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Analgesic efficacy and respiratory effects of butorphanol and morphine in turtles

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Objective—To test the hypothesis that butorphanol or morphine induces antinociception with minimal respiratory depression in conscious red-eared slider turtles.

Design—Prospective crossover study.

Animals—37 adult male and female red-eared slider turtles (*Trachemys scripta*).

Procedures—Antinociception (n = 27 turtles) and respiratory (10 turtles) experiments were performed. Infrared heat stimuli were applied to the plantar surface of turtle limbs. Thermal withdrawal latencies were measured before and at intervals after SC administration of physiologic saline (0.9% NaCl) solution, butorphanol tartrate (2.8 or 28 mg/kg [1.27 or 12.7 mg/lb]), or morphine sulfate (1.5 or 6.5 mg/kg [0.68 or 2.95 mg/lb]). Ventilation was assessed in freely swimming turtles before and after SC administration of saline solution, butorphanol (28 mg/kg), or morphine (1.5 mg/kg).

Results—For as long as 24 hours after injection of saline solution or either dose of butorphanol, thermal withdrawal latencies among turtles did not differ. Low- and high-dose morphine injections increased latencies significantly by 8 hours. Ventilation was not altered by saline solution administration, was temporarily depressed by 56% to 60% for 1 to 2 hours by butorphanol (28 mg/kg) administration, and was significantly depressed by a maximum of 83 ± 9% at 3 hours after morphine (1.5 mg/kg) injection. Butorphanol and morphine depressed ventilation by decreasing breathing frequency.

Conclusions and Clinical Relevance—Although widely used in reptile species, butorphanol may not provide adequate antinociception for invasive procedures and caused short-term respiratory depression in red-eared slider turtles. In contrast, morphine apparently provided antinociception but caused long-lasting respiratory depression. (*J Am Vet Med Assoc* 2007;230:1356–1362)

Analgesic drug administration under conditions considered painful in humans is regarded as the standard of veterinary practice for all vertebrate species.[1,2] In mammals, peri- and postoperative management of pain facilitate recovery and healing, reduce morbidity and death, and contribute to rapid return to normal activities.[3,4] With respect to analgesic drugs, relevant data for reptiles are lacking even though reptiles are commonly maintained as companion animals and heavily represented in zoologic and scientific laboratory collections. Thus, there is a fundamental need for studies evaluating species-relevant nociceptive behaviors and analgesic drug efficacy and pharmacodynamics, as well as mechanisms underlying nociception in reptiles.[1,5]

Opioids, the most effective drugs for controlling pain in mammals, are classified according to receptor subtypes—μ, κ, and δ. For pain management in mammals, many clinicians prefer administering either a μ-opioid receptor agonist (eg, morphine) or a mixed-opioid κ-receptor agonist–μ-receptor antagonist (eg, butorphanol); the latter is favored because of efficacy and relative safety. For postoperative pain control in reptiles, anecdotal reports recommend administration of butorphanol at mammalian-derived dosages. However, to our knowledge, there are no clinical data to substantiate that butorphanol is an effective analgesic drug in reptiles. In contrast, there is minimal evidence that morphine provides antinociception in lizards and crocodiles,[6,7] but there is limited information with respect to interspecies differences; dose-dependent effects; duration of drug efficacy; and potentially fatal drug-related adverse effects, such as respiratory depression. In mammals, μ- and δ-opioid receptor activation in the brainstem causes respiratory depression.[8,9] Similarly, μ-opioid receptor activation abolishes respiratory motor output in isolated turtle brainstems in vitro.[10] However, little is known about opioid receptor activation and subsequent alteration of breathing in awake reptiles after administration of clinically relevant drugs, such as butorphanol or morphine.

Abbreviations

$\dot{V}_E$	Ventilation
V_T	Tidal volume

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The purpose of the study reported here was to test the hypothesis that butorphanol or morphine induces antinociception with minimal respiratory depression in conscious red-eared slider turtles. The study was undertaken to determine whether butorphanol or morphine (at low and high doses) provides antinociception (determined by use of the thermal hind limb withdrawal latency test) and assess the extent and time course of any drug-associated respiratory depression. In the thermal hind limb withdrawal latency test, noxious thermal stimuli are applied to the hind limbs of study animals; this well-established behavioral model for assessing pain and analgesia[11] allows rapid application and decay of stimuli (without lasting inflammation), instant latency quantification, and unambiguous behavior after stimulus exposure (either the animal does or does not withdraw its limb). Semiaquatic turtles were chosen for investigation because they are common companion and research animals; analgesic information for chelonians is sparse; and analgesic drug-induced adverse effects (eg, respiratory depression) can be easily measured in awake, freely swimming turtles.[12]

Materials and Methods

All procedures were approved by the Animal Care and Use Committee at the University of Wisconsin, Madison, School of Veterinary Medicine.

Turtles—Thirty-seven adult red-eared slider turtles (*Trachemys scripta*) were obtained from a commercial supplier[a] for use in the study. The mean ± SEM weight of the turtles was 721 ± 12 g (1.59 ± 0.026 lb). Among the group, there were 19 males and 18 females, the mean weight of which was 732 ± 19 g (1.61 ± 0.042 lb) and 710 ± 20 g (1.56 ± 0.044 lb), respectively. Turtles were kept in 1,800-L open tanks (5 to 10 turtles/tank), in which they had access to dechlorinated water for swimming and dry areas for basking. Room temperature was set at 27° to 28°C (80.6° to 82.4°F; close to their preferred temperature range[13]) with light provided for 14 h/d. Turtles were fed floating food sticks[b] 3 to 4 times/wk.

Study design—A crossover experimental design was used to evaluate opioid receptor–dependent changes in thermal antinociception and respiration in the turtles. A complete crossover design was achieved for the antinociception experiments by use of physiologic saline (0.9% NaCl) solution, low- and high-dose butorphanol, and low-dose morphine injections, as well as for the respiratory experiments. Eleven turtles were used in the antinociceptive experiments, and 10 turtles were used in the respiratory experiments; each turtle was exposed to each treatment condition with a minimum washout interval of 2 weeks. An incomplete crossover design was used for the antinociception experiments with high-dose morphine injections and naloxone pretreatment experiments because drug effects were robust and reproducible. The observer in the antinociceptive experiments was unaware of treatments administered to each turtle.

Thermal analgesia experiments—Analgesimetry consisted of measurement of latency of the hind limb withdrawal reflex in response to a noxious infrared radiant heat stimulus applied to the plantar surface of the hind limb by use of a standard apparatus.[c] Turtles were placed in plastic boxes (17 × 69 × 14 cm) on an elevated acrylic plastic surface with opaque barriers that prevented visual contact with each other. Once a turtle placed 1 or both hind limbs onto the acrylic plastic surface, heat was applied to the plantar surface of 1 limb. The increasing temperature caused the turtle to withdraw that limb, and the time to withdrawal was automatically measured. Stimulation strength was adjusted to attain baseline latencies of 16 to 18 seconds (corresponding to 45° to 47°C [113.0° to 116.6°F]); a maximum duration of 32 seconds was used to prevent prolonged heat exposure. Baseline mean withdrawal latency was calculated for each turtle from data obtained by application of 1 stimulus on 3 occasions at 5-minute intervals. Injections consisted of either physiologic saline solution (equivalent volume to opioid volumes), butorphanol tartrate[d] (2.8 or 28 mg/kg [1.27 or 12.7 mg/lb]), or morphine sulfate[e] (1.5 or 6.5 mg/kg [0.68 or 2.95 mg/lb]). All drugs were administered SC between the neck and 1 forelimb of each turtle. At 1, 2, 4, 8, and 24 hours after injection, withdrawal latencies were calculated from data obtained by application of 1 stimulus on 3 occasions at 5-minute intervals. Turtles were administered saline solution to establish the time course of hind limb withdrawal latency variation.

Afterwards, turtles were randomly assigned to receive low- or high-dose butorphanol or low-dose morphine injections. Turtles were also randomly assigned to receive high-dose morphine injections, but only 5 turtles were used in these experiments because of concerns for opioid-induced morbidity and death. Separate experiments were performed to test whether the antinociceptive effects of butorphanol (2.8 mg/kg) or morphine (1.5 mg/kg) could be abolished via opioid receptor blockade; to this end, naloxone[f] (0.2 mg/kg [0.09 mg/lb], SC) was injected immediately following the baseline time point and 1 hour prior to administration of saline solution or drug. Sixteen turtles were randomly assigned to be used in an incomplete, prospective crossover study with 10 receiving a combination of more than 1 drug (ie, naloxone and saline solution, naloxone and butorphanol, or naloxone and morphine).

Respiratory experiments—Ventilation in awake, freely swimming turtles was measured by use of established methods.[12,14] Turtles were placed in a plastic container (16 × 42 × 42 cm) filled to the top with water at room temperature (**Figure 1**). A circle (diameter, 8.0 cm) was cut in the top, and a plastic container (volume, 250 mL) was inverted and sealed over the hole. This inverted plastic breathing chamber provided the only location within the tank where the turtles could breathe. Flow meters maintained gas flow (room air) into the chamber at approximately 500 mL/min. A pneumotachograph[g] was attached to the breathing chamber exit hole to measure airflow.

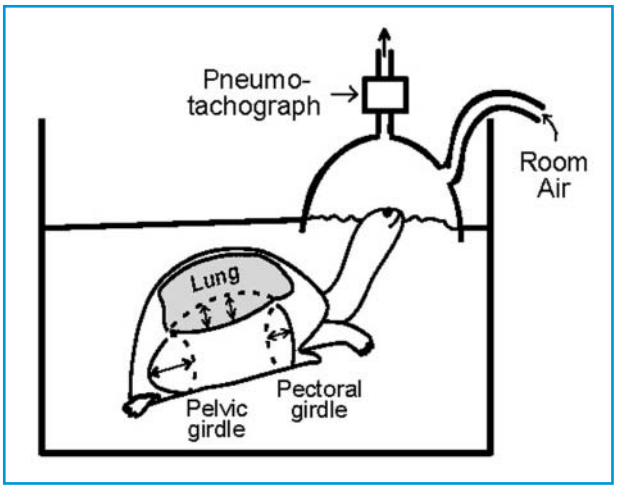

Figure 1—Diagram of a freely swimming turtle breathing in the breathing chamber, which is attached to a pneumotachograph.

The pneumotachograph was calibrated by connecting a 25-mL glass syringe to the breathing chamber. The syringe was set at different volumes (range, 3.5 to 26 mL) and rhythmically moved back and forth at cycle periods of 1.5 to 3.5 seconds (similar to the duration of 1 turtle expiratory-inspiratory cycle). The amplitude of these simulated breaths was used to calibrate pneumotachograph signals produced by turtle breathing. After 1 day of conditioning (eg, 6 hours in chamber), 10 turtles were each placed in the chamber to breathe room air for 2 hours; baseline $\dot{V}_E$ was established during the 60-minute period prior to injection.

Turtles were removed from the chamber; injected SC (between the neck and a forelimb) with saline solution, butorphanol (28 mg/kg), or morphine (1.5 mg/kg); and returned to the tank to breathe room air for an additional 4 hours.

Electrical signals from the pneumotachograph were saved to a computer database by use of a data acquisition system[h] and analyzed off-line with computer software.[i] Expiratory (upward traces) and inspiratory signals (downward traces) primarily occurred in pairs with expiration preceding inspiration. Both expiratory and inspiratory signals were analyzed separately and added together to obtain the mean V_T per breath (mL/kg). Ventilation (mL/min/kg) was calculated by multiplying the V_T per breath value with breathing frequency.

At the conclusion of both antinociception and respiratory experiments in which turtles were given butorphanol or morphine, turtles received naloxone (0.2 mg/kg, SC) to reverse potential opioid-induced respiratory depression and facilitate recovery. Each turtle was allowed a period of a minimum of 2 weeks between experiments.

Data analysis—For the antinociceptive experiments, withdrawal latencies at each time point were averaged together. For the respiratory experiments, data during the first hour in the tank were discarded as each turtle adjusted to handling and breathing in the chamber.[13] Data for the subsequent 5 hours were averaged into 60-minute periods; the second hour in the tank (eg, 1 hour prior to drug or saline solution administration) represented baseline breathing levels, and each datum point was the mean for the previous 60-minute period. With commercially available software,[j] a 2-way, repeated-measures ANOVA was used to analyze all data in which there was a complete crossover design (eg, antinociception experiments involving administration of saline solution, low- and high-dose butorphanol, or low-dose morphine injections and all respiratory experiments). A 2-way ANOVA was used to analyze data in which there was incomplete crossover (eg, antinociception experiments with high-dose morphine injections and naloxone pretreatment). If the normality assumption was not satisfied, data were ranked and the ANOVA was performed on the ranked data. Post hoc comparisons were made by use of the Student-Newman-Keuls test. All sex comparisons were analyzed by use of a standard *t* test. All data are expressed as mean ± SEM. A value of $P < 0.05$ was considered significant.

Results

To establish baseline thermal withdrawal latencies, saline solution injections were given to 11 turtles prior to starting the drug experiments. After performing several drug experiments, a second saline solution injection was administered (mean interval, 3.5 ± 0.4 months after the first saline solution injections) to each turtle to determine whether thermal withdrawal latencies had changed over time. Because withdrawal latencies from both sets of saline solution injections were identical ($P = 0.097$), all saline solution data were pooled. Thus, for the saline solution experiments, baseline withdrawal latency was 16.9 ± 1.2 seconds and latencies decreased over time to 11.8 ± 1.2 seconds at 24 hours ($P < 0.05$ for all data from 1 to 24 hours after injection; **Figure 2**). In the same 11 turtles, mean baseline withdrawal latencies after low- (2.8 mg/kg; n = 11) and high-dose (28 mg/kg; 11) butorphanol injections were 17.4 ± 0.7 seconds and 15.6 ± 0.7 seconds, respectively. Compared with the data obtained after saline solution injection, withdrawal latencies following butorphanol treatment decreased in a nearly identical manner, with respect to time. There was a time-dependent effect for the saline solution and butorphanol data ($P < 0.001$), but there were no differences ($P = 0.072$) in the withdrawal latencies after butorphanol and saline solution treatments. For the low-dose morphine treatment (1.5 mg/kg; n = 11) experiments, mean baseline withdrawal latency was 18.5 ± 1.0 seconds; latencies remained near baseline level for 1 to 4 hours and increased thereafter to 24.9 ± 2.3 seconds and 19.1 ± 2.0 seconds at 8 and 24 hours after injection, respectively ($P < 0.001$ for drug effect). In a subset of turtles (n = 5) to which a high-dose morphine injection (6.5 mg/kg) was administered, mean baseline withdrawal latency was 16.6 ± 2.0 seconds. Mean withdrawal latencies were unaltered at 1 hour after injection, increased to 5.5 to 6.7 seconds greater than baseline at 2 and 4 hours after injection, and reached 24.6 ± 2.8 seconds at 8 hours after injection (which was nearly identical to the value at this time point obtained in the low-dose morphine injection experiments; $P < 0.001$

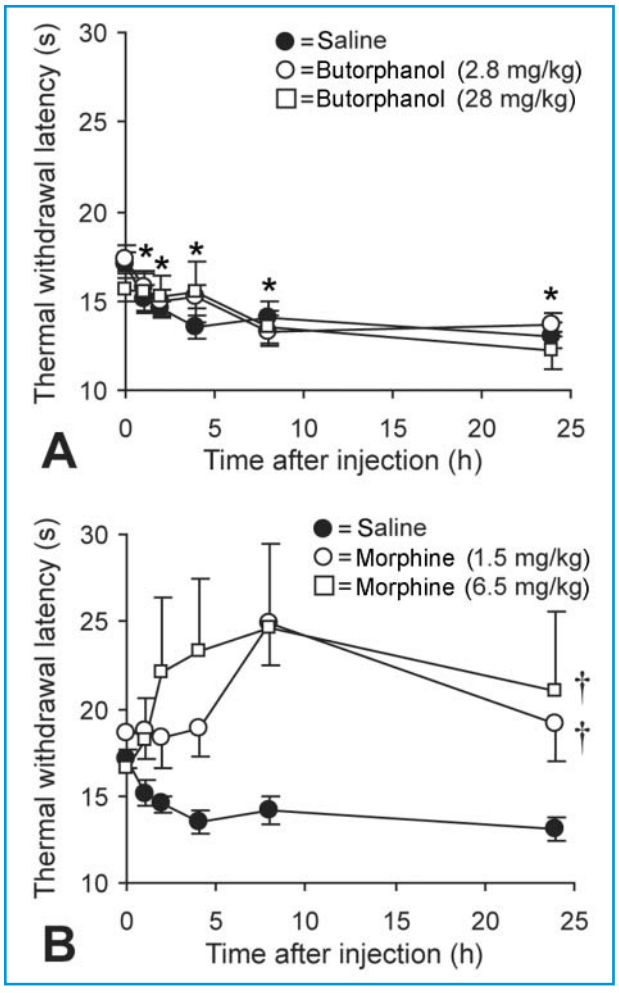

Figure 2—Mean ± SEM thermal hind limb withdrawal latencies in conscious red-eared slider turtles treated SC with saline (0.9% NaCl) solution, butorphanol, or morphine. A—At 1 to 24 hours after injection, low (2.8 mg/kg [1.27 mg/lb]; n = 11) and high (28 mg/kg [12.7 mg/lb]; 11) doses of butorphanol did not alter mean thermal hind limb withdrawal latencies, compared with the effect of saline solution administration. B—Low (1.5 mg/kg [0.68 mg/lb]; n = 11) and high (6.5 mg/kg [2.95 mg/lb]; 5) doses of morphine caused mean thermal withdrawal latencies to remain above latencies for saline solution at 1 to 4 hours after injection but were increased by 34% to 48% after 8 hours. *Significant ($P < 0.05$) time-dependent effect on all data at this time point, compared with baseline values. †Significant ($P < 0.05$) drug effect for all data from 0 to 24 hours, compared with saline solution treatment.

for drug effect). At 24 hours after injection of low and high doses of morphine, withdrawal latencies remained 7.2 to 9.3 seconds above that achieved after saline solution injection but were not significantly different than saline solution values.

When naloxone (0.2 mg/kg) was given 1 hour prior to administration of saline solution (n = 10) or butorphanol (2.8 mg/kg; 10) injections, withdrawal latencies were similar and decreased in a time-dependent manner similar to latencies following saline solution injections alone (**Figure 3**). Baseline withdrawal latency was 17.1 to 17.5 seconds, and mean withdrawal latencies decreased (albeit not significantly [$P = 0.635$]) to 15.6 to 16.0 seconds at 24 hours after injection. These experiments revealed that naloxone did not have any time-dependent effects on withdrawal latencies. In contrast,

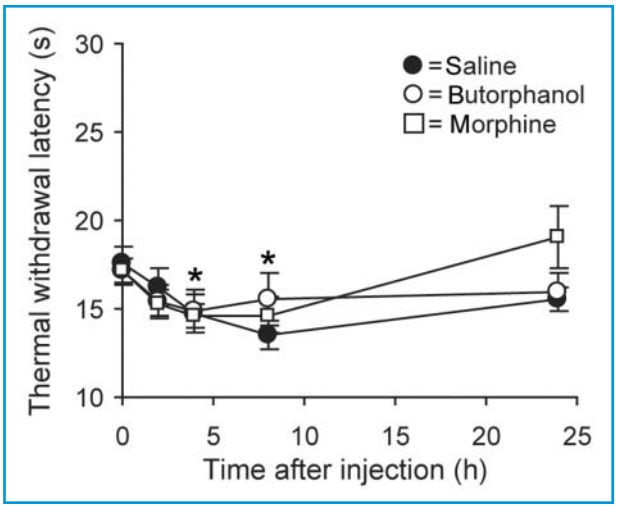

Figure 3—Mean ± SEM thermal hind limb withdrawal latencies in conscious red-eared slider turtles treated with naloxone (0.2 mg/kg [0.09 mg/lb]) injections SC prior to receiving saline solution (n = 10), butorphanol (2.8 mg/kg; 9), or morphine (1.5 mg/kg; 10) injections SC. As a result of pretreatment with naloxone, mean thermal hind limb withdrawal latencies remained similar to those achieved following administration of saline solution, regardless of opioid treatment. *See* Figure 2 for key.

administration of naloxone prior to low-dose morphine injections (1.5 mg/kg; n = 9) completely abolished the latency increase.

With respect to respiration, saline solution injections in another group of 10 turtles did not alter $\dot{V}_E$, frequency of breathing, or V_T during the 4-hour period after injection ($P = 0.45$ to 0.59; Figures 2 and 3). High-dose butorphanol (28 mg/kg) injections in these turtles decreased mean $\dot{V}_E$ from 33 ± 4 mL/kg/min at baseline to 13 ± 4 mL/kg/min and 15 ± 3 mL/kg/min at 1 and 2 hours after injection ($P < 0.001$), but $\dot{V}_E$ then returned to near baseline value (26 ± 4 mL/kg/min) at 4 hours after injection ($P = 0.93$; **Figure 4**). The butorphanol-dependent decrease in $\dot{V}_E$ was attributed to an approximately 63% decrease in breathing frequency from 1.5 ± 0.2 breaths/min at baseline to 0.5 ± 0.2 breaths/min at 1 to 2 hours after injections ($P < 0.001$). Tidal volume increased by approximately 28% at 2 to 3 hours after high-dose butorphanol (28 mg/kg) injection ($P = 0.001$ to 0.006), compared with baseline V_T. In contrast, morphine administered to these turtles depressed $\dot{V}_E$ significantly ($P < 0.001$) from 29 ± 5 mL/kg/min at baseline to 5 ± 1 mL/kg/min at 3 hours after injection (range, 5 to 7 mL/kg/min at 2 to 4 hours after injection) because of an 81% to 86% decrease in breathing frequency ($P < 0.001$) with no change in V_T ($P = 0.056$ to 0.938).

For the antinociception experiments, an effort was made to test similar numbers of male and female turtles (matched for size and weight) to determine whether sex influenced the responses to butorphanol and morphine. In all experiments, there were no obvious differences in data obtained from males and females. For the hind limb thermal latency (morphine) and ventilation (butorphanol and morphine) data at baseline and 4 hours after injection, *P* values for *t* test comparisons of males versus females ranged from 0.17 to 0.91 (mean, 0.54). Thus, there was no indication that sex influences the response to morphine or butorphanol with respect to antinociception and respiratory depression.

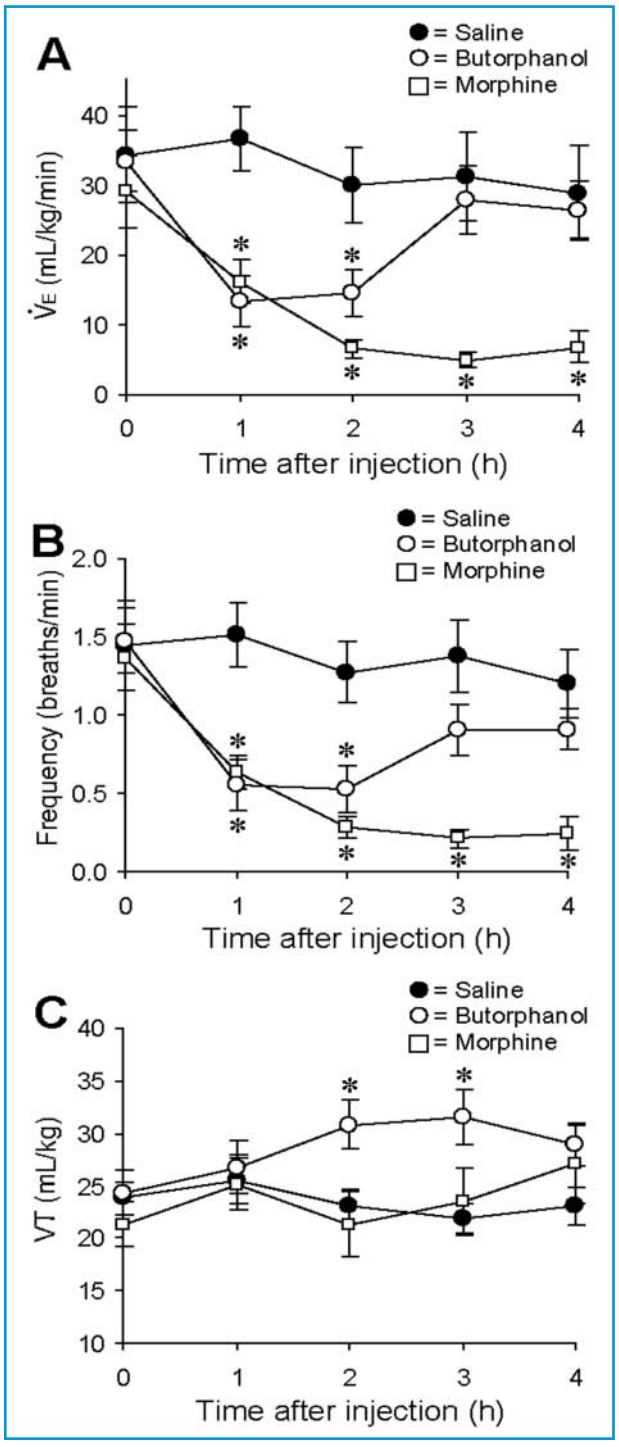

Figure 4—Respiratory effects in conscious red-eared slider turtles treated SC with saline solution (n = 10), butorphanol (28 mg/kg; n = 10), or morphine (1.5 mg/kg; 10). Data are given as mean ± SEM. A—Change in $\dot{V}_E$ following treatment with saline solution or an opioid injection. Butorphanol and morphine injections induced short-term and long-term respiratory depression, respectively, but saline solution injections had no effect on respiration. Mean baseline is the 0 time point on the x-axis. B—Respiratory frequency following treatment with saline solution or an opioid injection. Respiratory frequency decreased within 1 hour after morphine and butorphanol administration. Frequencies remained decreased for turtles receiving morphine, whereas values increased toward baseline after 3 hours for those receiving butorphanol. C—Mean V_T following treatment with saline solution or an opioid injection. Compared with the effect of saline injection, V_T increased by 2 hours after injection in turtles receiving butorphanol but was unchanged after morphine administration. *Value significantly ($P < 0.05$) different from baseline value for the saline solution treatment.

Discussion

Results of the present study in red-eared slider turtles indicated that butorphanol, the most widely used analgesic opioid drug in reptiles, had no apparent thermal antinociceptive effects 1 to 24 hours after injection in this species, although administration of the drug caused mild respiratory depression. By contrast, morphine provided antinociception in these turtles but caused marked and prolonged respiratory depression. To the authors' knowledge, this is the first study to quantify the magnitude and initial 24-hour time course of antinociception and respiratory depression in turtles after administration of low and high doses of opioid drugs.

Understanding species-typical behavior and having the ability to discriminate behavior indicative of pain are crucial to the study of nociception in animals. Unfortunately, few methods are available for assessing nociception in reptiles. Application of a noxious thermal stimulus provides a well-established behavioral model for assessing pain and analgesia in rodents.[15] In the present study, we successfully adapted this model to turtles and determined that turtles had unambiguous, easily quantifiable limb withdrawal responses. This model has many advantages over other noxious stimulus models, including rapid application and decay of the noxious stimulus (thereby not causing long-lasting inflammation), instant latency quantification, and unambiguous behavior after stimulus exposure (either the animal does or does not withdraw its limb). Most importantly, the animal can escape the noxious stimulus by simply withdrawing its limb. However, there are also a few weaknesses associated with this method. The plantar thermal apparatus uses only a radiant heat stimulus at a single intensity and with a steep rate of heating (heating slope).[15] There is evidence that a steep heating slope may preferentially activate A-δ fibers, whereas a slow heating slope activates C fibers; it is believed that morphine may have a more profound affect when C fibers are activated.[15] In addition, the temperature at the skin surface may be only an approximation of the temperature reached at the level of the nociceptors located at the dermoepidermal junction.[15] Nevertheless, to our knowledge, the present study is the first application of the plantar thermal apparatus in turtles from which unambiguous data were obtained. It appears to be a method that could be successfully used in future antinociceptive research. The high number of antinociception tests in our study was necessary, however, because there is no detailed information on the pharmacodynamics of butorphanol and morphine in red-eared slider turtles.

Butorphanol is considered the most commonly administered analgesic drug in reptiles.[16] In a published survey[16] of veterinary clinicians, the dosage range for butorphanol in reptiles varied from 0.02 to 25 mg/kg (0.009 to 11.36 mg/lb). We chose a low dose of butorphanol (2.8 mg/kg) on the basis of its analgesic efficacy in avian species,[17] and a high dose (28 mg/kg) to maximize a quantifiable effect, although we recognized that butorphanol has an analgesic ceiling effect in mammals. In the present study, those 2 doses of butorphanol administered SC did not alter hind limb withdrawal latencies to noxious thermal stimuli in turtles, which was surprising given the efficacy of butorphanol in mammals and birds. An alternative explanation is that butorphanol did not cross the

blood-brain barrier in the study turtles. However, butorphanol readily crosses the blood-brain barrier in birds,[18] and there was significant short-term respiratory depression, consistent with the hypothesis that butorphanol is able to alter central respiratory rhythm-generating neural circuits. It is also possible that butorphanol may be effective only at higher thermal intensities or only for other forms of noxious chemical, electrical, or physical stimuli (eg, cutting the skin). Nevertheless, it would be unusual for a drug to be effective for only 1 particular type of noxious stimulus. Finally, it is possible that butorphanol is more effective during certain times of the year in turtles. Although seasonal effects on nociception were not rigorously examined, these studies were conducted on turtles throughout the year with no obvious seasonal effects.

Alternatively, it may be that morphine, but not butorphanol, is an effective analgesic in reptiles. For example, IV administration of butorphanol (1 mg/kg [0.45 mg/lb]) has no isoflurane-sparing effect in green iguanas,[19] whereas treatment with morphine increases tail flick latencies in anole lizards[7] and hot-plate withdrawal latencies in crocodiles.[20]

Unlike butorphanol, morphine administered at low and high doses significantly increased hind limb withdrawal latencies in turtles in the present study, suggesting that opioid receptor activation induces antinociception in turtles. The opioid receptor gene family is highly conserved across multiple vertebrate orders (eg, bovids, chickens, bullfrogs, and teleost and elasmobranch fishes),[21] but there is limited information on opioid receptors in reptiles. For example, μ- and δ-opioid receptors are located throughout the brain in aquatic turtles, and δ-opioid receptors are more abundant than μ-opioid receptors.[22] However, results of our study did not determine the location and distribution of μ- and δ-opioid receptors in the spinal cord (where nociceptive inputs enter the CNS) of turtles, nor were κ-opioid receptors examined anywhere in the CNS. With respect to endogenous opioid-related neurotransmitters, proenkephalin-derived peptides are present in turtles with a distribution similar to that in mammals and birds.[23] Given that butorphanol (a mixed-opioid κ-receptor agonist–μ-receptor antagonist) was ineffective, we hypothesize that morphine-induced antinociception in turtles is likely to be attributable to μ-opioid receptor activation within the CNS. Consistent with this hypothesis, preliminary experiments in our laboratory show that DAMGO, a specific μ-agonist, induces antinociception in turtles. In contrast, κ-opioid receptors are quantitatively and qualitatively more important than μ-opioid receptors in avian species; therefore, butorphanol is an effective analgesic drug in psittacines.[17] Thus, opioid-dependent antinociception in different phyla may require activation of different opioid-receptor subtypes.

Respiratory depression can be a limiting factor when opioid drugs are used to provide analgesia. In the present study, both butorphanol and morphine caused notable respiratory depression; of the 2 drug effects, morphine-associated respiratory depression was longer lasting. It is unlikely that drug-induced respiratory depression was attributable to severely sedated turtles merely sitting at the bottom of the tank. In separate pilot experiments involving the same morphine dose, turtles moved about the water-filled tank in a manner similar to turtles injected with saline solution (data not shown). For both butorphanol and morphine, the decrease in ventilation was a result of a decrease in breathing frequency; V_T was maintained by both drugs and even augmented slightly with butorphanol. The morphine-induced respiratory depression was probably a result of activation of μ-opioid receptors in the brainstem because DAMGO abolishes fictive breathing in isolated turtle brainstems.[10] For aquatic turtles, the physiologic and clinical consequences of opioid-induced respiratory depression are not well understood. Healthy turtles are able to compensate for prolonged periods of anoxia[24] and easily survive relatively short periods (4 to 10 hours) of apnea.[25] In an injured or diseased turtle, however, the ability to survive respiratory depression is likely to be compromised; therefore, caution is required when administering opioid drugs in a clinical setting. Recent evidence suggests that it may be possible to administer opioid drugs with other drugs (eg, a serotonin 5-HT_4 receptor agonist or dopamine D1 receptor agonist) to reverse respiratory depression and preserve antinociception.[26,27] Future studies will be required to test this hypothesis in reptiles.

We believe that objectively derived methods for evaluation of pain in animals are critical, but these methods must be species- and context-specific. Morphine appears to be a better choice than butorphanol for antinociception in turtles, but respiration must be monitored closely. One cannot assume a single opioid drug is effective in all species, and the long-standing belief that butorphanol is the best choice for pain relief in reptiles is apparently not warranted. Future research directions should include evaluation of other clinically relevant analgesic drugs, which may induce antinociception without respiratory depression, and assessment of postsurgical opioid efficacy in reptiles.

a. Niles Biological Supply, Sacremento, Calif.
b. ReptoMin, Tetra, Blacksburg, Va.
c. Ugo Basile plantar analgesia instrument (Hargreaves' apparatus), Model 37370, Ugo Basile Co, Comerio VA, Italy.
d. Torbugesic-SA, Fort Dodge Animal Health, Fort Dodge, Iowa.
e. Baxter Healthcare Corp, Deerfield, Ill.
f. Hospira Inc, Lake Forest, Ill.
g. Godart, Gould Electronics, Eastlake, Ohio.
h. LabPro, Vernier Software & Technology, Beaverton, Ore.
i. Clampfit software, Axon Instruments Inc, Union City, Calif.
j. SigmaStat, Jandel Scientific Software, San Rafael, Calif.

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