The four-toed or African pygmy hedgehog (*Atelerix albiventris*) is a popular household pet and is commonly kept in zoological facilities. Although hedgehogs are frequently presented for veterinary care, there is little information regarding analgesia in this group of animals, which can lead to inappropriate pain management and an increased risk of adverse effects or reduced efficacy when extrapolating drug choice and dosing from other species.

Administering oral medications can be challenging in hedgehogs due to their distinctive anatomy and defensive behavior, making injectable analgesic agents particularly useful when working with these species. Additionally, in the authors’ experience, relatively small muscle bellies and the inability to access the limbs in awake, defensive hedgehogs make SC injections more practical than IM and IV injections. Recent studies have demonstrated that SC opioid administration can provide long-lasting antinociception in hedgehogs.

Methadone is a potent, moderately lipophilic, mu-agonist opioid with N-methyl-D-aspartate (NMDA) receptor antagonist effects that is widely used in veterinary medicine to treat severely painful conditions. In addition to its NMDA activity, another benefit of methadone in dogs and cats is...

**OBJECTIVE**
To evaluate the efficacy and safety of SC methadone in four-toed hedgehogs.

**ANIMALS**
9 to 12 healthy adult four-toed hedgehogs (7 to 9 males and 3 females).

**METHODS**
Hedgehogs underwent 3 randomized, blinded, placebo-controlled, complete crossover studies. Hind limb withdrawal latencies in response to an acute thermal noxious stimulus were measured to evaluate the antinociceptive efficacy of methadone. Single doses of SC methadone were evaluated at 0.5 and 1 mg/kg for dose-dependent effects. Additionally, methadone (1.5 mg/kg) was administered at different concentrations to assess the effect of injection volume on antinociceptive efficacy. Finally, the safety of multiple doses of methadone (1.5 mg/kg, SC, q 2 h, for 3 doses) was also evaluated. In addition to monitoring behavior during latency measurements, animals were assessed for overt sedation. Food intake, body weight, and running wheel activity were assessed daily for 6 days following methadone administration to evaluate for adverse effects.

**RESULTS**
Methadone at 1 and 1.5 mg/kg provided antinociception lasting < 2 hours, and injection volume had no significant effect on efficacy. Methadone at 0.5 mg/kg did not induce antinociception. Methadone produced transient abnormal behaviors in all hedgehogs, with more animals affected at the 1.5-mg/kg dose. Behaviors included periods of standing motionless, vocalization, chewing motions, and paw raising. Single- or multiple-dose administration of methadone had no significant effect on total food intake, body weight, or running wheel activity.

**CLINICAL RELEVANCE**
The results of this study provide additional information on providing analgesia to hedgehogs. Subcutaneous methadone (1 to 1.5 mg/kg) can be used for short-term antinociception in hedgehogs.

**Keywords:** analgesia, antinociception, *Atelerix*, hedgehog, methadone

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The efficacy and safety of methadone in hedgehogs are currently unknown, and suggested dosing varies widely between animal species. Recommended SC doses for methadone in cats range from 0.05 to 0.5 mg/kg and 0.1 to 1.0 mg/kg for dogs. In rodents, reported SC doses range from 0.5 to 4.0 mg/kg. The objective of this study was to evaluate the efficacy and safety of SC methadone hydrochloride in four-toed hedgehogs.

Methods

The study protocol was approved by the University of Wisconsin-Madison School of Veterinary Medicine Institutional Animal Care and Use Committee (protocol V005874). Twelve captive-bred four-toed hedgehogs (9 males and 3 females) aged approximately 8 months old with a mean ± SD weight of 350 ± 100 g were obtained from a commercial breeder. Animals were kept individually in ventilated plastic enclosures (84 X 51 X 36 cm) within a climate-controlled room maintained at 27 °C with a 12:12-hour light cycle. Each enclosure was lined with a thin cardboard sheet and contained an exercise wheel and a hide box filled with shredded paper for burrowing. Hedgehogs were maintained on a commercial low-fat cat kibble (Adult Weight Management Chicken & Rice Formula; Purina Pro Plan) and had access to a bowl of fresh water ad libitum. All hedgehogs were acclimated to the housing conditions and the analgesimetry device (Hargreaves apparatus) for several weeks before study onset. Hedgehogs were allowed to explore the apparatus chambers for 15 to 30 minutes 4 to 6 times per week to help acclimation to the sounds and surfaces of the apparatus. Hedgehogs were deemed healthy based on repeated physical examinations and long-term monitoring of body weight, food intake, and activity level. Trials occurred in a separate climate-controlled room maintained within 3 °C (5 °F) of the housing room.

Methadone (Mylan Institutional LLC) or an equivalent volume of sterile saline (0.9% NaCl) solution was administered SC at a depth of 1.3 cm with an insulin syringe (U-100 insulin syringe [29 gauge, 0.3 mL]; UltiCare VetRx) under the dorsal-spined skin (ie, mantle) in the area over the right scapula. The depth and location of injection were chosen to target intra-adipose deposition of the drug.

Analgesimetry was performed using a Hargreaves apparatus (Plantar Analgesia Meter: ITTC Life Science). Briefly, hedgehogs were placed in ventilated, opaque measurement chambers (22 X 17 X 13.5 cm) placed on a heated glass surface maintained at 29 °C. A noxious, infrared radiant heat stimulus (50% maximum intensity, maximum stimulus temperature of 120 °C) was applied to the metatarsal pad of a pelvic limb, and the thermal stimulus was immediately terminated once the hedgehog moved the targeted limb. If the hedgehog did not exhibit a withdrawal response, the thermal stimulus was turned off after 20 seconds to prevent tissue damage. The latency of limb withdrawal was recorded in seconds. The same metatarsal pad was used for all treatments and was examined for gross abnormalities before each measurement. If a hedgehog rolled up into a defensive posture that prevented access to the metatarsal pad for measurement, the hedgehog was gently removed from the chamber and then replaced to promote unrolling. Hedgehogs were allowed to acclimate for a minimum of 1 minute after being placed back into the chamber before the antinociceptive measurement was reattempted. Thermal withdrawal latency measurements for baseline measurements and for each time point following the injection were performed in duplicate 5 minutes apart. If the 2 measurements varied by more than 20%, a third measurement was performed, and the 3 latencies were averaged. Hedgehogs were placed into the testing chamber at least 5 minutes before the first measurement and were removed from the chamber immediately after the 2 to 3 measurements were completed. A single observer who was blinded to treatment performed all antinociceptive measurements (MLP).

Hedgehogs were evaluated for overt evidence of sedation using a previous scoring system when handled for each thermal latency measurement, including before injection. A single observer who was blinded to treatment performed all sedation scoring (MLP). To assess safety, changes in food intake, running wheel activity, and body weight were assessed daily and compared to baseline values. Baseline values for food intake, running wheel activity, and body weight consisted of an average of measurements collected in the morning over the 3 days immediately before injection. For running wheel activity, wired bicycle odometers (model DCY-16; Dream Sport) were attached to commercial running wheels designed for four-toed hedgehogs (Carolina Storm Wheels) and calibrated using the circumference of the running wheel. The distance ran in miles was recorded for each 24-hour period. A single observer who was blinded to treatment performed all food intake, running wheel activity, and body weight measurements (MLP).

Experimental design

The study consisted of 3 distinct study trials to evaluate the antinociceptive efficacy and safety of SC methadone. Three of the hedgehogs failed to acclimate to the Hargreaves apparatus chambers, despite repeated acclimation attempts, and were therefore not included in the latency trials due to concerns for inaccurate latency measurement. Seven-day minimum washout periods were utilized between all trials, including pilot trials and the 3 final trials.

Dose-finding pilot trials—Subcutaneous methadone was initially evaluated in 9 hedgehogs (7 male and 2 female) with a starting dose of 0.2 mg/kg, based on dosing recommendations for dogs and cats. Each methadone dose was evaluated in a minimum of 2 to 3 hedgehogs, and dose escalation was performed to find doses that were both efficacious...
based on thermal latency measurements and had minimal adverse effects. Doses evaluated included 0.2 mg/kg, 0.6 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, and 5 mg/kg. There was a minimum of a 7-day washout period between treatments. Pilot trials identified that doses of methadone at approximately 0.6 to 5 mg/kg produced measurable thermal antinociception, but a high prevalence of adverse effects, including excessive vocalization, hyperactivity, ataxia, and vomiting, were noted with doses of 2 to 5 mg/kg. Additionally, pilot trials identified a possible effect of injection volume on the duration of the antinociceptive effect. Based on the pilot data, methadone doses of 0.5 to 1.5 mg/kg were considered suitable for further evaluation.

**Antinociceptive efficacy study: dose effect**—In a randomized, blinded, placebo-controlled, complete crossover study, 9 hedgehogs (7 male and 2 female) were administered a single SC injection of 0.5 mg/kg methadone, 1 mg/kg methadone, or sterile saline at a dose of 0.5 mL/kg (equivalent volume to a 1-mg/kg methadone dose). A methadone concentration of 10 mg/mL was used. There was a minimum of a 7-day washout period between treatments.

Baseline thermal withdrawal latencies and sedation scores were obtained before injection and measured again at 0.5, 1, 2, 4, 8, and 12 hours following injection. Hedgehogs were housed in plastic laboratory rodent enclosures in between latency measurements, where they were monitored during the trials for the development of adverse behavioral effects. Food intake and body weight data were collected in the morning for 6 days following methadone administration.

**Antinociceptive efficacy study: volume effect**—The effect of methadone injection volume was evaluated in 9 adult hedgehogs (7 male and 2 female) in a randomized, blinded, placebo-controlled, complete crossover study. Methadone was administered as a single SC injection at a low-volume dose of 1.5 mg/kg (10 mg/mL methadone), a high-volume dose of 1.5 mg/kg (2 mg/mL), or a sterile saline dose of 0.75 mL/kg (equivalent to high-volume methadone dose). The 2 mg/mL methadone was prepared by diluting commercial 10 mg/mL methadone in sterile saline immediately before injection. Methadone remains stable for at least 10 days following dilution with sterile saline.9 There was a minimum of a 7-day washout period between treatments. Baseline thermal withdrawal latencies and sedation scores were obtained and then collected at 0.5, 1, 2, 4, and 8 hours after drug administration. Hedgehogs were housed in plastic laboratory rodent enclosures in between latency measurements, where they were monitored during the trials for the development of adverse behavioral effects. Food intake, running wheel activity, and body weight data were collected in the morning for 6 days following injection.

**Multidose adverse effect study**—The effects of multiple SC doses of methadone were assessed in a randomized, blinded, placebo-controlled, complete crossover study in 12 hedgehogs (9 male, 3 female). Hedgehogs were administered 1.5 mg/kg methadone SC, q 2 h, for 3 doses) or an equivalent volume (0.15 mL/kg) of sterile saline SC, q 2 h, for 3 doses. A single observer blinded to treatment (MLP) performed all the sedation scores and monitored the hedgehogs during the trials for the development of adverse behavioral effects. Following a short period of handling for the injections, hedgehogs were placed back into their normal housing for observation. Food intake, running wheel activity, and body weight data were collected in the morning for 6 days following the trial day when the injections were administered.

**Statistical analysis**

Treatment order was randomized for each experimental trial using free online software (Research Randomizer, version 4.0; Social Psychology Network), and treatments were balanced between trial days. Commercial statistical software (SigmaPlot, version 13; Systat Software) was used for all data analysis. Normal distribution was evaluated using the Shapiro-Wilk test and the Brown-Forsythe test used for testing the equality of group variances. Data were naturally log transformed, if necessary. The data were analyzed for effects of treatment and time with repeated-measures ANOVA, and the Holm-Sidak method was utilized for post hoc analyses. Values of $P < .05$ were considered statistically significant. Data are reported as mean ± SD unless otherwise indicated.

**Results**

**Antinociceptive efficacy study: dose effect**

Methadone at 0.5 mg/kg did not produce a statistically significant increase in thermal withdrawal latency measurements at any time point when compared to the control treatment (Figure 1). In
contrast, 1 mg/kg of methadone resulted in a statistically significant increase in thermal withdrawal latencies at the 1-hour time point compared to the control treatment. There was no statistically significant difference in the mean total food intake or body weight between treatments (Table 1). The sedation score was 0 for all time points for all animals, regardless of treatment.

**Antinociceptive efficacy study: volume effect**

High-volume methadone (1.5 mg/kg and 0.75 mL/kg) resulted in a statistically significant increase in thermal withdrawal latency measurements at the 0.5-hour time point (Figure 2). Low-volume methadone (1.5 mg/kg and 0.15 mL/kg) produced a statistically significant increase in thermal withdrawal latency measurements up to and including the 1-hour time point. There were no statistically significant differences in mean total food intake, body weight, or wheel running activity between treatments. The sedation score was 0 for all time points for all animals, regardless of treatment.

**Multidose adverse effect study**

There were no statistically significant differences in mean total food intake, body weight, or wheel running activity between treatments administered 3 doses of 1.5 mg/kg methadone. The sedation score was 0 for all time points for all animals, regardless of treatment.

**Observed behavioral effects in all studies**

Adverse effects were observed in hedgehogs administered methadone, regardless of dose (Supplementary Table S1). Abnormal behaviors noted were periods of standing motionless while appearing to be “staring into the distance,” mild ataxia, paw raising, chewing-like motions of the mandible, increased snout licking, vocalization, and hyperactivity (Supplementary Videos S1–S3). One hedgehog exhibited behavior where it rubbed the side of its body against adjacent structures following low-volume, high-volume, and multiple doses of methadone. The observed adverse effects did not last longer than 2 hours following methadone injection in all hedgehogs.

**Adverse effects: antinociceptive efficacy studies**—Adverse effects were noted in 1/9 (11%) hedgehogs administered 0.5 mg/kg methadone, in 3/9 (33%) hedgehogs administered 1.0 mg/kg methadone, and in 1/9 (11%) hedgehogs following saline administration. Adverse effects were noted in 6/9 (67%) hedgehogs administered a low volume of 1.5 mg/kg methadone, in 7/9 (78%) hedgehogs administered a high volume of 1.5 mg/kg methadone, and in 2/9 (22%) hedgehogs administered saline.

**Adverse effects: multidose adverse effect study**—Several hedgehogs (5/12, 42%) began using their running wheels immediately following placement back in their home enclosure. This behavior was not observed following the control treatment.
Adverse effects were noted in 7/12 (58%) hedgehogs administered 3 doses of methadone compared to 1/12 (8%) hedgehogs administered 3 doses of saline. Hedgehogs were returned to their enclosures immediately after each injection in the multidose trial to minimize any effect of handling on food intake or running wheel activity. Once returned, hedgehogs often retreated out of view into their hide box.

Discussion

Subcutaneous methadone administered at 0.5 mg/kg failed to produce antinociception, while higher doses of 1 to 1.5 mg/kg had short-lasting antinociceptive effects lasting less than 2 hours. This is slightly longer than the duration of effect noted in rats administered SC methadone, where doses of 1 and 3 mg/kg produced a significant increase in tail-flick latencies for up to 0.5 and 1 hours, respectively.10 This duration of effect is also comparable to SC methadone in cats (0.2 mg/kg, 1 to 3 hours).7 The limited duration of action found in this study limits methadone’s clinical usefulness in hedgehogs when compared to other analgesic options. For example, SC buprenorphine hydrochloride was found to have a greatly prolonged duration of action (36 to 48 hours) in hedgehogs.7 Additionally, hydromorphone produced antinociception lasting < 4 (0.15 mg/kg) or < 6 hours (0.3 mg/kg) when administered SC in hedgehogs.5 However, short-term use of SC methadone may be a beneficial option for moderately to severely painful conditions or procedures (eg, major surgery) where buprenorphine would not be the first analgesic choice and short-term frequent dosing is feasible.

Interestingly, the duration of the effect of methadone in this study is significantly shorter than for SC buprenorphine hydrochloride in hedgehogs, which produced an antinociceptive effect that lasted up to 48 hours after a single dose. Numerous factors could explain the discrepancy in duration between these opioids, including differences in lipid solubility as well as receptor activity and binding affinity.5 For example, methadone is less lipophilic than buprenorphine.5 Additionally, the receptor binding affinity of methadone is comparable to morphine,11 which is significantly weaker than buprenorphine.5 Methadone has a shorter duration of effect than buprenorphine in many species.4 Higher doses of methadone may produce longer lasting antinociceptive effects in hedgehogs. However, a 50% dose increase (1 to 1.5 mg/kg) failed to have an increased antinociceptive duration. Still, it did result in an increased frequency of adverse behaviors, making administration of higher doses not advisable until further data are available regarding efficacy and safety.

While there was a dose-dependent effect on efficacy duration with methadone in this study, an effect on magnitude of antinociceptive response was not apparent. This is distinct from SC buprenorphine administration, where the highest dose evaluated produced noticeably longer withdrawal latencies, indicating a greater magnitude of effect.7 It is possible that a dose-dependent effect on the magnitude of antinociceptive response could be identified with higher doses of methadone. Higher methadone doses were not further evaluated, however, due to the increase in the frequency of adverse effects noted with higher doses in the pilot trials.

A smaller injection volume of methadone produced a significantly longer increase in thermal withdrawal times compared to high-volume treatment. The reason behind this difference is unknown. However, it is possible that the difference in volumes led to varied drug absorption rates, producing differences in the duration of the antinociceptive effect.12 Typically, more rapid absorption occurs with smaller injection volumes, which is theorized to be secondary to several factors, including a disproportionate ratio between the increase in volume and increase in surface area, increased compression of adjacent capillaries, and larger distance the active drug molecules must travel from the center of the injectate to the absorptive periphery.12

Multiple doses of methadone at 1.5 mg/kg did not produce significant changes in food intake, wheel running activity, or body weight compared to the control treatment. This was also noted following multiple doses of buprenorphine in this species.2 Methadone produced transient adverse effects in hedgehogs at all doses evaluated. However, adverse effects were more commonly noted in the higher dose and multidose treatments. Additionally, when comparing the frequency of adverse effects between the trials utilizing a single dose of 1.5 mg/kg methadone versus multiple doses of 1.5 mg/kg methadone, the animals receiving a single dose of methadone appear to have a higher rate of occurrence of most adverse effects (Supplementary Table S1). This discrepancy is likely due to hedgehogs being immediately returned to their enclosures for the multidose safety trial compared to hedgehogs undergoing latency measurements, where hedgehogs were spending extended periods of time under unobstructed observation in an arguably more stressful environment (Hargreaves measurement chamber and plastic rodent enclosures). For the multidose safety trial, hedgehogs were returned to their enclosures immediately after injections were complete to minimize any effect of handling on food intake or running wheel activity, but this may have led to missing subtle behaviors as hedgehogs often retreated out of view in a hide box and fell asleep.

Some animals in the control group also displayed adverse effects. It is unclear why some animals administered saline displayed these behaviors, but a potential explanation is that these “abnormal” behaviors are normal in four-toed hedgehogs but simply displayed at a higher frequency or intensity in the hedgehogs administered methadone or following injections of saline. The periods of standing motionless and vocalization in the hedgehogs may reflect a dysphoric-like state, a documented effect of opioid administration in several species.6 Dysphoria and excitation are more commonly encountered when opioids are administered at higher doses and include vocalization and...
ataxia.4 High doses of SC methadone (3 to 5 mg/kg) were noted to have cataleptic-like effects in rats without affecting motor function.10 Subcutaneous methadone produced whining behavior in dogs compared to the saline control.6 Methadone produced vocalization in dogs when administered at doses of 0.5 to 1 mg/kg.11 Goats administered methadone SC (0.6 mg/kg) had a higher frequency of climbing behavior, described as lifting the thoracic limbs off the ground onto enclosure bars.14 It is possible that the mandibular chewing motions represent a unique behavior secondary to methadone administration in this species or could indicate nausea, which, along with emesis, is a common adverse effect of opioid use reported in numerous species.4

Interestingly, obvious adverse effects were not noted with the administration of antinociceptive doses of buprenorphine in hedgehogs.2 Conversely, a higher rate of adverse effects was noted in hedgehogs administered hydromorphone.2 This may be secondary to species-specific differences in tolerances for various opioid drugs. For example, buprenorphine may produce fewer adverse effects in cats compared to morphine, but the adverse effects produced by these opioids in horses are comparable.4

This study has some limitations. Healthy animals were used in this study, which does not reflect how debilitated hedgehogs would respond to methadone administration at the doses evaluated in this current study. Additionally, the thermal antinociceptive model, while noninvasive, does not reproduce the wide variety of nociceptive stimuli experienced by clinical hedgehog patients, and the results of this study must be interpreted with this noteworthy limitation in mind.

Subcutaneous methadone hydrochloride produced dose-dependent, short-lasting antinociception in hedgehogs and had no clinically relevant effect on food intake, wheel running activity, and body weight, following single- and multiple-dose administration. Adverse effects were noted at all doses tested but were short lived. Methadone (1 to 1.5 mg/kg) can provide short-term analgesia in hedgehogs.

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References


Supplementary Materials

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