Assessments of thermal antinociceptive effects of butorphanol and human observer effect on quantitative evaluation of analgesia in green iguanas (*Iguana iguana*)

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Objective—To determine whether butorphanol induces thermal antinociception in green iguanas (*Iguana iguana*) and assess the human observer effect on quantitative evaluation of butorphanol-induced analgesia.

Animals—6 juvenile green iguanas.

Procedures—Skin temperature was recorded, and then a direct increasing heat stimulus was applied to the lateral aspect of the tail base of each iguana. Temperature of the stimulus at which the iguana responded (thermal threshold) was measured before and for 8 hours after IM injection of either butorphanol tartrate (1.0 mg/kg) or an equal volume of saline (0.9% NaCl) solution. Six experiments (butorphanol [n = 3] and saline solution [3]) were conducted with the observer in the iguanas' field of vision, and 11 experiments (butorphanol [n = 5] and saline solution [6]) were conducted with the observer hidden from their view. The interval between treatments or tests was ≥ 1 month.

Results—Temperature difference between thermal threshold and skin temperature when iguanas were administered saline solution did not differ from temperature difference when iguanas were administered butorphanol regardless of whether the observer was or was not visible. Temperature difference between thermal threshold and skin temperature was significantly lower when iguanas were tested without the observer in visual range, compared with the findings obtained when iguanas were tested with an observer in view, at multiple times after either treatment.

Conclusions and Clinical Relevance—Intramuscular administration of 1.0 mg of butorphanol/kg did not induce thermal antinociception in juvenile green iguanas. The visible presence of an observer appeared to influence the results of noxious stimulus testing in this reptile species. (*Am J Vet Res* 2012;73:1507–1511)

Although analgesics or antinociceptive agents are now widely used in domestic animals, their usage has been very limited in reptiles, such as green iguanas (*Iguana iguana*).[1–3] The number of reports[4] of the use of analgesic agents in reptiles is increasing, but most have been based on anecdotal experiences, with dosages often extrapolated from other species, such as birds. Butorphanol, a κ-opioid receptor[a] agonist and a μ-opioid receptor[a] antagonist, has historically been used to provide analgesia in mammals and birds. In some mammalian and bird species, the presence, classification, and distribution of opioid receptors have been described.[5–7]

Abbreviations

ST	Skin temperature
TT	Thermal threshold

Of the > 7,200 known species of reptiles, only 30 to 50 are commonly kept in zoological collections or as pets.[8] Green iguanas represent one of the most common species in captivity. These reptiles often require veterinary care, and the use of analgesics or antinociceptive agents should be encouraged in reptiles that are injured or undergoing surgical procedures.

Signs of pain are difficult to recognize in reptiles. Reptiles do not display overt behaviors such as vocalization, and changes in behavior related to pain may be subtle and therefore overlooked.[1,9] Another challenge is the development of ethical and repeatable methods for assessment of signs of pain and its alleviation in reptiles so that analgesics can be objectively evaluated.[10]

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When developing a testing protocol for green iguanas, the normal behavior of this species must be considered.

Techniques for studying analgesia in reptiles, such as the tests involving use of hotplates or electrical stimulation, have been applied with variable success.[11,12] Thermal antinociception was demonstrated after administration of morphine and meperidine (pethidine) in red-eared slider turtles, Nile crocodiles, and anole lizards.[9,12,13] However, in red-eared sliders, bearded dragons, and corn snakes, butorphanol administration did not result in thermal antinociception.[9,14]

Historically, the anesthetic-sparing effects of analgesic drugs have been used as an indirect measure of their analgesic efficacy. In some species, opioids may decrease the amount of inhalation anesthetic (minimum alveolar or anesthetic concentration) required to prevent the response to a noxious stimulus.[15] In cats, remifentanil infusions resulted in no change in the minimum anesthetic concentration of isoflurane, but antinociception in conscious animals was clearly demonstrated as assessed by TT testing.[16] A relationship between anesthetic-sparing effects and analgesia should not be assumed in all species. Butorphanol did not have an anesthetic-sparing effect in green iguanas,[17] but to our knowledge, this opioid has not been evaluated as an analgesic in conscious green iguanas.

The primary objective of the study reported here was to assess the thermal antinociceptive effects of IM treatment with butorphanol tartrate in juvenile green iguanas. The null hypothesis was that there would be an increase in TT following a single IM injection of butorphanol (1.0 mg/kg) but not following a control treatment (single IM injection of an equal volume of saline [0.9% NaCl] solution). As the study progressed, additional experiments were included to investigate the effect of human presence (ie, an observer in the iguanas' field of view) on quantitative evaluation of butorphanol-induced analgesia.

Materials and Methods

Animals—Six juvenile green iguanas were obtained from a commercial supplier.[b] The sex of the iguanas could not be determined because they were not sexually mature. Body weights ranged from 30 to 78 g, and all iguanas gained weight during the course of the study. They were housed together in a 2.0 × 0.75 × 0.5-m cage, with newspaper substrate, natural branches, and a basking site. The ambient temperature was approximately 27°C, and the temperature in the basking spot was 43.3°C. Cage lighting was provided with commercially available full-spectrum bulbs[c] and a single incandescent bulb (100 W) for thermoregulation. The iguanas were fed a mixture of chopped leafy greens, vegetables, and fruit with a calcium supplement[d] daily and had free access to fresh water. Once a week, the iguanas were housed outside in a 0.5 × 0.5 × 2.0-m screened cage with access to unfiltered sunlight. The iguanas were acclimated to their housing for 8 weeks prior to study commencement. The study was approved by the Institutional Animal Care and Use Committee at the University of Florida. The iguanas were adopted following completion of the study.

Study design—A prospective, blinded study with 2 grouping factors (observation and treatment) and 1 repeat factor (time) was performed. Each iguana received the control treatment and treatment with butorphanol on different occasions, and testing was conducted with or without an observer visible to the animal. Allocation of the 6 iguanas to each of the 4 treatment groups was as follows: with an observer in the animals' field of view, iguanas 1, 2, and 3 were tested after receiving treatment with butorphanol and iguanas 4, 5, and 6 were tested after receiving treatment with saline solution; with no observer in the animals' field of view, iguanas 1, 2, 4, 5, and 6 were tested after receiving treatment with butorphanol and all iguanas were tested after receiving treatment with saline solution. Iguana 3 was found dead 3 weeks after the third trial, and necropsy did not determine a cause of death.

Thermal antinociceptive testing—A TT testing device developed for use in cats and horses[10,18] was adapted for the present study. A probe containing a heating element and a temperature sensor measuring 10 × 10 × 5 mm and weighing 5 g was attached to the left or right side of the base of each iguana's tail (selected side determined by the outcome of a coin toss), approximately 0.5 cm caudal to the hind limb and held in place with white adhesive tape and a layer of bandage material.[e] The probe was connected to the testing device via a lightweight flexible 2-m ribbon cable.

On the morning of each study, the probe was attached as described and the iguana was placed in a testing enclosure (0.6 × 0.6 × 0.6 m) with similar environmental conditions established for daily housing. Each iguana was free to move around the enclosure during the testing process. The ST was recorded, and then the heating probe was activated; the rate of increase in probe temperature was 0.6°C/s. A safety cutoff was set at 55°C. A toggle switch was pressed to initiate heating and then pressed again to terminate heating once a behavioral response was observed (kicking, flicking the tail, or jumping); this was recorded as the TT. If no response was observed, the probe heated to 55°C and then automatically shut off; the result was recorded as no response. Three baseline measurements were recorded at 15-minute intervals prior to treatment; the mean of these results was calculated to give 1 baseline reading.

After baseline data were collected, each iguana received its allocated treatment. Treatments were butorphanol tartrate[f] (1.0 mg/kg) or an equal volume of saline solution injected via a 15.8-mm 25-gauge needle into the quadriceps femoris muscle of a randomly selected hind limb (hind limb selection determined by the outcome of a coin toss). There was an interval of at least 1 month between treatments and thermal antinociceptive testing.

For each iguana, TT was evaluated every 15 minutes to 2 hours after injection, every 30 minutes from 2 to 6 hours after injection, then at 7 and 8 hours after injection. The observer who conducted the TT tests was blinded to the treatment for each iguana. Initially, testing of 3 iguanas after treatment with butorphanol and 3 iguanas after treatment with saline solution was

completed but with variable results, including many time points at which no response occurred before cutoff was reached. Upon review of the first 6 tests, we noticed that the iguanas became immobile if the observer was in their visual range. If the observer moved out of visual range, the iguanas became active and explored the enclosure. We concluded that the presence of the observer was influencing the behavior of the iguanas during testing. A visual barrier was erected and a video camera was inserted through this barrier to observe the response to TT testing. It was noticed immediately that with the visual barrier in place, the iguanas would move around the test cage. One month later, we repeated the testing of iguanas after treatment with butorphanol (n = 5) and after treatment with saline solution (6) when the observer was not visible to the animals.

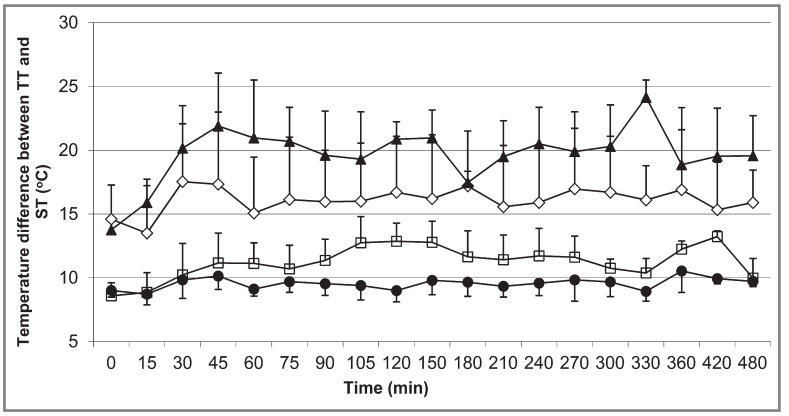

Figure 1—Mean ± SEM temperature difference between TT and ST (TT – ST) in 6 juvenile green iguanas (*Iguana iguana*) after administration of butorphanol tartrate (1 mg/kg, IM) when the observer was (n = 3 [triangles]) or was not (5 [circles]) in the iguanas' field of vision and after administration of an equal volume of saline (0.9% NaCl) solution when the observer was (5 [diamonds]) or was not (6 [squares]) in the iguanas' field of vision. A TT testing device developed for use in cats and horses was adapted for the present study; a probe containing a heating element and a temperature sensor was attached to the left or right side of the base of each iguana's tail, approximately 0.5 cm caudal to the hind limb. Rate of increase in probe temperature was 0.6°C/s; a safety cutoff was set at 55°C. Data points at 0 minutes represent baseline (pretreatment) values, after which the iguanas were treated and tested at intervals.

Statistical analysis—The factors that could affect the temperature difference between TT and ST were treatment (fixed; butorphanol or saline solution), time (fixed), observer visible to the iguanas versus observer not visible to the iguanas (fixed), and the random factor of iguana. Data were analyzed by means of a split-plot ANOVA with 2 grouping factors (treatment and observation) and 1 repeat factor (time).[g] Multiple comparisons over time were performed by means of a Bonferroni *t* test. Data are reported as mean ± SEM. Significance was set at a value of $P < 0.05$.

Results

Sham testing did not result in any response by any iguana. For either treatment (butorphanol or saline solution), there were no differences ($P = 0.67$) in the temperature difference between TT and ST recorded when the observer was visible to the iguanas or when the observer was not visible to the iguanas (**Figure 1**). There was a significant ($P = 0.002$) difference in the temperature difference between TT and ST under conditions when the observer was visible to the iguanas (17.85 ± 1.58°C) and conditions when the observer was not visible to the iguanas (10.39 ± 1.17°C). The temperature difference between TT and ST did not change over time following butorphanol treatment when the observer was not visible to the iguanas and following the saline solution treatment when the observer was or was not visible to the iguanas. There was a significant increase in the difference between TT and ST, compared with the 0-minute value, following butorphanol treatment in iguanas when the observer was visible from 30 to 75 minutes, from 120 to 150 minutes, and at 240, 300, and 330 minutes after injection.

Discussion

The results of the present study, albeit derived from a small number of animals, indicated that butorphanol tartrate (1.0 mg/kg, IM) does not produce thermal antinociception within 8 hours after administration in juvenile iguanas. It is possible butorphanol at higher doses may induce antinociception or that the results would be different for adult iguanas. Doses of 0.1 to 0.8 mg of butorphanol/kg administered IV to cats result in significant but short periods of thermal antinociception,[19] suggesting differences in opioid efficacy between mammals and reptiles.

It has been suggested that injection of drugs into the musculature of the hind limbs or tail of reptiles should be avoided because of the potential for a renal first-pass effect. However, this may be a species-specific concern. In red-eared sliders, very little blood from the hind limbs or tail passes directly through the kidneys.[20,21] In that species, the femoral veins drain directly into the liver via the abdominal vein, resulting in hepatic excretion and decreased bioavailability of buprenorphine.[22] In green iguanas, very little blood from the hind limbs passes through the kidneys, whereas most blood from the tail does[23]; therefore, injection into the hind limbs should not result in a major renal first-pass effect.

The use of thermal stimulation or thermal latency testing in reptiles has been questioned because those animals are ectothermic.[3] In addition, captive reptiles are often treated clinically for thermal injuries caused by contact with hot surfaces (hot rocks and heat lamps),[3] suggesting heat under certain circumstances is not a noxious stimulus. Among the animals within the class Reptilia, different biological adaptations allow them to thermoregulate under a wide range of environmental temperatures, which may make thermal nociception species specific.[24,25] The various lifestyles of reptiles (eg, arboreal, terrestrial, subterrestrial, and aquatic) and related thermoregulatory adaptations may result in different locations and concentrations of nociceptors.[24,25] For example, a study of the distribution of thermal and mechanoreceptors in American alligators revealed that there were few thermal receptors; however, some mechanoreceptors did respond to rapid temperature changes.[26] Sladky et al[14] suggest that on the

basis of the variable response to thermal latency testing in corn snakes and bearded dragons, multiple methods of testing may be needed to fully assess nociception and analgesia in reptiles.

To determine how each iguana would react to a thermal stimulus, 3 baseline TT tests were performed for each animal before treatment. The iguanas consistently reacted to the thermal stimulus by kicking with the hind limb that was on the same side of the body as the thermal probe. These baseline responses were clear and consistent, indicating that TT testing was suitable for the purpose of the study reported here. Sham testing did not result in any observed responses, indicating that the iguanas did not learn to respond to visual or auditory signals related to the testing procedure.

The use of thermal latency testing (involving measurement of the interval between the application of a constant heat stimulus and the response to the stimulus) has been used successfully in many reptilian species to demonstrate the analgesic properties of several opioids.[9,14,27] By use of infrared thermal latency testing in red-eared sliders, butorphanol administered IM at doses of 2.8 and 28 mg/kg did not increase latency of the response to the thermal stimulus.[9] In the same study,[9] morphine administered IM at doses of 1.5 and 6.5 mg/kg increased response latency significantly but caused long-lasting respiratory depression. By means of the same methodology, morphine administered IM at doses of 10 and 20 mg/kg increased the time to response to a thermal stimulus in bearded dragons, but butorphanol administered IM at doses of 2 and 20 mg/kg did not result in an increased response time to a thermal stimulus.[14] However, the effects of morphine were delayed until 8 hours after administration.[14] Subcutaneous administration of tramadol, a drug that induces analgesia via both opioid and nonopioid pathways,[28] resulted in an increase in thermal latency in red-eared sliders.[27] On the basis of the findings of previous investigations and the present study, it appears that butorphanol (at the doses and within the time frames evaluated) is unlikely to provide analgesia in reptiles.

Early in the present study, the influence of an observer on iguanas' responses was suspected. It became apparent that when iguanas could see the observer during testing, they became immobile and often did not respond to the thermal stimulus prior to reaching the safety cutoff temperature. Consequently, the testing technique was changed. When the study was reinitiated, the observer was hidden from view by a large visual barrier and a video camera was placed through the barrier to observe the iguanas' responses to testing.

In the present study, there was a significant difference in the temperature difference between TT and ST following treatment with saline solution or butorphanol under conditions where the observer was visible to the iguanas and conditions where the observer was not visible to the iguanas, which suggested that stress-induced analgesia may have occurred when iguanas were observed. Similar results in other prey species, such as rats, have been reported.[13,29,30] Rats tested in the presence of a predator (a cat) had significant reduction in sensitivity to a formalin irritant, compared with findings for rats tested in the absence of a predator.[29] Other stressors, including physical restraint, have induced similar changes in several rodent species.[31] In the present study, iguanas were handled for application of the probe and IM injection when the observer was or was not in visual range, but only direct observation during testing resulted in a predator response. Only when iguanas received the butorphanol treatment and the observer was visible were there any changes over time; whether this was an effect of the combination of butorphanol and stress or a consequence of the small sample size used in the present study is unknown.

In rats, the opioid receptor antagonists naloxone or naltrexone given prior to nociceptive testing in the presence of a cat resulted in a reaction similar to that of a rat that was not in the presence of a cat, suggesting that endogenous opioids are responsible for so-called stress-induced analgesia in this species.[29,30] In both domestic fowl and Japanese quail,[32,33] the influence of stressors such as handling, restraint, and isolation on nociceptive testing have been emphasized. Evrard and Bathazart[33] reported that handling of Japanese quail may artificially alter the baseline withdrawal latency to noxious thermal stimuli and that naloxone decreases withdrawal latencies, suggesting that this may be an effect of endogenous opioids. In domestic fowl, social isolation during testing increased the latency of the response to thermal testing, again suggesting stress-induced analgesia.[32] Whether the findings for the iguanas when the observer was visible in the present study were related to endogenous opioid release was not determined. However, these data[29,30,32,33] indicate that factors other than the putative analgesic drugs being tested can influence the results of nociceptive tests and that these must be considered when planning a study.

It is hoped that further research including the identification of opioid receptors, development of additional testing methods, and testing of other classes of analgesic agents may identify effective drugs for the relief of signs of pain in green iguanas. The potential influence of an observer on responses to noxious stimuli in iguanas should be considered in future studies.

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c. ReptiSun 5.0 UVB fluorescent 1.25 m, Zoo Med Laboratories Inc, San Luis Obispo, Calif.
d. Rep-Cal calcium with vitamin D_3, phosphorus free, Rep-Cal Research Labs, Los Gatos, Calif.
e. Vetrap, 3M, Saint Paul, Minn.
f. Torbugesic, Fort Dodge Pharmaceuticals, Fort Dodge, Iowa.
g. PROC MIXED, SAS, SAS Institute Inc, Cary, NC.

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