Antinociceptive effects of nalbuphine hydrochloride in Hispaniolan Amazon parrots (*Amazona ventralis*)

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Objective—To evaluate the antinociceptive effects and duration of action of nalbuphine HCl administered IM on thermal thresholds in Hispaniolan Amazon parrots (*Amazona ventralis*).

Animals—14 healthy adult Hispaniolan Amazon parrots of unknown sex.

Procedures—3 doses of nalbuphine (12.5, 25, and 50 mg/kg, IM) and saline (0.9% NaCl) solution (control treatment) were evaluated in a blinded complete crossover experimental design by use of foot withdrawal threshold to a noxious thermal stimulus. Baseline data on thermal threshold were generated 1 hour before administration of nalbuphine or saline solution; thermal threshold measurements were obtained 0.5, 1.5, 3, and 6 hours after administration.

Results—Nalbuphine administered IM at 12.5 mg/kg significantly increased the thermal threshold (mean change, 2.4°C), compared with results for the control treatment, and significantly changed thermal threshold for up to 3 hours, compared with baseline results (mean change, 2.6° to 3.8°C). Higher doses of nalbuphine did not significantly change thermal thresholds, compared with results for the control treatment, but had a significant effect, compared with baseline results, for up to 3 and 1.5 hours after administration, respectively.

Conclusions and Clinical Relevance—Nalbuphine administered IM at 12.5 mg/kg significantly increased the foot withdrawal threshold to a thermal noxious stimulus in Hispaniolan Amazon parrots. Higher doses of nalbuphine did not result in significantly increased thermal thresholds or a longer duration of action and would be expected to result in less analgesic effect than lower doses. Further studies are needed to fully evaluate the analgesic effects of nalbuphine in psittacine species. (*Am J Vet Res* 2011;72:736–740)

Opioids are frequently used in veterinary medicine and are considered the most effective class of analgesic drugs for perioperative management of pain. Investigators in several studies[1–6] have validated the clinical use of opioids for birds, particularly opioids with affinity for κ-opioid receptors. Butorphanol tartrate, a κ-opioid receptor agonist and μ-opioid receptor antagonist, is currently considered the analgesic drug of choice for management of acute and chronic pain in birds.[1–6] However, an accepted dose for psittacines of 1 to 3 mg of butorphanol/kg has a short plasma half-life and may require repeated administration (every 2 to 3 hours).[7] Long-acting opioid drugs would address the issue of the need for frequent administration. This has been determined in studies[3–5] in which investigators found that liposomal-encapsulated butorphanol provided analgesia for up to 5 days in Amazon parrots. Unfortunately, that formulation currently is not commercially available and is not amenable to pharmaceutical compounding.

Nalbuphine ([-]-17-[cyclobutylmethyl]-4, 5 α-epoxymorphinan-3, 6α, 14-triol) is an opioid analgesic that is a κ-opioid receptor agonist and μ-opioid receptor antagonist. Nalbuphine has been used widely in humans as a treatment for acute and chronic pain.[8–15] Nalbuphine has a mechanism of action similar to that of butorphanol; therefore, it may have similar analgesic effects in birds. The commercially available formulation, nalbuphine HCl, has an antinociceptive effect for up to 6 hours in rats.[16] Nalbuphine HCl may provide a similar or longer period of analgesia than current butorphanol formulations. The use of nalbuphine HCl is not regulated by the Drug Enforcement Administration,

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but butorphanol is a schedule IV drug that requires a Drug Enforcement Administration license for prescription. To the authors' knowledge, there have been no studies conducted to evaluate the analgesic properties of nalbuphine HCl in avian species. The purpose of the study reported here was to evaluate the antinociceptive effects and duration of action of nalbuphine HCl administered IM on thermal thresholds in Hispaniolan Amazon parrots.

Materials and Methods

Animals—Fourteen adult (range, 5 to 21 years old; mean ± SD, 8.33 ± 4.76 years old) Hispaniolan Amazon parrots (*Amazona ventralis*) of unknown sex with a mean body weight of 287.7 ± 20.2 g were used in the study. All parrots were part of a research colony and were assessed as healthy on the basis of results of physical examination performed before and during the study. Parrots were maintained in flocks (4 to 6 parrots/room) in large rooms (11.2 m^2). During the study, parrots were housed in standard stainless steel laboratory cages (0.6 × 0.6 × 0.6 m) with a perch and hanging toy. They were maintained on a 12-hour light cycle (12 hours of light and 12 hours of darkness), fed a commercial pelleted diet[a] formulated for psittacine birds, and provided fresh water ad libitum. The Institutional Animal Care and Use Committee of the University of Wisconsin School of Veterinary Medicine approved the experimental protocol.

Experimental design—A within-subjects, complete crossover experimental design was used. Parrots were randomly assigned to 4 groups by drawing pieces of paper from a bag. Parrots received nalbuphine HCl[b] (12.5, 25, and 50 mg/kg) and a control treatment (saline [0.9% NaCl] solution). Nalbuphine or saline solution was administered IM in the left pectoral muscles, and observers were not aware of the treatment administered. The experiment was repeated such that each parrot received all 3 doses of nalbuphine HCl and the saline solution; there was a 21-day washout period between subsequent treatments. Because the 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 treatment in place of a negative control treatment[17] was not considered feasible for the evaluation of antinociceptive effects and duration of action of nalbuphine.

Testing procedures—Measurements of the thermal threshold (as determined by foot withdrawal to a thermal stimulus) were obtained for 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[18] by use of thermal microchips that resulted in rapid changes in the temperature of the perch. Parrots could escape the brief noxious thermal stimulus by lifting the foot, and the foot could be placed back on the perch within 2 to 3 seconds after the withdrawal response because the temperature of the perch decreased quickly. The test box had dark sides that inhibited the parrot from viewing its surroundings, including any observers, and a clear front that allowed observers to monitor behavioral responses by use of a remote video camera.

Before the start of each experiment, parrots were acclimated to the test box by mimicking an entire test-day observation. The thermal stimulus, generated by thermoelectric modules, ranged from 29° to 70°C and caused a rapid increase and decrease in perch temperature (rate of temperature increase and decrease, 0.3°C/s). The maximum temperature was 70°C to prevent soft tissue damage. A thermal threshold withdrawal response was defined as the perch temperature corresponding to foot withdrawal response.

A separate thermal withdrawal threshold value was recorded at baseline for each experiment by means of a single measurement obtained 1 hour prior to administration of nalbuphine or saline solution (time of treatment administration was designated as time 0). Measurements of thermal foot withdrawal threshold were obtained via a single measurement at 0.5, 1.5, 3, and 6 hours after IM administration of a treatment. All thermal thresholds were determined by the same observer (JMK), who was not aware of the treatment administered. All birds were monitored during the study for signs of adverse effects, including sedation, excitation, vomiting, and diarrhea.

Statistical analysis—Data were analyzed by use of statistical software.[c] The endpoint of interest was the difference between thermal threshold at any time point after treatment administration and the baseline thermal threshold for that specific parrot on that experimental day. A repeated-measures ANOVA was used, with fixed effects of dose, time, and period and all associated interactions. Correlation within parrots over time within a period was modeled by use of a spatial power structure. Residuals resulting from the fitted model were verified to be normally distributed and had no evidence of heteroscedasticity. The least squares means of changes in thermal threshold were obtained from the values generated by use of the fitted model. Pairwise comparisons of the least squares means for the treatment within each time period and over all time periods were performed by use of the Tukey *P* value correction to account for multiple comparisons. Values of $P < 0.05$ were considered significant.

Results

Baseline values for thermal withdrawal threshold for the thermal stimulus (n = 56) ranged from 43° to

Table 1—Estimated mean change in thermal threshold from baseline values in 14 Hispaniolan Amazon parrots (*Amazona ventralis*) administered saline (0.9% NaCl) solution (control treatment) and 12.5, 25, and 50 mg of nalbuphine HCl/kg.

		Nalbuphine		
Time (h)	Saline solution	12.5 mg/kg	25 mg/kg	50 mg/kg
0.5	1.10	2.64*	2.08*	2.67*
1.5	0.26	3.29*	3.88*	2.15*
3	0.41	3.78*	2.71*	0.33
6	–1.00	0.67	–0.25	–0.93

Baseline values (data not shown) were obtained 1 hour before IM administration of the treatments (time of IM administration was designated as time 0), and measurements were obtained at various time points for 6 hours after administration. There was a 21-day washout period between subsequent treatments. The estimated SEM is 0.85 for all means.

*Within a column, value differs significantly ($P < 0.05$) from the baseline value.

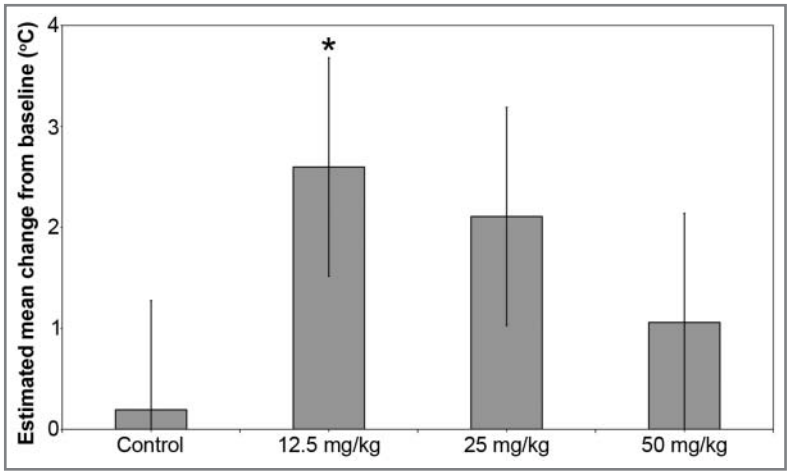

Figure 1—Estimated mean change in thermal threshold from baseline values in 14 Hispaniolan Amazon parrots (*Amazona ventralis*) administered saline (0.9% NaCl) solution (control treatment) and 12.5, 25, and 50 mg of nalbuphine HCl/kg. Baseline values were obtained 1 hour before IM administration of the treatments (time of IM administration was designated as time 0), and measurements were obtained at various time points for 6 hours after administration. There was a 21-day washout period between subsequent treatments. Error bars represent half of the Tukey honestly significant difference and are the same for all means. *Value differs significantly ($P < 0.05$) from the value for the control treatment.

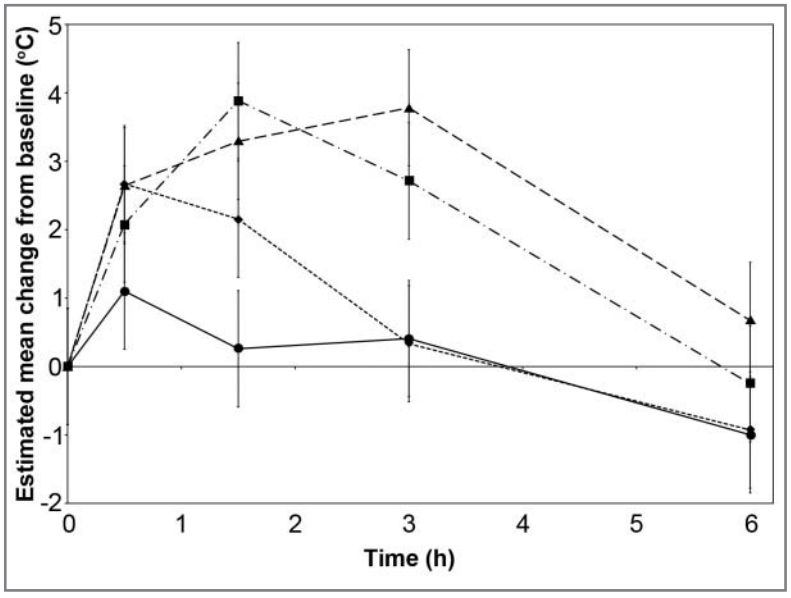

Figure 2—Estimated mean ± SEM change in thermal threshold from baseline values in 14 Hispaniolan Amazon parrots after IM administration of saline solution (circles with solid line) and nalbuphine HCl at 12.5 mg/kg (triangles with dashed line), 25 mg/kg (squares with dashed-and-dotted line), and 50 mg/kg (diamonds and dotted line). *See* Figure 1 for remainder of key.

59.8°C. The estimated mean change in thermal threshold from baseline values for the control (saline solution) and nalbuphine treatments over time was calculated (**Table 1**). There was no significant ($P = 0.19$ to 0.63) change in thermal threshold over time after administration of saline solution. Within-parrot SD over the 6-hour period after administration of saline solution ranged from 0.50 to 7.47; the SD for all parrots (except for 1) was < 4.34, and the SD for all parrots (except for 3) was < 2.44. The lack of a significant ($P = 0.171$) effect for repetition or any interactions with repetition supported the contention that there was no change in baseline values throughout the study. There was a significant overall effect of dose ($P = 0.023$) and time ($P < 0.001$). The lowest dosage of nalbuphine (12.5 mg/kg) resulted in significantly ($P = 0.024$) higher mean withdrawal temperatures, compared with results for the control treatment (mean difference, 2.4°C); results for the other 2 nalbuphine treatments did not differ significantly from results for the control treatment (**Figure 1**). For the control treatment, there was no significant difference between baseline values and values at 0.5, 1.5, 3, and 6 hours. Administration of nalbuphine at doses of 25 and 12.5 mg/kg significantly increased mean withdrawal temperatures, compared with withdrawal temperatures at baseline, for at least 3 hours, whereas administration of 50 mg of nalbuphine/kg significantly increased mean withdrawal temperatures, compared with withdrawal temperatures at baseline, for at least 1.5 hours (**Figure 2**). None of the parrots behaved abnormally throughout testing, and there were no adverse effects (including sedation) detected during the study.

Discussion

Nalbuphine HCl (12.5 mg/kg, IM) significantly increased thermal threshold values (mean change, 2.4°C), compared with values for the saline solution treatment, and also significantly changed the withdrawal threshold values for at least 3 hours, compared with baseline values. Higher doses of nalbuphine HCl (25 and 50 mg/kg, IM) did not significantly change thermal threshold values, compared with results for the control treatment, but did have a significant effect for 3 and 1.5 hours, respectively, compared with baseline values. Results of the study reported here are consistent with data from mammalian species that indicate nalbuphine HCl provides antinociception.[16,19,20] The dose-response antinociceptive effect observed in the present study has been reported for nalbuphine in other animals[20] and was consistent with a bell-shaped dose-responsive curve for analgesia in which there is a ceiling effect. The highest nalbuphine doses in the present study had the least effect on antinociception and had a shorter duration of analgesic action than did the lowest dose evaluated. The lowest dose in the present study (12.5 mg/kg) had the greatest antinociceptive effect; therefore, lower doses need to be evaluated for their antinociceptive effects.

The mechanism of action of nalbuphine is similar to that of butorphanol, which is currently used for management of acute pain in birds. Nalbuphine and butorphanol are κ-opioid receptor agonists and μ-opioid

antagonists and possess numerous pharmacological similarities, although there are some dissimilarities.[21] Nalbuphine administered parenterally is approximately one-fifth as potent as butorphanol.[21] The pharmacokinetics of nalbuphine and butorphanol are similar in mammalian and avian species.[21,22] In mammals, nalbuphine has a longer duration of action (3 to 6 hours) than does butorphanol (3 to 4 hours).[21] In the study reported here, nalbuphine administration to Hispaniolan Amazon parrots resulted in a duration of action of up to 3 hours, whereas in the authors' experience with the same species, butorphanol provides antinociception for up to 90 minutes, which was the last time point evaluated. On the basis of the results of the present study, nalbuphine HCl may provide a longer period of analgesia than do current commercial butorphanol formulations.

The thermal nociception response has been used to evaluate several opioids at various doses in various psittacine species, with varied results. In Cockatoos and Hispaniolan Amazon parrots, the response to the thermal stimulus was found to be a reliable measurement.[3,23] However, the response to the thermal stimulus was considered inconsistent in a study[18] in which investigators evaluated the antinociceptive effects of 1 mg of butorphanol/kg in African grey parrots. In other studies, thermal and electrical stimuli have been combined, but during a preliminary study conducted by our research group, we found that an electrical stimulus was a perturbing factor that caused signs of anxiety in birds when applied repeatedly at time points over a 6-hour period. The noxious thermal stimulation technique has a short period of stimulation and uses stimulation of skin rather than stimulation of visceral or muscular sites.[24] Nociception is the result of thermal stimuli activating thermal receptors. Thermal receptors are afferent Aδ and C fibers and transmit the nociceptive information to different areas of the midbrain and forebrain via ascending spinal pathways.[25] The use of thermal stimuli and the natural perching behavior of parrots is a noninvasive method for evaluation of nociceptive thresholds and analgesic modulation of those nociceptive thresholds, but further studies with other types of stimuli are necessary for a full evaluation.

Individual variability in the antinociceptive effects of opioids has been detected in many species and appears to be multifactorial, with genotype,[26–29] sex,[30] type of noxious stimulus,[30] receptor,[30] and relative efficacy of the agent[30] all affecting the individual response. The variation in individual response to the treatments resulted in a large SD when individual results were grouped by treatment. Although all nalbuphine treatments caused an increase in thermal threshold, compared with the baseline values, the variance in individual response precluded the ability to detect significant differences between the control treatment and the nalbuphine treatments at 25 and 50 mg/kg. The sample size (n = 14 parrots) for the study was selected on the basis of previous experiments that used a similar design and yielded significant results. A retrospective power analysis determined that approximately 106 parrots would have been needed to detect (with 80% power) the difference of 0.86°C observed between the 50 mg/kg and control treatments, whereas 23 parrots would have been needed to detect the difference of 1.91°C observed between the 25 mg/kg and control treatments.

The therapeutic plasma concentration varies with species and with the method used to provide a painful stimulus, so caution should be used when plasma concentration alone is used to predict analgesia.[31] The antinociceptive effects are likely determined by the concentration at the receptor, which lags behind the plasma concentration.[31] The antinociceptive effect at 12.5 mg/kg lasted for 3 hours, compared with the thermal threshold at baseline. Mean ± SD plasma concentrations at 3 hours in another study[22] in Hispaniolan Amazon parrots in which nalbuphine was administered at 12.5 mg/kg were 27.76 ± 20.38 ng/mL. Furthermore, 4 of 8 parrots in that study[22] had plasma concentrations < 1 ng/mL at 3 hours after administration. The affinity of the drug for the receptor may account for a longer duration of action than is predicted by the half-life.[31]

Adverse effects, including sedation, were not observed in the parrots of the present study. Nalbuphine has a low incidence of undesirable effects in mammals, with sedation being the most common; however, sedation can be advantageous in clinical settings.[21] Nalbuphine is considered superior to butorphanol in mammals because nalbuphine does not increase cardiac oxygen requirements and cardiac work in cardiac-compromised patients, nor does it prolong the duration of respiratory depression with higher doses, as is seen with butorphanol.[21] There is a plateau in respiratory depression with butorphanol and nalbuphine in mammals.[21] The results of the study reported here and in other reports support that both drugs are safe and effective opioid receptor agonist-antagonist analgesics, but further studies are needed to evaluate cardiovascular and analgesic effects of nalbuphine in parrots.

We concluded that nalbuphine HCl (12.5 mg/kg, IM) significantly increased the thermal threshold to a noxious thermal stimulus in Hispaniolan Amazon parrots and may provide clinical analgesia for up to 3 hours. Higher doses of nalbuphine HCl did not result in an increase in thermal threshold or a longer duration of action and may not have the analgesic benefit of lower doses. Additional studies to evaluate doses < 12.5 mg/kg and other types of noxious stimuli are needed to fully characterize the analgesic effects of nalbuphine HCl in psittacine species.

a. Exact, Kaytee Products Inc, Chilton, Wis.
b. Nalbuphine hydrochloride, Barr Laboratories, Pomona, NY.
c. MIXED PROC, SAS, version 9.1.3, SAS Institute Inc, Cary, NC.

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