_T in MOR rats, although they never fell below pre-values. These decreases were still present at the end of each hypoxic challenge (e.g., H1 at +15 min, −0.55 ± 0.11 °C, $P < 0.05$). B_T returned to pre-hypoxia levels upon return to room air, such that the morphine-induced hyperthermia was sustained throughout the study.

4. DISCUSSION

Despite resolution of the morphine-induced changes in ABG chemistry, A-a gradient and V_M, the first episode of hypoxia elicited smaller ventilatory responses than in VEH rats. Moreover, the pattern of ventilatory responses during subsequent episodes of hypoxia changed in MOR rats. As such, morphine and/or metabolites have latent deleterious effects on ventilatory responses elicited by hypoxic challenge. These latent effects may involve direct effects on carotid body and/or brain mechanisms processing the hypoxic responses rather than changes in metabolism.

4.1. Analgesia and body temperature

The ability of 5 mg/kg morphine to elicit a long-lasting analgesia validated our choice of this dose for the ventilatory studies. Our finding that a 5 mg/kg dose of morphine elicited a minor sustained hyperthermia of ≈ 0.4 °C is consistent with findings from other laboratories [34,35]. Exposure of VEH rats to 15 min episodes of hypoxia (10% O_2, 90% N_2) elicited

minor decreases in B_T of 0.4 °C, consistent with evidence that exposure of rats to 10–12% O_2 for 15 min elicits no effects [36] or a decrease of ≈ 0.5°C [37,38]. Since each episode of hypoxia elicited very similar arithmetic falls in B_T in VEH and MOR rats, it appears that despite elevating B_T, morphine does not impair the metabolic processes that cause hypoxia-induced hypothermia. Taken together, the morphine-induced changes in BT are likely to have had minimal effects on the ventilatory responses to hypoxia.

4.2. Morphine-induced changes in ABG chemistry, A-arterial gradient and ventilation

The effects of morphine on ABG chemistry (i.e., decreases in pH, pO_2 and sO_2, increases in pCO_2) are consistent with hypoventilation (i.e., decreases in V_T) whereas the increases in A-a gradient (mismatch of ventilation-perfusion) may have arisen via increases in pulmonary airways resistance [18,19], pulmonary vasoconstriction reducing arterial blood flow to alveoli [20,39,40] and/or exacerbated hypoxic pulmonary vasoconstriction via morphine-induced reductions in arterial pO_2. Although, morphine minimally affected f_R, it lengthened Ti and EIP and so obviously affected inspiratory control mechanisms. Morphine also elicited (1) decreases in V_M via reductions in V_T that resolved by 15 min (i.e., the time of exposure to H1), (2) increases in T_I and EIP still evident at 15 min, (3) decreases in V_T/T_I and PIF still evident at 15 min, and (4) a transient decrease in PEF. The decreases in V_T and PIF suggest that morphine impaired ventilatory mechanical efficiency via decreases in neural drive to the intercostal muscles and diaphragm. Since the morphine-induced reductions in V_T/T_I had not resolved by 15 min, it appears that the negative influence on Respiratory Drive was present despite the resolution of ABG chemistry, A-a gradient and V_M. Morphine also elicited sustained decreases in T_E (i.e., expiration was faster), which may have been due to diminished V_T requiring less time to exhale. However, the decreased T_E may have involved direct (presumably central) effects of morphine on pathways driving expiration.

4.3. Ventilatory responses to hypoxic challenges in vehicle-treated rats

In VEH rats, the hypoxic challenges elicited prompt and sustained ventilatory responses including (1) increases in V_T/T_I and V_M (via increases in f_R and V_T), (2) decreases in T_I and T_E but minor decreases in EIP, and (3) increases in PIF and PEF. The rates of increase in f_R, PIF, PEF, and V_T/T_I were slower during H2 and H3 than H1. This suggests that the mechanisms responsible for these rapid responses had “adapted” to hypoxic challenge whereas those involved in maintaining the responses did not. Whether this adaptation occurred in response elements sensitive to reductions in blood pO_2 and in the carotid bodies [41–43], or within the brain is unknown. Ventilatory drive can diminish during exposures to severe hypoxia or to longer duration exposures to mild hypoxia. This “ventilatory roll-off” involves neurochemical processes in the NTS under mild hypoxia [44,45], and the direct depressive effects of severe hypoxia on brain neurons regulating ventilation [45,46]. However, in the present study, none of the key ventilatory parameters (e.g., f_R, V_T, Inspiratory Drive) displayed of roll-off indicating that 15 min exposures to 10% O_2 did not engender this phenomenon in our conscious Sprague-Dawley rats.

4.4. Ventilatory responses to hypoxic challenges in morphine-treated rats – episode H1

H1 elicited markedly smaller increases in f_R in MOR rats although resting f_R was not diminished. As such, morphine elicited latent effects on systems including those within the

carotid bodies that drive the hypoxic responses. Our data are consistent with evidence that the negative effects of opioids in humans may be latent since the morphine metabolite, M6G, does not affect resting ventilatory parameters whereas it substantially blunts the ventilatory response to hypercapnic challenge [5]. Since morphine did not markedly blunt the increase in V_T during H1, it is evident that it did not negatively affect neural drive to the chest muscles or diaphragm. This is supported by the finding that Ti in MOR rats (elevated immediately prior to exposure to hypoxia) decreased substantially during H1. Taken together, our data support the concept that morphine affected brainstem centers responsible for generating breathing such as the respiratory pattern central generator, including the pre-Botzinger complex [47,48], rather than affecting peripheral motor/skeletal muscle components driving breathing. Nonetheless, it was evident that H1 elicited substantially smaller increases in PIF and PEF in MOR rats than in VEH rats thereby compromising the dynamics of active inspiratory/expiratory ventilation. It should be noted that resting PIF was still depressed in MOR rats (−24 ± 7%, $P < 0.05$) prior to H1 whereas resting PEF was not (+8 ± 6%, $P > 0.05$). In addition, T_E was diminished prior to H1 in MOR rats (−23 ± 5%, $P < 0.05$) but actually rose appreciably during H1, suggesting that morphine inhibited the hypoxia-induced stimulation of active expiration.

4.5. Ventilatory responses upon reintroduction of room-air in vehicle-treated rats

In humans, V_M falls below pre-hypoxia levels upon return to room-air via falls in f_R and V_T [49,50], 1994). In rats, f_R falls below resting levels upon return to room-air via an increase in T_E [51,52,53]. This post-hypoxia depression of f_R (post-hypoxic f_R decline. PHFD) [54], is an active neural process that depends on the ventrolateral pons [52,53,55,56]. In VEH rats, Ti, EIP, and PEF returned to pre-values upon return to room-air whereas f_R, V_T, V_M and PIF declined below pre-levels. Consistent with previous findings [52,53], this PHFD was associated with an elongation in T_E. The finding that the pattern of ventilatory responses was similar for each of the three normoxic episodes indicates that adaptations in carotid body or brainstem circuitry that may affect the normoxia responses did not occur during repeated exposure to hypoxia.

4.6. Temporal changes in ventilatory responses to hypoxia-normoxia in morphine-treated rats

The pattern of ventilatory responses in MOR rats changed during the study. Although f_R during each episode of hypoxia was depressed in MOR rats, baseline f_R gradually rose during N1–N3 such that f_R was higher than in VEH rats during N3. Since the PHFD observed in VEH rats was absent in MOR rats, morphine may have directly interfered with pontomedullary systems responsible for this phenomenon [52,53,55,56]. Although resting V_T returned to baseline levels during N1–N3 in MOR rats, the responses during H1–H3 changed in that the increases in V_T following H1 were slightly greater than those of VEH rats whereas they were greater than VEH rats during H2 and H3. As a result of the temporal patterns of changes in f_R and TV, resting V_M in MOR rats was substantially above that of VEH rats during N2 and N3. Moreover, although the peak increases in V_M in MOR rats were less than in VEH rats during H1, the increases in V_M in MOR rats were equal to those in VEH rats during H2 and reached higher values (perhaps due to elevated starting baselines) during H3. Although the PHFD in V_M appeared qualitatively similar in both

groups, PHFD in VEH rats was due to a decline in f_R and to a lesser degree a decrease in V_T whereas it was due solely to a decrease in TV in MOR rats. The prolongation of T_I by morphine was essentially resolved when N3 was applied. Although each episode of hypoxia decreased T_I in the MOR rats, none of the decreases were as pronounced as those in VEH rats. Moreover, T_I promptly returned to elevated levels during N1 and N2 but not N3. Although morphine elicited reductions in V_T/T_I and PIF, which had not resolved prior to H1 or H2, these challenges still elicited robust increases in these parameters. In addition, although morphine elicited a sustained increase in EIP that was evident during N1–N3, each episode of hypoxia elicited robust decreases in EIP. As such, it appears that whereas morphine reduced EIP, V_T/T_I and PIF, it did not depress the mechanisms by which the carotid bodies responded to hypoxia or central/efferent processing of this input. The findings that the PEF during N2 and N3 were elevated and that the post-hypoxia increases in T_E in VEH rats (N1–N3) were absent in MOR rats are suggestive of facilitatory effects of morphine and/or metabolites on expiratory control.

4.7. Mechanisms responsible for the delayed effects of morphine - morphine metabolites

The presence of (+)-morphine metabolites [57–60] may explain the delayed effects of the opioid on the ventilatory responses to hypoxia. In rats, maximal plasma levels of (+)-morphine occur within 10 min and the elimination half-life is approximately 45–50 min. As such, pharmacologically active plasma concentrations of morphine were likely present in our rats during H1 and N1 (15 to 45 min post-morphine injection), during H2 and N2 (46–75 min post-morphine) but perhaps less so during H3 and N3 (76–105 min post-morphine). It is feasible that morphine sequestered into relevant tissues may have effects when plasma levels are minimal. Obvious tissues would be (1) carotid bodies and neuromuscular elements of the chest wall [61], (2) brainstem nuclei receiving/processing carotid body chemoafferent input [62], and (3) brain structures devoid of blood-brain barriers [63]. It may also be possible that concentrations of morphine that are insufficient to affect central and/or peripheral drive may still be able to blunt the mechanisms responsible for sensing/expressing ventilatory responses to hypoxia. In humans, (+)-morphine is metabolized to M6G [57,60], which relatively more selective for μ-opioid receptors (μ-ORs) than δ- or κ-ORs and elicits pronounced analgesia and respiratory depression [58,64]).

In rats, (+)-morphine is metabolized primarily to M3G [57,59,60]. Maximal plasma levels of M3G occur within 30 min of morphine administration and the elimination half-life is over 200 min. Unlike M6G, M3G has a low affinity for ORs [65,66] and minimal analgesic or respiratory depressant activity [65,67]. However, M3G is not inert since (1) intracerebroventricular [68,69] or intrathecal [70,71] injections of M3G cause behavioral excitation, (2) rats develop hyperaesthesia and allodynia after intrathecal M3G [70], and (3) intracerebroventricular injections of M3G elicit electroencephalographic spiking, excessive grooming, and epiliptoform discharges [68,72]. Taken together, the latent effects of morphine on the ventilatory responses to hypoxia may be due to the actions of M3G in the brain and/or periphery via mechanisms other than the activation of ORs.

5. CONCLUSIONS

The ventilatory responses during hypoxic challenge were depressed in MOR rats even though its effects on ABG chemistry, A-a gradient and V_M had fully subsided before challenge. Whether these latent effects are due to morphine or a metabolite are yet to be determined. Opioid-induced hypoventilation, upper airway obstruction, and destabilization of breathing during sleep are major concerns in humans [73,74]. Adults with OSA are at risk for respiratory complications to opioids given post-operatively [73]. Moreover, children undergoing adenotonsillectomy for relief of OSA symptoms have a higher sensitivity to the ventilatory depressant effects of μ-OR agonists, and higher post-operative difficulty in breathing compared to those without OSA [73,74]. There may be many patients with undiagnosed OSA [75], which are at risk for impaired breathing postoperatively [76–78]. The present study suggests that even normal patients may be under threat from opioids that blunt ventilatory responses to hypoxia even when monitoring suggests that the effects of opioids on ABG chemistry and V_M had subsided. Considering the ability of opioids to destabilize breathing during sleep, the latent deleterious actions of opioids on ventilatory responses to hypoxia, may be especially dangerous when the patient is asleep. Our studies suggest that M6G in humans and M3G in rats should be further investigated with respect to their potential ability (and mechanisms of action) to exert deleterious effects on the hypoxic ventilatory response.

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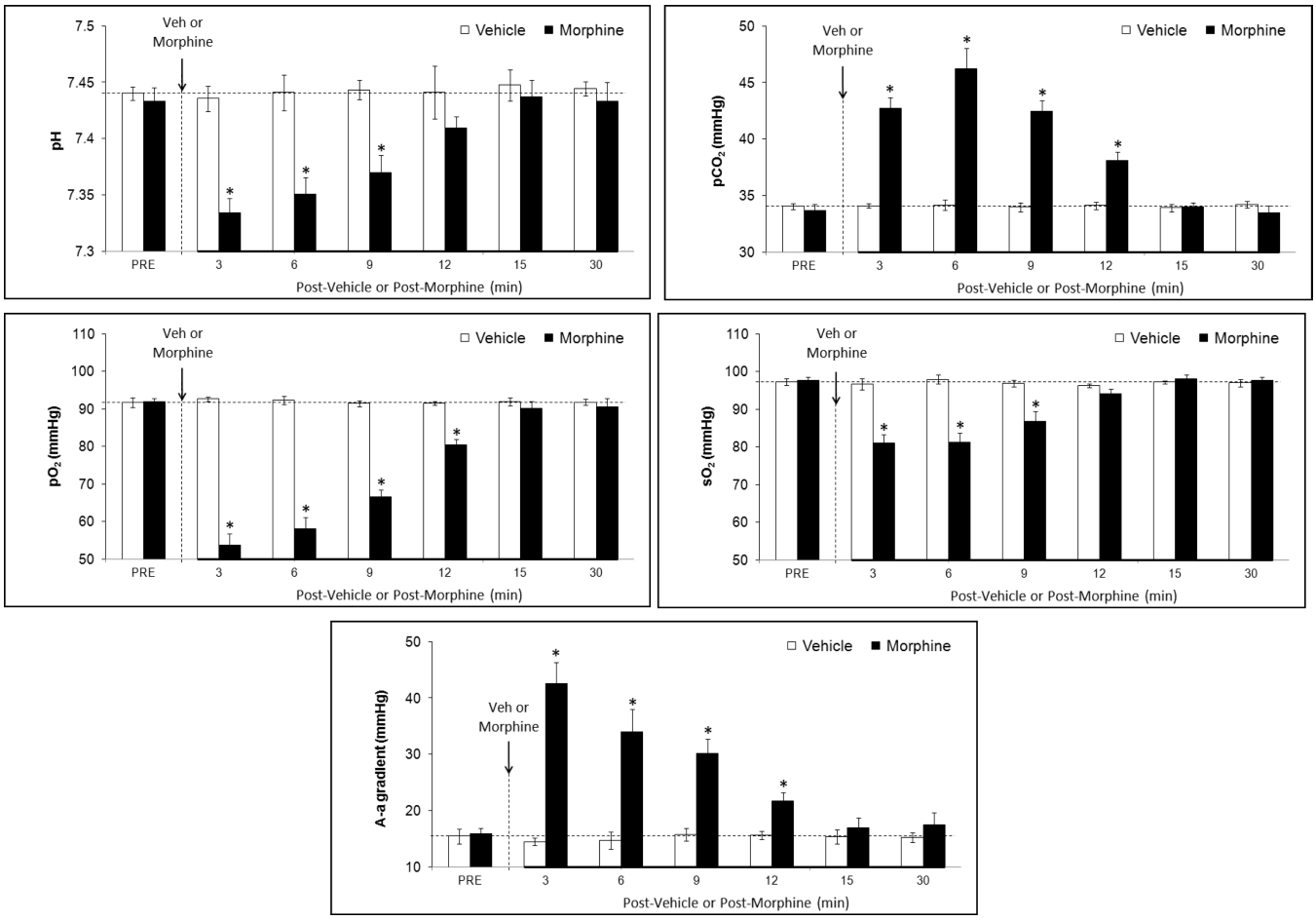

Figure 1.

Temporal changes in arterial blood gas chemistry values and Alveolar-arterial (A-a) gradient elicited by bolus injections of vehicle or morphine (5 mg/kg, i.v.) in conscious rats. There were 8 rats in each group. The data are mean ± SEM. *$P < 0.05$, significant change from Pre values.

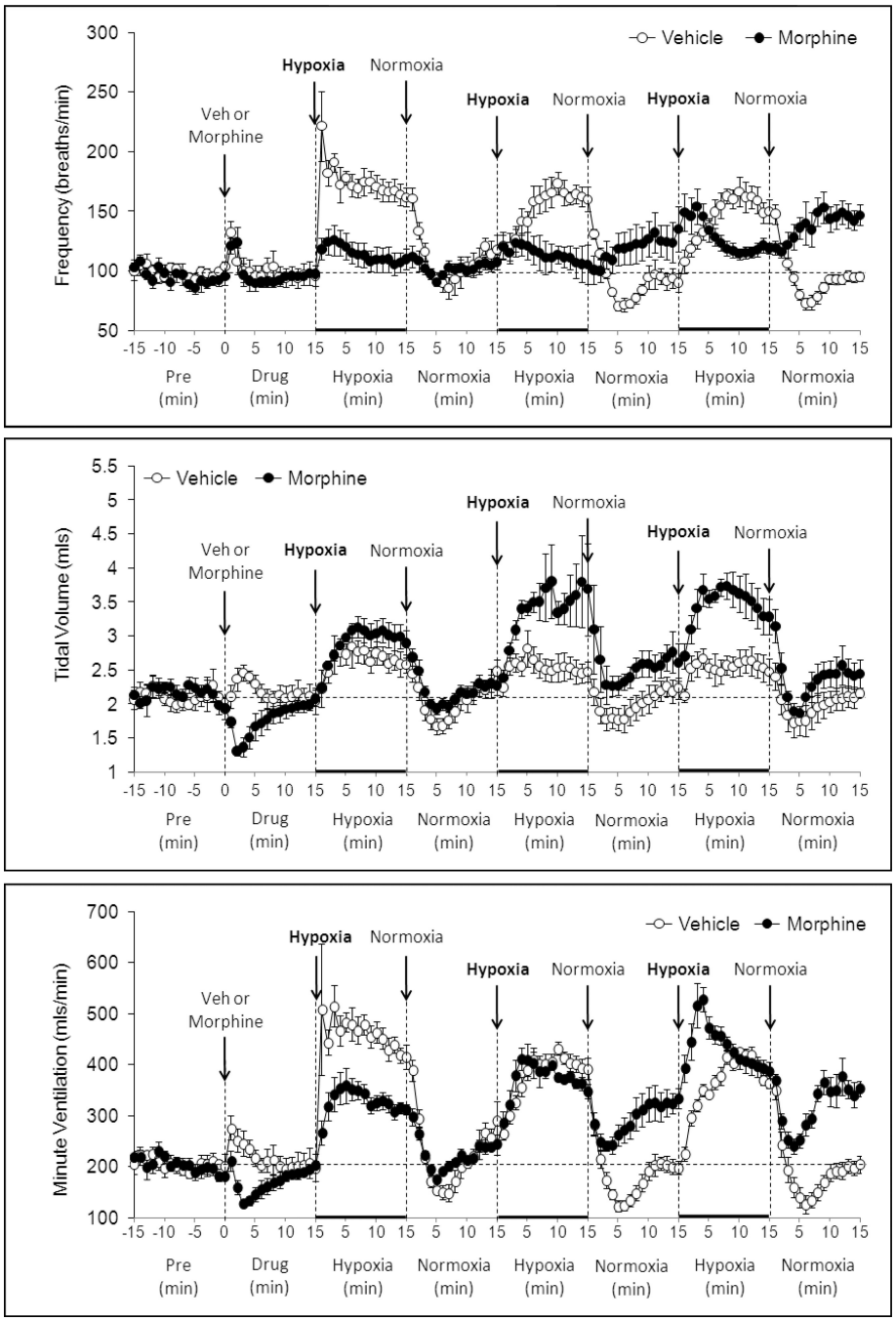

Figure 2.

Changes in frequency of breathing (upper panel), tidal volume (middle panel) and Minute Volume (lower panel) elicited by injections of vehicle or morphine (5 mg/kg, i.v.) and subsequent exposure to three 15 min episodes of hypoxia (10% O_2, 90% N_2) each of which was followed by a 15 min period of normoxia (room-air). The first episode of hypoxia began 15 min after injection of vehicle or morphine. There were 6 rats in each group. Data are mean ± SEM.

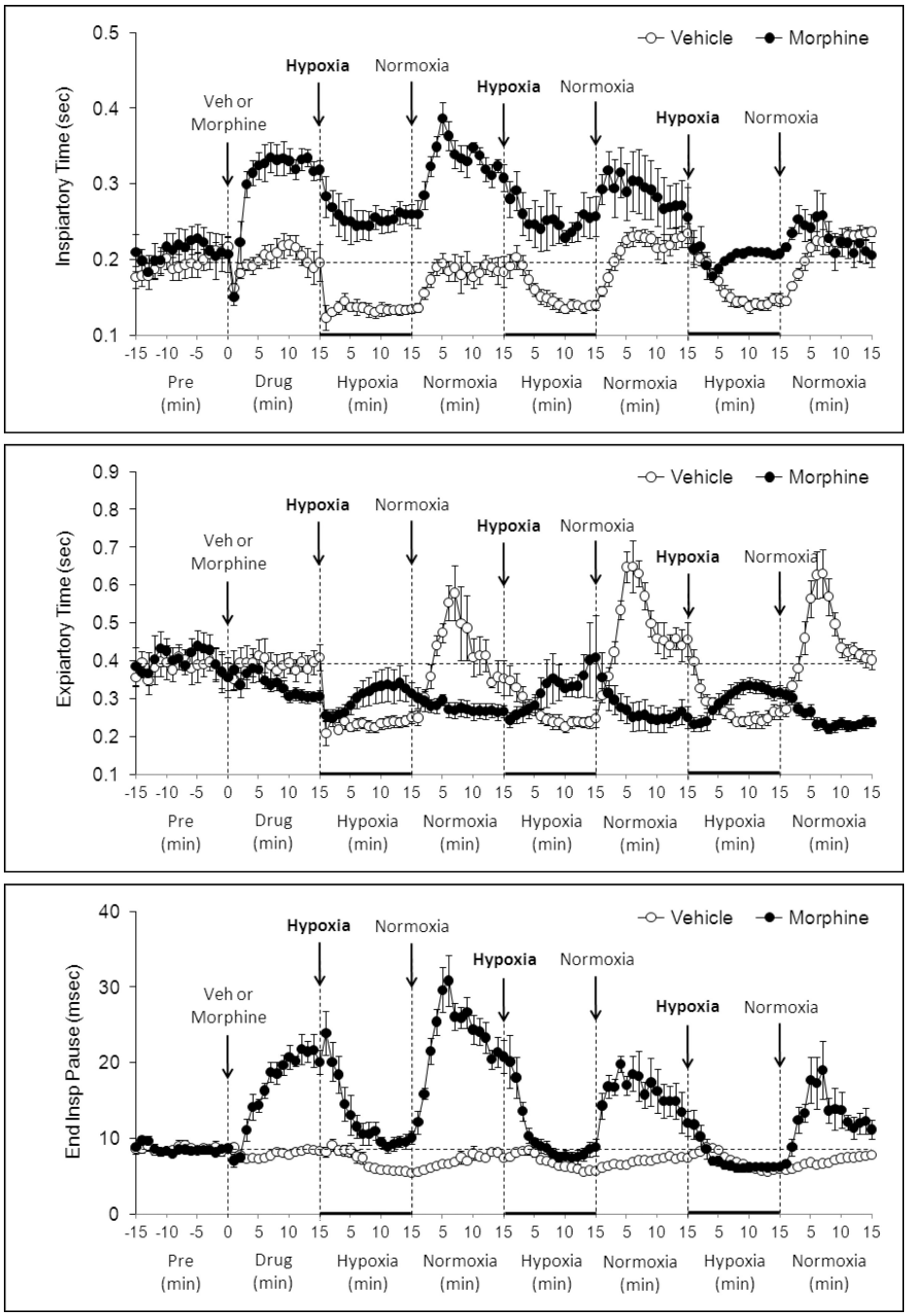

Figure 3.

Changes in inspiratory time (top panel), expiratory time (middle panel) and end inspiratory pause (bottom panel) elicited by injections of vehicle or morphine (5 mg/kg, i.v.) and subsequent exposure to three 15 min episodes of hypoxia (10% O_2, 90% N_2) each of which was followed by a 15 min period of normoxia (room-air). The first episode of hypoxia began 15 min after injection of vehicle or morphine. There were 6 rats in each group. Data are mean ± SEM.

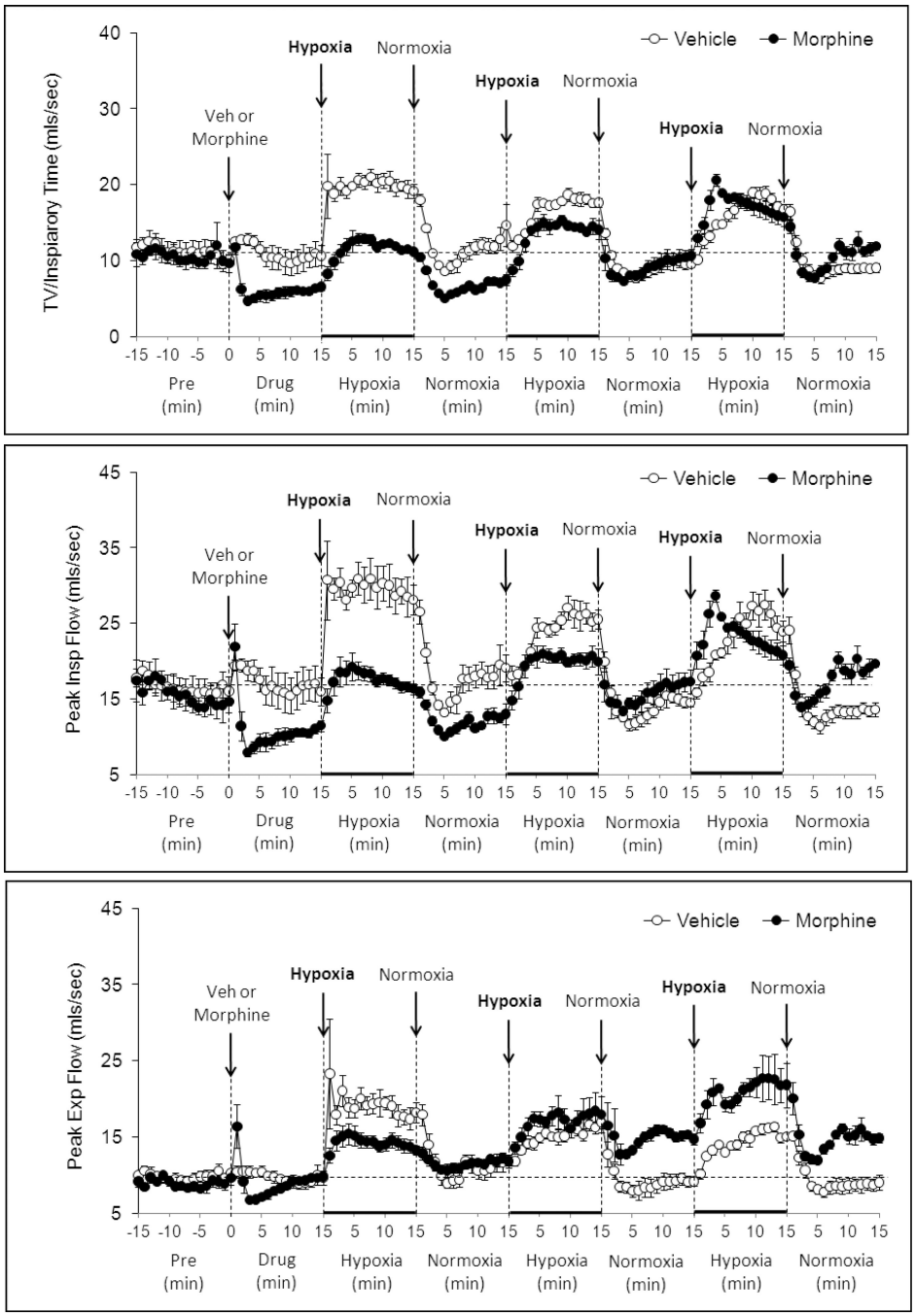

Figure 4.

Changes in tidal volume/inspiratory time (upper panel), peak inspiratory flow (middle panel), and peak expiratory flow (bottom panel) elicited by injections of vehicle or morphine (5 mg/kg, i.v.) and subsequent exposure to three 15 min episodes of hypoxia (10% O_2, 90% N_2) each of which was followed by a 15 min period of normoxia (room-air). The first episode of hypoxia began 15 min after injection of vehicle or morphine. There were 6 rats in each group. Data are mean ± SEM.

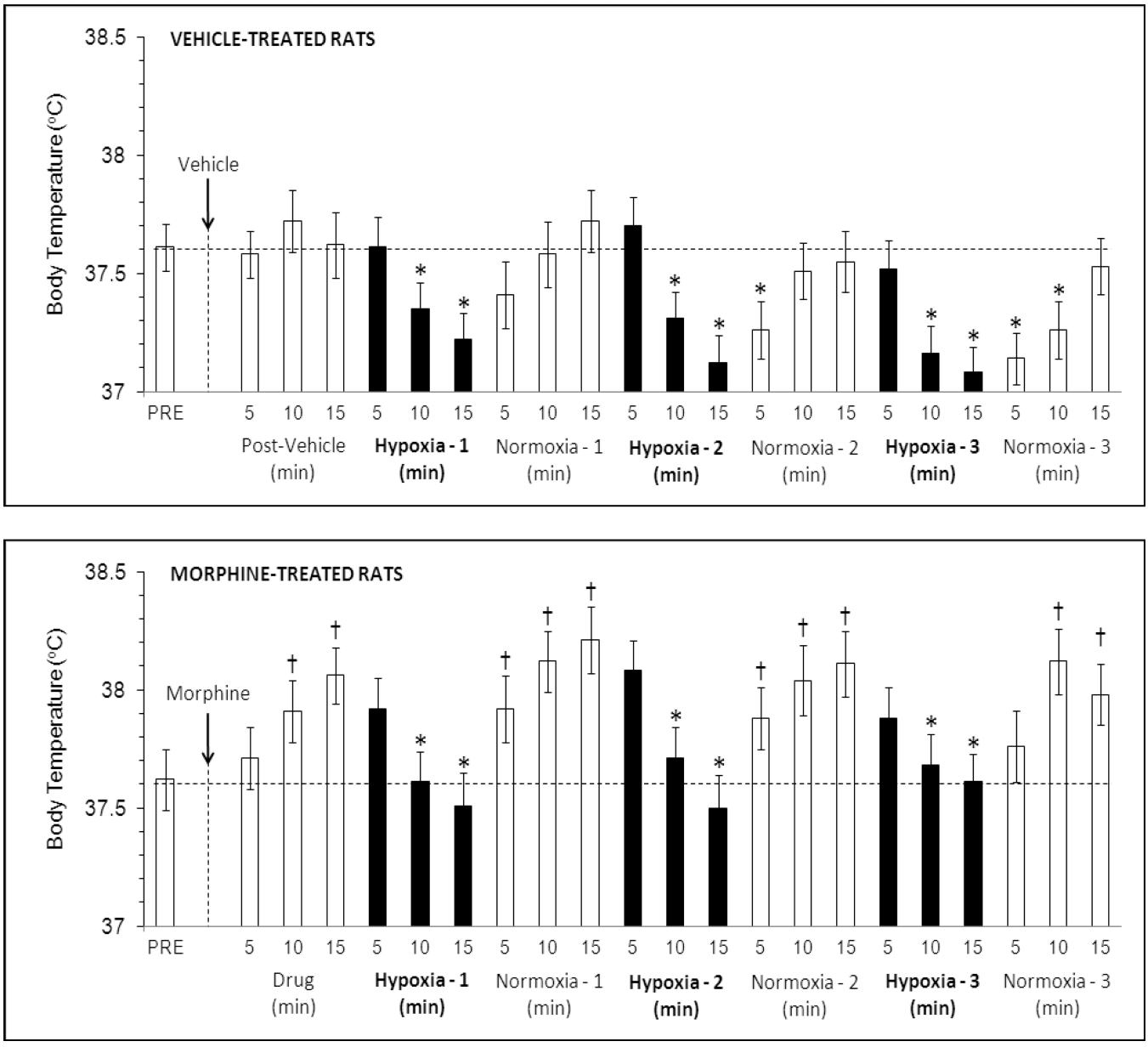

Figure 5.

Changes in body temperature elicited by injections of vehicle (upper panel) or morphine (5 mg/kg, i.v., lower panel) and subsequent exposure to three 15 min episodes of hypoxia (10% O_2, 90% N_2) each of which was followed by 15 min periods of normoxia (room-air). The first episode of hypoxia began 15 min after injection of vehicle or morphine. There were 8 rats in each group. Data are mean ± SEM.

Table 1

Percent changes in arterial blood gas chemistry and Alveolar-arterial gradient elicited by injections of vehicle or morphine

		Percent Changes					
Parameter	**Group**	**+3 min**	**+6 min**	**+9 min**	**+12 min**	**+15 min**	**+30 min**
pH	Vehicle	0.0 ± 0.1	0.0 ± 0.2	0.0 ± 0.1	0.0 ± 0.3	0.0 ± 0.1	0.0 ± 0.1
	Morphine	−1.3 ± 0.2*	−0.9 ± 0.2*	−0.8 ± 0.2*	−0.3 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
pCO_2	Vehicle	+0.2 ± 0.7	+0.3 ± 0.7	−0.2 ± 0.9	+0.2 ± 0.4	−0.4 ± 0.4	+0.5 ± 0.3
	Morphine	+27.1 ± 2.6*	+37.2 ± 3.9*	+26.2 ± 2.4*	+13.3 ± 2.2*	+1.2 ± 1.5	−0.5 ± 0.7
pO_2	Vehicle	+1.0 ± 1.2	0.7 ± 0.8	−0.2 ± 0.7	−0.2 ± 1.0	+0.3 ± 0.4	+0.1 ± 0.5
	Morphine	−41.5 ± 3.1*	−36.9 ± 2.9*	−27.6 ± 1.6*	−12.5 ± 1.0*	−1.8 ± 1.1	−1.5 ± 2.0
sO_2	Vehicle	−0.6 ± 1.4	+0.7 ± 1.4	−0.4 ± 1.2	−1.0 ± 0.8	0.0 ± 1.0	−0.2 ± 1.1
	Morphine	−17.1 ± 2.4	−16.7 ± 2.6	−11.1 ± 2.0	−3.6 ± 0.8	+0.4 ± 1.1	+0.1 ± 0.8
A-a gradient	Vehicle	−3 ± 9	−5 ± 6	+3 ± 5	+3 ± 5	−0 ± 2	−1 ± 4
	Morphine	+173 ± 31*	+111 ± 15*	+92 ± 16*	+38 ± 6*	+7 ± 7	+9 ± 13

All data are mean ± SEM. There were 8 rats in each group. A-a gradient, Alveolar-arterial gradient.

* $P < 0.05$, significant percent change from Pre values.

A-a gradient = $PAO_2 - PaO_2$, where PAO_2 is the partial pressure of alveolar O_2 (calculated from the alveolar gas equation) and PaO_2 is the partial pressure of O_2 in arterial blood. PAO_2 is determined by the formula, $PAO_2 = [(FiO_2 \times (P_{atm} - P_{H2O}) - (PaCO_2/\text{respiratory quotient})]$, where FiO_2 is the fraction of O_2 in inspired air; P_{atm} is atmospheric pressure; P_{H2O} is the partial pressure of water in inspired air; and $PaCO_2$ is the partial pressure of CO_2 in arterial blood. We took FiO_2 of room-air to be 21% = 0.21; respiratory quotient to be 0.8; P_{atm} to be 760 mmHg; and P_{H2O} to be 47 mmHg [see 27,28].

Table 2

Ventilatory responses elicited by vehicle or morphine

			Percent changes from Pre values				
Parameter	**Group**	**Pre**	**+3 min**	**+6 min**	**+9 min**	**+12 min**	**+15 min**
Frequency (breaths/min)	Vehicle	99 ± 4	+1 ± 8	−4 ± 6	−5 ± 6	−6 ± 6	−3 ± 8
	Morphine	95 ± 4	+1 ± 6	−4 ± 5	−3 ± 5	+1 ± 4	+3 ± 4
Tidal Volume (mls)	Vehicle	2.09 ± 0.12	+18 ± 2*	+3 ± 5	+2 ± 5	+3 ± 5	−3 ± 5
	Morphine	2.15 ± 0.12	−37 ± 6*,†	−24 ± 5*,†	−17 ± 3*,†	−8 ± 6	−3 ± 6
Minute Volume (mls/min)	Vehicle	206 ± 12	+16 ± 11	−1 ± 2	+3 ± 6	−3 ± 6	−5 ± 11
	Morphine	203 ± 8	−37 ± 3*,†	−24 ± 6*,†	−15 ± 3*,†	−7 ± 4	0 ± 3
Inspiratory Time (sec)	Vehicle	0.20 ± 0.01	+1 ± 7	+6 ± 5	+12 ± 5	+6 ± 6	+3 ± 14
	Morphine	0.21 ± 0.02	+47 ± 15*,†	+61 ± 19*,†	+64 ± 20*,†	+64 ± 18*,†	+57 ± 15*,†
Expiratory Time (sec)	Vehicle	0.38 ± 0.03	+2 ± 8	+5 ± 6	+1 ± 6	+3 ± 3	+6 ± 6
	Morphine	0.40 ± 0.03	−9 ± 6	−13 ± 3*,†	−18 ± 3*,†	−23 ± 3*,†	−23 ± 3*,†
End Inspiratory Pause (msec)	Vehicle	8.6 ± 0.4	−14 ± 4*	−13 ± 4*	−6 ± 6	−2 ± 7	−3 ± 5
	Morphine	8.6 ± 0.3	+30 ± 18*	+92 ± 17*,†	+130 ± 19*,†	+154 ± 22*,†	+134 ± 21*,†
Tidal Volume/Inspiratory Time (mls/sec)	Vehicle	11.2 ± 1.2	+18 ± 11	−6 ± 3	−13 ± 6*	−8 ± 8	−4 ± 6
	Morphine	10.6 ± 1.1	−55 ± 3*,†	−49 ± 4*,†	−45 ± 5*,†	−42 ± 5*,†	−37 ± 5*,†
Peak Inspiratory Flow (mls/sec)	Vehicle	16.8 ± 1.6	+14 ± 9	−4 ± 4	−8 ± 7	−1 ± 7	−5 ± 6
	Morphine	15.6 ± 1.4	−48 ± 3*,†	−41 ± 4*,†	−35 ± 5*,†	−30 ± 7*,†	−25 ± 5*,†
Peak Expiratory Flow (mls/sec)	Vehicle	9.8 ± 0.6	+8 ± 10	+1 ± 6	−6 ± 4	−9 ± 6	−1 ± 8
	Morphine	9.0 ± 0.4	−24 ± 5*,†	−17 ± 4*,†	−4 ± 4	+5 ± 7	+11 ± 6

All data are mean ± SEM. There were 6 rats in each group.

*$P < 0.05$, significant percent change from Pre values.

†$P < 0.05$, Morphine *versus* Vehicle.

Table 3

Cumulative responses elicited by vehicle or morphine

Parameter	Vehicle	Morphine
Frequency (breaths/min) × min	+24 ± 80	+37 ± 63
Tidal Volume (mls) × min	+1.3 ± 0.4*	−5.5 ± 1.9*,†
Minute Volume (mls/min) × min	+172 ± 103	−462 ± 94*,†
Inspiratory Time (sec) × min	+0.1 ± 0. 2	+1.4 ± 0.3*,†
Expiratory Time (sec) × min	+0.1 ± 0.3	−1.0 ± 0.2*,†
End Inspiratory Pause (msec) × min	−9.0 ± 6.8	+124.5 ± 11.5*,†
Tidal Volume/Inspiratory Time (mls/sec) × min	−3.2 ± 8.0	−66.3 ± 11.9*,†
Peak Inspiratory Flow (mls/sec) × min	+4.8 ± 11.0	−71.6 ± 17.4*,†
Peak Expiratory Flow (mls/sec) × min	−1.0 ± 7.5	−1.0 ± 6.1

All data are mean ± SEM. There were 6 rats in each group.

*$P < 0.05$, significant Cumulative Response.

†$P < 0.05$, Morphine *versus* Vehicle.

Table 4

Values (expressed as %change from Pre values) recorded at the 15 min time-point during each episode of hypoxia or normoxia

		+15 min values expressed as percent of pre values					
Parameter	**Group**	**Hypoxia-1**	**Hypoxia-2**	**Hypoxia-3**	**Normoxia-1**	**Normoxia-2**	**Normoxia-3**
Frequency (breaths/min)	Vehicle	+64 ± 7*	+62 ± 10*	+52 ± 10*	+19 ± 12	−9 ± 7	−3 ± 5
	Morphine	+15 ± 5*,†	+9 ± 14	+26 ± 5*,†	+13 ± 3*	+40 ± 15*,†	+54 ± 4*,†
Tidal Volume (V_T, mls)	Vehicle	+24 ± 6*	+18 ± 5*	+18 ± 5*	+19 ± 6*	+7 ± 7	+2 ± 2
	Morphine	+35 ± 4*	+69 ± 23*,†	+52 ± 5*,†	+6 ± 3	+21 ± 9*	+14 ± 7
Minute Volume (mls/min)	Vehicle	+101 ± 3*	+91 ± 5*	+78 ± 6*	+44 ± 24*	−3 ± 7	−1 ± 5
	Morphine	+55 ± 6*,†	+72 ± 10*	+91 ± 14*	+20 ± 6*	+66 ± 14*,†	+76 ± 11*,†
Inspiratory Time (T_I, sec)	Vehicle	−30 ± 6*	−26 ± 8*	−22 ± 9*	−5 ± 12	+24 ± 14*	+24 ± 10
	Morphine	+26 ± 10*,†	+24 ± 12*,†	+2 ± 9	+50 ± 10*,†	+21 ± 13*	0 ± 7
Expiratory Time (sec)	Vehicle	−34 ± 6*	−34 ± 9*	−31 ± 6*	−6 ± 13	+20 ± 15	+5 ± 6*
	Morphine	−22 ± 6*	−2 ± 10†	−19 ± 8*	−33 ± 4*,†	−36 ± 10*,†	−40 ± 4*,†
End Inspiratory Pause (msec)	Vehicle	−36 ± 3*	−33 ± 3*	−30 ± 4*	−14 ± 3*	−13 ± 3*	−8 ± 4*
	Morphine	+18 ± 12†	+3 ± 13†	−27 ± 6*	+144 ± 28*,†	+38 ± 17*,†	+29 ± 11*,†
V_T/T_I (mls/sec)	Vehicle	+78 ± 18*	+64 ± 16*	+55 ± 14*	+34 ± 26	−11 ± 11	−17 ± 6*
	Morphine	+9 ± 8†	+40 ± 12*	+55 ± 19*	−28 ± 4*,†	+4 ± 12	+17 ± 13*,†
Peak Inspiratory Flow (mls/sec)	Vehicle	+73 ± 18*	+58 ± 16*	+47 ± 15*	+15 ± 10	−10 ± 10	−17 ± 7*
	Morphine	+7 ± 6†	+32 ± 17*	+37 ± 12*	−17 ± 3*,†	+14 ± 14	+30 ± 12*,†
Peak Expiratory Flow (mls/sec)	Vehicle	+86 ± 10*	+74 ± 10*	+56 ± 12*	+20 ± 6*	−6 ± 5	−9 ± 5
	Morphine	+50 ± 10*,†	+103 ± 30*	+146 ± 36*,†	+32 ± 6*	+67 ± 15*,†	+68 ± 11*,†

All data are mean ± SEM. There were 6 rats in each group.

*$P < 0.05$, significant response.

†$P < 0.05$, Morphine *versus* Vehicle.

Table 5

Cumulative responses during exposures to hypoxia and normoxia

		Cumulative Responses during Episodes of Hypoxia and Normoxia					
Parameter	**Group**	**Hypoxia-1**	**Hypoxia-2**	**Hypoxia-3**	**Normoxia-1**	**Normoxia-2**	**Normoxia-3**
Frequency (breaths/min) × min	Vehicle	+1147 ± 143*	+798 ± 146*	+711 ± 126*	+152 ± 70*	−122 ± 47*	−64 ± 36
	Morphine	+285 ± 88*,†	+283 ± 112*,†	+492 ± 109*,†	+111 ± 24*	+364 ± 147*,†	+644 ± 99*,†
Tidal Volume (V_T, mls) × min	Vehicle	+8.4 ± 1.2*	+6.6 ± 1.6*	+6.6 ± 1.4*	−0.5 ± 0.8	−1.3 ± 1.2	−1.5 ± 1.3
	Morphine	+11.4 ± 1.0*	+18.7 ± 2.8*,†	+19.6 ± 1.8*,†	+0.7 ± 0.6	+5.9 ± 1.7*,†	+3.2 ± 0.7*,†
Minute Volume (mls/min) × min	Vehicle	+3819 ± 241*	+2599 ± 102*	+2399 ± 103*	+291 ± 101*	−374 ± 89*	−251 ± 72*
	Morphine	+1876 ± 304*,†	+2536 ± 224*	+3489 ± 320*,†	+329 ± 61*	+1351 ± 250*,†	+1764 ± 236*,†
Inspiratory Time (T_I, sec) × min	Vehicle	−0.9 ± 0.3*	−0.6 ± 0.2*	−0.6 ± 0.2*	−0.2 ± 0.3	+0.3 ± 0.3	+0.3 ± 0.3
	Morphine	+0.7 ± 0.2*,†	+0.6 ± 0.2*,†	−0.1 ± 0.3	+1.8 ± 0.2*,†	+1.2 ± 0.3*,†	+0.3 ± 0.2
Expiratory Time (sec) × min	Vehicle	−2.3 ± 0.3*	−1.8 ± 0.3*	−1.7 ± 0.4*	0.5 ± 0.6	+1.6 ± 0.7*	+1.1 ± 0.4*
	Morphine	−1.5 ± 0.3*,†	−1.2 ± 0.4*	−1.5 ± 0.6*	−1.9 ± 0.3*,†	−2.0 ± 0.3*	−2.3 ± 0.4*,†
End Inspiratory Pause (msec) × min	Vehicle	−25 ± 8*	−26 ± 5*	−24 ± 5*	−24 ± 5*	−24 ± 6*	−25 ± 7*
	Morphine	+62 ± 21*,†	+24 ± 10*,†	−22 ± 10*	+219 ± 22*,†	+112 ± 21*,†	+67 ± 22*,†
V_T/T_I (mls/sec) × min	Vehicle	+131 ± 17*	+80 ± 10*	+74 ± 15*	+9 ± 14	−27 ± 17	−25 ± 12
	Morphine	+16 ± 11†	+46 ± 8*,†	+97 ± 22*	−56 ± 12*,†	−21 ± 14	0 ± 19
Peak Inspiratory Flow (mls/sec) × min	Vehicle	+193 ± 35*	+104 ± 24*	+95 ± 17*	+15 ± 16	−39 ± 23	−40 ± 10*
	Morphine	+29 ± 15*,†	+62 ± 17*	+117 ± 27*	−51 ± 15*,†	+2 ± 19	+29 ± 15†
Peak Expiratory Flow (mls/sec) × min	Vehicle	+140 ± 14*	+79 ± 8*	+68 ± 8*	+24 ± 7*	−10 ± 9	−7 ± 5
	Morphine	+79 ± 15*,†	+121 ± 16*,†	+179 ± 24*,†	+39 ± 10*	+88 ± 13*,†	+87 ± 11*,†

All data are mean ± SEM. There were 6 rats in each group.

*$P < 0.05$, significant Cumulative Response.

†$P < 0.05$, Morphine *versus* Vehicle.