Journal of the American Association for Laboratory Animal Science
Copyright 2018
by the American Association for Laboratory Animal Science

Vol 57, No 3
May 2018
Pages 286–290

Analgesic Efficacy and Safety of Buprenorphine in Chinchillas (*Chinchilla lanigera*)

Lana Fox and Christoph Mans*

Buprenorphine is routinely used in chinchillas at reported doses of 0.01 to 0.1 mg/kg IM or SC. However, these dose recommendations are based on anecdotal reports or extrapolation from studies in other species. Therefore, the purpose of this study was to evaluate the analgesic efficacy and safety of subcutaneously administered buprenorphine in chinchillas. Using a randomized, blind, controlled, complete crossover design, we evaluated buprenorphine at a single dose of 0.05, 0.1 or 0.2 mg/kg SC (experiment A) and 0.2 mg/kg SC (experiment B). Analgesic efficacy was determined by measuring limb withdrawal latencies in response to a thermal noxious stimulus (Hargreaves method) at 0, 3, 6, 12, and 24 h (experiment A) and at 0, 1, 2, 4, and 8 h (experiment B). In a third experiment, food intake and fecal output were monitored after repeated administration of buprenorphine (0.2 mg/kg SC every 6 h for 3 doses). Buprenorphine at 0.2 mg/kg SC, but not at 0.05 or 0.1 mg/kg SC, significantly increased limb withdrawal latencies for less than 4 h. Self-limiting reduction in food intake and fecal output occurred after administration at the 0.2-mg/kg dose in animals undergoing algesiometry. In chinchillas not undergoing algesiometry, the administration of 3 doses at 0.2 mg/kg SC every 6 h did not reduce food intake but significantly decreased fecal output for the first 24 h. Additional studies are needed to evaluate buprenorphine in different algesiometry models and to establish its pharmacokinetic profile in chinchillas.

DOI: 10.30802/AALAS-JAALAS-17-000108

Chinchillas (*Chinchilla lanigera*) are a popular animal model, particularly for otologic research, and are increasingly maintained as companion animals.[16,21] Chinchillas require effective analgesic protocols for a variety of indications, including experimental surgery or for treatment of traumatic injuries or after therapeutic surgical interventions, such as fracture repair, and C-sections.[16,17] Buprenorphine is currently the most commonly used opioid analgesic in chinchillas. This partial-μ opioid agonist is widely used in rodents because it is potent (25 to 50 greater than morphine), and has a relatively long duration of action.[13,19] Extensive research has been performed to evaluate the efficacy and safety of buprenorphine in mice and rats.[8,14] However, no research has been published regarding the safety and efficacy of buprenorphine in chinchillas. In rats, buprenorphine at a dosage of 0.05 mg/kg SC is effective for thermal pain.[11] In addition, buprenorphine is effective in both acute and chronic pain models in mice.[3] Extrapolation from these studies and many others has suggested that the dose for chinchillas likely is between 0.01 and 0.05 mg/kg.[22] Anecdotally, doses as large as 0.1 mg/kg have been recommended.[17] The currently recommended administration frequency of buprenorphine in chinchillas is every 6 to 12 h.[17,22] Recent studies have evaluated the pharmacokinetics and analgesic effects of buprenorphine in guinea pigs, a related hystricomorphic rodent species. At a dose of 0.05 mg/kg SC, plasma levels in guinea pigs remained above 1 ng/mL for a maximum of 3 h and correlated with increased paw pressure measurements between 1 and 3 h but not at 6 or 12 h.[19] In another study in guinea pigs, plasma levels above 1 ng/mL were maintained for 7 h after administration at 0.2 mg/kg IV and for 4 h after oral–transmucosal administration. At the 0.2 mg/kg doses sedation was reported in guinea pigs.[15]

Buprenorphine is favored over other opioids because it has fewer cardiovascular and respiratory side effects.[14] Adverse effects in rodents after buprenorphine administration include decreased gastrointestinal motility, pica behavior, and rebound hyperalgesia.[5,6,14] Studies in rats found that multiple doses of buprenorphine decreased food intake by 52% and that hyporexia can last for as long as 6 d.[2,12] The frequent use of buprenorphine in chinchillas at currently recommended doses (0.01 to 0.1 mg/kg) suggests that no overt clinical adverse effects are commonly seen.[17,22] However, no systemic evaluation of the effects of buprenorphine at different dosages and after single and repeated administration has been published.

The objective of the current study was to investigate the analgesic efficacy and safety of subcutaneously administered buprenorphine in chinchillas. We hypothesized that buprenorphine would result in dose-dependent effective analgesia, demonstrated by increased limb withdrawal latencies in response to a noxious thermal stimulus, and that the effects on food intake and fecal output would be dose-dependent and clinically irrelevant.

Materials and Methods

This study was approved by the University of Wisconsin-Madison School of Veterinary Medicine Animal Care and Use Committee. Adult chinchillas were obtained from a commercial breeder (R and R Chinchillas, Jenera, OH) and were housed in a climate-controlled room with a 12:12-h light:dark cycle (lights on, 0700 to 1900). Room temperature was maintained between 21 and 23 °C, and relative humidity ranged between 40% and 55%. The chinchillas were maintained in individual cages (0.69 m × 0.69 m × 0.46 m, Allentown 6-cage Rabbit Housing Unit, Allentown Caging, Allentown, NJ) with perforated plastic pans.

Received: 04 Sep 2017. Revision requested: 31 Oct 2017. Accepted: 17 Jan 2017.
Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, Wisconsin
**Corresponding author. Email: christoph.mans@wisc.edu*

Each cage contained a plastic hide box, as well as cardboard tubes and a piece of natural manzanita wood for foraging. An opportunity to exercise and socialize in a playpen (1.8 m × 0.9 m), which contained a dust bath, was provided at least once weekly. The chinchillas were offered a commercial pelleted rabbit diet (MannaPro Rabbit pellets, MannaPro Products, Chesterfield, MO) and tap water from a rabbit ball-tipped water bottle. All chinchillas were habituated to the housing conditions for at least 2 wk prior to starting the experiments and were deemed to be healthy in light of repeated physical examinations and monitoring of food intake, fecal output, and body weights.

Two algesiometry experiments, designed according to the Hargreaves method, were performed by using testing devices.[10] Prior to starting each experiment, all chinchillas were habituated to the Hargreaves apparatus for 15 min daily for 2 wk. For an additional 2 wk, baseline limb withdrawal latency was measured, ranging from 2 to 4 times per foot, for as many as 3 times per day, to determine the most consistent testing method. On each experimental day, the chinchillas were placed in the Hargreaves apparatus and allowed to acclimate for 10 min prior to starting measurements. Measurements were taken 5 min apart at each time point. The same limb was used in each animal throughout each experiment. Limb latency measurements began between 0800 and 0900 on each experimental day. For all experiments, the fur was clipped in the area over the shoulder blades to facilitate subcutaneous drug administration and to avoid inadvertent injection failures, due to the presence of the dense fur.

Experiment A. This initial experiment was performed by using a standard Hargreaves apparatus (Ugo Basile, Gemino, Italy). Measuring hindlimb withdrawal latencies consistently was not feasible, because hindlimb placement could not be visualized reliably and therefore the infrared heat source could not be placed correctly on the plantar aspect of the hindpaw. Instead, withdrawal latencies were obtained from the forelimbs. In a randomized, blind, complete crossover design, 13 adult chinchillas (7 male, 6 female; body weight [mean ± 1 SD], 0.72 ± 0.08 kg; range, 0.54 to 0.79 kg) were used to evaluate the analgesic efficacy of buprenorphine (Buprenex 0.3 mg/mL, Hospira, Lake Forest, IL) at 0.05, 0.1, and 0.2 mg/kg SC. Saline was administered at 0.2 mL/kg SC in the control group. Forelimb withdrawal latencies were measured at 0 (baseline) 3, 6, 10, and 24 h after drug administration. Four forelimb withdrawal latencies were recorded, and the average of the last 3 latencies used for data analysis.[1,4] The cut-off time was set as 25 s and the infrared intensity setting at 90. Testing chamber size was 22 × 17 × 13.5 cm. Body weight and 24-h food intake and fecal output were measured the day prior to starting each treatment (baseline) and daily for 6 d after drug administration. All spilled food was taken into account. Only the food ingested (that is, not spilled) was measured. The washout period between treatments was at least 7 d.

Experiment B. After the completion of experiment A, a different plantar testing device (ITTC Life Science, Woodland Hills, CA) was acquired and, due to the presence of a mirror and guide light, enabled correct plantar placement of the infrared heat source in chinchillas. Therefore, we used this plantar testing device to measure hindlimb withdrawal latencies during experiment B. In a randomized, blind, complete crossover design, we used 11 adult chinchillas (6 male, 5 female; body weight, 0.65 ± 0.12 kg; range, 0.51 to 0.89 kg) to evaluate the analgesic efficacy of a single dose of buprenorphine at 0.2 mg/kg SC. Saline was administered at 0.66 mL/kg SC in the control group. Hindlimb withdrawal latencies were measured at 0 (baseline), 1, 2, 4, 6, and 8 h after drug administration. Two hindlimb withdrawal latencies were recorded at each time point. If these latencies varied by more than 20%, a third measurement was recorded, and the average of all 3 latencies used for data analysis. Cut-off time was set as 25 s The active intensity (high beam) of the heat source was set at 50%, and the idle intensity (low beam) was set to 5%. The dimensions of the testing chamber were 19.5 × 19.5 × 12 cm. Body weight and 24-h food intake and fecal output were measured the day prior to starting the treatment (baseline) and daily for 6 d after drug administration. The washout period between treatments was at least 7 d.

Experiment C. To assess the effects of repeated administration of buprenorphine at 0.2 mg/kg SC on food intake and fecal output, we used 8 chinchillas (2 male, 6 female; body weight: 0.70 ± 0.08 kg, range: 0.54-0.79 kg) in a randomized, blind, complete crossover experiment. Buprenorphine (0.2 mg/kg SC) or saline (0.66 mL/kg SC) was administered every 6 h for a total of 3 doses. Food intake, fecal output, and body weight were measured every 24 h, starting 1 d prior to drug administration (baseline) and for 6 d after. The washout period between treatments was at least 7 d.

Statistical analysis. Commercial software (SigmaPlot 13, Systat Software, San Jose, CA) was used to perform the data analysis. Food intake and fecal output data were analyzed as g/kg body weight. The data were evaluated for normal distribution by using the Shapiro–Wilk test and for equal distribution by using the Brown–Forsythe test. Data were transformed or ranked, as necessary. The data were analyzed for effects of drug and time by using repeated-measures 2-way ANOVA, with Holm–Sidak posthoc analysis. A P value less than 0.05 was considered statistically significant. Data are reported as mean ± 1 SD unless otherwise indicated.

Results

Analgesic efficacy. *Experiment A*. Baseline forelimb withdrawal latency was 10.8 ± 2.5 s in the control group, with no significant difference between groups (Figure 1). Compared with saline, buprenorphine administered at 0.05 and 0.1 mg/kg SC did not significantly change forelimb withdrawal latencies at any time point. However, buprenorphine at 0.2 mg/kg SC resulted in a significant (P = 0.018) increase in withdrawal latencies at 3h after administration.

***Experiment B*.** Baseline hindlimb withdrawal latency was 9.5 ± 1.7 s in the control group, with no significant difference compared with the buprenorphine group's baseline values (Figure 2). Administration of buprenorphine at 0.2 mg/kg SC increased hindlimb withdrawal latency nonsignificantly at 1 h (P = 0.2); this effect reached statistical significance at 2 h (P = 0.005) and decreased again to near baseline levels by 4 h.

Effect of buprenorphine on food intake. In experiment A, food intake was reduced in the control group and all buprenorphine groups in a dose-dependent manner over the first 24 h after drug administration (Figure 3 A). Compared with the control group, food intake was not significantly affected at any time point after the administration of buprenorphine at 0.05 mg/kg SC and only on day 4 at 0.1 mg/kg SC. In contrast, administration of a single dose of buprenorphine at 0.2 mg/kg SC significantly reduced food intake in both experiment A (P = 0.04 for overall effect, Figure 3 A) as well as in experiment B (P = 0.003 for overall effect, Figure 3 B). The greatest reduction in food intake occurred during the first 24 h in both experiment A (24.9% ± 15.9%; control group, 9.0% ± 7.2%; P = 0.01, Figure 3 A) as well as in experiment B (39.2% ± 24.8%; control group, 4.4% ± 11.0%; P < 0.001, Figure 3 B). However, chinchillas that received 3 doses of buprenorphine at 0.2 mg/kg SC every 6 h

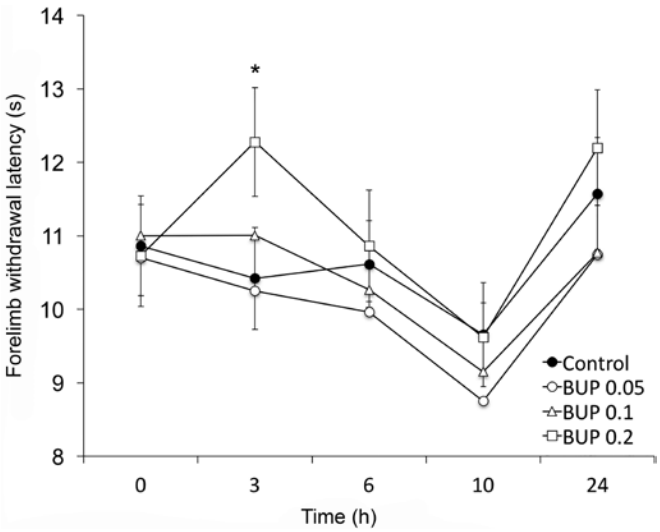

Figure 1. Forelimb withdrawal latencies (mean ± SEM) in response to a thermal noxious stimulus in 13 chinchillas treated with a single dose of buprenorphine 0.05, 0.1, and 0.2 mg/kg SC (BUP 0.05, BUP 0.1, and BUP 0.2 respectively) in a randomized, blind, controlled, complete cross-over experiment (experiment A). Error bars are shown for the control group and 0.2-mg/kg buprenorphine group only. Saline was administered subcutaneously in the control group. *, Significantly ($P < 0.05$) different from control group at the same time point.

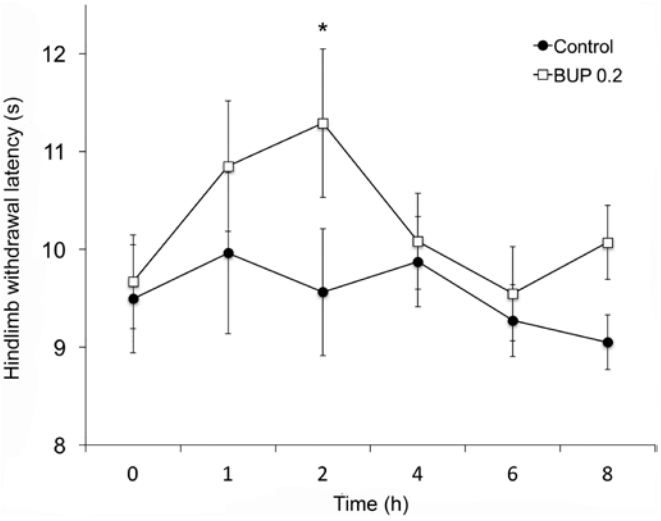

Figure 2. Hindlimb withdrawal latencies (mean ± SEM) in response to a thermal noxious stimulus in 11 chinchillas treated with a single dose of buprenorphine at 0.2 mg/kg SC (BUP 0.2) in a randomized, blind, controlled, complete cross-over experiment (experiment B). Saline was administered subcutaneously in the control group. *, Significantly ($P < 0.05$) different from control group at the same time point.

(experiment C) and that did not undergo algesiometry had no significant decrease in food intake, compared with the control group (Figure 3 C).

Effect of buprenorphine on fecal output. In experiment A, fecal output was reduced dose-dependently in all buprenorphine groups over the first 24 h (Figure 3 D).

Administration of a single dose of buprenorphine at 0.2 mg/kg SC resulted in a significant reduction in fecal output in experiment A ($P = 0.004$ for overall effect, Figure 3 D). The greatest reduction in fecal output occurred during the first 24 h in both experiment A (29.2% ± 17.5%; control group, 5.5% ± 15.5%; Figure 3 D) as well as experiment B (42.2% ± 37.4%; control group, 13.9% ± 14.9%; Figure 3 E). After the administration of 3 doses of buprenorphine at 0.2 mg/kg SC every 6 h, fecal output was significantly reduced for the first 24 h after drug administration in animals not undergoing algesiometry (12.5% ± 20.3%), whereas control animals showed an increase in fecal output (7.3% ± 18.5%; $P = 0.015$, Figure 3 F).

Body weight was not significantly affected by any treatment in any of the experiments.

Discussion

In this study, buprenorphine at 0.05 or 0.1 mg/kg SC did not provide antinociception in chinchillas, in contrast to the currently recommended dosages in chinchillas of 0.01 to 0.1 mg/kg.[17] However, buprenorphine at 0.2 mg/kg SC resulted in an increase in limb withdrawal latencies, indicating analgesic efficacy at this dose.[23] This finding is consistent with a pharmacokinetic study in guinea pigs, which showed that a dose of 0.2 mg/kg IV was necessary to achieve plasma levels considered analgesic (greater than 1 ng/mL) in other species.[15] In contrast, another study in guinea pigs showed that buprenorphine at 0.05 mg/kg SC resulted in a significant increase in paw withdrawal pressure for a maximum of 3 h and plasma levels greater than 1ng/mL for 3 h.[19] Buprenorphine has been evaluated in several algesiometry models in rats and mice and has been shown to have a broad analgesic profile in many species.[8] However, although thermal nociception models are accepted methods to evaluate analgesic efficacy in animals, they may overestimate drug dose requirements to provide analgesia for other types of pain stimuli (for example, surgical pain).[14] Therefore, buprenorphine might be effective clinically at dosages lower than 0.2 mg/kg SC to alleviate visceral, traumatic, or postsurgical pain in chinchillas.[9] Additional studies should investigate the analgesic efficacy of buprenorphine in nociception models that better simulate clinically relevant situations, such as surgical models.

The duration of effect of buprenorphine in rodents is typically considered to be lengthy, and the currently recommended administration frequency of buprenorphine in chinchillas is every 6 to 12 h.[17,22] However, in our current study, buprenorphine at 0.2 mg/kg SC increased limb withdrawal latencies for less than 4 h. Our finding is consistent with a recent study in guinea pigs, in which a significant increase in paw withdrawal pressure was reported between 1 to 3 h after administration of buprenorphine at 0.05 mg/kg SC but not at 6 or 12 h.[19] A shorter duration of effect of buprenorphine has been reported in other species. Cats that had undergone ovariohysterectomy needed a second dose of buprenorphine after only 4 h.[20] In mice, the duration of effect of buprenorphine is 3 to 5 h.[7] In guinea pigs, the administration of buprenorphine at 0.2 mg/kg resulted in plasma levels that exceeded 1 ng/mL for 7 h after intravenous administration and for 4 h after oral–transmucosal administration.[15] Pharmacokinetic studies in chinchillas are needed to evaluate the buprenorphine plasma levels achieved after subcutaneous administration.

Buprenorphine has an unusually high therapeutic index, and the ratio of lethal dose to the effective dose is at least 3 times greater that of morphine.[8] In the current study, repeated administration of buprenorphine at high dosage (0.2 mg/kg SC for 6 h) did not decrease food intake but significantly decreased fecal output. This discrepancy may indicate that repeated dosing of high-dose buprenorphine causes gastrointestinal ileus. In rats, buprenorphine at 0.01 to 0.1 mg/kg SC slowed gastrointestinal transit times.[5] The decrease in food intake after a single dose but not after multiple doses of 0.2 mg/kg buprenorphine may be explained by the stress of handling associated with measuring limb

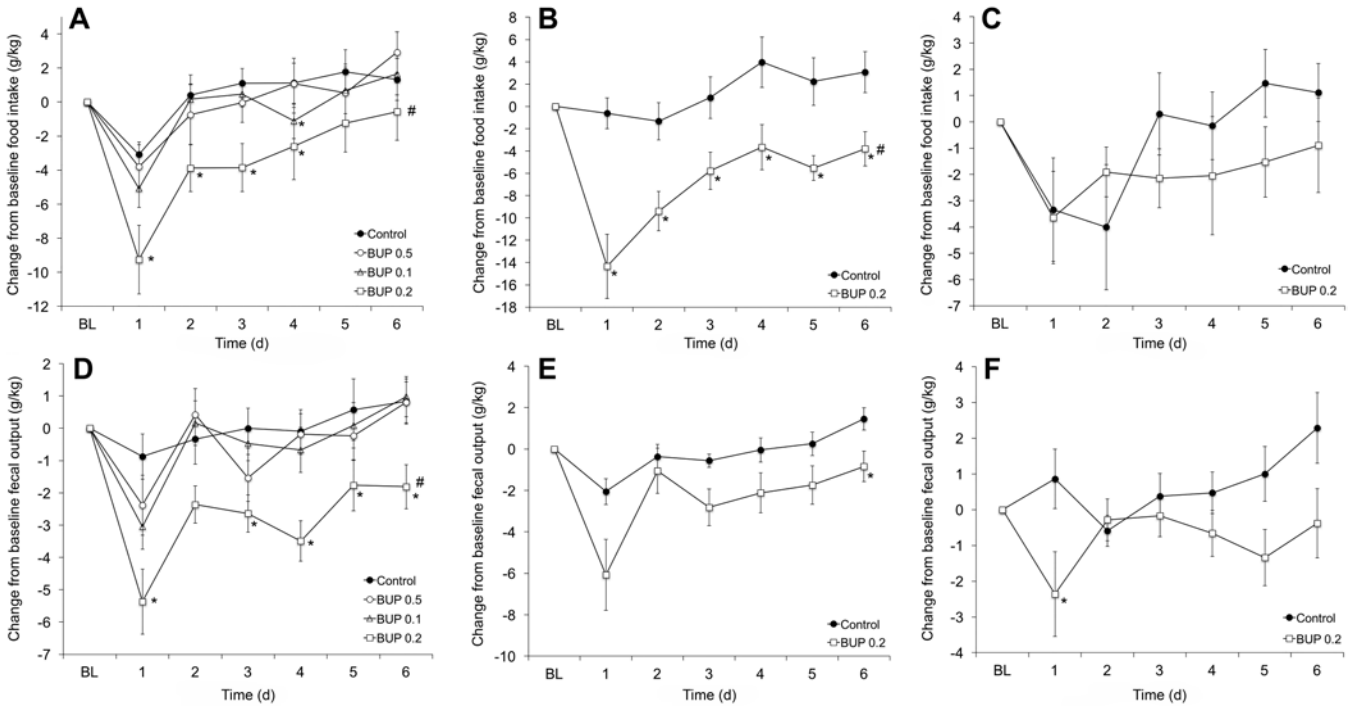

Figure 3. Food intake and fecal output (mean ± SEM) in chinchillas after administration of subcutaneous buprenorphine in randomized, blind, controlled complete cross-over experiments. (A) Food intake and (D) fecal output after a single subcutaneous dose of buprenorphine at 0.05, 0.1, and 0.2 mg/kg (BUP 0.05, BUP 0.1, and BUP 0.2, respectively) in 13 chinchillas (experiment A). (B) Food intake and (E) fecal output after a single dose of buprenorphine of at 0.2 mg/kg SC (BUP 0.2) in 11 chinchillas (experiment C). (C) Food intake and (F) fecal output after administration of buprenorphine at 0.2 mg/kg SC (BUP 0.2) every 6 h for 3 doses in 8 chinchillas (experiment C). Saline was administered subcutaneously in all experiments in the control group. *, Significantly ($P < 0.05$) different from control group at the same time point; #, significant ($P < 0.05$) effect of treatment group in the overall model.

withdrawal latencies in the single-dose algesiometry experiments (experiment A and B). Although a dose-dependent decrease in food intake and fecal output occurred after drug administration, the effects of buprenorphine were self-limiting, and therefore buprenorphine should be considered safe to administer in healthy chinchillas at the doses we evaluated in this study.

In experiment A, we used the forelimbs to measure withdrawal latencies, because of the inability to place the radiant heat source reliably on the plantar aspects of the hindlimbs. However, because chinchillas often did not place their forepaws on the glass surface through which the radiant heat was transmitted, measurements were often challenging to obtain and associated with delays. Therefore a 1-h time point was deemed infeasible, and only a 3-h time point was used. Evaluating chinchillas at 1-h time points in experiment A would have been preferable, to determine the duration of action and onset time. This same problem was encountered in a mouse study, with mice having increased locomotor activity for at least 4 h, complicating measurement efforts.[18] This limitation of experiment A was overcome in experiment B by using a different plantar tester which, due to the presence of a mirror and a guide light, allowed for reliable placement of the radiant heat source on the plantar surfaces.

In conclusion, buprenorphine at previously recommended doses does not provide antinociception in the chinchilla thermal algesiometry model we used. At 0.2 mg/kg SC, buprenorphine provided antinociception in chinchillas for less than 4 h. Repeated administration of 0.2 mg/kg resulted in a self-limiting reduction in fecal output but did not decrease food intake in healthy chinchillas.

Acknowledgments

Funding for this study was provided by the University of Wisconsin-Madison School of Veterinary Medicine Companion Animal Fund.

References

1. **Barrot M.** 2012. Tests and models of nociception and pain in rodents. Neuroscience **211:**39–50. https://doi.org/10.1016/j.neuroscience.2011.12.041.
2. **Bourque SL, Adams MA, Nakatsu K, Winterborn A.** 2010. Comparison of buprenorphine and meloxicam for postsurgical analgesia in rats: effects on body weight, locomotor activity, and hemodynamic parameters. J Am Assoc Lab Anim Sci **49:**617–622.
3. **Christoph T, Kogel B, Schiene K, Meen M, De Vry J, Friderichs E.** 2005. Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. Eur J Pharmacol **507:**87–98. https://doi.org/10.1016/j.ejphar.2004.11.052.
4. **Chum HH, Jampachairsri K, McKeon GP, Yeomans DC, Pacharinsak C, Felt SA.** 2014. Antinociceptive effects of sustained-release buprenorphine in a model of incisional pain in rats (*Rattus norvegicus*). J Am Assoc Lab Anim Sci **53:**193–197.
5. **Cowan A, Doxey JC, Harry EJ.** 1977. The animal pharmacology of buprenorphine, an oripavine analgesic agent. Br J Pharmacol **60:**547–554. https://doi.org/10.1111/j.1476-5381.1977.tb07533.x.
6. **Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LB, Kristal MB.** 2009. Evaluation of buprenorphine in a postoperative pain model in rats. Comp Med **59:**60–71.
7. **Gades NM, Danneman PJ, Wixson SK, Tolley EA.** 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Contemp Top Lab Anim Sci **39:**8–13.
8. **Guarnieri M, Brayton C, DeTolla L, Forbes-McBean N, Sarabia-Estrada R, Zadnik P.** 2012. Safety and efficacy of buprenorphine for analgesia in laboratory mice and rats. Lab Anim (NY) **41:**337–343. https://doi.org/10.1038/laban.152.
9. **Jessen L, Christensen S, Bjerrum OJ.** 2007. The antinociceptive efficacy of buprenorphine administered through the

drinking water of rats. Lab Anim **41:**185–196. https://doi.org/10.1258/002367707780378131.

10. **Le Bars D, Gozariu M, Cadden SW.** 2001. Animal models of nociception. Pharmacol Rev **53:**597–652.
11. **Leach MC, Forrester AR, Flecknell PA.** 2010. Influence of preferred foodstuffs on the antinociceptive effects of orally administered buprenorphine in laboratory rats. Lab Anim **44:**54–58. https://doi.org/10.1258/la.2009.009029.
12. **Liles JH, Flecknell PA.** 1992. The effects of buprenorphine, nalbuphine, and butorphanol alone or following halothane anaesthesia on food and water consumption and locomotor movement in rats. Lab Anim **26:**180–189. https://doi.org/10.1258/002367792780740558.
13. **Martin LB, Thompson AC, Martin T, Kristal MB.** 2001. Analgesic efficacy of orally administered buprenorphine in rats. Comp Med **51:**43–48.
14. **Roughan JV, Flecknell PA.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating postoperative pain in animals. Lab Anim **36:**322–343. https://doi.org/10.1258/002367702320162423.
15. **Sadar MJ, Knych H, Drazenovich TL Paul-Murphy JR.** 2018. Pharmacokinetics of buprenorphine after intravenous and oral transmucosal administration in guinea pigs (*Cavia porcellus*). Am J Vet Res **79:**260–266. doi:10.2460/ajvr.79.3.260.
16. **Saunders R.** 2009. Veterinary care of chinchillas. In Pract **31:**282–291. https://doi.org/10.1136/inpract.31.6.282.
17. **Saunders R, Harvey L.** 2012. Anaesthesia and analgesia in chinchillas. In Pract **34:**34–43. https://doi.org/10.1136/inp.d7730.
18. **Schildhaus N, Trink E, Polson C, Detolla L, Tyler BM, Jallo GI, Tok S, Guarnieri M.** 2014. Thermal latency studies in opiate-treated mice. J Pharm Bioallied Sci **6:**43–47. https://doi.org/10.4103/0975-7406.124316.
19. **Smith BJ, Wegenast DJ, Hansen RJ, Hess AM, Kendall LV.** 2016. Pharmacokinetics and paw withdrawal pressure in female guinea pigs (*Cavia porcellus*) treated with sustained-release buprenorphine and buprenorphine hydrochloride. J Am Assoc Lab Anim Sci **55:**789–793.
20. **Steagall PV, Monteiro-Steagall BP, Taylor PM.** 2014. A review of the studies using buprenorphine in cats. J Vet Intern Med **28:**762–770. https://doi.org/10.1111/jvim.12346.
21. **Wang AY, Shen Y, Wang JT, Friedland PL, Atlas MD, Dilley RJ.** 2014. Animal models of chronic tympanic membrane perforation: a 'time-out' to review evidence and standardize design. Int J Pediatr Otorhinolaryngol **78:**2048–2055. https://doi.org/10.1016/j.ijporl.2014.10.007.
22. **Wenger S.** 2012. Anesthesia and analgesia in rabbits and rodents. Journal of exotic pet medicine **21:**7–16. https://doi.org/10.1053/j.jepm.2011.11.010.
23. **Yeomans DC, Proudfit HK.** 1994. Characterization of the foot withdrawal response to noxious radiant heat in the rat. Pain **59:**85–94. https://doi.org/10.1016/0304-3959(94)90051-5.