Ventilation and the Response to Hypercapnia after Morphine in Opioid-naive and Opioid-tolerant Rats

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ABSTRACT

Background: Opioid-related deaths are a leading cause of accidental death, with most occurring in patients receiving chronic pain therapy. Respiratory arrest is the usual cause of death, but mechanisms increasing that risk with increased length of treatment remain unclear. Repeated administration produces tolerance to opioid analgesia, prompting increased dosing, but depression of ventilation may not gain tolerance to the same degree. This study addresses differences in the degree to which chronic morphine (1) produces tolerance to ventilatory depression versus analgesia and (2) alters the magnitude and time course of ventilatory depression.

Methods: Juvenile rats received subcutaneous morphine for 3 days (n = 116) or vehicle control (n = 119) and were then tested on day 4 following one of a range of morphine doses for (a) analgesia by paw withdraw from heat or (b) respiratory parameters by plethysmography–respirometry.

Results: Rats receiving chronic morphine showed significant tolerance to morphine sedation and analgesia (five times increased ED$_{50}$). When sedation was achieved for all animals in a dose group (lowest effective doses: opioid-tolerant, 15 mg/kg; opioid-naive, 3 mg/kg), the opioid-tolerant showed similar magnitudes of depressed ventilation (−41.4 ± 7.0%, mean ± SD) and hypercapnic response (−80.9 ± 15.7%) as found for morphine-naive (−35.5 ± 16.9% and −67.7 ± 15.1%, respectively). Ventilation recovered due to tidal volume without recovery of respiratory rate or hypercapnic sensitivity and more slowly in morphine-tolerant.

Conclusions: In rats, gaining tolerance to morphine analgesia does not reduce ventilatory depression effects when sedated and may inhibit recovery of ventilation. (Anesthesiology 2016; 124:945-57)

TREATMENT of chronic pain with opioids has greatly increased in recent years and will likely continue to increase as the population ages, and chronic pain becomes more prevalent.1–3 Unfortunately, expanded treatment with opioids has been closely associated with an alarming increase in unexpected opioid-related deaths.1,4–7 Although the majority of patients are safely treated with these drugs, prescription opioid-related deaths are currently a leading cause of accidental death in the United States.1,5,8

It has long been known that opioid administration leads to depression of ventilation and hypoxia,9,10 including decreased depth (tidal volume $[V_T]$) and rate (frequency $[f]$) of breathing and decreased ventilation responses to hypercapnia and hypoxia.11,12 Chronic opioids are known to increase sleep-disordered breathing, including irregular breathing patterns and central and obstructive apneas,13 and most opioid-related deaths are presumed due to cardiovascular collapse secondary to hypoventilation and hypoxia.14 Other respiratory effects of opioids include reduced pulmonary system compliance,10 which may add to normal age-related reductions in chest wall and lung compliance15 to increase the risk for depressed ventilation in the elderly. In addition to effects on ventilation, there is evidence suggesting that some long-acting opioids (including methadone) promote cardiac conduction defects that can result in fatal arrhythmias.16

Mechanisms responsible for increased risk of death during chronic opioid therapy likely include differences in the degree to which tolerance develops to reduce their analgesic ventilation depressant effects.6,13,14 Opioid-induced analgesia and depression of ventilation seem to be tightly

What We Already Know about This Topic

- Chronic opioid use induces tolerance to opioid analgesia
- We know little about influence of chronic opioid use on ventilatory response to opioid administration

What This Article Tells Us That Is New

- In morphine-sedated rats after chronic opioid administration, hypercapnic ventilatory response remained depressed despite gaining tolerance to morphine analgesia
- This animal study suggests a possible increased risk of severe ventilatory depression in chronic opioid patients receiving sufficient opioid for postoperative analgesia

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linked in patients not receiving chronic opioids, with predictable levels of both with increased dose, but this relationship may be uncoupled with chronic treatment. Increasing tolerance to opioid analgesic effects with chronic use has been well described in laboratory animals and humans, with larger doses being needed to maintain similar levels of pain relief. However, few well-controlled studies of respiratory system adaptation to chronic opioids have been reported, with only one in tandem with measurement of analgesic adaptation. The one previous study that directly compared analgesic and respiratory effects of chronic opioids (in mice) found substantially greater tolerance to analgesia versus depression of ventilation, but pre-exposure to chronic hypercapnia may have limited general relevance of some results.

To better understand how chronic opioid administration affects ventilation, we studied the unanesthetized rat to address two questions: (1) To what degree does tolerance to morphine-induced ventilatory depression differ from analgesic tolerance? (2) Does chronic morphine administration modify the magnitude and time course of morphine-induced ventilatory depression? Juvenile rats were separated into two groups with one made tolerant to morphine analgesia by a previously described 3-day regimen of dose-escalating morphine treatments, whereas the other group remained opioid-naive using vehicle control. The groups were further divided on treatment day 4 so that a portion of both received testing premorphine and postmorphine for analgesia, whereas the others received testing during natural rest (no morphine) or under morphine sedation for ventilation and the ventilatory response to hypercapnia or oxygen uptake (VO₂).

### Materials and Methods

**Animals and Study Design**

This study was approved by the Institutional Animal Care and Use Committee at the University of Washington (Seattle, Washington), and all procedures were conducted in accordance with institutional guidelines. Two hundred thirty-five Sprague-Dawley rats of either sex were delivered to the animal housing facility with their mothers on postnatal day 11 (P11). In addition to the following text, figure 1 shows the experiment design (A) and test protocol timelines (B). To produce populations of opioid-tolerant (n = 116) and opioid-naive (n = 119) animals, pretreatments were given consisting of twice daily (morning and evening) injections of morphine or equal volume saline vehicle, respectively, on days P17 through P19 with morphine dose increased each day (15, 30, and 45 mg/kg). All study injections were subcutaneous. On P20, a portion of both groups received one of three test protocols in a lighted room during the light phase of their normal light/dark cycle, including (1) testing for pain sensitivity before and after morphine at one of the test doses (naive, n = 67; tolerant, n = 80), (2) testing for ventilation parameters including the ventilatory response to hypercapnia during natural rest or after morphine at one of the test doses (naive, n = 46; tolerant, n = 30), or (3) VO₂ before and after morphine at one of the test doses (naive, n = 6; tolerant, n = 6). Each animal received only one test protocol after one dose of morphine, except for the animals tested during natural rest where no morphine was given. The testing protocol and morphine dose given in consecutive experiments (i.e., consecutive animals) was chosen in a random manner. Logistical considerations required that students performing the injections and test protocols were not blinded to the given doses, but data analysis was performed by a senior personnel (ventilation results) or reviewed by a senior personnel (pain results).

Previous studies have shown that maximal opioid analgesic tolerance is achieved in rats after 3-day exposure regimens similar to these studies, but it is not known how well that time course describes chronic opioid effects on ventilation. Juvenile rats were used instead of adults because complimentary studies of neural structure versus function are often performed on juvenile rats. In general, developmental nervous system changes are complete in rats at this age, including those affecting expression and binding of primary opioid receptors (µ, δ, and κ) and function of descending inhibitory pathways, and ventilatory responses to hypoxia and hypercapnia.

**Testing for Pain Sensitivity**

Pain testing was performed using methods similar to the Hargreaves paw withdrawal test. Briefly, the freely moving rat was placed on a glass surface within a 9-cm diameter and 7-cm tall plastic cylinder, and a movable radiant heat source (light) was aimed at the rat’s hind paw from beneath the glass surface (with surface height adjusted to optimize the light focus). In preliminary studies, light intensity was adjusted to yield withdrawal latencies of 4 to 5 s, with a 10-s limit of heat exposure to avoid tissue damage. The time required for a rat to withdraw its paw after application of heat (latency) was measured as an indicator of pain sensitivity. The pain sensitivity protocol included (1) baseline testing (before morphine injection) including a series of five paw-withdrawal trials at 1-min intervals, with the mean of the last three trials defining the latency value, then (2) administration of morphine at the selected dose, and (3) repeated series of five paw-withdrawal trials at 30 and 60 min post-injection before being returned to their home cage. The rats were tested for pain sensitivity when awake before receiving morphine and then under the state of wakefulness or sedation that was present after morphine administration.

The morphine dose range and minimal dose–group size for pain sensitivity testing were defined by consideration of previous experience in this laboratory, and more recent preliminary work showing that opioid-tolerant rats received no analgesia with less than or equal to 3 mg/kg and that opioid-naive rats received maximal analgesia (maximal heat exposure time) more than or equal to 10 mg/kg. To limit
unnecessary testing of animals to the maximal heat exposure, we limited the number of opioid-naive rats that received doses greater than 10 mg/kg. Therefore, the opioid-tolerant rats received a somewhat higher range of morphine dose (5, 10, 15, 30, 45, and 60 mg/kg) than was given to the opioid-naive rats (1, 3, 5, 10, 15, 30, and 35 mg/kg).

**Testing for Ventilation, the Response to Hypercapnia, and Oxygen Consumption**

**Apparatus and Measurement of Ventilation.** Ventilation parameters were assessed utilizing a previously described whole-body plethysmography method where the test chamber is continuously replenished with fresh gas to allow for long-term recording and also serves as a respirometer to measure $V_{O_2}$. The apparatus consists of two identical rigid cylindrical and transparent plastic chambers (internal diameter, 8 cm; height, 9 cm) mounted side by side on a single rigid plastic sheet, with one chamber serving as the animal chamber and the other as the reference chamber (Clear Cut Plastics, USA). Each chamber is sealed with a rigid transparent plastic lid containing access holes for tubing and instrumentation that is easily removed to allow for transfer of animals.

By this method, pressure fluctuations in the animal chamber produced by warming and humidification of inhaled gas (opposite for exhaled gas) are continuously monitored with a low-pressure differential transducer between the chambers.

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**Fig. 1.** (A) Study flow chart showing the path for each juvenile rat, including receiving one of two pretreatments (morphine or vehicle sham) over 3 postnatal days (P17–P19) and then testing with one of three protocols on the fourth day (P20). Littermates were assigned to the pretreatments and testing protocols in a random manner. (B) Timelines for each of the study test protocols. $CO_2$ = carbon dioxide.
(model DLP 2.5, Hugo Sachs Elektronik - Harvard Apparatus, Germany), whereas pressure changes outside of the system affect both chambers equally and cancel. Differential pressure is recorded for later assessment of tidal volume ($V_t$, in milliliter), respiratory frequency (f, per minute), and minute ventilation (f$ \cdot V_t$) using pressure/volume calibration information and the ideal gas laws. Other parameters recorded for this quantitative assessment of minute ventilation included barometric pressure (daily), chamber gas temperature and humidity (continuous), and body temperature ($T_a$, rectal, measured the end of test procedures).

The two chambers were surrounded by a heated water bath to maintain air temperature within the animal chamber at approximately 29° ± 1°C. Unless otherwise stated, total gas flow through the test system was fixed at 2.5 l/min (1.25 l/min, each) using a mass flow controller (0 to 5 standard liter per minute, Cole-Palmer, USA), which was adequate to maintain oxygen more than 20.0% and carbon dioxide less than 0.5% during room air breathing. Gas from the animal chamber was continuously monitored for fraction of carbon dioxide (model 602–3, Criticare Systems, USA) and oxygen (model S-3A/I, AEI Technologies, USA).

Ventilation is best measured by this method when animals have little or no nonventilatory movements, as during non–rapid eye movement (REM) sleep and morphine sedation, because such movements show up as “noise” on the differential pressure record and partially obscure ventilation-related pressure changes. Differences in the pattern of ventilation between quiet (non-REM) and active (REM) sleep have been noted, with quiet sleep characterized by an absence of body movements, low amplitude and invariant $V_t$, and invariant f, and active sleep characterized by small body movements and variable $V_t$ and f. In our studies, testing was carried out during the light phase of their light/dark cycle when rats normally sleep and when the patterns of body movement and ventilation suggested quiet sleep. However, no electroencephalogram measurements were made to confirm a state of sleep, and we instead identify that they were tested when resting quietly under natural circumstances (without morphine) or during morphine sedation.

During morphine sedation, ventilation was not equivalent to that observed during quiet rest without morphine, with low amplitude but variable $V_t$ and variable f, but as with quiet rest, there were minimal body movements. We also observed that although an animal’s eyes were always closed during quiet natural rest, their eyes were often open during morphine sedation. As with testing for analgesia, time points for testing ventilation after morphine administration were chosen to be within the time window of peak effects for these animals as determined by preliminary testing in our laboratory. Minute ventilation during natural quiet rest and morphine sedation was quantified for approximately 2 min just before testing for the ventilatory response to hypercapnia, by assessing the average frequency and amplitude of several groups of continuous waveforms (each group, 5 to 10 breaths, inhalation and exhalation).

The morphine dose range and minimal dose–group size for testing of ventilation and the response to hypercapnia were defined after consideration of previous experience by one senior investigator in a different laboratory and more recent preliminary work. That work showed that opioid-tolerant rats were never sedated after receiving less than or equal to 5 mg/kg morphine and that for all rats, ventilation effects of morphine were found to be maximal after receiving 15 to 30 mg/kg (defining our upper range of dose). Therefore, the opioid-tolerant rats received a much more limited range of morphine dose (10, 15, and 30 mg/kg) than was given to the opioid-naive rats (1, 3, 5, 10, 15, and 30 mg/kg).

**Testing the Ventilatory Response to Hypercapnia.** The ventilatory response to hypercapnia was assessed when the animals were in natural quiet rest or when morphine sedated, including 46 rats that were opiate naive and 30 that were morphine tolerant. This normoxic, hypercapnic challenge involved introducing a ramp increase in the chamber CO$_2$ concentration from 0.5 to 5.0% over 2 min while maintaining normal oxygen. The hypercapnic challenge was stopped when the animal awoke or CO$_2$ reached 5.0%, and the challenge was considered adequate to assess response to CO$_2$ if the concentration reached 3.5%. Minute ventilation was assessed at each 0.5% increase in CO$_2$ by quantifying the frequency and average amplitude of several continuous waveforms. To establish the level of minute ventilation and the ventilatory response to hypercapnia during natural quiet rest, eight morphine-tolerant and six morphine-naive animals were observed and tested during two or three periods of such rest, within a 60-min window on the test day (the natural quiet rest ventilation test protocol). For animals that received morphine before testing, observation of minute ventilation and testing for the ventilatory response to hypercapnia during sedation was attempted at 30, 45, and 60 min after drug administration (the postopioid ventilation test protocol). A group of rats was considered consistently sedated if all individuals in that group were sedated for all three postmorphine test periods.

With these methods, we did not attempt to measure the true carbon dioxide stimulus for breathing, arterial carbon dioxide, because it has long been known that ventilation and control of breathing are easily altered by physical manipulations required for such measurements (i.e., restraint, indwelling catheters, blood draw, anesthesia, etc.). However, meaningful conclusions concerning similarities and differences in morphine effects on ventilation and the hypercapnic response between opioid-naive and opioid-tolerant rats require that the carbon dioxide stimulus be similar for both groups. If certain conditions can be met, one can assume that arterial carbon dioxide was not substantially different between the test groups during resting breathing and the hypercapnic challenge, including
the following: (1) minute ventilation and $\dot{V}_O_2$ are similar between the groups so that $P_{C02}$ is also likely similar and (2) a similar stimulus of inhaled hypercapnia is applied to the groups. In the case of these experiments, those conditions were met to a large degree (see Results).

Testing for Oxygen Consumption. $\dot{V}_O_2$ was quantified in six opioid-naive and six opioid-tolerant rats during natural quiet rest before morphine and 30 and 60 min after morphine sedation at a dose range of 15, 30, or 45 mg/kg (two naive and two tolerant rats in each dose group). This protocol was designed to allow for simple observation (but not testing) of potential morphine dose-dependence of $\dot{V}_O_2$, in addition to testing for any morphine effects on $\dot{V}_O_2$. To assess $\dot{V}_O_2$, total gas flow through the test system was reduced to 1.75 l/min (each chamber, 0.875 l/min) to increase oxygen concentration differences between test chamber inflowing and outflowing gas, while still maintaining chamber $C02$ less than 0.5% during natural rest and morphine sedation. For each animal, four to six measurements of $O_2$ concentration (approximately 1 to 5 min, each) in the outflowing gas were made during periods of natural quiet rest before removal from the chamber for morphine injection and then returned to the chamber for 5 min of continuous measurements beginning at 30 and 60 min postmorphine (the $\dot{V}_O_2$ test protocol).

Data Analysis and Statistics. Excel (Microsoft, USA) and Kaleidagraph software (Synergy Software, USA) were used to input and graph data, and SPSS software (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

Analgesia was quantified for each animal as the area under the curve (AUC) of paw withdrawal latency, $L$ (s), measured premorphine ($L_{baseline}$) and postmorphine plus 30 and 60 min ($L_{30}$ and $L_{60}$, respectively), as follows:

$$AUC = 0.5 \left( L_{30} - L_{baseline} \right) \cdot 30 \text{min} + 0.5 \left( L_{60} + L_{30} \right) - L_{baseline} \cdot 30 \text{min}.$$

Baseline (natural sleep) assessments of minute ventilation, $V_{p}$, and $f$ results were averaged for the available test periods. The hypercapnic ventilatory response was quantified for minute ventilation (fig. 2), $V_{p}$, and $f$, as the computer-generated linear regression best-fit slope of those values over the range of increased carbon dioxide (minute ventilation×$C02$%−1, $V_{p}$×$C02$%−1, and $f$×$C02$%−1). The rate of postmorphine ventilation recovery was quantified as the linear regression best-fit slope of change in minute ventilation, $V_{p}$, and $f$, over the 30-min time of postmorphine testing (minute ventilation [per minute], $V_{p}$/[per minute], and $f$/[per minute]), but only for dose groups with consistent sedation (over all three test periods: 30, 45, and 60 min). $\dot{V}_O_2$ was quantified as chamber flow rate multiplied by average difference in oxygen concentration between inside and outside the animal test chamber during the sample periods.

**Fig. 2.** Examples of minute ventilation responses to hypercapnia (ramp increase; 0 to 5% inspired carbon dioxide ($C02$) in 2 min) in two 20-day-old opioid-naive rats (similar weight), including one tested during natural quiet rest (no morphine) and the other tested under morphine sedation 30 min after 5 mg/kg subcutaneous administration of morphine. Also shown are equations that describe the best-fit linear solutions of the data. When compared with the rat resting quietly without morphine, minute ventilation was substantially depressed (−46%) before exposure to carbon dioxide in the morphine-treated rat (see 0% $C02$), and the normal response of increased minute ventilation in response to increased carbon dioxide was essentially eliminated.

Statistical analysis of results was performed primarily using two-way ANOVA (group by dose; group by time with one repeated measure was used for $\dot{V}_O_2$ results) with the Student’s Newman–Keuls test for appropriate post hoc comparisons. Due to the fact that opioid-tolerant rats were not sedated over the lower range of morphine doses (1, 3, and 5 mg/kg) and, therefore, not available for ventilation testing, two-way ANOVA was only conducted for doses where data from both opioid-tolerant and opioid-naive groups could be collected (0, 10, 15, and 30 mg/kg). Additional one-way ANOVA was also run for ventilation data from opioid-naive rats that received 0, 1, 3, and 5 mg/kg morphine to allow better dose–response assessment for that group. For all comparisons, $P < 0.05$ was considered statistically significant and represents all smaller $P$ values that were found in the analyses.

**Results**

Unless otherwise indicated, results are given as mean ± SEM, and minute ventilation, $V_{p}$, and $\dot{V}_O_2$ refer to body weight-normalized values. All animals entered into the test protocols survived, and their results were entered into the data analysis.

**Weight and $T_{\dot{V}}$**

Weight and $T_{\dot{V}}$ were only recorded for rats in the ventilation studies. Average body weight of opioid-tolerant animals on
the test day (31.3 ± 0.55 g, n = 30) was lower (approximately 18%) than found for the opiate-naive animals (38.1 ± 0.51, n = 47; \( P < 0.05 \)). \( T_a \) was found to be similar for unmedicated, opioid-naive and opioid-tolerant rats (fig. 3), and for both groups, \( T_a \) was generally increased with increasing dose when measured approximately 1 h after morphine administration.

### Pain Sensitivity after Morphine

Paw withdrawal latencies were increased in both groups after administration of all doses of morphine on the test day, but rats previously exposed to morphine showed less analgesia than opioid-naive rats after any dose (fig. 4A). At approximately half of the maximum analgesic response, there was no significant difference between opioid-naive rats receiving 3 mg/kg morphine compared with opioid-tolerant rats receiving 15 mg/kg morphine (fig. 4B), demonstrating approximately fivefold shift in the analgesic ED\(_{50}\).

### Efficacy of Morphine Sedation

Efficacy of morphine sedation was greatly reduced in the opioid-tolerant versus opioid-naive groups (table 1). All opioid-naive rats in dose groups more than or equal to 3 mg/kg were consistently sedated, but the opioid-tolerant groups were consistently sedated only after receiving 15 and 30 mg/kg morphine.

### Resting Ventilation during Natural Quiet Rest and after Morphine

Figure 5 shows minute ventilation (fig. 5, A–C) measured during natural quiet rest (no morphine) in opioid-naive and opioid-tolerant rats, and 30, 45, and 60 min (respectively) after receiving morphine at several doses in other groups of rats, and components \( V_{\text{ET}} \) (fig. 5, D–F) and \( f \).
In general, minute ventilation and its components measured from the quietly resting opioid-naive rats were in good agreement with other published values determined for rats of similar age, including minute ventilation (930 ± 70 µl min⁻¹ g⁻¹), Vₚ (7.08 ± 0.64 µl/g), and f (128 ± 6/min). Although Vₚ during natural quiet rest was similar between the groups, f was significantly depressed to a large degree at 30, 45, and 60 min after administration of all morphine doses. Depression of minute ventilation at 30 min after receiving 15 and 30 mg/kg morphine was significantly depressed compared with natural quiet rest and similar to the level of hypventilation found for opioid-naive rats that received those doses. However, due to the lower average value of minute ventilation measured during natural quiet rest in the opioid-tolerant versus opioid-naive rats, the average degree of ventilatory depression in the opioid-tolerant groups after receiving 15 and 30 mg/kg morphine (−34 and −30%, respectively) was less than found for the opioid-naive groups after receiving the same doses (−48%, for both). Dose-dependent decreases in Vₚ and f contributed uniquely to depression of minute ventilation when tested 30 min after morphine administration. In the opioid-naive groups, Vₚ (fig. 5D) showed substantial dose-dependent depression over the lower range of morphine dose (to 5 mg/kg) and similar values with all higher doses. For the opioid-tolerant group, significant depression of Vₚ compared with natural quiet rest was found 30 min after 15 mg/kg morphine administration but not 30 mg/kg morphine (and variability substantially increased in the latter), although Vₚ was similar to the values found for the opioid-naive groups after the same doses. A dose-dependent decrease of f was observed 30 min after morphine in most opioid-naive and opioid-tolerant groups over the higher range of doses (fig. 5G).

Recovery of Resting Ventilation after Morphine

For both opioid-naive and opioid-tolerant rats, minute ventilation had recovered toward natural sleep levels to a large degree by 60 min after morphine at all doses although average values tended to remain somewhat reduced (fig. 5C). Recovery of minute ventilation by 60 min after morphine was dominated by increased Vₚ (fig. 5F) with little contribution from increased f, which remained similar to the values obtained at 30 min (fig. 5I).

The rate of minute ventilation recovery (minute ventilation min⁻¹) in the opioid-naive groups increased substantially with increasing dose (fig. 6A) due to increased Vₚ min⁻¹ (fig. 6B) and not f min⁻¹ (fig. 6C), whereas opioid-tolerant groups showed a much reduced rate of recovery.

Ventilation Response to Hypercapnia during Natural Quiet Rest and after Morphine

Figure 7 shows the minute ventilation response to hypercapnia measured during natural quiet rest in opioid-naive and opioid-tolerant rats, and 30, 45, and 60 min (respectively) after morphine at several doses including minute ventilation CO₂%⁻¹ (fig. 7, A–C), Vₚ CO₂%⁻¹ (fig. 7, D–F), and fCO₂%⁻¹ (fig. 7, G–I). Carbon dioxide response values were similar for opioid-naive and opioid-tolerant rats when measured during natural quiet rest and were greatly depressed to a large degree at 30, 45, and 60 min after administration of all morphine doses. Depression of minute ventilation CO₂%⁻¹ was due to substantial depression of both Vₚ CO₂%⁻¹ and fCO₂%⁻¹ in most dose groups.
Fig. 5. Ventilation in 20-day-old rats during natural quiet rest (no morphine) or during sedation at 30 min (A, D, G), 45 min (B, E, H), and 60 min (C, F, I) after receiving subcutaneous morphine at one of several doses. The rats were either tolerant to opioid analgesia after a previous 3-day pretreatment with increasing, twice-daily doses of morphine (15, 30, and 45 mg/kg) or opioid-naïve due to no previous exposure to morphine. All rats received one dose or no morphine before the observation period. Shown are weight-normalized minute ventilation (ml min⁻¹ g⁻¹; A–C) and components tidal volume (Vₜ; ml/g; D–F) and respiratory frequency (f; per minute; G–I). Rats previously treated with morphine were only observed for ventilation parameters after administration of 10 mg/kg morphine and higher due to preliminary work that showed these animals to not be sedated after lower doses. ¹The no morphine group values are the average of two or three measures made during periods of quiet rest within a 60-min window. §Groups containing animals that did not have consistent sedation for the entire 30-min period of observation and testing (30, 45, and 60 min postmorphine). *Significant differences between morphine-dose versus no morphine groups. †Significant differences between morphine-naïve versus morphine-tolerant groups at various doses (P < 0.05, between-group ANOVA with the Student’s Newman–Keuls test for post hoc analyses).
Oxygen Consumption during Natural Sleep and after Morphine

During natural quiet rest, $\dot{V}O_2$ was similar for opioid-naive and opioid-tolerant rats (table 2) and similar to values obtained from previous studies of rats. $\dot{V}O_2$ were often increased at 30 and 60 min after morphine in both groups, but no potential trends were observed regarding dose-dependence. With dose groups combined, $\dot{V}O_2$ at 30 and 60 min after morphine showed no significant changes compared with natural quiet rest for both opioid-naive and opioid-tolerant, but potential increases compared with natural quiet rest were apparent.

Discussion

Overview of the Novel Findings

These studies revealed a number of novel findings regarding morphine depression of ventilation and other parameters and how those effects are modified in rats with substantial tolerance to morphine analgesia after chronic treatments: (1) When compared with natural quiet rest (no morphine), depression of ventilation after morphine was not due to reduced metabolism. (2) Gaining tolerance to morphine analgesic effects does not result in tolerance to morphine-induced hyperthermia. (3) Minute ventilation and the response to carbon dioxide during natural quiet rest were not significantly different for opioid-naive versus opioid-tolerant rats. (4) Substantial depression of ventilation only occurred when the morphine dose was sufficient to produce consistent sedation, and then the level of minute ventilation was very similar for the opioid-naive and opioid-tolerant rats. But importantly, chronic morphine treatments produced substantial tolerance to morphine sedation effects. (5) Therefore, tolerance to morphine depression of ventilation independent of sedation was not found in this study. (6) Rats made tolerant to morphine analgesia showed no tolerance to the profound morphine-induced loss of hypercapnic ventilatory response. (7) For both opioid-naive and opioid-tolerant rats, recovery of minute ventilation in the hour after morphine was entirely due to increased VT and (8) recovery of minute ventilation occurred without any recovery of the hypercapnic ventilatory response. (9) However, the rate of increasing VT after morphine was substantially slower in the opioid-tolerant versus opioid-naive rats after receiving the same doses.

Mechanisms Underlying Hypoventilation after Morphine

Metabolism and Temperature Regulation. Reduced metabolism is expected to decrease ventilation independent of other opioid effects, as when active wakefulness is replaced with sedation. However, in this study, $\dot{V}O_2$ during natural quiet rest was very similar to that measured after morphine administration, and a tendency for $\dot{V}O_2$ to increase after morphine administration was apparent (table 2). We are not aware of any previous studies that measured metabolism parameters...
Fig. 7. Ventilation response to a ramp increase in inspired carbon dioxide (0 to 5% in 2 min) in 20-day-old rats during natural quiet rest (no morphine) or during sedation at 30 min (A, D, G), 45 min (B, E, H), and 60 min (C, F, I) after subcutaneous administration of morphine at one of several doses. The rats were either opioid tolerant after a previous 3-day pretreatment with increasing twice-daily doses of morphine or opioid naive. All rats received one dose or no morphine before testing. Shown are slopes of increasing or decreasing weight-normalized minute ventilation (ml·min⁻¹·g⁻¹·CO₂%⁻¹; A–C) and components tidal volume (Vₜ; ml·g⁻¹·CO₂%⁻¹; D–F) and respiratory frequency (f; min⁻¹·CO₂%⁻¹; G–I). Rats previously treated with morphine were only tested for ventilation parameters after administration of 10 mg/kg morphine and higher due to preliminary work that showed these animals not to be sedated after lower doses. *The no morphine group values are the average of two or three measures made during periods of quiet rest within a 60-min window. §Groups containing animals that did not have consistent sedation for the entire 30-min period of observation and testing (30, 45, and 60 min postmorphine). *Significant differences between morphine-dose versus no morphine groups. †Significant differences between morphine-naive versus morphine-tolerant groups at various doses (P < 0.05, between-group ANOVA with the Student’s Newman–Keuls test for post hoc analyses).
increased VO2.42,43 Other studies of rats have found that rats before and after subcutaneous morphine†‡ found in opioid-related deaths.4,5,7,8,14,48 These study results induced hyperthermia,40,41 which may be related to opioid-ventilation.

This study confirms previous observations of morphine-induced hyperthermia,40,41 which may be related to opioid-increased VO2.42,43 Other studies of rats have found that lower morphine doses produce hyperthermia and increased metabolism, but higher doses result in loss of thermal autoregulation (poikilothermia), with hypermetabolism and hyperthermia in warm environments and hypometabolism and hypothermia in cool environments.42,43 The lack of tolerance to hyperthermic or hypermetabolic effects of opioids is relevant, in that any increase in metabolic rate when ventilation is depressed will increase the risk for hypoxemia/hypercapnia by effectively increasing the degree of hypoventilation.

Sedation. Rats were considered to be morphine sedated when they showed minimal or no nonventilatory body movements during testing, perhaps limiting comparisons with other studies that utilized more subtle neurologic measurements. The current results showing substantial tolerance to morphine sedation after chronic morphine treatments are in agreement with earlier findings regarding tolerance to fentanyl sedative versus analgesic effects.44 In contrast, chronic treatments did not influence the ability of opioid-tolerant rats to achieve natural quiet rest without morphine, and their ventilation was essentially normal in that state. The fact that both opioid-naive and opioid-tolerant rats had normal ventilation during rest without morphine and substantially increased ventilatory depression with increased efficacy of morphine sedation, but at different doses, suggests that some minimum level of opioid sedation is required for, or associated with, substantial depression of ventilation.

These findings may have clinical importance if sedation is an opiate effect that patients try to achieve, which is likely considering that sleep disturbances are associated with chronic pain and chronic opioids.35-48 Indeed, pursuit of sedation effects that are lost to tolerance likely contribute to the high rate of combined chronic sedative and opioid use found in opioid-related deaths.4,5,7,8,14,48 These study results suggest that tolerance to opiate sedation increases the effective dose, so that when sedation is achieved, the degree of reduced ventilation may be more pronounced.

### Suppression of Chemosensitivity and Rhythm Generation.

As with studies of normal humans, these results from rats show that VT and f are uniquely influenced by morphine over the range of given doses (fig. 5, D and G), with VT most sensitive over the lower range and depression of f over the higher range.33,49,50 This is in contrast to studies of anesthetized and otherwise reduced animal preparations, where f is most sensitive to low-dose opioids while amplitude of brainstem respiratory-related neural outflow (sometimes interpreted to indicate VT) is decreased with higher doses.51,52 Morphine depression of f and loss of hypercapnic ventilatory response are consistent with opioid depression of central (brainstem) chemosensitivity and rhythm generator output as shown by many previous investigations.51-55 However, the profound and prolonged loss of hypercapnic sensitivity (fig. 7A), even at doses that produced substantially submaximal analgesia (fig. 4A) and sedation, was surprising. These results support the results of other studies that found opioids to affect central chemosensitivity and rhythm generation uniquely,53 because the hypercapnic response decreased substantially with increasing dose before depression of f. Given this clear evidence for increasingly severe reduction of central chemosensitivity after increasing morphine dose, the nature of mechanisms that preserved VT and minute ventilation over the higher range of dose is of considerable interest.

Significant depression of f during natural quiet rest that was found for opioid-tolerant versus opioid-naive rats (fig. 5G) may reflect chronic depression of f with repeated opioid exposure. A similar effect was found for awake and resting humans who had been receiving chronic opioid therapy (methadone), where chronic hypoventilation was due to depressed f and not VT.33,50 Chronic hypercapnia is expected under such circumstances, as found for some humans receiving chronic methadone.56

### Mechanisms Underlying Recovery of Ventilation after Morphine.

Independent of the previously discussed evidence, other results from this study argue against the widely held view that central carbon dioxide sensitivity supports ventilation after opioid administration.52 Minute ventilation recovered due to increased VT without any recovery of hypercapnic ventilatory sensitivity (figs. 5 and 6), which is not evidence for increased hypoxia or hypercapnia increasing ventilatory drive because ventilation had increased to near-normal levels. We are not aware of previous well-controlled studies that focused on mechanisms for recovery of ventilation after acute opioid administration in the opioid-naive versus opioid-tolerant.

Perhaps most relevant for addressing increased risk of death with chronic opioid treatment, are our results showing that VT increased at a much lower rate from 30 to 60 min in opiate-tolerant versus opiate-naive groups after receiving the same doses (fig. 6B). Any influence that inhibits or prolongs
recovery from hypoventilation directly increases the risk for prolonged hypoxemia and hypercapnia and their consequences. These results may indicate that chronic exposure to morphine produces a functional abnormality that delays recovery of ventilation (assuming the change is not due to altered pharmacokinetics, which would also be functionally meaningful).

**Further Clinical Implications**

These results suggest that the best clinical strategy to avoid hypoxemia after increasing opioid dose may focus on altered $V_T$ and carbon dioxide, but current monitoring outside of surgical/intensive care environments is often limited to $f$ and peripheral oxygen saturation. End-tidal carbon dioxide will increase before oxygen saturation declines. However, because a common finding after administration of low-dose opioids is mild hypoxemia and increased alveolar carbon dioxide without substantially altered ventilation, end-tidal carbon dioxide would likely provide the earliest indication of hypoventilation.

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**Competing Interests**

The authors declare no competing interests.

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