Subcutaneous administration of hydromorphone (0.2 mg/kg) provides antinociception in ferrets (*Mustela putorius furo*)

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O**B**JECTIVE
To evaluate antinociceptive efficacy of SC administration of hydromorphone hydrochloride and buprenorphine hydrochloride in ferrets (*Mustela putorius furo*).

A**N**IMALS
14 healthy adult ferrets (6 neutered males, 8 spayed females).

M**E**THODS
In a randomized, blind, controlled, complete crossover design, all 14 ferrets received a single, SC injection of hydromorphone low dose (0.1 mg/kg), hydromorphone high dose (0.2 mg/kg), buprenorphine low dose (0.02 mg/kg), buprenorphine high dose (0.04 mg/kg), or saline solution (0.2 mL/kg). Sedation and forelimb withdrawal latency from a noxious thermal stimulation were evaluated, and behavior was recorded for a total of 8 hours postinjection.

R**E**SULTS
Compared to saline, administration of hydromorphone at 0.2 mg/kg resulted in an estimated increase of withdrawal latencies of 7.4 seconds (95% CI, 3.2 to 11.6) at 60 minutes, of 6.6 seconds (2.4 to 10.8) at 90 minutes, of 6.0 seconds (1.8 to 10.2) at 120 minutes, of 7.0 seconds (2.9 to 11.1) at 180 minutes, and of 4.5 seconds (0.5 to 8.6) at 240 minutes. These differences were statistically significant. Hydromorphone administered at a lower dose and buprenorphine at either dose did not increase withdrawal latencies compared to saline. Based on the sedation score used in this study, signs of sedation increased over time in a similar fashion with all treatments, including saline. Erratic dysphoric-like behaviors occurred in all groups except for saline.

C**L**INICAL RELEVANCE
SC administration of hydromorphone at a dose of 0.2 mg/kg provided antinociception from 1 to 4 hours postinjection. Further validation of sedation scores in ferrets is warranted.

K**e**ywords: ferrets, buprenorphine, hydromorphone, opioid, antinociception

Ferrets (*Mustela putorius furo*) are commonly kept pets regularly presented for veterinary care with potentially painful conditions such as foreign bodies, neoplastic disease, or trauma.1 Despite their popularity, there is a surprising lack of information regarding the effectiveness of analgesic agents in ferrets. Indeed, no studies evaluating the effectiveness of analgesic drugs in ferrets are published, other than 1 study2 evaluating the postoperative analgesic effect of morphine administered epidurally. Analgesic agents (e.g., NSAIDs or opioids) and their suggested dosages and routes of administration are therefore often extrapolated from other species, such as dogs or cats.3-7 Considering that opioids may have species-specific effects,8 administration of opioids in a species without previous research may result in a lack of effectiveness or adverse effects.

Buprenorphine is classified as a partial μ-opioid receptor agonist. It is available worldwide and is commonly used in veterinary medicine, including in small exotic mammals. Its use has been recommended in ferrets specifically,3-7 and the recommended...
ranges from 0.01 to 0.06 mg/kg by SC, IM, IV, or transmucosal route, with a dosing interval of 4 to 12 hours. A study\(^6\) evaluated the pharmacokinetics of buprenorphine at a dose of 0.04 mg/kg, IM, in ferrets. The pharmacokinetic profile of buprenorphine was found to differ substantially from dogs or cats, with a half-life of approximately 3.6 hours in ferrets. The antinociceptive or analgesic potential of buprenorphine was not assessed. Anecdotal reports\(^5,6\) mention possible adverse effects, such as profound sedative effects at the higher dose range.

Hydromorphone is a \(\mu\)-opioid receptor agonist reported to be 5 times more potent than morphine,\(^10\) and it is suitable to manage more intense pain levels. It is commonly used as a nociceptive agent for which dosages and routes of administration have been recommended in ferrets specifically.\(^3,6\) The recommended dose ranges from 0.025 to 0.2 mg/kg, SC, IM, or IV, with a dosing interval of 1 to 8 hours. A study\(^6\) evaluated the pharmacokinetic profile of hydromorphone at a dose of 0.1 mg/kg, SC, in ferrets. Based on that study, the dosage might have been insufficient to reach serum hydromorphone concentrations associated with antinociception in cats for more than an hour.\(^1,3\) The antinociceptive or analgesic potential of hydromorphone was not assessed. Anecdotal reports\(^5,6\) mention possible adverse effects, such as profound sedation, vomiting, bradycardia, and respiratory depression.

The objective of the present study was to determine the antinociceptive efficacy of 2 doses of buprenorphine and 2 doses of hydromorphone administered SC in ferrets, using a plantar testing device based on the Hargreaves method. Our hypotheses were that both doses of SC buprenorphine would provide antinociception for up to 6 hours, while only the higher dose of hydromorphone (0.2 mg/kg, SC) would provide antinociception for up to 4 hours, with possible sedation. It was anticipated that these hypotheses would be accepted based on the reported dosages, dosing intervals,\(^3,6\) and pharmacokinetics\(^9\) of buprenorphine and hydromorphone in ferrets.

### Methods

#### Animals

The study protocol was approved by the University of Saskatchewan Western College of Veterinary Medicine Ethics Boards under the Animal Use Protocol No. 20200091. A power study was performed using an online tool (https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html), and the data were drawn from similar studies\(^1,10,12,13\) conducted in other species. The means and standard deviations of withdrawal times for these studies range from approximately 8 ± 1.7 to 11 ± 2.1 seconds for saline groups and 11.2 ± 1.7 to 15 ± 2.2 seconds for treated groups. Based on these values, the number of animals needed to obtain a statistical power of 80% ranged from 5 to 15 per treatment group. We therefore elected to use 15 ferrets.

Fifteen purpose-bred adult ferrets (7 neutered males, 8 spayed females) were obtained from a commercial breeder (Triple F Farms). The age ranged from 2 to 3 years old and the mean ± SD body weight was 1.106 ± 0.439 kg (range, 0.67 to 1.55 kg). Animals were housed in a climate-controlled room with a 12:12-h light cycle (07:00 to 19:00). Room temperature was maintained at 19 to 21 °C and relative humidity was 30 to 52%. The ferrets were group-housed communally in a room with various hides, food/water bowls, and toys. A 3-tiered ferret cage was also always accessible for ferrets to play or sleep in. They were fed a commercial pelleted ferret diet (Mazuri Exotic Nutrition Ferret Diet; 5M08; PMI Nutrition International) ad libitum and received tap water from rabbit ball-tipped water bottles and free-flowing water dishes without restriction. Food was offered in multiple plastic bowls on the room floor and in cage-mounted food dispensers and was not withheld before the trials. The location of the water bottle and food bowls was the same throughout acclimatization and the study period. All ferrets were acclimated to the housing conditions for at least 2 weeks before starting the experiments. All animals were deemed to be clinically healthy based on repeated physical examinations, daily visual examinations, monitoring of food intake and fecal output throughout the study, and weekly weight monitoring. Trials occurred in a separate climate-controlled room next door to the housing room, with a similar temperature setup.

#### Antinociceptive evaluation

Analgesimetry experiments were performed by using a plantar testing device (Plantar Test with Heated Base; IITC Life Science), designed based on the Hargreaves method, as described in other species.\(^10,12,14\) The animals were placed in ventilated measurement chambers (33 X 22.86 X 30.48 cm, 13 X 9 X 12 inches) positioned on a heated glass surface maintained at 32 °C. A noxious infrared radiant heat stimulus was applied to the palmar surface of the manus, on the torus metacarpus (metacarpal pad). The radiant heat beam active intensity was set at 65%, which correlates with a surface temperature of 50 °C, and an idle intensity of 5%. The idle intensity did not exceed the heated glass base's temperature of 32 °C, and the radiant heat beam produced an almost–immediate increase in temperature from 32 °C to 50 °C when activated. Forelimb withdrawal latencies were recorded in seconds, and both forelimbs were used alternately to prevent sensitization. The skin of the torus metacarpus used for thermal testing was examined for abnormalities, such as erythema, swelling, or erosion, daily during the study period. The thermal stimulus was immediately terminated when the ferret moved the limb to which the stimulus was applied in response to the stimulus. A stimulus cut-off time was set at 25 seconds to prevent tissue damage, even if no withdrawal response had occurred. Two forelimb withdrawal latencies were recorded at each time point, including baseline, and the median was used for analysis. When these 2 latencies varied by more than 20%, a third measurement was recorded, and the median of all 3 latencies was used for data analysis, as previously described in other species.\(^10,11\) The ferrets

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were placed in the Hargreaves apparatus chamber and allowed to acclimate for 1 to 2 minutes before starting measurements, and an interval of at least 1 min was maintained between each measurement to provide rest while limiting the total time spent in the chamber at each time point. The ferrets spent a maximum of 10 minutes in the chamber at each time point. After that time, the ferrets were removed from the chamber and the median of measurements obtained during that time were used for analysis. If no measurement could be obtained within 10 minutes, “missing data” were documented for that ferret and time point. The measurements were only acquired when the ferrets were awake and undistressed. The ferrets were removed from the chamber immediately after the last measurement of that time point was obtained. A single observer (AC) performed all measurements and was blinded to the treatment group.

One of the male ferrets failed to acclimatize to the testing chamber and appeared constantly stressed and restless while in it, making measurement impossible. He was therefore excluded from the study after his first treatment, and only 14 ferrets (6 neutered males and 8 spayed females) were used for the rest of the study.

**Sedation score**

Immediately before each thermal withdrawal latency measurement, each ferret was evaluated for signs of sedation using a scoring system modified from Philips et al (Table 1). A baseline sedation score was determined before injection. Sedation scores ranged from 0 to 10. In addition to the sedation scoring, during each measurement event, the behavior of the ferret in the measurement chamber was recorded.

**Experimental protocol**

In a randomized, blind, controlled, complete crossover design, all 14 ferrets received a single SC injection of hydromorphone (hydromorphone hydrochloride injection USP, 2 mg/mL) at a dose of 0.1 or 0.2 mg/kg, SC; buprenorphine (Vetergesic Multidose, 0.3 mg/mL; Ceva Animal Health Inc) at a dose of 0.02 or 0.04 mg/kg, SC; or saline (sodium chloride, 0.9%; BD PosiFlush; Beckon-Dickinson Company) at a dose 0.2 mL/kg, SC. All SC injections were performed using a 22-G needle and 1-mL syringe, and they were administered between the scapulae by one person while the other person manually restrained the ferret. Treatments were administered in a randomly determined order with a minimum washout period of 7 days. Randomization was achieved using online software (www.randomizer.org). Baseline thermal withdrawal latencies and sedation score were obtained immediately before injection. Forelimb withdrawal latencies were then measured 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours after each injection.

**Statistical analysis**

Continuous variables were reported as median and range. Generalized linear mixed models were built for the primary outcome (withdrawal latency, continuous) and the secondary outcome (sedation score, multinomial), accounting for repeated ferret analyses. For each outcome, treatment, time, and the interaction between treatment and time were included as fixed effects, while the individual ferret was included as a random effect. The random effect block included the intercept and used the variance component as the random effect covariance type. To report the estimated variation over time of the sedation score for each treatment, 4 additional generalized linear mixed models were built with each 1 of the treatments as the referent category. Statistical significance was considered $P < .05$. Analysis was performed with commercial software (SPSS Statistics V.24.0).

**Results**

**Withdrawal latency**

Summary results of withdrawal latency are reported (Table 2). There were no significant differences in withdrawal latency at baseline in ferrets
administered different treatments (Supplementary Table S1). There were no significant changes in withdrawal latency over time in ferrets administered saline. Compared to saline, there was a significant increase in withdrawal latency in ferrets administered hydromorphone at 0.2 mg/kg starting from 60 minutes and ending at 240 minutes from the administration (Supplementary Figure S1). Administration of hydromorphone at 0.2 mg/kg resulted in an estimated increase of withdrawal latency of 7.4 seconds (95% CI, 3.2 to 11.6) at 60 minutes, of 6.6 s (2.4 to 10.8) at 90 minutes, of 6.0 seconds (1.8 to 10.2) at 120 minutes, of 7.0 seconds (2.9 to 11.1) at 180 minutes, and of 4.5 s (0.5 to 8.6) at 240 minutes, compared to administration of saline. The other treatments did not result in significant changes in withdrawal latency compared to saline.

Sedation score
There were no significant differences in sedation score at baseline in ferrets administered different treatments (Supplementary Table S2). Sedation scores increased over time in ferrets administered any treatment, including saline (Supplementary Table S3). There were no significant differences in how the sedation scores changed depending on the treatment administered.

Behavior
In all groups except for saline, changes in behavior were observed, including lip licking, attempts at chewing the enclosure, frantic digging, rolling, shuffling, aggression toward handlers, or holding forepaws together and shaking the forearms. All these behaviors were characterized by being repeated and

| Table 2—Summary results of withdrawal latency medians and minimum and maximum values. |
|---|---|---|---|---|
| Time (min) | Saline | Buprenorphine 0.02 mg/kg | Buprenorphine 0.04 mg/kg | Hydromorphone 0.1 mg/kg | Hydromorphone 0.2 mg/kg |
| Time 0 | 10.4 (4.2–23.2) | 11.8 (5.1–17.1) | 10.0 (5.8–14.4) | 10.7 (5.2–17.1) | 7.7 (4.7–13.2) |
| Time 30 | 13.1 (7.3–20.0) | 11.5 (6.3–24.1) | 10.5 (5.7–23.0) | 11.5 (4.5–20.0) | 11.8 (9.1–25.0) |
| Time 60 | 11.8 (5.8–16.6) | 13.7 (8.6–25.0) | 10.7 (5.6–18.6) | 13.7 (6.7–21.8) | 17.5 (8.1–25.0) |
| Time 90 | 11.2 (7.4–18.4) | 13.4 (7.9–22.7) | 9.3 (5.9–25.0) | 11.6 (6.5–21.0) | 15.7 (7.6–25.0) |
| Time 120 | 11.2 (8.5–20.8) | 13.0 (5.7–18.6) | 9.9 (5.5–24.6) | 10.2 (5.8–20.0) | 14.3 (7.6–25.0) |
| Time 180 | 11.2 (6.6–16.2) | 13.7 (9.3–22.7) | 13.8 (4.8–21.0) | 12.5 (7.9–20.0) | 14.8 (7.5–25.0) |
| Time 240 | 11.7 (6.5–20.0) | 13.2 (5.5–23.4) | 10.5 (7.6–25.0) | 11.9 (9.8–17.3) | 11.7 (8.2–24.2) |
| Time 360 | 10.8 (5.8–18.9) | 13.9 (5.6–20.7) | 13.9 (6.3–24.2) | 11.7 (9.3–20.0) | 10.3 (6.1–24.1) |
| Time 480 | 11.5 (6.9–16.6) | 13.5 (6.5–22.9) | 9.7 (5.6–17.1) | 12.5 (5.7–19.6) | 11.2 (5.2–17.1) |

Results of withdrawal latency medians are expressed in seconds, with minimum and maximum values in parenthesis. The withdrawal latencies were obtained in a total of 14 ferrets using a thermal plantar test based on the Hargreaves method to determine the antinociceptive properties of 2 different doses of SC hydromorphone and 2 different doses of SC buprenorphine compared to SC saline administration.

| Table 3—Number of ferrets out of 14 that displayed behavioral changes for each treatment group and at each time point. |
|---|---|---|---|---|---|---|---|---|
| Time point (min) | Number of behavioral changes events per group and time point (number of missing data in parentheses) | Total number of behavioral changes events | Total number of missing data occurrences |
| | 0 30 60 90 120 180 240 360 480 | 0 30 60 90 120 180 240 360 480 |
| Saline | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 0 0 0 0 |
| Buprenorphine at 0.02 mg/kg | 0 10 (1) 12 (1) 10 (1) | 5 (1) 5 (1) 2 1 2 | 47 5 |
| Buprenorphine at 0.04 mg/kg | 0 12 (1) 10 7 | 6 6 3 2 1 | 47 1 |
| Hydromorphone at 0.1 mg/kg | 0 8 (1) 10 (1) 9 (2) | 6 1 2 1 2 | 39 4 |
| Hydromorphone at 0.2 mg/kg | 0 10 (1) 8 (3) 6 (2) 6 (1) | 5 1 0 0 0 | 36 7 |
| Total number of behavioral changes events | 0 40 40 32 25 17 8 5 4 | 171 |
| Total number of missing data occurrences | 0 4 5 5 2 1 0 0 0 | 17 |

The number of occurrences of missing data for each time point is indicated in parentheses.
stereotypic, with a complete inability for the handler to distract the ferret by the means of a sharp sound (eg, clapping hands), offering food, or opening the enclosure. These behaviors started as early as 30 minutes postinjection and persisted intermittently up to 8 hours postinjection. They appeared very mild in some ferrets and more pronounced in others, without correlation with a specific treatment group, time point, or sex. When including all 5 treatment groups and all 14 ferrets, 70 measurement events occurred at each time point (2 to 3 withdrawal latencies obtained for each measurement event). When including all 9 time points, 630 measurements events occurred throughout the study. The behavioral changes were never observed in the saline group or during the baseline measurement event, and they were observed during 171 measurement events throughout the study, leading to the inability to obtain a measurement (missing data) at 17 times points. The number of ferrets that displayed those behavioral changes per time point and per treatment group are summarized (Table 3).

Discussion

Results of the present study indicated that in ferrets, SC administration of hydromorphone at a dose of 0.2 mg/kg increased the thermal withdrawal latency from 1 hour after injection up to 4 hours postinjection, thus suggesting that this treatment produced antinociception. Administration of SC buprenorphine (0.02 mg/kg and 0.04 mg/kg) or hydromorphone at a dose of 0.1 mg/kg, however, did not increase the thermal withdrawal latency in this study. For hydromorphone, this finding could be due to the dosage being insufficient. A single-dose study9 describes the pharmacokinetics of hydromorphone administered SC at a dose of 0.1 mg/kg in ferrets. The results of this study showed that hydromorphone only reached the plasma concentrations associated with analgesia in other species, which are reported to be 4 ng/mL in humans16 and between 2 and 3 ng/mL in dogs,17 for a short period of time. The half-life of SC hydromorphone in ferrets also appeared much shorter than in dogs (24.7 vs 39.6 minutes).9,18 and the mean plasma concentrations of hydromorphone decreased rapidly below the concentrations associated with analgesic effect in other species. These inadequate plasma levels associated with the short half-life suggested that ferrets may have a higher hydromorphone dose requirement than dogs. A study describing the pharmacokinetics of higher doses of SC hydromorphone would be required to investigate this hypothesis.

The duration of antinociception after SC injection of hydromorphone in this study was approximately 4 hours, with an onset at 1 hour postinjection, which is consistent with what has been reported in other species.19 A gradual increase in sedation score was observed over time in all groups, including saline. This observation suggests that the sedation score used may not be adequate. A possible contributing factor to this observation may be the higher frequency of measurements in the morning. This may have led to a higher level of stimulation at that time and lower level of stimulation later in the day in all groups, creating a longer resting period and more quiet animals in the afternoon. Ferrets also display circadian variations in their activity levels, which may have affected sedation scoring. Mean baseline activity levels tend to drop during and just after the transition from darkness to light, and activity levels are the highest immediately before and after this period,18 which would correspond to the morning measurement period in our study. Their natural activity level is then lower in the afternoon and may contribute to the gradual increase in sedation score observed in all groups in this study. More studies are necessary to establish a more appropriate sedation scoring system in ferrets.

The antinociceptive effect of buprenorphine was inconsistent in the present study, regardless of the dose administered. In cats, SC buprenorphine has been shown to have erratic absorption and disposition, which did not allow for pharmacokinetic modeling.20 A pharmacokinetic study9 was performed in ferrets using a dose of buprenorphine of 0.04 mg/kg, IM, and no erratic absorption or disposition was observed, supporting the theory of a route-dependent issue. A pharmacokinetic study of SC buprenorphine in ferrets will help determine if this also occurs in that species. While this could explain the lack of increase in thermal withdrawal in the present study, another possibility is that the buprenorphine dosages used were insufficient to produce reliable antinociception in this model. Thermal nociception models are widely accepted methods to evaluate antinociceptive efficacy in animals,14 but they are not validated in ferrets and may not predict accurately the potential analgesic effects of a drug for other type of pain stimuli, such as surgical or visceral pain.10,12 Thermal nociception models also tend to overestimate drug dose requirements to provide analgesia for other types of stimuli, and the dosages used in the present study might demonstrate analgesic properties in a different analgesia model.21

When receiving buprenorphine or hydromorphone, most ferrets displayed behavioral alterations, including overall increased and uncoordinated locomotor activity, lip licking, or digging. In several species, “dysphoric” behaviors have been described following opioid administration.8,22-28 Dysphoria is a known possible side effect of opioid administration,27-30 but it is not consistently defined, and the expressed behaviors vary with and within species.27 In dogs, panting, whining/whimpering, agitation, biting, uncoordinated behavior or thrashing have been associated with opioid-induced dysphoria.21,22,29-31 Increased locomotor activity, restlessness, pacing, or body-swinging was included in the description of opioid-induced dysphoria in horses.25,30 Dysphoric behaviors have been reported in either dogs, cats, or horses following administration of morphine, remifentanil, fentanyl, and
hydromorphone, and they are reported as less frequent after administration of buprenorphine, especially in the presence of pain.\textsuperscript{22,23,26,27,28,31} In these species, the contributing factors to opioid-induced dysphoria are not fully determined, but higher dose and SC administration seem to play a role.\textsuperscript{22,23}

In the present study, the altered behaviors observed in the ferrets that received an opioid could be attributed to opioid-induced dysphoria, but the reversal of abnormal behaviors after administration of naloxone or a sedative would have been necessary to prove this theory.\textsuperscript{22,23} The SC route of administration in animals that were only exposed to a transient noxious stimulus and the absence of these abnormal behaviors in the saline group support this theory, and further studies are necessary to better document the occurrence of opioid-induced dysphoria or neuroexcitation in ferrets.

Limitations of this study include the use of a non-validated method to assess nociceptive response to heat, as mentioned previously. Unexpected behavior changes caused difficulties in acquiring some of the measurements, but only 17 events of missing data occurred as the behaviors remained intermittent and ferrets would settle down most of the time. No measurements were acquired when the ferrets were distracted or sleeping to prevent bias. The ferrets were also moved in and out of the chamber before and after each measurement and stimulated by sedation scoring before each measurement, which contributed to some level of excitement and stress. This could have had an impact on measurement quality, especially since the ferrets may have been stressed while in the chamber despite being used to being handled. The sedation score used before each measurement was adapted from a study assessing a sedation protocol and may therefore not be useful to detect subtle changes, which limits its usefulness in the present study.

In conclusion, SC administration of hydromorphone at a dose of 0.2 mg/kg provided antinociception from 1 to 4 hours postinjection in ferrets. Dysphoria or neurostimulation associated with hyperactive and uncoordinated behaviors may occur and warrant more investigation. Further validation of sedation scores in ferrets may be needed. Administration of SC buprenorphine failed to provide antinociception in this study, and further studies are warranted to assess different dosages or routes of administration.

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References


**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org