Effects of three fentanyl plasma concentrations on the minimum alveolar concentration of isoflurane in Hispaniolan Amazon parrots (Amazona ventralis)

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OBJECTIVE
To determine effects of 3 plasma concentrations of fentanyl on the minimum alveolar concentration of isoflurane (MAC$_{iso}$) and cardiovascular variables in Hispaniolan Amazon parrots (Amazona ventralis).

ANIMALS
6 adult parrots.

PROCEDURES
In phase 1, anesthesia was induced and maintained with isoflurane; intermittent positive-pressure ventilation was provided. The MAC$_{iso}$ was determined for each bird by use of a bracketing method and supramaximal electrical stimulus. Fentanyl (20 µg/kg) was administered IV, and blood samples were collected over time to measure plasma fentanyl concentrations for pharmacokinetic calculations. In phase 2, pharmacokinetic values for individual birds were used for administration of fentanyl to achieve target plasma concentrations of 8, 16, and 32 ng/mL. At each concentration, MAC$_{iso}$ and cardiovascular variables were determined. Data were analyzed with mixed-effects multilevel linear regression analysis.

RESULTS
Mean ± SD fentanyl plasma concentrations were 0 ng/mL, 5.01 ± 1.53 ng/mL, 12.12 ± 3.58 ng/mL, and 24.93 ± 4.13 ng/mL, and MAC$_{iso}$ values were 2.09 ± 0.17%, 1.45 ± 0.32%, 1.34 ± 0.31%, and 0.95 ± 0.14% for fentanyl target concentrations of 0, 8, 16, and 32 ng/mL, respectively. Fentanyl significantly decreased MAC$_{iso}$ in a dose-dependent manner. Heart rate and blood pressure significantly decreased at all fentanyl doses, compared with values for MAC$_{iso}$ at 0 ng of fentanyl/mL.

CONCLUSIONS AND CLINICAL RELEVANCE
Fentanyl significantly decreased the MAC$_{iso}$ in healthy Hispaniolan Amazon parrots, but this was accompanied by a depressive effect on heart rate and blood pressure that would need to be considered for application of this technique in clinical settings. (Am J Vet Res 2018;79:600–605)

Amazon parrots and other parrot species frequently require general anesthesia to enable veterinarians to perform medical or surgical treatments. General anesthesia of birds is most commonly accomplished with inhalation agents such as isoflurane and sevoflurane. Inhalation anesthetic agents have the advantage of allowing rapid changes in anesthetic depth, and recovery from anesthesia is generally rapid because inhalation anesthetics are eliminated via the respiratory system. However, inhalation anesthetic agents can induce cardiovascular and respiratory depression, which may be life-threatening, especially in compromised patients.

Balanced anesthesia techniques, which are achieved by combining inhalation anesthetics with other drugs such as parenterally administered opioids, are commonly used in mammals to reduce cardiovascular depression by reducing the necessary concentrations of the inhalation anesthetics. Fentanyl citrate, a synthetic injectable µ-opioid receptor agonist, has been used extensively in both human and veterinary medicine because of its rapid onset and short duration of action. Studies in humans, dogs, rats, and red-tailed hawks (Buteo jamaicensis) have revealed that fentanyl significantly reduces the MAC$_{iso}$, and greater hemodynamic stability is achieved when fentanyl is used as part of a balanced anesthetic technique, compared with results when an inhalation agent is used alone. However, responses differ among species. A steep reduction (63%) in MAC$_{iso}$ is evident in people when a fentanyl plasma concentration of 3 ng/mL is achieved, and increases in plasma concentrations up to 10 ng/mL only lead to a further reduction of...
19% in MAC_{iso}.^{4} In contrast, no significant change in MAC_{iso} was detected in horses after fentanyl administration.^{8}

Birds have opioid receptors,^{9-11,a} but the quantity and expression of each receptor type have not been reported, and evaluation of the antinociceptive properties and MAC_{iso}-sparking properties of opioids, especially μ-opioid receptor agonists, has been limited. Administration of a single dose of morphine sulfate or fentanyl citrate causes a dose-dependent decrease in MAC_{iso} in chickens.^{12,13} Administration of methadone (6 mg/kg, IM) significantly reduces MAC_{iso} by 30% in hens 15 minutes after administration, but it also causes mild respiratory acidosis and an increase in systemic blood pressure. Atroventricular block was induced in 3 birds, and ventricular premature contractions were induced in 2 birds in another study.^^14 In red-tailed hawks, fentanyl citrate at mean ± SD plasma concentrations of 8.51 ± 4 ng/mL, 14.85 ± 4.82 ng/mL, and 29.25 ± 11.52 ng/mL decreased MAC_{iso} by 31%, 44%, and 55%, respectively.^^15 However, to our knowledge, no other studies on MAC reduction with these or other μ-opioid receptor agonists for any species of psittacine birds have been published. The antinociceptive effects of a single dose of fentanyl citrate have been evaluated in only 1 study in psittacine birds. In that study,^^16 a higher tolerance to a thermal noxious stimulus was identified when a single high dose of fentanyl citrate (200 μg/kg) was administered SC to conscious white cockatoos (Cacatua alba), but lower doses (10 and 20 μg/kg) administered IM had no significant effect. The maximal fentanyl plasma concentration achieved with the dose of 20 μg/kg was 3.33 ng/mL, which is within the range considered analgesic in humans but lower than the target plasma concentration for other species. The objective of the study reported here was to determine the efficacy of fentanyl citrate to decrease the anesthetic concentrations of isoflurane needed to block the response to an electrical noxious stimulus in Hispaniolan Amazon parrots (Amazona ventralis).

Materials and Methods

Animals
Six Hispaniolan Amazon parrots (3 males and 3 females) were used in the study. Birds were 12 to 28 years old and had a body weight of 258 to 316 g (mean ± SD, 280 ± 16 g). These birds have been used previously in a number of other studies;^{16-18} however, they had not been used in the 90 days preceding this study. Health of each bird was assessed on the basis of results of a physical examination, CBC, and plasma biochemical analysis. Food and water were not withheld before the experiments were performed; instead, the birds were moved from the overnight housing room to the study area immediately after the lights were turned on in the morning, which minimized food consumption before the initiation of each phase of the study. The crop of each bird was palpated before the beginning of each experiment to ensure it was empty before anesthesia was initiated. At the conclusion of the study, the birds were returned to the research colony. The study was approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Study design
The study was conducted at sea level and comprised 2 phases. In phase 1, the birds were anesthetized with isoflurane, and the MAC_{iso} was determined. Once the MAC_{iso} was determined, fentanyl citrate was administered (20 μg/kg, IV). Blood samples were obtained before (time 0) and 1, 5, 10, 15, 30, 60, 120, and 180 minutes after fentanyl administration. Plasma was harvested; samples were analyzed to determine the fentanyl concentration, and the pharmacokinetic variables of fentanyl were calculated for each bird.

After a washout period of ≥ 4 weeks, the birds were again anesthetized with isoflurane for phase 2. The birds were instrumented, and the isoflurane concentration was decreased to approximately 0.75 times the individual MAC_{iso}. Fentanyl then was administered to achieve targeted plasma concentrations of 8, 16, and 32 ng/mL. The MAC_{iso} was determined at each fentanyl concentration.

Data collection
The birds were weighed immediately before each experiment. During phase 1, anesthesia was induced with isoflurane in 95% to 98% oxygen via a face mask with a specially designed laboratory animal circle system.^^5 After an appropriate depth of anesthesia was achieved, the birds were orotracheally intubated with a 3.0-mm uncuffed endotracheal tube, and anesthesia was maintained with isoflurane in oxygen. Ventilation was controlled with intermittent positive-pressure ventilation set at 10 breaths/min. The side port on the endotracheal tube was connected to a multiparameter monitor for continuous measurement of concentrations of inspired and end-tidal oxygen and isoflurane and the PetCO₂. The port was also used to manually obtain samples of end-tidal gas over a period of 3 breaths; the concentration of isoflurane for those samples was measured by use of a separate infrared analyzer.^^6 The analyzer was calibrated before each experiment by use of 2 concentrations of isoflurane (2.55% and 1.42%) in nitrogen, and a 3-point curve (which included 0%) generated with these measurements was used to adjust the measured concentrations. A thermistor was placed in the esophagus at the level of the heart and connected to the aforementioned multiparameter monitor for continuous monitoring of body temperature. External heat (warm water blankets or forced-air blankets [or both]) was used to maintain core body temperature at 37.4° to 40.2°C. A Doppler ultrasonographic probe was placed over the ulnar artery, and a neonatal No. 1 cuff was placed over the humerus (cuff width was 40% of the circumference of the wing). Catheters were placed percutaneously into the ulnar vein of the wing contralateral to the Doppler ultrasonographic...
probe and into a jugular vein. Lactated Ringer solution was administered through the ulnar venous catheter at a rate of 3 mL/kg/h throughout the anesthesia period. An ECG was attached to each bird by use of a 3-lead configuration; the lead then was connected to the multiparameter monitor. Heart rate (from the ECG), blood pressure (measured indirectly by Doppler ultrasonography), PetCO₂, inspired oxygen concentration, body temperature, and respiratory rate were recorded every 5 to 10 minutes.

Two insulated needles were placed into the subcutaneous tissues on the medial side of the tibia and connected to an electrical stimulator. These were used to provide a supramaximal stimulus at 30 V and 30 Hz with a duty cycle of 75 milliseconds. After the catheters and needles were placed, the end-tidal isoflurane concentration was stabilized and maintained at that concentration for 15 minutes. An electrical stimulus was applied and the bird observed for signs of movement. Any gross movement (eg, partial wing flap) was considered a positive result. Increases in respiration or heart rate and vocalization were not regarded as a positive reaction. The stimulus was applied for 60 seconds, unless the bird moved prior to that time, in which case the stimulus was terminated. After a positive reaction was detected, the end-tidal isoflurane concentration was increased by 10%, and the concentration was decreased by 10% after a negative reaction (no movement). Each MACiso was the mean value of the concentration at which the bird moved and the concentration at which it did not move. Testing was repeated, with 15-minute equilibration periods, to obtain 3 MACiso values. The final MACiso value for each bird was calculated as the mean of these 3 values.

After the MACiso was determined, the end-tidal isoflurane concentration was decreased to 0.75 times the MACiso, and 15 minutes was allowed for birds to stabilize at the new concentration. A bolus of fentanyl (20 µg/kg, IV) then was administered through the medial ulnar venous catheter over a 20-second period. Venous blood samples (0.3 mL) were collected from the jugular vein catheter immediately before (time 0) and 1, 5, 15, 30, 60, 120, and 180 minutes after fentanyl administration. Samples were transferred into sterile tubes containing lithium heparin and immediately centrifuged at 3,900 X g for 10 minutes. Plasma was harvested and frozen at -80°C until the samples were analyzed.

After the final sample was obtained from each bird, all instrumentation was removed, and administration of isoflurane was discontinued. Meloxicam (1 mg/kg, IM) was administered IM to each bird after the 180-minute sample had been collected but before recovery from anesthesia. All birds were monitored for evidence of pain (altered behavior, wing droop, or lameness) for 48 hours after the procedures.

**Pharmacokinetic analysis**

Individual pharmacokinetic data generated from these same birds in another study were used to determine fentanyl infusion rates. Selected volume and rate-constant variables were entered into a computer program and used to determine the delivery of fentanyl as target-controlled infusions for the pharmacodynamic evaluation.

For phase 2 of the study, birds were anesthetized and instrumented in the same manner as for phase 1, and the end-tidal concentration of isoflurane was stabilized at approximately 0.75 times the previously determined MACiso. Fentanyl citrate (50 µg/mL) was administered IV via the medial ulnar catheter by use of a target-controlled infusion system consisting of a syringe pump and computer program to rapidly achieve the desired plasma concentrations. Target fentanyl plasma concentrations of 8, 16, and 32 ng/mL were evaluated in ascending order to decrease the duration of the experiment. The infusion rate was updated every 10 seconds to maintain pseudo-steady-state plasma concentrations. During the infusion, the amount of fentanyl delivered that was displayed by the pump was frequently compared with the computer calculation of the amount delivered and the actual amount left in the syringe. The MACiso was determined in duplicate by use of the previously described method for each fentanyl concentration. Immediately before the second and third MACiso stimulation points, a blood sample (0.3 mL) was collected from the jugular vein and processed as in phase 1 for measurement of the plasma fentanyl concentration. Once MACiso was determined at each fentanyl concentration, the next concentration of fentanyl was established and maintained for 15 minutes before the subsequent electrical stimulation. After all data were recorded, the fentanyl infusion was discontinued, all instrumentation was removed, and isoflurane administration was discontinued. Meloxicam was administered (1 mg/kg, IM), and the birds were allowed to recover from anesthesia. The birds were constantly monitored until they were able to stand. All birds were monitored for evidence of pain (altered behavior, wing droop, or lameness) for 48 hours after the procedures.

**Statistical analysis**

The D'Agostino-Pearson omnibus test was used to assess normality of data at each concentration. Data were analyzed by use of mixed-effects model regression analysis to evaluate the association between plasma fentanyl concentration and MACiso, body temperature, heart rate, PetCO₂, and blood pressure. The analysis controlled for time, and bird was included as a random effect. Standardized residuals were calculated for each model and used in probability plots to verify the assumption of normality. Values for body temperature, heart rate, PetCO₂, and blood pressure obtained before each positive and negative point around the MACiso determinations were used in the analysis; values obtained during phase 1 were compared with those obtained at each subsequent tar-
Results

After phase 1 was completed, 1 bird died as a result of trauma unrelated to the present study. That bird was replaced by another Hispaniolan Amazon parrot, which was subjected to and completed the testing for phase 1. Thus, the 6 birds reported here completed both phases of the study. Mean ± SD time to determine MACiso in phase 1 was 183 ± 37 minutes, and the birds were anesthetized for a total of 372 ± 34 minutes. In phase 2, the birds were anesthetized for 409 ± 64 minutes. Mean ± SD duration of the infusions for the 3 targeted concentrations was 135 ± 39 minutes, 95 ± 20 minutes, and 142 ± 58 minutes for 8, 16, and 32 ng/mL, respectively. Recovery from anesthesia was rapid, with extubation of most birds within 15 minutes after discontinuation of isoflurane. None of the birds had signs of pain that required additional analgesics after the experiment.

Mean ± SD MACiso for the 6 parrots in phase 1 was 2.09 ± 0.17%. In phase 2, mean MACiso for the targeted concentrations of 8, 16, and 32 ng/mL was decreased to 1.45 ± 0.32%, 1.34 ± 0.31%, and 0.95 ± 0.14%, respectively, which resulted in MACiso reductions of 31%, 36% and 54%, respectively. The MACiso values for phase 2 were all significantly (P < 0.001) less than the MACiso value for phase 1. Mean ± SD actual plasma concentrations measured at the targeted values of 8, 16, and 32 ng/mL were 5.01 ± 1.55 ng/mL, 12.12 ± 3.58 ng/mL, and 24.93 ± 4.13 ng/mL, respectively.

Body temperature measured during the doses of 8 and 32 ng/mL was significantly higher than the value measured during the MACiso determinations for phase 1 (Table 1). Heart rate and blood pressure were significantly lower for all 3 fentanyl doses, compared with the value for the MACiso determination in phase 1. The PETCO2 was not different at any dose.

Discussion

Results of the study reported here indicated that a µ-opioid receptor agonist can have a significant effect on MACiso in Hispaniolan Amazon parrots. These results were specific for this species and these drugs, but it is quite reasonable to believe that they could be generalized to other inhalation agents and other µ-opioid receptor agonists. Although there are differences in the spectrum of activities associated with inhalation agents and opioids, they exert their effects through similar pathways in most species. Dose requirements for inhalation agents are similar between birds and mammals, with minimal species variation, despite big differences in metabolic activity and body size. In contrast, the MAC-sparing effect of fentanyl on inhalation agents is quite different among species, with up to a 70% reduction for some species (eg, dogs), whereas there is almost no change for other species (eg, horses). A significant MACiso reduction in the Hispaniolan Amazon parrots was likely attributable to the activity of fentanyl at µ-opioid receptors, but this does not necessarily mean that fentanyl would be antinociceptive in Hispaniolan Amazon parrots. The µ-opioid receptor agonists have analgesic effects in conscious horses but have a minimal effect on MACiso. However, there are no reports for other species that fentanyl has a significant effect on MACiso but not a demonstrable antinociceptive effect (when both have been evaluated).

The MACiso-sparing effects in the present study were extremely similar to those for red-tailed hawks; however, the infusion rates necessary to achieve these effects were much higher in the Hispaniolan Amazon parrots than in the red-tailed hawks. The final fentanyl plasma concentrations in the parrots of the present study were lower than those in red-tailed hawks. When the actual infusion rates were back-calculated as the volume delivered by the syringe pump and duration of the actual infusions by use of pharmacokinetic parameters for individual parrots (clearance X plasma concentration), the resulting infusion rates necessary to achieve almost identical MACiso-sparing effects at the low and high fentanyl dose were approximately 17 times those for red-tailed hawks, as determined by one of the investigators (PJP). In several parrots of the present study, there appeared to be no change in MACiso associated with the middle fentanyl dose; thus, the results indicated a smaller effect with the dose of 16 ng/mL in Hispaniolan Amazon parrots than in red-tailed hawks.

For the study reported here, we chose to use an electrical stimulus of lower intensity (30 V and 30 Hz with a duty cycle of 7.5 milliseconds) because there was some tissue injury with the higher stimulus intensity used in a study of red-tailed hawks (50 V

<table>
<thead>
<tr>
<th>Target fentanyl concentration (ng/mL)</th>
<th>Heart rate (beats/min)</th>
<th>Blood pressure (mm Hg)</th>
<th>Body temperature (°C)</th>
<th>PETCO2 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>359 ± 81</td>
<td>144 ± 38</td>
<td>38.2 ± 0.3</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>302 ± 48*</td>
<td>124 ± 31*</td>
<td>38.5 ± 0.3*</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>16</td>
<td>281 ± 60*</td>
<td>106 ± 24*</td>
<td>38.4 ± 0.4</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>32</td>
<td>312 ± 69*</td>
<td>103 ± 34*</td>
<td>38.5 ± 0.3*</td>
<td>47 ± 3</td>
</tr>
</tbody>
</table>

*Within a variable, value differs significantly (P ≤ 0.05) from the value for the control MACiso determination (target fentanyl concentration of 0 ng/mL).
and 50 Hz with a duty cycle of 75 milliseconds). The control MAC\textsubscript{50} in that study\textsuperscript{7} of red-tailed hawks was 2.05 ± 0.45%, which is extremely similar to the control value determined in phase 1 of the present study (2.09 ± 0.17%), which suggested that the stimulus had a similar effect to that for red-tailed hawks. A voltage as low as 15 V in rats resulted in MAC values for several anesthetics that were indistinguishable from MAC values obtained by use of a tail clamp.\textsuperscript{25}

We chose to provide intermittent positive-pressure ventilation to the birds of the present study to ensure a stable PET\textsubscript{CO\textsubscript{2}}. Increases in CO\textsubscript{2} concentration have been associated with decreases in blood pressure in birds,\textsuperscript{24,25} but controlled ventilation has been associated with decreases in heart rates, including in Hispaniolan Amazon parrots.\textsuperscript{26} A reduction in heart rate of approximately 20% was detected in association with fentanyl administration, but the relationship to ventilation was not evident because the birds were ventilated during both phases of the study. Changes in heart rate identified in the present study could be clinically important in highly compromised patients. We also recorded a reduction in blood pressure that appeared to have some relationship to dose, although it was unlikely that there would be a difference in blood pressure between the 2 higher doses of fentanyl (data not evaluated). There was poor agreement between arterial blood pressures measured directly and with Doppler ultrasonography in another study.\textsuperscript{26} However, sequential measurements on the same bird may reflect patterns, even if they are not representative of actual pressures. We were unable to measure cardiac output in these small birds, so the changes in heart rate and blood pressure cannot necessarily be interpreted as a decrease in blood flow, but some of the values recorded during administration of the highest fentanyl dose were quite low. For example, 8 of 18 blood pressure values were ≤ 80 mm Hg during the dose of 32 ng/mL, compared with only 2 of 23 and 2 of 18 low values for the 8- and 16-ng/mL doses, respectively. In the previously mentioned study\textsuperscript{26} in which blood pressure was measured in Hispaniolan Amazon parrots, the directly measured systolic pressure probably would have been higher, but that study did not include pressures < 100 mm Hg, so we cannot be sure of the relationship at these lower pressures. It is also possible that this was merely a reflection of time because infusion of the highest dose was started 4 to 5 hours after the induction of anesthesia. However, the birds in phase 1 were also anesthetized for long periods without a similar decrease in blood pressure. These clinical observations would suggest that the highest fentanyl dose may not be extremely useful in this species because of increasingly profound effects on heart rate and blood pressure. However, bradycardia associated with infusions of fentanyl to dogs can be reversed with an anticholinergic drug, with the effect that cardiac output, blood pressure, and heart rate are significantly increased.\textsuperscript{2} Use of anticholinergic drugs was not attempted in the present study because we did not want to have any influence of other drugs in the birds. In red-tailed hawks\textsuperscript{27} and chickens,\textsuperscript{13} there are no significant cardiovascular changes associated with fentanyl administration.

Body temperature can affect MAC, and the 8- and 32-ng/mL doses caused significantly higher body temperatures. However, on the basis of results of a study of dogs,\textsuperscript{27} this difference in body temperature would represent an increase in MAC of approximately 2% (eg, the value for 32 ng/mL would have been 0.97% instead of 0.95%). To our knowledge, temperature effects on MAC have not been reported for birds.

In the present study, fentanyl had a significant effect on the MAC\textsubscript{50} of healthy Hispaniolan Amazon parrots. This MAC-sparing effect was accompanied by a depressive effect on heart rate and blood pressure that would need to be considered if this technique were to be applied in clinical settings.

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**Footnotes**


b. Hospira Inc, Lake Forest, Ill.


d. GE/Datex-Ohmeda 5/Scompact, GE Healthcare Technologies, Madison, Wis.

e. Beckman medical gas analyzer LB-2, Beckman Instruments Inc, Schiller Park, Calif.

f. Model 811-BTS, Parks Medical Electronics, Aloha, Ore.

g. Pedisphyg neonatal cuffs, Artemis Medical Ltd, Kent, England.

h. 26-gauge, Insyte, Becton Dickinson Infusion Therapy Systems Inc, Sandy, Utah.

i. 22-gauge, Insyte, Becton Dickinson Infusion Therapy Systems Inc, Sandy, Utah.

j. Grass S88 stimulator, Grass Technologies Corp, Quincy, Mass.

k. Metacam, 5 mg/mL, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.

l. Rugloop I, Demed, Tense, Belgium.


n. Prism, version 6, Graphpad Software Inc, La Jolla, Calif.

o. Stata, version 13, StataCorp LLC, College Station, Tex.

**References**


