Judicious use of analgesic drugs in all species is critical to the practice of clinical veterinary medicine.1–3 Conditions considered painful in humans and other mammals should be assumed to be painful in all vertebrate species.4–7 Although our understanding of pain control in domestic mammals and humans is considerable, methods for measuring pain and analgesia in nondomestic species require further development.4–7 Information regarding pain control is particularly scarce for reptiles, even though reptiles are frequently maintained as companion animals and in zoologic and scientific laboratory facilities.3,5,6,8–10 In addition, reptiles are one of the most phylogenetically diverse animal classes with 3 main Orders: Chelonia (turtles and tortoises), Sauria (lizards), and Serpentes (snakes). Thus, it is important and necessary to identify means by which analgesia can be effectively provided for representative species from each reptile taxon.

In mammals, opioid receptor agonists or partial agonist-antagonists are commonly administered and considered the most effective drugs for controlling pain.11 However, the lack of experimentally derived data regarding use of opioid drugs in reptiles is a major clinical disadvantage. The gene family for opioid receptors (μ, κ, and δ) is highly conserved across multiple vertebrate orders, although specific information on reptilian endogenous ligands and opioid receptors is sparse.12,13 For example, 2 snake species have endogenous brain opiates14,15 and red-eared slider turtles have both proenkephalin-derived peptides and functional μ- and δ-opioid receptors in the brain.16,17 Although opioid receptors are expressed in reptiles, the efficacy of commonly used opioid drugs remains unknown in reptiles.

Anecdotally, it is recommended to administer butorphanol to reptiles at mammalian-derived dosages.9 However, by use of a noxious thermal stimulus method, we recently determined that butorphanol administered SC at doses of 2 and 20 mg/kg (0.91 and 9.1 mg/lb) has no antinociceptive efficacy in red-eared slider turtles.7 Consistent with these findings, butorphanol administered IM at a dose of 1 mg/kg (0.45 mg/lb) has no antinociceptive effects (determined by use of a thermal noxious stimulus method)18 and no isoflurane-sparing effect19 in green iguanas. In contrast, morphine increases limb withdrawal latencies in turtles2 and crocodiles20,21 and increases tail flick latencies in anole lizards,22 thereby indicating that morphine provides antinociception in certain reptile species. Thus, there is a need for systematic evaluation of different opioid drugs in several reptile species to assess species differences.

### Objective
To test the hypothesis that administration of butorphanol or morphine induces antinociception in bearded dragons and corn snakes.

### Design
Prospective crossover study.

### Animals
12 juvenile and adult bearded dragons and 13 corn snakes.

### Procedures
Infrared heat stimuli were applied to the plantar surface of bearded dragon hind limbs or the ventral surface of corn snake tails. Thermal withdrawal latencies (TWDLs) were measured before (baseline) and after SC administration of physiologic saline (0.9% NaCl) solution (equivalent volume to opioid volumes), butorphanol tartrate (2 or 20 mg/kg [0.91 or 9.1 mg/lb]), or morphine sulfate (1, 5, 10, 20, or 40 mg/kg [0.45, 2.27, 4.5, 9.1, or 18.2 mg/lb]).

### Results
For bearded dragons, butorphanol (2 or 20 mg/kg) did not alter hind limb TWDLs at 2 to 24 hours after administration. However, at 8 hours after administration, morphine (10 and 20 mg/kg) significantly increased hind limb TWDLs from baseline values (mean ± SEM maximum increase, 2.7 ± 0.4 seconds and 2.8 ± 0.9 seconds, respectively). For corn snakes, butorphanol (20 mg/kg) significantly increased tail TWDLs at 8 hours after administration (maximum increase from baseline value, 3.0 ± 0.8 seconds); the low dose had no effect. Morphine injections did not increase tail TWDLs at 2 to 24 hours after administration.

### Conclusions and Clinical Relevance
Compared with doses used in most mammalian species, high doses of morphine (but not butorphanol) induced analgesia in bearded dragons, whereas high doses of butorphanol (but not morphine) induced analgesia in corn snakes. (J Am Vet Med Assoc 2008;233:267–273)

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**Analgesic efficacy of butorphanol and morphine in bearded dragons and corn snakes**

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dose-dependent effects, and duration of drug efficacy. The purpose of the study reported here was to test the hypothesis that administration of butorphanol or morphine induces antinociception in bearded dragons and corn snakes (as representative species of the Sauria and Serpentes).

Materials and Methods

The Animal Care and Use Committee at the School of Veterinary Medicine, University of Wisconsin, Madison, approved all experimental procedures.

Animals—Twelve juvenile and adult bearded dragons (Pogona vitticeps [6 males and 6 females]) and 13 corn snakes (Elaphe guttata [7 males and 6 females]) were obtained from commercial suppliers. Mean ± SEM initial weight was 57.9 ± 7.9 g (0.127 ± 0.017 lb) for the bearded dragons and 72.5 ± 15.1 g (0.160 ± 0.03 lb) for the corn snakes. All animals were considered healthy during the entire experimental period on the basis of results of physical examinations and routine hematologic and serum biochemical assessments. Room temperature was set at 28° to 29°C (82.4° to 84.2°F). Bearded dragons were housed individually in 38-L aquariums equipped with fresh water, a climbing branch, a hide box, and newspaper substrate. Daily, bearded dragons were fed a mixed salad composed of fresh, dark, leafy greens, sliced carrots, and strawberries; calcium carbonate powdera was applied to the salad mix twice weekly, and a multivitamin powderb was applied twice monthly. Bearded dragons were offered live crickets 3 times/wk. A broad-spectrum UV light bulbc was suspended above each lizard and kept illuminated for 14 h/d. A ceramic heat bulb for basking was also suspended above each aquarium. Snakes were housed in standard rodent cages equipped with a hide box, fresh water, and newspaper substrate. Snakes were fed thawed mice once per week.

Study design—A crossover experimental design was used to evaluate opioid-dependent changes in thermal antinociception in the bearded dragons and corn snakes, in which each reptile was exposed to each treatment condition with a minimum washout period of 2 weeks between treatments. Each bearded dragon (n = 12) received physiologic saline (0.9% NaCl) solution as a control treatment and 2 doses of butorphanol (2 and 20 mg/kg). A subset of bearded dragons received 4 doses of morphine (1, 5, 10, and 20 mg/kg [0.45, 2.27, 4.5, and 9.1 mg/lb; n = 10, 11, 11, and 6, respectively]). A subset of corn snakes (n = 7) received physiologic saline solution as a control treatment and 2 doses of butorphanol (2 and 20 mg/kg). In a separate experiment, a subset of snakes (n = 13) received physiologic saline solution as a control treatment and 5 doses of morphine (1, 5, 10, 20, and 40 mg/kg [0.45, 2.27, 4.5, 9.1, and 18.2 mg/lb; n = 7, 3, 3, 9, and 3, respectively]). The observer in the antinociceptive experiments was unaware of the animals’ treatments.

Thermal analgesia experiments—Analgesimetry consisted of measuring the latency of the hind limb or tail withdrawal reflex in response to a noxious infrared radiant heat stimulus applied by use of a standard apparatusb to the plantar surface of the hind limb of bearded dragons or to the ventral tail surface of corn snakes (approx 1 to 2 cm caudal to the vent). Animals were placed in plastic boxes (17 X 13 X 14 cm) on an elevated acrylolic plastic surface with opaque barriers that prevented visual contact with each other. When infrared heat was applied to the limbs or tails through the acryllic surface, the increasing temperature caused the animal to either lift their limb in the case of the lizards or move their tails in the case of the snakes and the time to withdrawal (ie, latency) was automatically measured. Stimulation strength was adjusted to attain baseline latencies of approximately 5 to 10 seconds (corresponding to 45° to 47°C [113° to 116°F]); a maximum duration of 32 seconds was used to prevent prolonged heat exposure.

Figure 1—Mean ± SEM hind limb withdrawal latency assessed in response to a noxious thermal stimulus (A) and change in withdrawal latency from baseline (time, 0 hours [before treatment]) value (B) in 12 bearded dragons at 2, 4, 8, and 24 hours after IM administration of a low dose (2 mg/kg [0.91 mg/lb]; white circles) of butorphanol, a high dose (20 mg/kg [9.1 mg/lb]; white squares) of butorphanol, and an equivalent volume of saline (0.9% NaCl) solution (black circles) in a crossover study. For the high dose of butorphanol, baseline latency was greater than values for the other treatments; however, latencies did not change significantly from baseline values following any of the 3 treatments. *All values for the high-dose butorphanol treatment are significantly (P < 0.05) greater than those for saline solution treatment (drug effect).
Mean baseline withdrawal latency was established before each experimental treatment (drug or saline solution treatment) for each animal (time, 0 hours) from data obtained by application of 1 stimulus on 3 occasions at 5-minute intervals. Following determination of the baseline value, treatment consisted of an SC injection of physiologic saline solution (equivalent volumes to opioid volumes), butorphanol tartrate (2 or 20 mg/kg; designated as high and low doses, respectively), or morphine sulfate (1, 5, 10, or 20 mg/kg); during the study, each treatment was administered to each animal. Snakes also received morphine sulfate at a dose of 40 mg/kg. All drugs were administered IM in the cranial, epaxial muscles of bearded dragons and corn snakes. At 2, 4, 8, and 24 hours after injection, withdrawal latencies were determined from data obtained by application of 1 stimulus on 3 occasions at 5-minute intervals. All animals were conditioned to the chamber, and all drug and saline solution treatments were administered in a random order.

Data analysis—For a given treatment, the mean thermal withdrawal latency for bearded dragons or corn snakes at each time point was calculated. Commercially available software was used to analyze all data and perform 2-way ANOVAs. If normality or equal variance assumptions were not satisfied, data were ranked and the 2-way ANOVA was recalculated on the ranked data. Post hoc comparisons were made by use of the Student-Newman-Keuls test. All data are expressed as mean ± SEM. Values of $P < 0.05$ were considered significant.

Results

Findings in bearded dragons—To establish that hind limb thermal withdrawal latencies did not change
over time without drug administration, bearded dragons (n = 12) were administered a saline solution injection prior to starting the drug experiments. For the saline solution treatment, mean baseline withdrawal latency was 7.7 ± 0.4 seconds; at 2 to 24 hours after injection, withdrawal latency remained at 7.8 ± 0.4 seconds to 8.3 ± 0.4 seconds (Figure 1). After administration of the low dose of butorphanol (2 mg/kg [n = 12]), mean withdrawal latencies for butorphanol were almost identical to the values obtained after treatment with saline solution at the 2- through 24-hour time points (P = 0.86). Prior to administration of the high dose of butorphanol (20 mg/kg [n = 11]), mean baseline withdrawal latency was 10.0 ± 0.8 seconds. This value was more than 2.0 seconds greater than baseline values for saline solution and low-dose butorphanol treatments, which resulted in a spurious significant (P < 0.001) drug effect because otherwise latencies did not change with time from baseline values, regardless of treatment. It is possible that the bearded dragons were actively shedding at certain times during this study. However, we avoided use of reptiles that were actively shedding or preparing to shed so that the confounding influence of limited nociception during the shedding process could be avoided. Thus, there is no obvious scientific, technical, or seasonal explanation for the higher mean baseline withdrawal latency associated with the high-dose butorphanol treatment. Apparently, in the bearded dragons of the present study, mean baseline hind limb withdrawal latencies had considerable variability (Figure 2). Nevertheless, the high dose of butorphanol (20 mg/kg) did not significantly (P = 0.98) alter withdrawal latencies from 2 to 24 hours after injection, compared with the baseline value.

In bearded dragons, morphine injections at doses of 1 and 5 mg/kg (n = 10 and 11) had significant (P < 0.001) drug effects, compared with findings after injection of saline solution, but these effects were attributable to differences in baseline hind limb values (Figure 2). When the change in latency from the respective baseline values was assessed, morphine injections at either of those doses had no effect (P = 0.13) on withdrawal latency, despite a change in latency of approximately 2 seconds at the 2-, 4-, and 8-hour time points following injection (before returning to baseline values at 24 hours). In contrast, the changes in latency from the respective baseline values for each of the higher doses of morphine (10 or 20 mg/kg; n = 11 or 6) were almost identical results; withdrawal latency increased significantly (P < 0.001) by 2.2 to 2.8 seconds for 2 to 8 hours after injection. At 24 hours after injection of morphine at a dose of 10 mg/kg, latencies were only 1.2 ± 0.7 seconds greater (P = 0.14) than the baseline value, indicating that the duration of drug effects was < 24 hours at that dose.

Findings in corn snakes—For corn snakes, treatment with saline solution (n = 7) did not alter tail withdrawal latencies from the baseline value of 5.6 ± 0.9 seconds at 2, 4, 5, and 24 hours after injection (Figure 3). Likewise, treatment with butorphanol (2 mg/kg [n = 7]) did not (P = 0.98) alter tail withdrawal latency from the baseline value. After administration of the high butorphanol dose (20 mg/kg [n = 7]), baseline withdrawal latency (8.3 ± 0.7 seconds) was greater than that achieved after administration of the low dose; values increased to a maximum of 11.3 ± 0.4 seconds at 8 hours after injection, which resulted in a significant drug effect (P < 0.001), even after the change in latency from baseline value versus time was calculated (P = 0.01).

In corn snakes, tail withdrawal latency increased from the baseline value to a maximum of 2.3 ± 0.9 seconds at 4 hours after morphine injections at doses of 1 or 5 mg/kg (n = 10 or 11) were administered (Figure 4). Because mean baseline withdrawal latency was higher for the 5 mg/kg dose of morphine, there was a spurious significant drug effect for these data (P < 0.001) but not for the 1 mg/kg dose of morphine (P = 0.65). On assessment of the change in latency from the respective baseline...
line values, there were no significant (P = 0.38) drug effects. Morphine injections (10, 20, and 40 mg/kg, [n = 3, 9, and 3, respectively]) resulted in no significant drug effects.

**Discussion**

To our knowledge, this is the first study to systematically evaluate the magnitude and extent of the antinociceptive effects of butorphanol and morphine (administered IM) during a 24-hour period in lizards and snakes by use of a well-established thermal withdrawal latency test. Butorphanol, the most widely used analgesic opioid drug in reptiles, had no antinociceptive effects in bearded dragons but did have antinociceptive effects at a high dose in corn snakes in the present study. In contrast, morphine had antinociceptive effects in bearded dragons but not in corn snakes. However, the antinociceptive doses of morphine (10 and 20 mg/kg) in bearded dragons and butorphanol (20 mg/kg) in corn snakes were substantially greater than the doses of those drugs that are typically administered in clinical settings and may cause severe respiratory depression. Thus, caution is warranted before administering these high doses of opioid drugs to lizards and snakes to provide analgesia within a clinical setting.

The finding that morphine but not butorphanol provided analgesia in bearded dragons is similar to results of a study\(^\text{7}\) in which the same drugs were administered SC in red-eared slider turtles. However, the magnitude of the morphine-associated increase in hind limb withdrawal latency in bearded dragons was much smaller than that detected in turtles treated with the drug. For example, hind limb withdrawal latency increased from baseline value (albeit not significantly) by 2.0 ± 0.9 seconds following IM injection of 5 mg of morphine...
morphine/kg in bearded dragons in our study, whereas latency increased significantly from baseline value by 8.0 ± 2.8 seconds following SC injection of 6.5 mg of morphine/kg (2.95 mg/lb) in turtles.7 Even high doses of morphine (10 and 20 mg/kg) resulted in increases in hind limb withdrawal latency of only 2 to 3 seconds in bearded dragons. Surprisingly, tail withdrawal latency in corn snakes was not altered despite IM administration of morphine doses ranging from 1 to 40 mg/kg. Thus, morphine efficacy appears to vary widely among reptile species or within individual snakes. Because injection of 1.5 mg of morphine/kg (0.68 mg/lb) depressed breathing by 83% in red-eared slider turtles,7 high doses of morphine may also depress breathing to a similar degree in bearded dragons. In contrast, butorphanol provided mild analgesia in corn snakes but had no effect on hind limb withdrawal in bearded dragons in our study. On the basis of these findings, it appears that the role of opioid receptor activation in providing analgesia against noxious thermal stimuli may not be conserved across reptile Classes and species. This variability may be attributable to species-dependent differences in the mechanisms by which opioid receptor activation modulates noiception, differences in the binding of drugs to opioid receptors, or differences in opioid drug metabolism and pharmacodynamics.

The ability to discriminate and quantify behavior that is indicative of pain from species-typical behavior is crucial to the study of pain and analgesia.1 Once pain-related behaviors are reliably quantified, it is possible to evaluate antinociceptive drug efficacy. Unfortunately, there are few methods available for quantitatively assessing species- and context-specific pain-related behavior in reptiles and other nondomestic species.1,2 In the present study, noxious thermal stimuli were used in an adaptation of a classic method for measurement of withdrawal responses in rodents.21 Application of a noxious thermal stimulus is a well-established behavioral technique for assessing pain and analgesia. Compared with the use of other types of noxious stimulus, a noxious thermal stimulus technique has the advantages of rapid application and decay of the noxious stimulus (so as not to cause 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 or tail.

Although we successfully adapted the noxious thermal stimulus method to bearded dragons and corn snakes in the present study, this technique may not always be ecologically or physiologically relevant for all reptile species. This fact may explain the unexpected findings of our study, such as the large variability in baseline withdrawal latencies, lack of consistent antinociceptive effects of the opioid drugs between the 2 species, and relatively small increases (from baseline values) in withdrawal latencies after administration of high doses of the opioid drugs. For example, aquatic red-eared slider turtles, like rodents, respond consistently and predictably by withdrawing a hind limb after application of noxious thermal stimuli and morphine administration results in large increases (from baseline values) in hind limb withdrawal latencies.7 Because the water temperature in a typical aquatic ecosystem suitable for red-eared sliders may reach a maximum of 30°C (86°F), it is reasonable to assume that a hot stimulus would elicit avoidance. On the other hand, bearded dragons live in different habitats, such as open woodlands and deserts in Australia or arboreal habitats, suggesting that exposure to hot surfaces is not necessarily a constant feature of bearded dragons' environments.24 Signs of discomfort are apparent in bearded dragons after exposure to ambient temperatures > 40°C (104°F), suggesting that extreme heat may be aversive to this species.25 Nevertheless, some bearded dragons are adapted to warm, arid environments and prefer ambient temperatures that range from 30° to 40°C.25 Thus, unlike aquatic turtles and mammals, adaptation to warm environmental temperatures may preclude bearded dragons from responding to a noxious thermal stimulus after opioid administration.

Because corn snakes inhabit a wide variety of temperate ecosystems, they would also be expected to withdraw their tails in response to hot noxious stimuli. By use of a noxious thermal stimulus technique, the antinociceptive effects of opioid drugs should be apparent in this species. In the corn snakes of our study, however, baseline tail withdrawal latencies were highly variable, butorphanol-associated increases (from baseline value) in withdrawal latency were evident only following administration of markedly high doses, and antinociceptive effects of morphine were not detected. These findings were surprising and raise important questions regarding how snakes process different nociceptive sensory afferent inputs. For example, captive snakes may develop thermal burns if allowed to bask on faulty, overheated, in-cage heating units. On the basis of such anecdotal observations, we hypothesize that snakes may process sensory inputs from noxious thermal stimuli and those from noxious stimuli such as electric shock, surgical incision, visceral tissue damage, or inflammation differently. Alternatively, the evolution of limblessness in snakes may have dramatically altered spinal opioid receptor expression and function. However, to our knowledge, there is no information in the veterinary medical literature regarding the presence or function of spinal opioid receptors in any snake species.

Measurement of pain in reptiles is complex, and the expansion of methodologies used in our study is critical for analyzing nociception and analgesia across reptile species and within different contexts (eg, hospital cage vs home environment). On the basis of changes in withdrawal latencies to noxious thermal stimuli, µ-opioid receptor agonists, such as morphine, appear to be more efficacious in bearded dragons and red-eared slider turtles than butorphanol, which is currently the analgesic drug of choice for all reptile species. Because of the variability in response to noxious thermal stimuli before and after administration of morphine or butorphanol in corn snakes in the present study, we were unable to determine which opioid analgesic is more effective in this species. Application of multiple methods for assessment of nociception and analgesia in reptiles may be more appropriate than sole use of a thermal stimulus technique.
References