Evaluation of self-injurious behavior, thermal sensitivity, food intake, fecal output, and pica after injection of three buprenorphine formulations in rats (*Rattus norvegicus*)

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OBJECTIVE
To assess effects of buprenorphine hydrochloride (BH), sustained-release buprenorphine (SRB), and high-concentration buprenorphine (HCB) formulations in healthy rats.

ANIMALS
8 Sprague-Dawley rats.

PROCEDURES
In a crossover-design study, rats received BH (0.05 mg/kg), SRB (1.2 mg/kg), HCB (0.30 mg/kg), or 5% dextrose solution (0.2 mL/kg), SC, once. Self-injurious behavior and thermal sensitivity (hind limb withdrawal latencies) were assessed prior to injection (time 0) and 1, 4, 8, 12, and 24 hours after injection. Food intake, kaolin intake, and fecal output were measured over 12-hour light and dark periods before and after each treatment. Values were compared among treatments and time points.

RESULTS
Self-injurious behavior was detected with all buprenorphine treatments; scores were greater at all time points during the 12 hours after HCB and 24 hours after SRB administration than at time 0. Percentage change in hind limb withdrawal latencies from time 0 was higher with BH and HCB 1 hour after injection than at other time points. Postinjection light-period food intake was higher (BH and HCB) and dark-period food intake was lower (BH, HCB, and SRB), compared with preinjection values for the same treatments. For SRB, postinjection light-period kaolin intake was greater than the preinjection value, and postinjection light- and dark-period kaolin intake was greater than that for other treatments.

CONCLUSIONS AND CLINICAL RELEVANCE
Hypoalgesic effects were briefly observed after administration of BH or HCB in healthy rats; adverse effects were detected in some rats with all buprenorphine formulations. Studies comparing effects of BH, SRB, and HCB in rats undergoing surgery or other noxious stimuli are indicated to determine clinical benefits in this species. (*Am J Vet Res* 2018;79:697–703)

Buprenorphine is a semi-synthetic, partial μ-opioid-receptor agonist drug that is commonly used for perioperative analgesia in rats.[1,2] The drug has a half-life of 2.1 to 3 hours after IV bolus administration.[1,3] The standard dosage of BH in rats (0.05 mg/kg, SC, q 6 to 8 hours)[2,4–6] requires injections at a frequency associated with signs of stress, pica, reduced food intake, altered locomotor activity, and hyperalgesia.[7–10] Results of previous studies[11,12] to assess the effects of SRB in rats revealed that the treatment prolonged hypoalgesia (up to 72 hours), but had adverse effects of skin irritation (erythema and scabbing),[11] reduced activity, and respiratory depression.[13]

ABBREVIATIONS

BH	Buprenorphine hydrochloride
HCB	High-concentration buprenorphine
IQR	Interquartile (25th to 75th percentile) range
SRB	Sustained-release buprenorphine

Pica, the consumption of nonnutritive substrates (eg, bedding), is an adverse effect of buprenorphine administration that can result in reduced food intake and low weight gain, gastrointestinal obstruction, and even death in rats.[9,14,15] Kaolin, a nonnutritive substance composed of acacia gum, has been safely used for repeatable measurement of pica behavior in rats.[16–20]

Commercially available HCB (1.8 mg/mL) is hypoalgesic for up to 24 hours in cats when administered at 0.24 mg/kg.[21] However, when this same formulation was tested in healthy rats at 0.075, 0.15, and 0.30 mg/kg, only the highest dose was associated with hypoalgesic effects, which were detectable for a short duration (1 hour); all doses were associated with alterations in food intake and with self-injurious behavior.[22] This finding, in a study[22] performed by our group, led to speculation that an inability to express pica behavior as a manifestation of nausea

might induce self-injurious behavior, because pica was prevented by housing the rats in wire-bottomed cages without bedding during the experiments.

The purpose of the study reported here was to evaluate self-injurious behavior, thermal sensitivity, food intake, fecal output, and pica after SC administration of BH, SRB, or HCB in healthy rats that did not undergo surgery or other potentially painful procedures. Kaolin was provided to the rats to allow pica behavior. We hypothesized that no significant self-injurious behavior would occur; that postinjection hind limb thermal withdrawal latencies would be higher than preinjection values for all buprenorphine formulations, with this increase lasting longer for SRB than for BH and HCB treatments; that postinjection food intake and fecal output would be reduced during the active (dark) 12-hour period, compared with that during the preinjection dark period; and that kaolin intake would be observed after all 3 buprenorphine treatments, with SRB associated with a more prolonged decrease in food intake and fecal output and a more prolonged increase in kaolin intake than the other formulations.

Materials and Methods

Animals

All experiments were approved by the University of Wisconsin Animal Care and Use Committee, and all rats were treated in compliance with Institute for Laboratory Animal Research guidelines.[23] Eight adult male Sprague-Dawley rats (*Rattus norvegicus*[a]; mean ± SD body weight at the start of the study, 339 ± 11 g) were used. Rats were housed in pairs on corn cob bedding in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility with a 12-hour light (6:00 AM to 6:00 PM) and 12-hour dark cycle (6:00 PM to 6:00 AM). A commercial pelleted rat feed[b] and water were provided with access ad libitum.

Rats were allowed 7 days to acclimatize to the animal holding facility prior to testing and 48 hours to acclimatize to wire-bottomed cages before experiments. For 48 hours before and 24 hours after injection, rats were housed singly on wire-bottomed cages, with free access to the same commercial rat feed[b] and water. Rats were returned to their corn cob bedding for each drug washout period. The rats were certified (by the commercial supplier) free from ectromelia virus, Hantaan virus, murine pneumotropic virus, Kilham rat virus, lactic dehydrogenase elevating virus, lymphocytic virus, minute virus of mice, mouse adenoviruses type 1 and 2, mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, mouse polyoma virus, mouse rotavirus, mouse thymic virus, murine norovirus, pneumonia virus of mice, rat minute virus, rat parvovirus, rat Theiler virus, respiratory enteric virus III, Sendai virus, sialodacryoadenitis virus, Theiler murine encephalomyelitis, Toolan H1 parvovirus, *Bordetella bronchiseptica*, *Helicobacter* spp, *Mycoplasma pulmonis*, *Pasteurella multocida*, dermatophytes, ectoparasites, and endoparasites. The rats were euthanized by CO_2 inhalation at the end of the study in accordance with AVMA euthanasia guidelines.[24]

Study design

In a blinded, randomized,[c] crossover design, all rats received each of 4 treatments as a single SC injection (BH,[d] 0.05 mg/kg; SRB,[e] 1.2 mg/kg; HCB,[f] 0.30 mg/kg; or 5% dextrose solution,[g] 0.20 mL/kg). The investigator who performed all injections (MA) was blinded to treatment (opaque tape was used to obscure the injectate in each syringe). The volume of dextrose solution corresponded to the highest injectate volume, which was associated with the SRB treatment. Injections were given between 6:00 AM and 7:00 AM (immediately after the dark cycle). The dose for HCB was determined from a previous investigation by our group,[22] in which only the selected dose (0.30 mg/kg) was associated with hypoalgesia. The doses for BH and SRB were determined from previously published studies.[2,4–6,11,12] Rats underwent a 1-week washout period between treatments.

Self-injurious behavior

Self-injurious behavior (self-biting and cage-biting) was quantified before injection (time 0) and at 1, 4, 8, 12, and 24 hours after injection. Each observation period had a 2-minute duration. A descriptive scale adapted from previous studies[10,25] and used in a recent study of HCB in rats[22] was applied, where 0 = no cage-biting or self-biting; 1 = occasional cage-biting and no self-biting; 2 = frequent cage-biting, occasional to frequent self-biting with no visible evidence of skin trauma, or both; 3 = frequent self-biting with mild to moderate visible evidence of skin trauma (inflammation); and 4 = frequent self-biting with moderate to severe visible evidence of skin trauma (bleeding). Occasional cage-biting or self-biting was defined as occurring for ≤ 30 seconds (continuous or intermittent) during a given observation period. Frequent cage-biting or self-biting was defined as occurring for > 30 seconds during a single observation period.

Withdrawal responses to thermal stimuli

Hypoalgesia was assessed by measuring latency of hind limb withdrawal to a radiant heat stimulus[26] applied with a commercial testing device.[h] Rats were allowed to acclimatize to the chambers for 15 minutes prior to testing. The intensity of the heat stimulus and rate of heating were kept constant throughout the study to establish a target withdrawal latency range of 7 to 9 seconds for preinjection measurements, with a maximum latency limit of 20 seconds to avoid thermal injury. An infrared heat stimulus was applied to a plantar surface, and the time to withdrawal of the paw in response to the stimulus was defined as the hind limb withdrawal latency. The latencies were measured before injection (time 0) and at 1, 4, 8, 12, and 24 hours after injection. Each rat was tested 3 times at each time point with ≥ 5 minutes between trials on alternating paws (≥ 10 minutes between tri-

als on the same paw), and the mean latency was calculated. This pattern was chosen to minimize hyperalgesia secondary to repeated noxious stimuli.[13,26,27] Hind limb withdrawal latencies were performed on both right and left hind limbs, and a mean value for both hind limbs was calculated, similar to previous studies using withdrawal latencies.[22,28–32] Data were summarized as the percentage change from preinjection values (percentage change at time 0 = 0%), similar to previously published studies.[13,22]

Food intake, fecal output, and kaolin intake

Rats were provided free access to a premeasured amount of commercial rat feed[b] (250 ± 3 g) in a removable wire rack and a premeasured amount of kaolin pellets[i] (32 ± 3 g) in a polystyrene Petri dish when they were housed singly in wire-bottomed cages 48 hours before each treatment. Paper liners were placed on cage bottoms to absorb urine and water so that spilled food and kaolin would not become saturated. The mass of food and kaolin remaining in the wire rack and Petri dish, respectively, and that spilled on the cage floor was measured every 12 hours from 48 hours before until 24 hours after injection. Spilled food and kaolin were separated prior to measurement. Fecal pellets were collected and weighed every 12 hours for the same time period. Measurements for individual rats were expressed as an index of body weight (mg/kg) because body weights varied over the course of the study (range, 315 to 402 g). Similar to the methods in a previous study,[22] the mean food intake, kaolin intake, and fecal output for two 12-hour light and two 12-hour dark periods before injection (preinjection values) and one 12-hour light and one 12-hour dark period after injection (postinjection values) were calculated for each treatment to assess changes in food intake, kaolin intake (pica), and fecal output associated with circadian rhythms.[33–37]

Statistical analysis

All 8 rats received each treatment and had data collected at each predetermined time point. The Shapiro-Wilk test was used to determine whether data were normally distributed. Hind limb withdrawal latencies were compared between the left and right hind limbs with a Mann-Whitney rank sum test because of nonnormal distribution; the values were not significantly different, and data were subsequently combined, with mean values for the 2 limbs at each time point used for further analysis, similar to previous studies.[28–32] Self-injurious behavior scores were expressed as median and IQR (25th to 75th percentile) and were analyzed with a Friedman repeated-measures ANOVA on ranks, with time (1, 4, 8, 12, and 24 hours) and treatment as independent factors. The percentage change in hind limb withdrawal latency from preinjection (time 0) values (normal distribution), food intake (mg/kg/12 hours; normal distribution), fecal output (mg/kg/12 hours; nonnormal distribution), and kaolin intake (mg/kg/12 hours; nonnormal distribution) measurements were expressed as mean ± SEM and were analyzed with a 2-way repeated-measures ANOVA, with time (1, 4, 8, 12, and 24 hours for hind limb withdrawal latency data; preinjection light or dark cycle or postinjection light or dark cycle for food intake, fecal output, and kaolin intake) and treatment as independent factors. The Student-Newman Keuls post hoc test was used to detect differences among the 4 treatments. Values of $P \leq 0.05$ were considered significant. Statistical analysis was performed with commercially available software.[j]

Results

Self-injurious behavior

Self-injurious behavior (ie, a score ≥ 1/4) was absent in all preinjection (time 0) assessments. Median self-injurious behavior scores 1, 4, 8, and 12 hours after injection of SRB or HCB were significantly ($P \leq 0.001$ for all comparisons) higher than preinjection scores for the same treatments. Self-injurious behavior scores were also significantly ($P = 0.024$) higher than preinjection scores 24 hours after SRB administration **(Figure 1)**. No rats had to be removed from the study because of injury.

Differences were also observed among study treatments. Median self-injurious behavior scores for the SRB treatment were significantly ($P \leq 0.014$ for all comparisons) higher than for the dextrose, BH, and HCB treatments at the 8- and 12-hour time points (Figure 1).

Hind limb withdrawal latencies

No significant (all $P \geq 0.75$) differences in hind limb withdrawal latencies were found among treatments at preinjection (time 0) assessments. No significant (all $P \geq 0.14$) differences in percentage change from preinjection values for hind limb withdrawal latencies were found among treatments from 1 to 12 hours after injection **(Figure 2)**. However, at the 24-hour time point, the percentage change in hind limb withdrawal latencies was significantly ($P = 0.014$) higher for the SRB treatment than for the BH treatment. When compared within treatments over time, the percentage change from preinjection values in hind limb withdrawal latencies was significantly higher for the BH (all $P \leq 0.015$) and HCB (all $P \leq 0.006$) treatments 1 hour after injection, compared with all other time points for those treatments.

Food intake

There were no differences (all $P \geq 0.50$) in food intake among treatments during the preinjection light period or the preinjection dark period **(Figure 3)**. Preinjection food intake was significantly (all $P < 0.001$) higher during the dark period than during the light period for all treatments (with mean ± SD differences of 127 ± 25%).

Postinjection light-period food intake was significantly (all $P \leq 0.028$) higher for the BH and HCB (all $P = 0.002$) treatments than for the dextrose treatment (with differences of 34% and 48%, respectively) or the SRB treatment (with differences of 37% and 51%, respectively). The postinjection light-period values for the BH and HCB treatments were also significantly ($P \leq 0.002$ for both) greater than the preinjection light-period values for the same treatments (with differences of 68% and 58%, respectively; Figure 3).

Postinjection dark-period food intake was significantly (all $P \leq 0.004$) lower for the BH, SRB, and HCB treatments than for the dextrose treatment (with differences of 21%, 24%, and 33%, respectively). Post-injection dark-period food intakes for the BH, SRB, and HCB treatments were also significantly (all $P < 0.001$) lower than the preinjection dark-period values for the same treatments (with differences of 28%, 35%, and 41%, respectively; Figure 3).

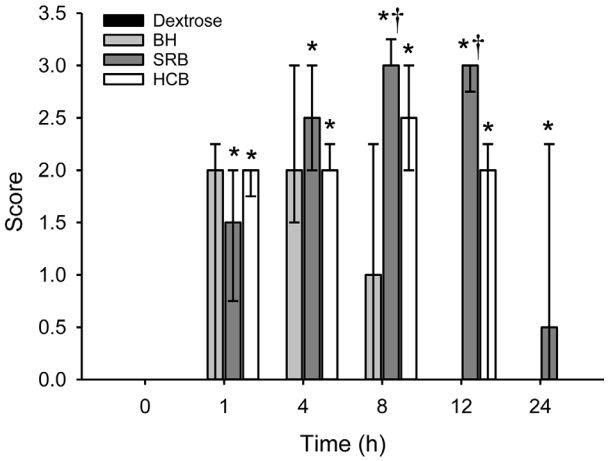

Figure 1—Median and IQR (25th to 75th percentile) self-injurious behavior scores for 8 healthy adult male Sprague-Dawley rats (*Rattus norvegicus*) before (time 0) and after (1, 4, 8, 12, and 24 hours) SC administration of each of 4 treatments (BH at 0.05 mg/kg, SRB at 1.2 mg/kg, HCB at 0.30 mg/kg, or the drug carrier [5% dextrose solution] at 0.20 mL/kg) in a blinded, randomized, crossover-design study with a 1-week washout period between experiments. A descriptive scale adapted from previous studies[10,25] and used in a recent study[22] of HCB in rats (from 0 [no cage-biting or self-biting] to 4 [frequent self-biting with moderate to severe visible evidence of skin trauma]) was used for assessments. *Significantly different, compared with the value prior to injection (time 0) for the same treatment. †Significantly different, compared with the values for dextrose, BH, and HCB treatments within a time point. Values of $P < 0.05$ were considered significant.

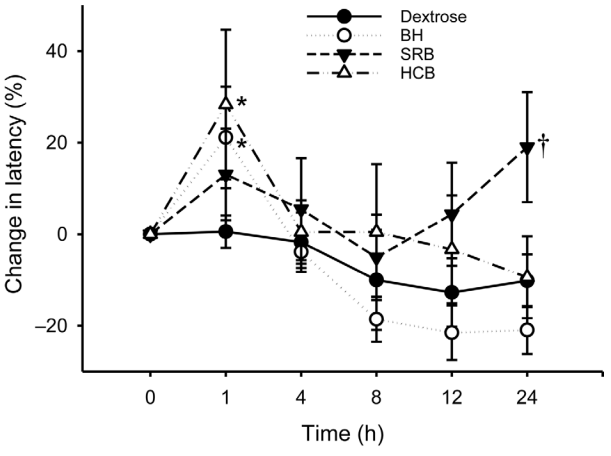

Figure 2—Mean ± SEM percentage change in hind limb withdrawal latency (response to a thermal stimulus) compared with the preinjection (time 0) value for the same rats as in Figure 1. Latencies did not differ significantly between left and right hind limbs and were combined for the analysis. *Significantly different from the values at time 0 and 4, 8, 12, and 24 hours for the same treatment. †Significantly different from the value for BH treatment at the same time point.

Fecal output

No significant (all $P > 0.65$) differences in fecal output were found among treatments at any time **(Figure 4)**. Fecal output was significantly (all $P \leq 0.001$) higher for all treatments during the dark period than during the light period for both preinjection (with differences of 77%, 81%, 47%, 57% for dextrose, BH, SRB, and HCB, respectively) and postinjection (62%, 128%, 200%, and 176% for dextrose, BH, SRB, and HCB, respectively) measurements.

Kaolin intake

Preinjection kaolin intake did not differ significantly (all $P \geq 0.08$) among treatments or between light and dark periods **(Figure 5)**. Postinjection light-period kaolin intake was significantly ($P \leq 0.021$) higher for the SRB treatment than for the dextrose, BH, and HCB treatments (with differences of 3,445%, 134%, and 298%, respectively). Postinjection light-period kaolin

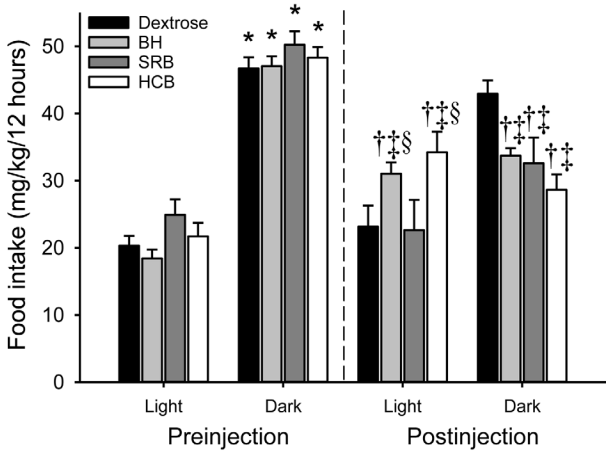

Figure 3—Mean ± SEM food intake before (preinjection) and after (postinjection) each of 4 treatments for the same rats as in Figure 1. Data were separated into 12-hour light and dark periods (2 of each cycle before and 1 of each cycle after injection) for analysis. *Significantly different from the light-period value for the same treatment and measurement type (preinjection or postinjection). †Significantly different from the preinjection value for the same treatment and light-dark period. ‡Significantly different from the value for dextrose treatment within a light-dark period and measurement type. §Significantly different from the value for SRB treatment within a light-dark period and measurement type.

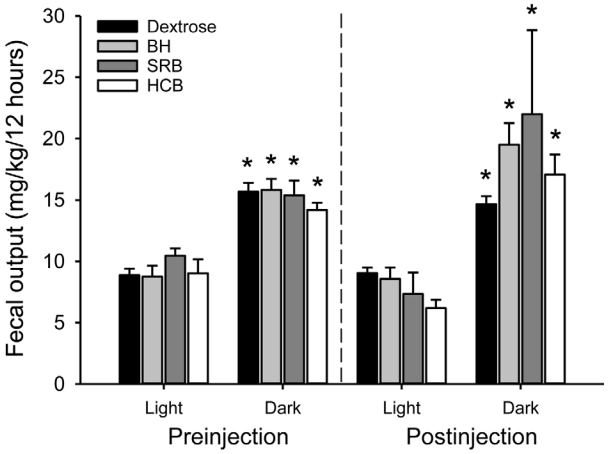

Figure 4—Mean ± SEM preinjection and postinjection fecal output during light and dark periods for the same rats as in Figure 1. ***See*** Figure 3 for key.

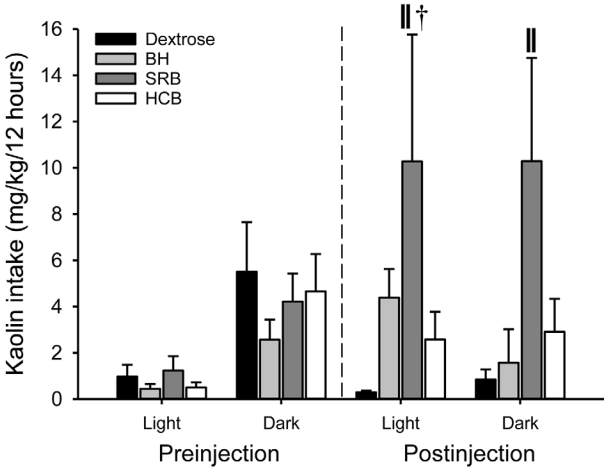

Figure 5—Mean ± SEM preinjection and postinjection kaolin intake (used as a measure of pica) during light and dark periods for the same rats as in Figure 1. ||Significantly different from the values for all other treatments within a light-dark period and measurement type. ***See*** Figure 3 for remainder of key.

intake was also significantly ($P = 0.002$) higher for the SRB treatment, compared with preinjection light-period intake for the same treatment (with a difference of 736%). Postinjection dark-period kaolin intake was significantly ($P \leq 0.008$) higher for the SRB treatment than the dextrose, BH, and HCB treatments (with differences of 1,110%, 551%, and 254%, respectively).

Discussion

To our knowledge, the present study was the first to compare HCB with other commercially available buprenorphine formulations with regard to hypoalgesia, self-injurious behavior, food intake, fecal output, and pica behavior in rats. Each drug was administered SC, once, at a single predetermined dose (0.30, 1.2, and 0.05 mg/kg for HCB, SRB, and BH, respectively) in this crossover-design study. Although hypoalgesia (as determined by increased hind limb withdrawal latency in response to a thermal stimulus, relative to that prior to injection) was detected for a short period after administration of some buprenorphine formulations, self-injurious behavior in all buprenorphine-treated rats suggested that all of the tested formulations have the potential to cause unwanted effects when administered to healthy rats with no evidence of pain. Specifically, self-injurious behavior was not seen in rats that received the vehicle treatment (5% dextrose solution, 0.2 mL/kg, SC) but was observed in all rats during the first 8 hours after injection of BH, HCB, or SRB, with significantly increased median self-injurious behavior scores persisting for 12 hours in HCB-treated rats and for 24 hours in SRB-treated rats. The availability of kaolin pellets did not prevent self-injurious behavior, thus refuting 1 of the study hypotheses. Self-injurious behavior is observed in rats after treatment with neurostimulants such as amphetamines and morphine, with dopaminergic stimulation implicated as a potential cause.[10,38–44] Self-injurious behavior associated with buprenorphine administration, however, appears to be rare or at least not well documented, with the exception of our previously described HCB study[22] and a recent letter describing excessive licking and chewing after treatment with an extended-release formulation of buprenorphine,[45] in which the authors equated the behavior with nausea. The low frequency of such reports is potentially attributable to most studies that involve the use of buprenorphine in rats being performed in animals undergoing surgery or other potentially painful procedures, unlike the rats in the present study.

Significant hypoalgesia was observed only with the BH and HCB treatments in the present study, and the results differed from preinjection (time 0) values only at the 1-hour time point. Our results are similar to previous investigations of BH and HCB in rats, in which hypoalgesia was detected for only 1 hour.[13,22] In the present study, the percentage change from preinjection values was higher in SRB-treated rats than in BH-treated rats at 24 hours, but it was not significantly different from time 0 values or values at other time points for the SRB treatment. Similar to our findings, results of a study[11] comparing effects of BH (at 0.2 mg/kg) and SRB (at 1.2 mg/kg) in rats revealed no significant difference between treatments in hind limb withdrawal latencies measured by means of thermal analgesiometry until 48 hours after injection. In that study,[11] plasma concentrations of BH and SRB were similar until 8 hours after administration, and after that time point, only circulating SRB concentrations remained above the presumed therapeutic threshold of 1 ng/mL until 72 hours after injection.[11] Overall, given that adequate plasma drug concentrations and hypoalgesia associated with SC administration of BH and SRB in rats[2,4,5,11–13] and HCB in cats[21] have been demonstrated previously, it is unclear why we did not detect significant changes from time 0 in hind limb withdrawal latencies with SRB and more prolonged hypoalgesic effects with BH and HCB.

Our use of a thermal stimulus to assess the hypoalgesic effects of buprenorphine in animals that did not

undergo surgery likely influenced the results. Various types of noxious stimuli produce diverse responses, and it is possible that inflammatory conditions or the use of mechanical stimuli might have revealed evidence of hypoalgesia with all buprenorphine preparations. Furthermore, the observed responses to a thermal stimulus could potentially have been altered by opioid-induced effects, including pica, self-injurious behavior, hyperalgesia, sensitization, and tolerance, overriding any hypoalgesic effect. The first 2 factors were observed in the present study. The latter 3 effects, especially sensitization from repeated testing, were not measured and could not be ruled out. However, these influences should have been minimized by the study design. For example, hyperalgesia appears to be associated with repeated dosing, ultra-low doses, and low plasma drug concentrations, and occurs several hours to days after acute opioid treatment.[8,46] In addition, testing intervals were chosen on the basis of information in previous studies[26,27] to avoid sensitization, although this effect could not be completely ruled out. Finally, single dose administration, randomization of treatment order, and a 1-week washout period were chosen to minimize opioid tolerance. However, tolerance has been reported for up 10 days after a single postsurgical dose of buprenorphine > 0.05 mg/kg in rats.[4] Therefore, this effect might have been further minimized by extending the washout period to 2 weeks, although this would have doubled the study duration.

In the present study, we chose to separate food intake, fecal output, and kaolin intake data into light and dark periods to account for circadian rhythms.[33–37] In previous studies,[22,34,35] food intake in rats was highest during the 12-hour dark period when they are most active. Our study confirmed that in untreated rats, food intake was higher during the dark period than during the light period. After buprenorphine administration, this relationship was altered, with food intake becoming higher during the light period (BH and HCB), and lower during the dark period (BH, SRB, and HCB) compared with the dextrose treatment and with preinjection values. These findings suggested that rats became more active after buprenorphine administration and that caloric requirements were met by consuming more food during the light period. Given that postinjection dark-period food intake for rats with the SRB treatment was lower than that for the dextrose treatment and preinjection dark-period food intake for the SRB treatment (similar to BH and HCB treatments), the authors speculate that the lack of a significant increase in postinjection light-period food intake for this treatment (unlike BH and HCB treatments) might have been attributable to the high kaolin intake observed with that treatment or to small sample size.

Preinjection and postinjection fecal output was significantly higher during the dark period than during the light period when all treatments were combined for analysis. In a previous study[22] by the authors investigating HCB in rats, fecal output was altered during the first 12 hours (light period) after treatment, which the authors attributed to the inhibitory effects of buprenorphine on gastrointestinal motility, an effect of buprenorphine described in other studies.[1,47] The lack of a significant alteration in the relationship between light-period and dark-period fecal output after treatment in the present study, despite altered food intake patterns, may have resulted from kaolin intake in the present study. Kaolin passes easily through the gastrointestinal tract,[16–20] and, indeed, many large, white fecal pellets were collected throughout the experiments (separation of lighter [primarily kaolin-containing] vs darker, normal-appearing fecal pellets was not performed).

Preinjection kaolin intake was similar among all treatments during the light and dark periods. Postinjection kaolin intake was higher for the SRB treatment than for all other treatments during both light and dark periods. Postinjection kaolin intake during the light period for the SRB treatment was also higher than preinjection light period values for the same treatment. On the basis of these findings, we speculate that SRB may have greater nausea-inducing effects in healthy rats with no evidence of pain than the other tested buprenorphine formulations.

Overall, hypoalgesic effects in response to a thermal noxious stimulus were observed for only 1 hour after administration of 2 of the 3 buprenorphine formulations tested, and self-injurious behavior, pica, or both may have overridden hypoalgesic effects in these healthy laboratory rats. Opioid-induced hyperalgesia, sensitization, and tolerance could also not be completely ruled out. Further studies comparing the effects of BH, SRB, and HCB in rats undergoing surgery or exposed to noxious stimuli are indicated to elucidate whether these formulations have clinical benefits in this species.

Acknowledgments

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Footnotes

a. Harlan Laboratories, Indianapolis, Ind.
b. Teklad 6% fat mouse/rat diet, Envigo, Madison, Wis.
c. Randomizer.org. Research randomizer. Available at: www.randomizer.org. Accessed Apr 19, 2017.
d. Buprenex, 0.3 mg/mL, Reckitt Benckiser, Hull, England.
e. Buprenorphine SR Lab, 1 mg/mL, ZooPharm, Windsor, Colo.
f. Simbadol, 1.8 mg/mL, Zoetis Inc, Kalamazoo, Mich.
g. Baxter Healthcare, Deerfield, Ill.
h. Ugo Basile, Varese, Italy.
i. Research Diets, New Brunswick, NJ.
j. SigmaPlot version 12.0, Systat Software, San Jose, Calif.

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