Effects of opioid receptor activation on thermal antinociception in red-eared slider turtles (Trachemys scripta)

Kurt K. Sladky, MS, DVM; Matthew E. Kinney, BS; Stephen M. Johnson, MD, PhD

Objective—To determine the effects of μ-, δ-, and κ-opioid receptor (MOR, DOR, and KOR, respectively) activation on thermal antinociception in red-eared slider turtles Trachemys scripta.

Animals—51 adult turtles.

Procedures—Infrared heat stimuli were applied to the plantar surface of turtle hind limbs. Thermal hind limb withdrawal latencies (HLWLs) were measured before (baseline) and at intervals after SC administration of various doses of saline (0.9% NaCl) solution (SS), MOR, DOR, or KOR agonists (3 to 13 turtles/treatment). Treatment with a DOR antagonist SC prior to DOR agonist administration was also evaluated.

Results—Treatment with an MOR agonist (ID-Ala², N-Me-Phe⁴, Gly⁵-ol)-enkephalin acetate salt (DAMGO; 1.3 or 6.6 mg/kg) increased HLWLs (from baseline) at 2 to 8 hours after injection; at the higher dose, the maximum mean increase was 5.6 seconds at 4 hours. Treatment with a DOR agonist (ID-Ala², D-Leu⁴)-enkephalin acetate salt (DADLE; 25 mg/kg) increased mean HLWL by 11.3 seconds at 4 hours; however, treatment with DADLE (5.8 mg/kg) or with another DOR agonist (ID-Pen⁴⁵)-enkephalin hydrate (DPDPE; 1.2 or 6.3 mg/kg) did not alter HLWL, compared with SS effects. Administration of a DOR antagonist (naltrindole hydrochloride; 10 mg/kg) prior to DADLE administration (25 mg/kg) increased mean HLWL by 2.7 seconds at 4 hours. One KOR agonist, U50488 (−)-trans-[1S,2S]-U50488 hydrochloride hydrate; 6.7 mg/kg) decreased HLWL steadily from 2 to 24 hours (less than baseline value); another KOR agonist, U69593 ([−]-[5x, 7x, 8β]-N-Methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl]-benzene-acetamide; 6.7 or 26 mg/kg) did not alter HLWLs, compared with SS effects.

Conclusions and Clinical Relevance—Opioid-dependent thermal antinociception in turtles appeared to be attributable mainly to MOR activation with a relatively minor contribution of DOR activation. (Am J Vet Res 2009;70:1072–1078)

Analgesic drugs have become a mainstay of clinical veterinary medicine, and pain relief in vertebrates should be considered clinically imperative and ethically obligatory.1 Following surgery in mammals, alleviation of signs of pain facilitates recovery and healing, reduces morbidity, and contributes to a more rapid return to normal behavior.2,3 In reptiles, however, little is known about the mechanisms underlying nociception, analgesic drug efficacy and adverse effects, drug pharmacodynamics, and opioid receptor–binding characteristics. Nevertheless, under circumstances that would be considered painful in mammals, the lack of understanding of pain relief in reptiles should not preclude the use of analgesic drugs, nor should the potential for pain be disregarded at any time.

Morphine, which is primarily an MOR agonist (with DOR and KOR activities), induces antinociception in red-eared slider turtles during exposure to a noxious thermal stimulus.4 In contrast, butorphanol, which is primarily a KOR agonist and MOR antagonist, provides no thermal antinociception under the same experimental conditions.4 Additionally, both drugs cause profound respiratory depression in red-eared slider turtles.3 Because morphine also activates DORs and KORs, it is important to determine which opioid receptor subtypes are involved in morphine-dependent thermal antinociception and respiratory depression in turtles. With this information, it may be possible to identify a drug that induces antinociception with minimal or no respiratory depression.
The purpose of the study reported here was to determine the effects of MOR, DOR, and KOR activation on thermal antinociception in red-eared slider turtles. A thermal HLWL test that allows rapid application and decay of stimuli (without lasting inflammation), enables instant latency quantification, and results in unambiguous behavior after stimulus exposure was used to investigate the effects of specific opioid receptor agonists (DAMGO [MOR agonist], DADLE and DPDPE [DOR agonists], and U50488 and U69593 [KOR agonists]) on thermal antinociception in this species.

Materials and Methods

Turtles—Fifty-one adult red-eared slider turtles (Trachemys scripta) were obtained from a commercial supplier for use in the study. The group included 28 males and 23 females. The mean ± SEM weight of the turtles was 767 ± 14 g (mean weight of males, 756 ± 34 g; mean weight of females, 778 ± 31 g). The 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°C to 28°C (approximating their optimal body temperature), and light was provided for 14 h/d. Turtles were fed floating food sticks 3 to 4 times/wk. All procedures were approved by the Animal Care and Use Committee at the School of Veterinary Medicine, University of Wisconsin.

Study design—A crossover experimental design was used to evaluate opioid-dependent changes in thermal antinociception in the turtles. Experiments were first performed following administration of physiologic SS in all turtles; subsequently, experiments were performed following administration of the other drugs. Of the 51 turtles, 11 (6 females and 5 males) were treated with 2 drugs, and only 1 (female) was treated with 3 drugs; the remainder were treated with 1 drug. The observer (MEK) in the antinociceptive experiments was unaware of treatments administered to the turtles.

Thermal antinociception experiments—Analgesimetry experiments consisted of measuring the 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. Turtles were placed in plastic boxes (17 × 13 × 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 hind limb. The increasing temperature caused the turtle to withdraw the limb, and the time to withdrawal was measured automatically. Stimulation strength was adjusted to attain baseline latencies of 16 to 18 seconds (corresponds to 45° to 47°C); a maximum duration of 32 seconds was used to prevent prolonged heat exposure. A mean baseline HLWL was established for each turtle by application of 1 stimulus on 3 occasions at 5-minute intervals.

Following baseline assessments, turtles received treatments involving DAMGO (MOR agonist), DADLE (DOR agonist), DPDPE (DOR agonist), NH (DOR antagonist), U50488 (KOR agonist; [−]-trans-[1S,2S]-U50488 hydrochloride hydrate), and U69593 (KOR agonist; [3α,7α,8β]-N-methyl-N-[7-(1-pyrollidinyl)-1-oxaspiro(4.5)dec-8-yl]-benzene-acetamide).

The injections were administered SC in the pectoral girdle; the time of treatment administration was designated as 0 hours. Injection treatments were as follows: SS (volumes equivalent to those of the opioid receptor agonist treatments [n = 18]), DAMGO (1.3 mg/kg [12] or 6.6 mg/kg [13]), DADLE (5.8 mg/kg [5] or 25 mg/kg [6]), DPDPE (1.2 mg/kg [8] or 6.5 mg/kg [6]), NH (10 mg/kg given prior to injections of SS [11] or DADLE [8]), U50488 (6.7 mg/kg [3]), and U69593 (6.7 mg/kg [13] or 26 mg/kg [10]). At 1, 4, 8, and 24 hours after injection, HLWLs were calculated from data obtained following application of 1 stimulus on 3 occasions at 5-minute intervals. Because of the difference in results obtained after treatment with the 2 DOR agonists (DADLE and DPDPE), experiments were performed to test whether DADLE effects were attributable to DOR activation or mixed DOR-MOR activation. To address this question, 2-injection treatments were administered; the 2-injection treatments involved administrations of SS and SS (n = 12), NH (10 mg/kg) and SS (11), or NH (10 mg/kg) and DADLE (25 mg/kg [8]). The first injection was administered immediately after obtaining baseline data, and the second injection was administered 30 minutes later. Thermal HLWLs were then measured at 2, 4, and 6 hours after obtaining the baseline data. For each turtle that underwent any drug treatment, an interval of at least 2 weeks was allowed to elapse between experiments as a washout period.

Statistical analysis—For each turtle, the HLWLs measured at a given time point were averaged. These mean HLWLs were then averaged for all turtles given the same treatment. 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 a 2-way ANOVA was performed on the ranked data. Post hoc comparisons were made by use of a Student-Newman-Keuls test. All data are reported as mean ± SEM. Values of P < 0.05 were considered significant.

Results

Following SS administration (n = 18), mean ± SEM HLWL in the treated turtles decreased from 15.0 ± 0.3 seconds (baseline) to 13.2 ± 0.4 seconds at 8 hours; at 24 hours, the value was 14.4 ± 0.4 seconds (Figure 1). Following administration of DAMGO at a dose of 1.3 mg/kg (n = 13), HLWL increased from 15.0 ± 0.6 seconds (baseline) to a maximum value of 18.1 ± 2.2 seconds at 8 hours; at 24 hours, the value had decreased to 15.5 ± 1.5 seconds (P = 0.006 for drug effect). Likewise, following administration of DAMGO at a dose of 6.6 mg/kg (n = 12), HLWL increased from 14.8 ± 0.6 seconds (baseline) to a maximum value of 20.4 ± 2.0 seconds at 4 hours; at 24 hours, the value was 16.1 ± 2.1 seconds (P < 0.001 for drug effect). When the changes in HLWL from baseline were calculated, values following DAMGO injections were significantly increased, compared with values following SS injections, at 4 to
8 hours (treatment with 1.3 mg of DAMGO/kg) and at 2 to 8 hours (treatment with 6.6 mg of DAMGO/kg). Thus, administration of DAMGO induced dose- and time-dependent increases in thermal HLWL, and the duration of those increases was < 24 hours.

Following administration of DPDPE at a dose of 1.2 mg/kg (n = 8) or 6.3 mg/kg (6), HLWL did not differ from the value determined after treatment with SS (n = 13; P = 0.051 for drug effect; Figure 2). Similarly, assessment of the change in HLWL revealed no differences (P = 0.086 for drug effect). Administration of DADLE at a dose of 5.8 mg/kg (n = 5) resulted in no difference in HLWL, compared with the SS treatment value (n = 11; P = 0.147; Figure 3). Changes in HLWL from baseline for the low-dose DADLE and SS treatments also did not differ (P = 0.98). Following administration of DADLE at a higher dose of 25 mg/kg (n = 6), HLWL increased from 9.6 ± 1.0 seconds (baseline) to a maximum value of 20.9 ± 4.4 seconds at 4 hours; at 24 hours, the value decreased to 12.3 ± 2.6 seconds (P < 0.001 for drug effect). For this high-dose DADLE treatment, HLWLs at 2 to 8 hours were significantly different from SS treatment values, and there were significant differences in the change in HLWL from baseline (P < 0.001 for drug effect).

Because DADLE is only 10 times as selective for DORs as it is for MORs,7,8 DADLE may also activate MORs at relatively high doses. Given that DAMGO in-

---

**Figure 1**—Mean ± SEM thermal HLWLs in conscious red-eared slider turtles before (baseline; 0 hours) and at intervals after SC injection of DAMGO (MOR agonist) at doses of 1.3 (n = 13) or 6.6 (12) mg/kg or equivalent volumes of SS (18 [A]) and changes in HLWL from baseline value (horizontal dashed line) over time in those treatment groups (B). *Significant (P < 0.05) drug effect for 0- to 24-hour data, compared with corresponding SS treatment values. †At this time point, drug treatment value is significantly (P < 0.05) different from the SS treatment value.

**Figure 2**—Mean ± SEM thermal HLWLs in conscious red-eared slider turtles before (baseline; 0 hours) and at intervals after SC injection of DPDPE (DOR agonist) at doses of 1.2 (n = 8) or 6.3 (6) mg/kg or equivalent volumes of SS (13 [A]) and changes in HLWL from baseline value (horizontal dashed line) over time in those treatment groups (B). Administration of 1.2 or 6.3 mg of DPDPE/kg did not alter HLWLs or change in HLWL from baseline, compared with effect of SS treatment.
increased HLWL values, it is possible that DADLE cross-reacts with turtle MORs. Thus, to test whether the thermal antinociceptive effects of DADLE were attributable to DOR activation, the DOR antagonist NH (10 mg/kg) was administered immediately after obtaining baseline values in a separate set of experiments; DADLE (25 mg/kg) was administered 30 minutes later, and thermal HLWL was assessed at 2, 4, and 6 hours after obtaining the baseline measurements. Appropriate control experiments were performed either by administering an SS injection at each of the 2 time points or by administering an NH injection (10 mg/kg) followed by an SS injection. In turtles that received 2 injections of SS (n = 12), baseline HLWL was 11.0 ± 1.1 seconds; the value remained relatively constant, and at 6 hours, HLWL was 9.2 ± 0.8 seconds (Figure 3). Following administration of an NH injection and an SS injection (n = 11), HLWL increased (albeit not significantly [P = 0.504]) from 9.9 ± 0.8 seconds (baseline) to 12.4 and 12.1 seconds at 2 to 4 hours; this change was primarily a result of 1 of the 11 turtles having maximum HLWL values (ie, 32 seconds) at 2 to 6 hours. No other turtles that received this 2-injection treatment had similar HLWL values; the next highest HLWL in the other 10 turtles was only 14.3 seconds. Following administration of an NH injection and a DADLE injection (n = 8), HLWL increased from 11.1 ± 1.2 seconds (baseline) by only 2.7 seconds at the 2- through 6-hour time points (P = 0.01 for drug effect). Although there was a significant drug effect, the large HLWL increase (11.3 ± 4.4 seconds) detected following administration of 25 mg of DADLE/kg was attenuated. Assessments of the change in HLWLs revealed that both the NH-SS and NH-DADLE 2-injection treatments had small but significant drug effects.
The same results were obtained when the aforementioned potential outlier turtle that received the injections of NH and SS and had posttreatment HLWLs of 32 seconds was removed from the analysis.

Following administration of U50488 at a dose of 6.7 mg/kg (n = 3), HLWL in the treated turtles decreased from 18.2 ± 1.5 seconds (baseline) to 14.2 ± 0.1 seconds at 24 hours (P = 0.001 for drug effect; Figure 4). Assessments of the change in HLWLs revealed that U50488 significantly reduced HLWL from baseline at 4 to 24 hours, similar to the effects of SS treatment (13); comparison of HLWL values revealed significant drug effects (P = 0.028 and P = 0.002 for the low and high doses, respectively), but the change in HLWL following either treatment did not differ from the change in HLWL following SS treatment (P = 0.764 for drug effect).

**Discussion**

The main finding of the present study was that thermal antinociception in red-eared slider turtles was primarily attributable to MOR activation as evidenced by increases in HLWL (compared with baseline values and SS control data) after DAMGO administration. The DOR agonists (i.e., DPDPE and DADLE) were mostly
In mammals, antinociception can be elicited via administration of MOR and KOR agonists such as morphine (primarily an MOR agonist), hydromorphone (primarily an MOR agonist), buprenorphine (a partial MOR and KOR agonist), and butorphanol (a KOR agonist and MOR antagonist). To our knowledge, there are no clinically approved drugs that are DOR-specific agonists for mammals. Although MOR agonists are commonly used as analgesic drugs in mammals, other species are less dependent on MOR activation for analgesia. For example, psittacine birds display antinociceptive behavioral responses to thermal and electrical noxious stimuli only after administration of butorphanol (KOR agonist), but not after administration of MOR agonists. In support of these data, KORs are abundant in the brains of several avian species. Although snakes also display antinociceptive behavior in response to butorphanol, lizards and turtles display antinociceptive behavior after morphine, but not butorphanol, administration. Similarly, supraspinal administration of receptor-specific opioids in amphibians contributed to antinociception in the following decreasing rank order: morphine, DADLE, DPDPE, fentanyl, and U50488; this suggests that amphibian antinociception is associated with MOR activation.

Results of a previous study by our group indicated that morphine has antinociceptive properties in red-eared slider turtles and bearded dragons that are exposed to a noxious thermal stimulus. Consistent with those findings, morphine treatment increases tail flick latencies in anole lizards and hot-plate withdrawal latencies in crocodiles. Given that morphine is primarily an MOR agonist, antinociception in reptiles appears to be attributable to MOR activation in a dose-dependent manner. Even though there is an abundance of DORs in red-eared slider turtles, it appears that there is, at best, only a minor dose-dependent role for DOR-dependent antinociception in turtles. In the present study, DPDPE (a highly selective DOR agonist) and a low dose of DADLE did not alter HLWL values, whereas a high dose of DADLE induced thermal antinociception. Because DADLE is only 10 times as selective for DORs as it is for MORs, it is possible that DADLE also activated MORs. However, the DADLE-associated increase in HLWL was abolished by pretreatment with NH (a DOR antagonist), which suggests that DOR activation was involved. Alternatively, DOR-dependent thermal antinociception may be drug dependent, such that administration of some, but not all, DOR agonists may have a biological effect. In experiments performed in our laboratory (unpublished data), we have determined that several well-established 5-hydroxytryptamine-3 receptor agonists have different biological effects. Thus, subtle differences in turtle opioid receptors may cause drugs that are consistently effective in mammals to be variably effective in turtles.

With respect to the effect of KOR agonists on thermal antinociception in turtles, administration of U50488 or U69593 (at relatively high doses) did not affect HLWL; however, the U50488 data must be interpreted with caution because of the low number of turtles treated with this agonist. Nevertheless, this lack of effect is consistent with data from another study in which administration of butorphanol (a KOR agonist and MOR antagonist) had no thermal antinociceptive efficacy in red-eared slider turtles. Similarly, in green iguanas, administration of butorphanol has no antinociceptive effects and highly variable, but nonsignificant, isoflurane-sparing effects. A caveat to consider is whether DPDPE, U50488, and U69593 failed to cross the blood-brain barrier. In mammals in vivo or mammalian brain perfusion experiments, DPDPE weakly crosses the blood-brain barrier and has physiologic effects, whereas U50488 and U69593 more readily cross the blood-brain barrier and alter neuronal activity. Although little is known about the blood-brain barrier in turtles or other reptiles, it is unlikely that the negative results for DPDPE, U50488, and U69593 in the present study were attributable to lack of drug access to the CNS. In separate experiments involving red-eared slider turtles performed by one of the authors (SMJ), SC administration of DPDPE (3.0 mg/kg) rapidly decreased ventilation in awake turtles and decreased respiratory motor output in isolated turtle brainstems, which suggest that DPDPE crosses the blood-brain barrier to activate central DORs that regulate breath frequency.

Measurement of pain in reptiles is complex, and expansion of the methods used in our study is critical for analyzing nociception and antinociception among reptile species and within different contexts (eg, hospital cage vs home environment). Although the thermal HLWL test apparatus provides unambiguous results under controlled laboratory conditions, extrapolation of the test results to other painful stimuli must be made with some caution. Thus, another caveat is that application of noxious thermal stimuli to turtle hind limbs may not be ecologically or physiologically relevant. Also, noxious thermal stimuli may not be qualitatively equivalent to other noxious stimuli (eg, hypodermic needle insertion) or postsurgical pain. However, aquatic red-eared slider turtles behave similarly to rodents in response to noxious thermal stimulation, and DAMGO administration reliably and consistently increases HL威尔s. Because water temperature in a typical aquatic ecosystem suitable for red-eared sliders may be as high as 30°C (86°F), it is reasonable to assume that a hot stimulus would elicit avoidance. However, corn snakes are much more variable in their response to a thermal noxious stimulus. These findings raise important questions regarding how reptiles, particularly snakes, process different nociceptive sensory afferent inputs. Some captive snake and lizard species 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 some reptile species, especially terrestrial species, 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. Also, it seems probable the efficacy of analgesic drugs in reptiles may be highly variable, as evidenced by results of the present study (in which 1 turtle that received 2-injection treatment with NH and SS was insensitive to noxious thermal stimuli) and results of experiments in another laboratory.20

The results of the present study and previous investigations by our group confirm that MOR activation is critical for antinociception in turtles4 as well as in bearded dragons.14 A single dose of an MOR agonist (eg, DAMGO or morphine) may provide antinociception for a period greater than 24 hours in some reptile species, which has the advantage of decreasing the stress of handling and administering multiple injections for pain management. Although we determined a time course for the antinociceptive effect associated with MOR agonist administration, it will be necessary to characterize the pharmacokinetics and pharmacodynamics of opioid agonists in reptiles. Additionally, because deleterious adverse effects (eg, profound respiratory depression) are associated with MOR agonist administration,24,25 it will be important to identify opioid agonists that will provide antinociception but have fewer respiratory depressant effects in reptiles.

References