Antinociceptive effects of long-acting nalbuphine decanoate after intramuscular administration to Hispaniolan Amazon parrots (*Amazona ventralis*)

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Objective—To evaluate the thermal antinociceptive effects and duration of action of nalbuphine decanoate after IM administration to Hispaniolan Amazon parrots (*Amazona ventralis*).

Animals—10 healthy adult Hispaniolan Amazon parrots of unknown sex.

Procedures—Nalbuphine decanoate (33.7 mg/kg) or saline (0.9% NaCl) solution was administered IM in a randomized complete crossover experimental design (periods 1 and 2). Foot withdrawal threshold to a noxious thermal stimulus was used to evaluate responses. Baseline thermal withdrawal threshold was recorded 1 hour before drug or saline solution administration, and thermal foot withdrawal threshold measurements were repeated 1, 2, 3, 6, 12, 24, 48, and 72 hours after drug administration.

Results—Nalbuphine decanoate administered IM at a dose of 33.7 mg/kg significantly increased thermal foot withdrawal threshold, compared with results after administration of saline solution during period 2, and also caused a significant change in withdrawal threshold for up to 12 hours, compared with baseline values.

Conclusions and Clinical Relevance—Nalbuphine decanoate increased the foot withdrawal threshold to a noxious thermal stimulus in Hispaniolan Amazon parrots for up to 12 hours and provided a longer duration of action than has been reported for other nalbuphine formulations. Further studies with other types of nociceptive stimulation, dosages, and dosing intervals as well as clinical trials are needed to fully evaluate the analgesic effects of nalbuphine decanoate in psittacine birds. (*Am J Vet Res* 2013;74:196–200)

Opioid drugs are considered the most effective class of analgesic drugs for perioperative pain and are frequently used in veterinary medicine. Opioids, particularly those with κ-opioid receptor affinities,[1–7] have been validated for clinical use in birds. Butorphanol tartrate and nalbuphine hydrochloride are κ-opioid receptor agonists and μ-opioid receptor antagonists and currently are considered the opioid drugs recommended for acute pain management in psittacine birds.[1–7] The accepted dose range of 1 to 3 mg/kg for butorphanol and 12.5 mg/kg for nalbuphine hydrochloride results in a short period of action and requires repeated parenteral administration every 2 to 3 hours to maintain effects in psittacine birds.[1,6,8,9]

Long-acting opioid drugs would alleviate the need for frequent administration. Liposomal-encapsulated butorphanol provided analgesia for up to 5 days in psittacine birds.[2–4] Long-acting nalbuphine formulations, such as nalbuphine decanoate, may provide an alternative for long-term pain management in psittacine birds. Use of the custom-synthetized long-acting ester nalbuphine decanoate resulted in analgesia in rats for up to 60 hours[10,11] and rabbits for up to 48 hours.[12] Additionally, nalbuphine is currently not on the Drug Enforcement Administration list of scheduled substances because of its low abuse potential, which is advantageous in clinical settings. Butorphanol is a schedule IV drug that requires a Drug Enforcement Administration license for prescription. Clinicians are more likely to dispense nonscheduled analgesic drugs than controlled drugs.

To our knowledge, there have been no studies conducted to investigate the analgesic efficacy of long-

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acting nalbuphine esters in avian species. The purpose of the study reported here was to determine the antinociceptive effects and duration of action of nalbuphine decanoate administered IM on thermal thresholds of Hispaniolan Amazon parrots (*Amazona ventralis*). The hypothesis was that administration of nalbuphine decanoate to Hispaniolan Amazon parrots would result in significant antinociceptive effects to a noxious thermal stimulus for up to 48 hours.

Materials and Methods

Animals—Ten adult (range, 5 to 21 years old; median, 7.8 years old) Hispaniolan Amazon parrots of unknown sex were used in the study. Mean ± SD body weight of the parrots was 285.3 ± 18.5 g. All parrots were considered healthy prior to and during the study as determined on the basis of results of physical examinations. Parrots were maintained in flocks of 4 to 6 parrots in large rooms (11.2 m^2) between experimental periods. During the study, the parrots were housed in standard stainless steel laboratory cages (0.6 × 0.6 × 0.6 m) with a perch and hanging toy. Parrots were maintained on a light cycle of 12 hours of light and 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[13] was not considered feasible for the evaluation of the antinociceptive effects and duration of action of nalbuphine.

Experimental design—A within-subjects, complete crossover experimental design was used. Each bird received both nalbuphine decanoate and saline (0.9% NaCl) solution. Parrots were randomly assigned (as determined by drawing numbered pieces of paper from a bag) to 2 groups. During period 1, birds in group 1 received nalbuphine decanoate (33.7 mg/kg, IM), and birds in group 2 received an equivalent volume of saline solution IM. Treatments were administered in the pectoral muscles, and the observer was not aware of the treatment provided to each bird. After a 21-day washout period, birds received the alternate treatment during period 2.

Synthesis of nalbuphine decanoate—Nalbuphine decanoate was synthesized by modification of a method described elsewhere[14]; details on the synthesis are provided in a study[15] on the pharmacokinetics of long-acting nalbuphine decanoate in Hispaniolan Amazon parrots. Nalbuphine hydrochloride[b] (0.5315 g) was used as the base. A decanoate group was added by the use of decanoyl chloride.[c] The nalbuphine decanoate product was separated via thin-layer chromatography on a silica gel[d] with a solution of ethyl acetate and hexane (3:1) as the solvent. The crude product was collected in a round-bottom flask, evaporated, lyophilized, and weighed. The product was purified on a column, and fractions were collected, evaporated, and lyophilized. The nalbuphine decanoate was dissolved in 100% ethanol and analyzed for purity via mass spectroscopy. Prior to injection, nalbuphine decanoate was flash evaporated and then dissolved at the desired concentration in sterile sesame oil.

Nociception testing procedures—Measurements of thermal foot withdrawal threshold were collected on all parrots by use of a test box equipped with a testing perch. The test perch was designed to deliver a thermal stimulus to the left plantar surface of a parrot's foot[16] via thermal microchips that rapidly changed the temperature of the perch. Parrots could escape the brief noxious thermal stimulus by lifting the foot, and the foot could then be placed back on the perch within 2 or 3 seconds after the withdrawal response because the perch temperature decreased quickly. The test box had dark sides that inhibited the parrot from viewing its surroundings, including the investigators, and a clear front that allowed the investigator to monitor behavioral responses by use of a remote video camera. Prior to each experiment, each parrot was acclimated to the test chamber by mimicking a full test day. The thermal stimulus, which was 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 damage to soft tissues.

A thermal threshold was defined as the perch temperature that was concurrent with the foot withdrawal response. A separate baseline thermal withdrawal threshold was recorded for each experimental period via a single measurement obtained 1 hour before administration of the analgesic drug or saline solution. Measurements of thermal foot withdrawal threshold were performed via a single measurement 1, 2, 3, 6, 12, 24, 48, and 72 hours after IM administration of nalbuphine decanoate or saline solution. The birds were removed from the testing box between testing times. All thermal thresholds were determined by the same investigator (JMB), who was 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 statistical software.[e] The endpoint of interest was the difference between withdrawal temperature at any given time point after drug administration and the baseline withdrawal temperature for that bird in that period. A repeated-measures ANOVA was used, with fixed effects of dose, time, period, and all associated interactions. Correlations within birds over time within a period were modeled with a spatial power structure. Residuals resulting from the fitted model were verified to be acceptably normally distributed and had no evidence of heteroscedasticity. The least square means of changes in withdrawal temperature were obtained from the values generated with the fitted model. Pairwise comparisons of the least square means for the groups, both within each time point and over all times, were performed via

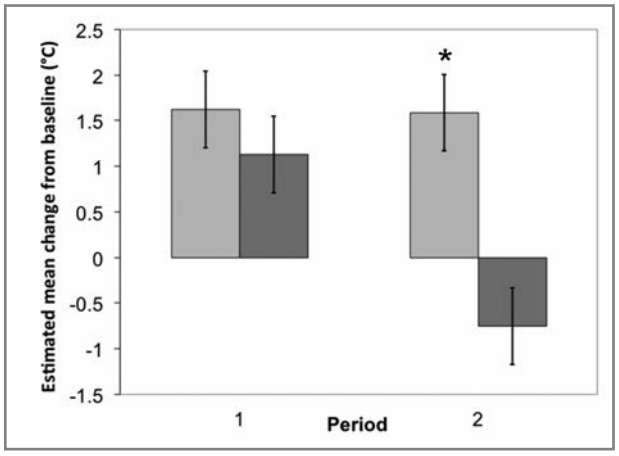

Figure 1—Mean change in thermal threshold from baseline values in 10 Hispaniolan Amazon parrots (*Amazona ventralis*) after IM administration of saline (0.9% NaCl) solution (control treatment; dark gray bars) and nalbuphine decanoate (33.7 mg/kg; light gray bars) for 8 time points during 72 hours after injection. Error bars represent one half of the Tukey honestly significant difference and are the same for all means. Birds were assigned to receive one of the treatments during period 1, and after a 21-day washout period, they received the alternate treatment during period 2. Thus, 4 birds received nalbuphine decanoate in period 1 followed by saline solution in period 2, and 6 birds received saline solution in period 1 and nalbuphine in period 2. *Within a period, value differs significantly ($P < 0.05$) from the value for the saline solution treatment.

the Tukey honestly significant difference method to account for multiple comparisons. For all analyses, significance was set at values of $P < 0.05$.

Results

Baseline thermal withdrawal threshold values (n = 20) for the thermal stimulus varied from 39.00° to 60.85°C. Four birds received nalbuphine decanoate in period 1 followed by saline solution in period 2, and 6 birds received the treatments in the opposite order. There were significant overall effects of treatment (P = 0.004), period (P = 0.040), and the treatment-by-period interaction (P = 0.045). Because of the treatment-by-period interaction, treatment effect was evaluated separately for each period (**Figure 1**).

In periods 1 and 2, there were no significant changes in thermal withdrawal threshold from baseline values for birds receiving saline solution at any times, except for period 1 at 72 hours (1.81°C [P = 0.040]; **Figure 2**). In period 1, birds receiving nalbuphine decanoate had a mean change in thermal threshold of 1.62°C throughout the entire testing period of 72 hours. This value was not significantly different (P = 0.846) from the value for birds receiving saline solution, which had a mean change in thermal threshold of 1.13°C. However, administration of nalbuphine decanoate resulted in a significant change from the baseline values in period 1 at 2 (2.57°C [P = 0.018]), 3 (2.64°C [P = 0.015]), 6 (2.26°C [P = 0.036]), and 12 (3.06°C [P = 0.005]) hours after drug administration. There was no significant (P values ranged from 0.055 to 0.833) change from baseline values at 1, 24, 48, or 72 hours after administration of nalbuphine decanoate. In period 2, birds receiving nalbuphine decanoate had a mean change in thermal threshold of 1.59°C. This differed significantly

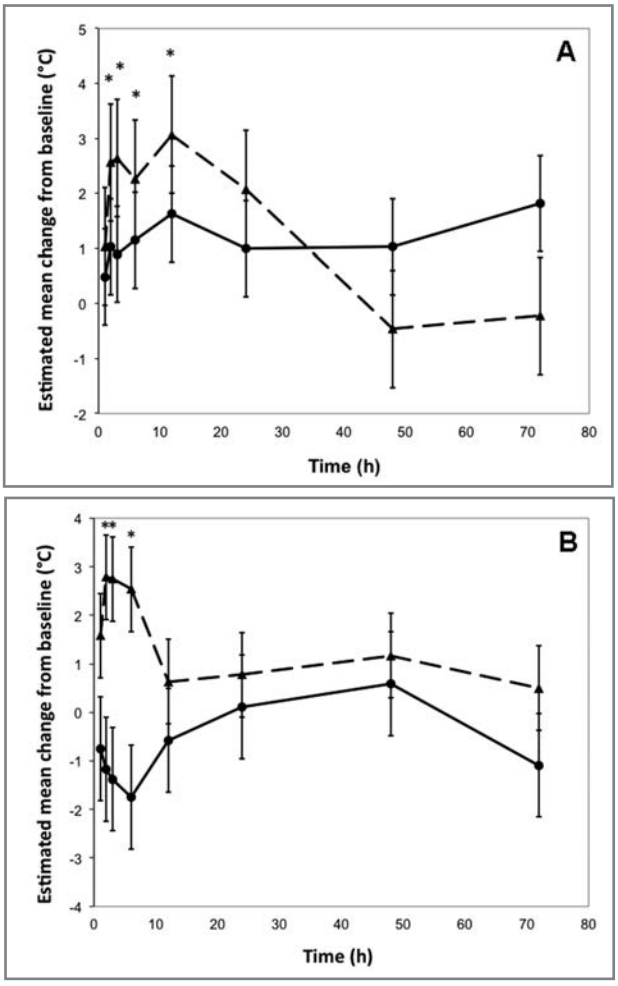

Figure 2—Mean ± SEM change in thermal threshold from baseline values in Hispaniolan Amazon parrots during periods 1 (A) and 2 (B) after IM administration of saline solution (control treatment; circles with solid line) or nalbuphine decanoate (33.7 mg/kg; triangles with dashed line) for 8 time points during 72 hours after injection. There was a 21-day washout period between periods. Four birds received nalbuphine decanoate in period 1 followed by saline solution in period 2, and 6 birds received saline solution in period 1 and nalbuphine in period 2. *Within a time point, the value for the nalbuphine decanoate treatment differs significantly ($P < 0.05$) from the baseline (time 0) value.

(P = 0.006) from the value for birds receiving saline solution, which had a mean change in thermal threshold of –0.75°C. In period 2, there was a significant change from the baseline value for nalbuphine decanoate–treated birds at 2 (2.79°C [P = 0.002]), 3 (2.75°C [P = 0.002]), and 6 (2.53°C [P = 0.004]) hours after administration. There was no significant (P values ranged from 0.072 to 0.568) change from the baseline value at 1, 12, 24, 48, or 72 hours after drug administration.

None of the parrots had abnormal behavior during the experiments. There were no adverse effects, including sedation, detected during the study.

Discussion

Nalbuphine decanoate administered at 33.7 mg/kg, IM, significantly increased thermal foot withdrawal threshold during both periods at 2, 3, and 6 hours after administration, compared with baseline values, and also

significantly changed the withdrawal threshold during period 1 for up to 12 hours, compared with baseline values. In both periods, there was not a significant increase in the thermal foot withdrawal threshold at 1 hour, compared with the baseline value, likely because of the slow absorption rate for nalbuphine decanoate.[15]

Results of the present study are consistent with data from mammalian species in which nalbuphine decanoate provided longer-acting antinociception than that provided by the standard nalbuphine hydrochloride.[10–12] The nalbuphine decanoate dose used in the present study was calculated on the basis of plasma concentrations of nalbuphine that were associated with antinociception and the pharmacokinetics of nalbuphine decanoate in Hispaniolan Amazon parrots receiving a slightly higher dose of the drug (37.5 mg/kg).

The mechanism of action for nalbuphine is similar to that of butorphanol, which is currently used for acute pain management in psittacine birds. Both opioids are κ-opioid receptor agonists and μ-opioid receptor antagonists, and they possess numerous pharmacological similarities and a few dissimilarities.[17] When administered parenterally, butorphanol is approximately 5 times as potent as nalbuphine,[17] and their pharmacokinetic properties are similar in mammalian and avian species.[9,17] In mammals, nalbuphine has a longer duration of action than does butorphanol (3 to 6 hours and 3 to 4 hours, respectively).[17] In Hispaniolan Amazon parrots, nalbuphine hydrochloride resulted in antinociceptive effects for up to 3 hours,[1] whereas in another study,[6] butorphanol provided antinociception for up to 90 minutes, which was the last time point evaluated. On the basis of results of the present study, nalbuphine decanoate provides analgesia in Hispaniolan Amazon parrots for up to 12 hours. This is longer than the reported duration for standard formulations of nalbuphine and butorphanol, but much shorter than the duration for liposome-encapsulated butorphanol formulations (which can provide analgesic effects for up to 5 days).[2–4]

The thermal nociceptive response has been used to evaluate several opioids at various doses in different psittacine species with differing results. In both cockatoos and Hispaniolan Amazon parrots,[1,18,19] the response to the thermal stimulus was found to be a reliable measurement. However, the response to the thermal stimulus in a study[16] conducted to evaluate the antinociceptive effects of butorphanol (1 mg/kg) in African grey parrots was considered inconsistent. Noxious thermal stimulation has a short period of stimulation and uses skin rather than visceral or muscular sites for stimulation.[20] Nociception is induced by thermal stimuli activating thermal receptors. Thermal receptors are afferent Aδ and C fibers and transmit the nociceptive information to various areas of the midbrain and forebrain via ascending spinal pathways.[21] 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 thresholds, but further studies with different types of stimuli are recommended for full evaluation of analgesic drugs. The control treatment in the present study was saline solution instead of sesame oil; however, sesame oil has not been found to have analgesic properties.[10–12]

Individual variation in the antinociceptive effects of opioids has been observed in many species, and the variation appears to be multifactorial, with sex,[22–25] genotype,[26] type of noxious stimulus,[26] receptor,[26] and relative efficacy of the drug all affecting the individual response.[26] In a previous study[1] that involved the use of nalbuphine hydrochloride, baseline values for thermal withdrawal threshold for the thermal stimulus (n = 56) ranged from 43° to 59.8°C, which are values similar to those obtained in the study reported here. 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 nalbuphine decanoate treatments caused an increase in thermal threshold, compared with baseline results, the variation in individual responses precluded the ability to detect significant differences in the mean change in period 1 between birds receiving the control and nalbuphine decanoate treatments, but there was a significant difference in period 2.

The data for the present study were analyzed 2 ways. Changes in values between the nalbuphine and control treatments were compared, as were changes for each treatment from the mean baseline values for that treatment. Both analyses were considered in the interpretation of the results. Despite a significant effect of period, analysis of the data suggested a possible duration of the antinociceptive effect for up to 12 hours. The significant mean change in temperature in the study was considered clinically relevant by the authors and likely was associated with mild analgesia. Because of limited availability of birds for the study, the sample size (n = 10) was the lowest for all the studies performed by our laboratory group during evaluation of analgesics in Hispaniolan Amazon parrots, and it likely was associated with the difference in results between periods. Results of a retrospective power analysis revealed that approximately 119 birds would have been needed to detect (with 80% power) the difference of 0.49°C observed between the nalbuphine decanoate and control treatment during period 1, whereas 7 birds would have been needed to detect the difference of 2.34°C observed between the groups during period 2.

Therapeutic plasma concentration differs among species, subjects within a species, and methods used for stimulating pain, so caution should be used when plasma concentration alone is used to predict analgesia.[27] The antinociceptive effects are likely determined by the concentration at the receptor, which lags behind the plasma concentration.[27] The antinociceptive effect of nalbuphine hydrochloride administered at 12.5 mg/kg lasted for 3 hours, compared with baseline values, at a mean plasma concentration of 27.76 ng/mL.[1,9] Mean plasma concentrations in Hispaniolan Amazon parrots at 6, 9, 12, and 24 hours after IM administration of nalbuphine decanoate at 37.5 mg/kg were 108.7, 46.4, 76.1, and 42.3 ng/mL, respectively.[15] The lower dose used in the present study to evaluate antinociceptive effects may also account for the shorter duration of action than was predicted in that previous study.[15]

Adverse effects, including sedation, were not observed in the parrots. These findings are consistent with results of a study[1] conducted to evaluate the anti-

nociceptive effects of nalbuphine hydrochloride in Hispaniolan Amazon parrots[1] and differ from findings in another study[4] conducted to evaluate liposome-encapsulated butorphanol in the same species. Nalbuphine has a low incidence of undesirable effects in mammals, with sedation being the most common, although sedation can be advantageous in clinical settings. There is plateau respiratory depression with both butorphanol and nalbuphine in mammals.[17] Results of the present study and those in previous reports[1–9] support the contention that both drugs are safe and effective agonist-antagonist analgesics, but further studies to evaluate cardiovascular and analgesic effects of nalbuphine in parrots are needed.

In the study reported here, nalbuphine decanoate administered IM at 33.7 mg/kg significantly increased the foot withdrawal threshold to a thermal noxious stimulus in Hispaniolan Amazon parrots for up to 12 hours. Further studies with other types of noxious stimulation, doses, and testing intervals are needed to fully evaluate the analgesic effects of nalbuphine decanoate in psittacine birds and their relevance in clinical settings.

a. Exact, Kaytee Products Inc, Chilton, Wis.
b. Spectrum Laboratories Inc, Gardena, Calif.
c. Fluka, St Louis, Mo.
d. EMD Chemicals, Gibbstown, NJ.
e. Mixed Procedure, SAS, version 9.1.3 for Unix, SAS Institute Inc, Cary, NC.

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