Antinociceptive effects after oral administration of tramadol hydrochloride in Hispaniolan Amazon parrots (*Amazona ventralis*)

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Objective—To evaluate antinociceptive effects on thermal thresholds after oral administration of tramadol hydrochloride to Hispaniolan Amazon parrots (*Amazona ventralis*).

Animals—15 healthy adult Hispaniolan Amazon parrots.

Procedures—2 crossover experiments were conducted. In the first experiment, 15 parrots received 3 treatments (tramadol at 2 doses [10 and 20 mg/kg] and a control suspension) administered orally. In the second experiment, 11 parrots received 2 treatments (tramadol hydrochloride [30 mg/kg] and a control suspension) administered orally. Baseline thermal foot withdrawal threshold was measured 1 hour before drug or control suspension administration; thermal foot withdrawal threshold was measured after administration at 0.5, 1.5, 3, and 6 hours (both experiments) and also at 9 hours (second experiment only).

Results—For the first experiment, there were no overall effects of treatment, hour, period, or any interactions. For the second experiment, there was an overall effect of treatment, with a significant difference between tramadol hydrochloride and control suspension (mean change from baseline, 2.00° and –0.09°C, respectively). There also was a significant change from baseline for tramadol hydrochloride at 0.5, 1.5, and 6 hours after administration but not at 3 or 9 hours after administration.

Conclusions and Clinical Relevance—Tramadol at a dose of 30 mg/kg, PO, induced thermal antinociception in Hispaniolan Amazon parrots. This dose was necessary for induction of significant and sustained analgesic effects, with duration of action up to 6 hours. Further studies with other types of noxious stimulation, dosages, and intervals are needed to fully evaluate the analgesic effects of tramadol hydrochloride in psittacines. (*Am J Vet Res* 2012;73:1148–1152)

Opioids are frequently used in veterinary medicine and are considered the most effective class of analgesic drugs for perioperative pain management. Previous studies[1–7] have validated the clinical use of opioids, particularly those with κ-opioid receptor affinities, for birds. However, studies[8] have also revealed that μ-opioid receptor agonists can provide analgesia to birds. Fentanyl, a μ-opioid receptor agonist, was analgesic when administered in high doses to white cockatoos, but some birds became hyperactive after administration.[8] Butorphanol tartrate and nalbuphine hydrochloride, a κ-opioid receptor agonist and a μ-opioid antagonist, respectively, are currently considered the opioid drugs of choice for pain management in birds.[1–7] The accepted dosages for butorphanol tartrate and nalbuphine hydrochloride in psittacines require frequent, parenteral administration.[7,9] The oral bioavailability of butorphanol tartrate is < 10% in Hispaniolan Amazon parrots (*Amazona ventralis*), which thus limits use of this drug.[10]

Abbreviation

NMDA *N*-methyl-D-aspartate

Tramadol is a centrally acting μ-opioid receptor agonist, and it also binds weakly to κ- and δ-opioid receptors.[11] The main metabolite, O-desmethyltramadol (ie, M1), has a higher affinity, approximately 200-fold as high, for μ-opioid receptors than does the parent compound. Tramadol and M1 also inhibit the reuptake of norepinephrine (noradrenaline) and serotonin, which are important activating components of the descending pain inhibitory system.[12–14] By inhibiting the reuptake of both norepinephrine and serotonin, tramadol activates the descending pain inhibitory system, thereby decreasing the sensation of pain. When the descending pain inhibitory system is activated, the transmission of

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painful stimuli through the dorsal horn of the spinal cord is inhibited by the action of endogenous opioids.[13] Other metabolites (N-desmethyltramadol [M2], N,N-didesmethyltramadol [M3], N,N,O-tridesmethyltramadol [M4], and N,O-didesmethyltramadol [M5]) have been described, but it is unknown whether they have analgesic properties.[15]

Tramadol has been used routinely for the relief of moderate to severe pain in humans for the past 2 decades.[16,17] In veterinary medicine, there is increasing information regarding the analgesic effects of this drug in rats,[18,19] dogs,[20] cats,[21,22] horses,[23] and red-eared sliders.[24] Studies have been conducted to evaluate the pharmacokinetic properties of tramadol in bald eagles[25] and peafowl,[26] but to the authors' knowledge, no studies have been conducted to evaluate the analgesic properties of tramadol in any avian species. In contrast to most other opioids, tramadol is not considered a controlled substance in many countries (including the United States and Australia) and is dispensed to companion animals via a veterinary prescription. The objective of the study reported here was to evaluate the antinociceptive effects and duration of action on thermal thresholds after oral administration of tramadol hydrochloride to Hispaniolan Amazon parrots.

Materials and Methods

Animals—Fifteen healthy adult (age range, 6 to 22 years; mean ± SD, 8.33 ± 4.76 years) Hispaniolan Amazon parrots of unknown sex with a mean weight of 287.7g ± 20.2 g were used in the study. All parrots were part of a research colony and were considered healthy on the basis of results of physical examinations performed before and during the study. Parrots were maintained in flocks of 4 to 6 parrots in large rooms (11.2 m^2) and were housed during the study in standard, stainless steel laboratory cages (0.6 × 0.6 × 0.6 m) with a perch and hanging toy. They were maintained on a cycle of 12 hours of light to 12 hours of darkness, fed a commercial pelleted diet[a] formulated for psittacine birds, and provided water ad libitum. The Institutional Animal Care and Use Committee at the University of Wisconsin School of Veterinary Medicine approved the experimental protocol. Because this study involved a species for which other common analgesics have not been adequately evaluated (eg, antinociceptive effect, duration of action, and interindividual variability), the use of a positive control group in place of a negative control group[27] was not deemed feasible for the evaluation of antinociceptive effects and duration of action of tramadol.

Experimental design—Two experiments were each conducted in accordance with a within-subjects, complete crossover design. For the first experiment, 15 parrots orally received 3 treatments: 10 mg of tramadol hydrochloride[b]/kg, 20 mg of tramadol hydrochloride/kg, and an equivalent volume of a control suspension.[c] Each bird received each of the 3 treatments in a random order with a washout period of 21 days between successive treatments. For the second experiment, 11 parrots were used, and there were 2 treatments administered orally: tramadol hydrochloride (30 mg/kg) and an equivalent volume of a control suspension.[c] Each bird received one of the treatments initially. After a 21-day washout period, each bird then received the other treatment. Tramadol hydrochloride was prepared in a 10-mg/mL solution as described elsewhere.[28] Treatments were administered into the crop with a metallic feeding tube in both experiments. There was a 1-year period between the 2 experiments.

Testing procedure—Thermal foot withdrawal threshold measurements were collected on all parrots by use of a test box equipped with a test perch. The test perch was designed to deliver a thermal stimulus to the left plantar surface of a parrot's foot[29] by use of thermal microchips to rapidly change the temperature of the perch. The birds could escape from the brief noxious thermal stimuli by lifting the foot, and the foot could then be placed back on the perch within 2 to 3 seconds after the withdrawal response because the temperature decreased rapidly. The test box had dark sides to inhibit the parrot from viewing its surroundings, including the observer, and a clear front that allowed the observer to monitor behavioral responses in real time with a remote video camera. Prior to the experiment, each parrot was acclimated to the test chamber, mimicking a full test day observation. The thermal stimulus, generated by thermoelectric modules, ranged from 29° to 70°C and caused a rapid increase and subsequent decrease in perch temperature (rate of temperature increase and decrease, 0.3°C/s). The cutoff temperature was 70°C to avoid tissue damage. A thermal threshold withdrawal response was defined as the perch temperature that was concurrent with a foot withdrawal response. A separate baseline thermal withdrawal threshold was recorded within each period by a single measurement obtained 1 hour prior to administration of the analgesic drug or control solution. Thermal foot withdrawal threshold measurements were performed as a single measurement at 0.5, 1.5, 3, and 6 hours after oral administration of the treatment for both experiments and also at 9 hours after oral administration for the second experiment. All thermal thresholds were determined by one observer in the first study and by another observer in the second study; observers were not aware of the treatment administered to each bird. All birds were monitored during the study for signs of adverse effects, including sedation, excitation, vomiting and diarrhea.

Statistical analysis—All data were analyzed with commercially available software.[d] The endpoint of interest was the difference between the thermal threshold at any given time point after drug administration and the baseline thermal threshold for that bird in the same period. A repeated-measures ANOVA was used, with fixed effects of dose, time, period, and all associated interactions. Correlation within birds over time within a period was modeled with a spatial power structure. Residuals resulting from the fitted model were verified to be normally distributed and had no evidence of heteroscedasticity. Least squares means of changes in thermal threshold were obtained from the fitted model. Pairwise comparisons of the least squares means for the various groups, both within each time point and over all times, were performed with the Tukey *P* value cor-

rection to account for multiple comparisons. Values of $P < 0.05$ were considered significant.

Results

All parrots behaved normally throughout the testing procedures. There were no adverse effects, including sedation, evident during the study.

Experiment 1 (10 and 20 mg/kg, PO)—Baseline thermal withdrawal thresholds (n = 30) ranged from 37.9° to 49.1°C. There were no significant overall effects of treatment ($P = 0.967$), hour ($P = 0.625$), period ($P = 0.169$), or any interactions (P value range, 0.056 to 0.473). There was a significant mean change from the baseline value for the control treatment, tramadol at 10 mg/kg, and tramadol at 20 mg/kg (1.75°C [$P = 0.002$], 1.56°C [$P = 0.005$], and 1.63°C [$P = 0.003$], respectively; **Table 1**). There was a significant difference from the baseline value for the control treatment at 0.5 (mean, 1.65°C; $P = 0.031$), 1.5 (mean, 1.77°C; $P = 0.020$), and 3 (mean, 2.13°C; $P = 0.006$) hours after administration. There was a significant difference from the baseline value for the 10 mg/kg treatment at 1.5 (mean, 1.52°C; $P = 0.046$), 3 (mean, 1.87°C; $P = 0.014$), and 6 (mean, 2.04°C; $P = 0.008$) hours after administration. There was a significant difference from the baseline value for the 20 mg/kg treatment at 0.5 (mean, 2.51°C; $P = 0.001$) and 3 (mean, 1.79°C; $P = 0.019$) hours after administration.

Table 1—Estimated mean change in thermal threshold from the baseline measurement (°C) in Hispaniolan Amazon parrots (*Amazona ventralis*; n =15) after administration of a control treatment and tramadol hydrochloride (10 and 20 mg/kg).

Time (h)	Control	Tramadol (10 mg/kg)	Tramadol (20 mg/kg)
0.5	1.65*	1.08	2.51*
1.5	1.77*	1.52*	1.42
3	2.13*	1.87*	1.79*
6	1.43	2.04*	0.49

Estimated SEM is 0.75 for all means.
Baseline values were obtained 1 hour before oral administration of the treatments; time of oral administration was designated as time 0.
*Value differs significantly ($P < 0.05$) from the baseline value.

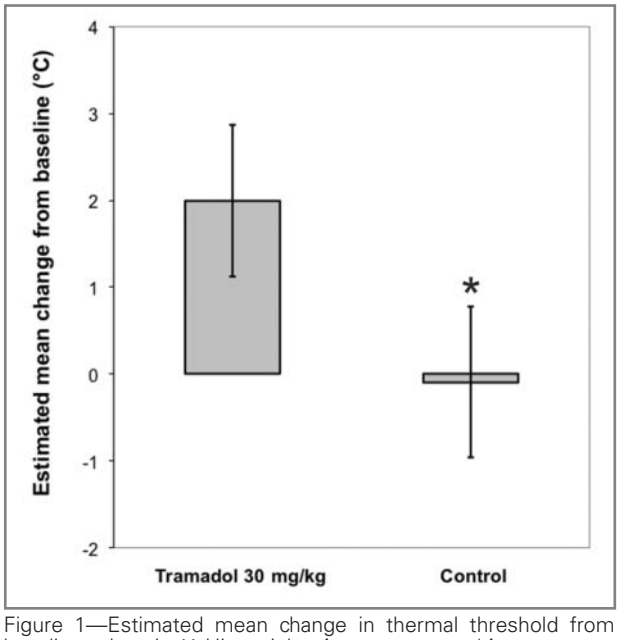

Figure 1—Estimated mean change in thermal threshold from baseline values in 11 Hispaniolan Amazon parrots (*Amazona ventralis*) after oral administration of a control suspension and tramadol hydrochloride at a dose of 30 mg/kg. Baseline values were obtained 1 hour before oral administration of the treatments. Error bars represent half of the Tukey honestly significant difference and are the same for both means. *Value differs significantly ($P < 0.05$) from the value for the tramadol treatment.

Experiment 2 (30 mg/kg, PO)—Baseline thermal withdrawal thresholds ranged from 39.1° to 53.0°C. Six birds initially received tramadol followed 21 days later by the control treatment, and 5 birds initially received the control treatment followed 21 days later by the tramadol treatment. There was an overall significant ($P = 0.031$) effect of treatment. There was a significant mean change from the baseline value for the tramadol treatment (2.00°C; $P = 0.005$) but not for the control treatment (–0.09°C; $P = 0.88$; **Figure 1**). There were no other significant effects for time, period, or any interactions. There was a significant change from the baseline value for the tramadol treatment at 0.5 (mean, 2.21°C; $P = 0.019$), 1.5 (mean, 2.23°C; $P = 0.018$), and 6 (mean, 2.61°C; $P = 0.006$) hours after administration (**Table 2**). However, there was no significant change from the baseline value for the tramadol treatment at 3 (mean, 1.64°C; $P = 0.078$) or 9 (mean, 1.30°C; $P = 0.162$) hours after administration. For the control treatment, there were no significant differences from baseline values at any time point.

Discussion

Tramadol hydrochloride administered orally at 30 mg/kg significantly increased thermal foot withdrawal threshold values (mean change from the baseline value, 2.00°C), compared with values for the control treatment (mean change from the baseline value, –0.09°C). Tramadol administered at lower doses (10 and 20 mg/kg) did not have a significant effect on thermal foot withdrawal threshold values, compared with results for the control treatment. The antinociceptive effect observed in the parrots of the present study is similar to that reported for the use of tramadol in other animals, including rats,[30] cats,[22] and red-eared sliders[24]; however, the effect differs from that reported in horses[23] and cats,[21] which did not induce antinociception. Only the highest tramadol dose evaluated in the present study had a significant effect on antinociception.

Table 2—Estimated mean change in thermal threshold from the baseline measurement (°C) in Hispaniolan Amazon parrots (n =11) after administration of a control treatment and tramadol hydrochloride (30 mg/kg).

Time (h)	Control	Tramadol (30 mg/kg)
0.5	0.82	2.21*
1.5	–0.31	2.23*
3	0.49	1.64
6	0.06	2.61*
9	–1.52	1.30

Estimated SEM is 0.92 for all means.
See Table 1 for remainder of key.

In the study reported here, 30 mg of tramadol/kg administered orally to Hispaniolan Amazon parrots had a duration of action of up to 6 hours, whereas previous research in the same species has revealed antinociception with butorphanol tartrate up to 90 minutes (unpublished data) and nalbuphine hydrochloride up to 3 hours.[7] In mammals, tramadol has a longer duration of action than does butorphanol.[31,32] Based on the results of the present study, tramadol hydrochloride could provide a longer period of analgesia than currently available commercial formulations of butorphanol and nalbuphine.

The 2 mechanisms of action of tramadol, binding to opioid receptors and activation of the descending pain inhibitory system, work synergistically to provide analgesia in vertebrate species. Authors of 1 study[33] also suggested that tramadol may provide analgesia by antagonizing NMDA receptors located throughout the nervous system as well as in the viscera. Antagonism of NMDA receptors reduces the hyperexcitability of nociceptive neurons in the dorsal horn of the spinal cord, which thereby decreases pain.[34,35] The NMDA receptors have been detected in birds,[36,37] and the use of ketamine for anesthesia in birds is well established,[9,38] although there are no controlled studies on the analgesic effects of NMDA-receptor antagonists in avian species. Serotonin and norepinephrine reuptake inhibitors have been studied in birds but not for their analgesic effects.[39–41] Selective serotonin reuptake inhibitors have been used clinically in psittacines to treat behavioral disorders but have yielded inconsistent results.[42] Whether the mechanism of action whereby tramadol provides analgesia in psittacine birds is via binding to different opioid receptors, antagonizing NMDA receptors, inhibiting reuptake of serotonin and norepinephrine, or a combination of these has not been determined.

The thermal nociception response has been used to evaluate several opioids and various doses in different psittacine species with varying results. In both cockatoos and Hispaniolan Amazon parrots, the response to the thermal stimulus was found to be a reliable measurement of antinociception.[3,8] However, the response to the thermal stimulus in a study[29] conducted to evaluate the antinociceptive effects of 1 mg of butorphanol/kg in African grey parrots was considered inconsistent. In previous studies, thermal and electrical stimuli were combined, but during a preliminary study conducted using nalbuphine, we found that electrical stimuli were a perturbing factor creating anxiety in the birds when applied repeatedly at various time points over an extended 6-hour period. The noxious thermal stimulation model has a short period of stimulation and uses skin rather than visceral or muscular sites of stimulation.[43] Nociception is induced by thermal stimuli activating thermal receptors. Thermal receptors are afferent Aδ or C fibers, and they transmit the nociceptive information to different areas of the midbrain and forebrain via ascending spinal pathways.[44] The use of thermal stimulation to affect the natural perching behavior of parrots is a noninvasive method for evaluation and modulation of nociceptive thresholds, but further studies with different types of stimuli are necessary.

Individual variability in the antinociceptive effects of opioids has been detected in many species, and the variability appears to be multifactorial, with sex, genotype, type of noxious stimulus, receptor, and relative efficacy of the agent all affecting the individual response.[45–49] The variation in individual response to the treatments in the present study resulted in a large SD when individual results were grouped by treatment. Although all tramadol treatments caused an increase in thermal threshold, compared with baseline results, the variance in individual responses precluded the ability to detect significant differences between the control treatment and the 10 and 20 mg/kg treatments. The sample size (n = 15 and 11) for the 2 experiments of this study were based on previous experiments that were conducted in accordance with a similar design and that yielded significant results.

Adverse effects, including sedation, were not observed in the parrots. Tramadol has a wide margin of safety in humans, with minimal respiratory, cardiovascular, or gastrointestinal adverse effects.[11] The administration of tramadol to dogs[28] or cats[50] does not induce adverse effects. Rapid IV administration of tramadol induced muscle twitching in 2 of 5 horses, but extending the IV administration time avoided this effect in other horses.[51] Opioid analgesics are generally associated with respiratory depression, but tramadol, in contrast to other opioids, does not cause clinically relevant respiratory depression or effects on heart rate in humans.[11] The abuse potential for tramadol is low in comparison to the risk for typical opioids such as morphine and not substantially greater than that for NSAIDs.[14,52] The minimal number of adverse effects observed in other vertebrate species make it a potentially useful analgesic in parrots. Results of the study reported here and previous studies support that both drugs are safe and effective agonist-antagonist analgesics, but further studies are needed to evaluate cardiovascular effects and analgesic effects of tramadol in parrots.

Tramadol hydrochloride administered orally at 30 mg/kg significantly increased the foot withdrawal threshold to a noxious thermal stimulus in Hispaniolan Amazon parrots and may provide clinical analgesia for up to 6 hours. Lower doses of tramadol hydrochloride did not result in an increased thermal threshold or longer duration of action and may not have the analgesic benefit seen with higher doses. Further studies to evaluate different types of noxious stimuli are needed to fully evaluate the analgesic effects of tramadol hydrochloride in psittacines.

a. Exact, Kaytee Products Inc, Chilton, Wis.
b. Spectrum Chemical Mfg Corp, New Brunswick, NJ.
c. Ora-Blend Paddock Laboratories Inc, Minneapolis, Minn.
d. PROC Mixed, SAS, version 9.1.3, SAS Institute Inc, Cary, NC.

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