Evaluation of thermal antinociceptive effects after intramuscular administration of buprenorphine hydrochloride to American kestrels (Falco sparverius)

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Objective—To evaluate the thermal antinociceptive effects and duration of action of buprenorphine hydrochloride after IM administration to American kestrels (Falco sparverius).

Animals—12 healthy 3-year-old American kestrels.

Procedures—Buprenorphine hydrochloride (0.1, 0.3, and 0.6 mg/kg) and a control treatment (saline [0.9% NaCl] solution) were administered IM in a randomized crossover experimental design. Foot withdrawal response to a thermal stimulus was determined 1 hour before (baseline) and 1.5, 3, and 6 hours after treatment administration. Agitation-sedation scores were determined 3 to 5 minutes before each thermal stimulus. Adverse effects were monitored for 6 hours after treatment administration.

Results—Buprenorphine hydrochloride at 0.1, 0.3, and 0.6 mg/kg, IM, increased thermal threshold for 6 hours, compared with the response for the control treatment. There were no significant differences among buprenorphine treatments. A mild sedative effect was detected at a dose of 0.6 mg of buprenorphine/kg.

Conclusion and Clinical Relevance—At the doses tested, buprenorphine hydrochloride resulted in thermal antinociception in American kestrels for at least 6 hours, which suggested that buprenorphine has analgesic effects in this species. Further studies with longer evaluation periods and additional forms of noxious stimuli, formulations, dosages, and routes of administration are needed to fully evaluate the analgesic effects of buprenorphine in American kestrels. (Am J Vet Res 2014;75:705–710)

Opioid drugs are used for their analgesic properties. These drugs act on μ-, κ-, and δ-opioid receptors as well as orphan opioid-like receptors in the CNS and peripheral nervous system. The action of opioid drugs on these receptors activates G-proteins, which leads to a reduction in transmission of nerve impulses and inhibition of neurotransmitter release. Buprenorphine is a slow-onset, long-acting, μ-opioid receptor agonist. However, there is not a consensus about buprenorphine's action on κ-opioid receptors. The current consensus in human medicine is that buprenorphine has full μ-opioid receptor agonistic activity and that its analgesic effect is dose dependent. Investigators of an early study of buprenorphine described a plateau or ceiling effect whereby increased dosages would not result in improved analgesia or a bell-shaped dose-response curve in which higher dosages would have a lower analgesic effect. Results of studies conducted since then and reevaluation of data have revealed that the concept of a ceiling effect may have been a misinterpretation of results and that there is only a ceiling effect for respiratory depression. Several opioid drugs have been evaluated to investigate their analgesic potential in American kestrels (Falco sparverius). Hydromorphone hydrochloride, a pure μ-opioid receptor agonist, has dose-responsive thermal antinociceptive effects when administered IM at 0.1, 0.3, and 0.6 mg/kg, which suggests analgesic properties. In contrast, butorphanol tartrate, a κ-opioid receptor agonist and μ-opioid receptor antagonist, did not have a significant thermal antinociceptive effect when administered IM at 1, 3, and 6 mg/kg. Tramadol hydrochloride significantly increased the foot withdrawal threshold when administered orally at 5 mg/kg, whereas oral administration of higher doses (15 and 30 mg/kg) resulted in a lower antinociceptive effect.
The minimal adverse effects observed in other vertebrate species\textsuperscript{17-19} and the ceiling effect of respiratory depression\textsuperscript{14-15} make buprenorphine a potentially useful analgesic in avian species. In African grey parrots (Psittacus erithacus), IM administration of 0.1 mg of buprenorphine/kg did not increase withdrawal thresholds when an electrical noxious stimulus was applied,\textsuperscript{19,20} and pharmacokinetic analysis revealed that this dose did not achieve plasma concentrations considered to be therapeutic in humans beyond 2 hours.\textsuperscript{21} Buprenorphine hydrochloride administered IM at 0.25, 0.5, and 0.75 mg/kg to pigeons increased the withdrawal latency to a noxious electrical stimulus, but the effect was smallest for the 0.75 mg/kg dose.\textsuperscript{22} Intra-articular injection of 0.05 to 1 mg of buprenorphine hydrochloride in domestic fowl did not decrease pain-related behaviors.\textsuperscript{13} To our knowledge, buprenorphine hydrochloride has not yet been investigated in American kestrels or any other raptor species.

The objective of the study reported here was to evaluate the thermal antinociceptive effects and duration of action of buprenorphine hydrochloride in American kestrels. The hypothesis was that buprenorphine would have dose-dependent thermal antinociceptive effects in American kestrels for 6 hours after administration.

\textbf{Materials and Methods}

\textbf{Animals}—Twelve captive-bred 3-year-old American kestrels (8 females and 4 males) were used in the study. The kestrels had been used before in similar studies.\textsuperscript{14-16} Mean ± SD body weight was 106.6 ± 6.3 g (median, 105.7 g; range, 93.8 to 126.4 g). All kestrels were part of a research colony at the University of California-Davis School of Veterinary Medicine and were assessed as healthy on the basis of results of physical examinations performed before and during the study. The kestrels were housed in small groups in rooms (2.5 × 2.5 × 3.2 m) equipped with several perches. Kestrels were fed killed, previously frozen adult mice and had ad libitum access to water. They were maintained on a cycle of 12 hours of light to 12 hours of darkness, and temperature was controlled at 20°C. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of California-Davis.

\textbf{Experimental design}—Four treatments were evaluated in a masked complete crossover experimental design. Treatments consisted of IM administration of 3 doses of buprenorphine hydrochloride (0.1, 0.3, and 0.6 mg/kg) and IM administration of an equivalent volume of saline (0.9% NaCl) solution\textsuperscript{2} as a control treatment. Birds were assigned by use of a random-number generator\textsuperscript{2} to each of 4 groups. On a test day, the birds in each group received a dose of buprenorphine hydrochloride or saline solution. Treatments were administered IM in the left or right pectoral muscle with a 30-gauge insulin syringe. After a 2-week washout period, the birds were administered the subsequent treatment in the contralateral pectoral muscle. Each kestrel received all 3 doses of buprenorphine and the saline solution. 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 was not considered feasible for the evaluation of antinociceptive effects and duration of action of buprenorphine.

\textbf{Testing procedure}—Measurements of the thermal threshold were obtained for all kestrels by use of a test box equipped with a test perch. Prior to the study, kestrels were allowed to acclimatize to the test box by spending approximately 30 minutes in the box on several days as well as an entire day that mimicked an experiment, which included heating of the perch but no administration of any medication.

The test perch was designed to deliver a thermal stimulus to the plantar surface of a bird’s right foot, as described elsewhere.\textsuperscript{20} Briefly, the thermal stimulus was generated by thermoelectric modules. The modules caused a rapid increase in perch temperature (rate of temperature increase, 0.3°C/s). Maximum temperature was set at 54°C to prevent soft tissue damage to the plantar surface. A kestrel could escape the brief noxious thermal stimulus by lifting the right foot, and the foot could be placed back on the perch within 2 to 3 seconds because of the rapid cooling of the perch. Thermal threshold was defined as the perch temperature corresponding to the foot withdrawal response recorded by an experienced observer (SMC) who was unaware of the treatment administered to each kestrel. Recordings were obtained by use of a remote video camera so that the kestrels did not see the observer. A uniform background sound was used to minimize effects of possible environmental disturbances.

A baseline value for thermal withdrawal threshold was determined for each bird in each experimental period by a single measurement obtained 1 hour before treatment (time of treatment administration was designated as time 0). Thermal foot withdrawal threshold was determined by a single measurement at 0.5, 1.5, 3, and 6 hours after IM administration of each treatment.

\textbf{Agitation-sedation score and adverse effects}—Throughout each test day, kestrels were housed in the same room with the observer in transport carriers (23 X 30.5 X 43 cm) that contained a perching brick. This allowed continuous monitoring for adverse effects, including sedation, agitation, vomiting, and diarrhea. During the 7-hour period of thermal data collection, each kestrel was placed in the test box and observed remotely for 3 to 5 minutes before each thermal test to enable the observer to record an agitation-sedation score. The score was determined by use of a scale previously used for kestrels\textsuperscript{14-16} (Appendix).

\textbf{Statistical analysis}—The endpoint of interest was the thermal threshold for each kestrel at each time point after each treatment administration. Longitudinal data analysis was performed with linear mixed modeling on the withdrawal temperature as the outcome variable. Time, treatment (saline solution and 0.1, 0.3, and 0.6 mg of buprenorphine/kg), treatment period, sex, baseline thermal threshold value, and all interactions were fixed effects, and kestrel was a random effect. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were also
created on the residuals, by treatment group, for assessment of normality. Residuals resulting from the fitted model were verified to be normally distributed and had no evidence of heteroscedasticity. Autocorrelation of the residuals over time was assessed by use of the autocorrelation function method, which did not reveal any significant autocorrelation of the first or second order. Various correlation structures for modeling dependence were used but did not significantly enhance the model fit as determined on the basis of Akaike information criterion. A type III ANOVA was performed on the fixed effects, and post hoc comparisons were performed with a Tukey adjustment. Data for sedation score were analyzed by use of an ordinal logit mixed model, with sedation score as the outcome ordinal categorical variable; time, sex, temperature, and all interactions as fixed variables; and kestrel as a random variable. Residuals were evaluated graphically. Data were analyzed by use of statistical software.44 Values of P < 0.05 were considered significant.

Results

Baseline values for thermal withdrawal threshold (n = 48) ranged from 42.3° to 50.1°C. Comparisons of thermal threshold between the control treatment and the 3 buprenorphine treatments over time were evaluated on the basis of sex, treatment order, and baseline thermal threshold value (Table 1). Individual variability in response accounted for 31.4% of the total variability of the model, and the SD was 0.8°C (total SD of the model, 2.5°C). There was a significant (P < 0.001) effect of treatment on thermal threshold (Figure 1). There also were significant effects for treatment order (P = 0.01), sex (P = 0.037), and baseline thermal threshold (P < 0.001). There was not a significant (P = 0.080) effect for time. All buprenorphine treatments (0.1, 0.3, and 0.6 mg/kg) induced significantly higher withdrawal temperatures than did saline solution for both male and female kestrels. Baseline values for thermal withdrawal threshold in 12 American kestrels (Falco sparverius) before and after IM administration of buprenorphine (0.1, 0.3, and 0.6 mg/kg).

Table 1—Mean ± SEM values for fixed effects on thermal threshold in 12 American kestrels (Falco sparverius) before and after IM administration of buprenorphine (0.1, 0.3, and 0.6 mg/kg).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Intercept</td>
<td>31.5</td>
</tr>
<tr>
<td>Dose 0.1 mg/kg</td>
<td>1.1</td>
</tr>
<tr>
<td>Dose 0.3 mg/kg</td>
<td>0.9</td>
</tr>
<tr>
<td>Dose 0.6 mg/kg</td>
<td>1.4</td>
</tr>
<tr>
<td>Time</td>
<td>0.1</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.3</td>
</tr>
<tr>
<td>Baseline value for thermal threshold</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Effect of treatment order is not shown. Values were considered significant at P < 0.05.

Table 2—Proportional OR that a dose of buprenorphine administered IM to 12 kestrels would cause an increase of 1 in the agitation-sedation score (ie, kestrels become more alert).

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>OR</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.48</td>
<td>0.14–1.61</td>
<td>0.230</td>
</tr>
<tr>
<td>0.3</td>
<td>0.39</td>
<td>0.12–1.35</td>
<td>0.140</td>
</tr>
<tr>
<td>0.6</td>
<td>0.68</td>
<td>0.02–0.32</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Discussion

In the study reported here, buprenorphine hydrochloride administered IM at 0.1, 0.3, and 0.6 mg/kg significantly increased thermal threshold in kestrels for 6 hours, compared with the response after IM administration of saline solution. The variability in responses for individual kestrels precluded the ability to detect differences among the 3 doses, which resulted in large SDs when results for individual kestrels were grouped by treatment. Individual variability in the antinocicept...
tive effects of opioids has been described in many species, and the variability appears to be multifactorial, with sex, genotype, type of noxious stimulus, type of receptor, and relative efficacy of the agent all affecting the response.\textsuperscript{24–27}

Mean withdrawal threshold was significantly higher in male kestrels than in female kestrels both before and after administration of buprenorphine. The mean effect of sex on the foot withdrawal temperature was of similar magnitude as the mean increase in temperature after administration of buprenorphine. However, the small number of males (n = 4) in the study prevented the authors from drawing a final conclusion concerning a potential difference attributable to sex.

The experimental treatment period also significantly affected thermal threshold. The first and third treatment periods had significantly higher thermal thresholds than did the fourth treatment period. Because there was a 2-week washout period between treatments, it is less likely that these differences were caused by a carryover effect and more likely that they represented a type I error.

The effect of baseline thermal threshold suggested that as the baseline threshold temperature for individual kestrels increased, a bird was more susceptible to withdrawing the foot at a smaller change in perch temperature. This implied that they became less tolerant to temperature change and a theoretical threshold was more quickly reached. The significant effect of baseline value on thermal withdrawal threshold also suggested that baseline nociception is a variable that should be included in subsequent statistical models because it significantly influences the response and may confound treatment effects.

We did not detect a significant effect of time on withdrawal temperatures, which indicated that buprenorphine’s initial effects were within 30 minutes after administration and lasted longer than the 6-hour postadministration period of the present study. Because there was no significant effect detectable, no reliable estimate can be provided for temporal analgesic effects of buprenorphine for different methods; thus, it was concluded that the analgesic efficacy of buprenorphine is effective in controlled clinical trials.\textsuperscript{32}

The dosages used in the present study were selected on the basis of doses in other avian species, which range from 0.1 to 0.75 mg/kg.\textsuperscript{19,20,22,28} The sample size (n = 12) for the study was selected on the basis of previous experiments that used a similar design and yielded significant results\textsuperscript{14–16} as well as the number of kestrels available at the time of the study. A sample size analysis was performed by use of the output of the linear mixed model reported here. If the size of the treatment effect were to remain the same in a subsequent study, approximately 30 to 40 kestrels would be needed to detect a 50% increase (with a power of 80% and α of 5%) in thermal threshold between the largest 2 doses of buprenorphine that were used in the study reported here.

Measuring antinociceptive effects by application of a noxious thermal stimulus is a method that involves cutaneous nociceptive thermal receptors, polymodal receptors,\textsuperscript{29} and afferent Aδ and C fibers that transmit nociceptive information to different areas of the midbrain and forebrain via ascending spinal pathways.\textsuperscript{30} The use of a phasic thermal noxious stimulus from which an animal can withdraw provides a noninvasive method for evaluation of nociceptive thresholds and analgesic modulation of those nociceptive thresholds, but further studies with other methods are necessary for a full evaluation of an analgesic drug.

Thermal stimuli appear to be appropriate for investigation of the potential antinociceptive effect of buprenorphine, although it was found that buprenorphine is much more potent (50 to 400 times) against nonthermal than against thermal stimuli in mice and rats.\textsuperscript{6} However, a more recent study\textsuperscript{9} involving mice and rats revealed comparable results on the efficacy of buprenorphine for different methods; thus, it was concluded that the analgesic efficacy of buprenorphine is not limited by the nature and intensity of the painful stimulus. Furthermore, μ-opioid receptor knockout mice had increased sensitivity to a thermal stimulus, which implicates the receptor in this mode of nociception.\textsuperscript{31} Regardless, the doses of buprenorphine required to induce antinociception in analgesiometric evaluations of rats by use of a thermal stimulus are higher than those that are effective in controlled clinical trials.\textsuperscript{32}

The thermal nociceptive response has been used to evaluate several opioids at various doses in several avian species. The response to a thermal stimulus was found to be a reliable measurement in African grey parrots,\textsuperscript{14} cockatoos,\textsuperscript{33} and chickens.\textsuperscript{34} The thermal nociceptive response has also been found to be a reliable measurement in previous studies in American kestrels.\textsuperscript{15–16} However, the response to a thermal stimulus was considered inconsistent in a study\textsuperscript{28} in which investigators evaluated the antinociceptive effects of 1 mg of butorphanol/kg in African grey parrots. An electrical stimulus has also been used in birds, but during a preliminary unpublished study conducted by our research group, we found that an electrical stimulus was a perturbing factor that led to unreliable measurements when applied repeatedly at various times during a 6-hour period.
Results of studies of American kestrels support the use of μ-opioid receptor agonists such as hydromorphone and buprenorphine, but they do not support the use of a κ-opioid receptor agonist and μ-opioid receptor antagonist such as butorphanol. These findings differ from those reported for psittacine birds. The reasons for these differences are unknown, but they may have been attributable to differences in the number and distribution of the various types of opioid receptors in the CNS and peripheral nervous system. Previous studies on the distribution of μ-, δ-, and κ-opioid receptors in the forebrains of humans, rats, monkeys, mice, guinea pigs, and pigeons revealed that there is remarkable similarity in the distribution of opioid receptors within the avian telencephalon but wide variation in the predominance of the various receptor types in the forebrains of different species. Pigeons predominantly have κ-opioid receptors (76%), whereas rats and mice have a low percentage of κ-opioid receptors (9% and 13%, respectively) with a predominance of δ-opioid receptors (50% and 62%, respectively). Further studies to compare the number and distribution of opioid receptors among various psittacine and raptor species would be needed to validate this theory.

In the present study, buprenorphine hydrochloride administered IM at 0.1, 0.3, and 0.6 mg/kg to American kestrels significantly increased the foot withdrawal threshold to a noxious thermal stimulus, when compared with the response for a saline solution control treatment. All doses of buprenorphine provided analgesia for at least 6 hours, although there was minimal sedation evident at the highest dose. Further studies with other types of noxious stimuli are needed to fully evaluate the analgesic and adverse effects of buprenorphine hydrochloride in kestrels and other avians.

References

5. Picker MJ. Kappa agonist and antagonist properties of mixed ac-

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**Appendix**

Agitation-sedation score used to assess behavioral effects after IM administration of buprenorphine hydrochloride to American kestrels (*Falco sparverius*).

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Kestrel does not remain on perch and constantly flies off the perch</td>
</tr>
<tr>
<td>2</td>
<td>Kestrel intermittently flies off perch but returns to the perch on its own</td>
</tr>
<tr>
<td>1</td>
<td>Kestrel remains on perch but constantly looks around</td>
</tr>
<tr>
<td>0</td>
<td>Kestrel remains on perch, is calm, and does not look around but is extremely reactive to movement that takes place in front of the test box</td>
</tr>
<tr>
<td>–1</td>
<td>Kestrel remains on perch, is calm, and has only a sluggish response to movement that takes place in front of the test box</td>
</tr>
<tr>
<td>–2</td>
<td>Kestrel does not react to movement that takes place in front of the test box and only reacts if the back of the test box is opened and a hand is inserted into the box</td>
</tr>
<tr>
<td>–3</td>
<td>Kestrel is only responsive when touched</td>
</tr>
<tr>
<td>–4</td>
<td>Kestrel is unresponsive to any visual or tactile stimulus</td>
</tr>
</tbody>
</table>