Evaluation of self-injurious behavior, food intake, fecal output, and thermal withdrawal latencies after injection of a high-concentration buprenorphine formulation in rats (*Rattus norvegicus*)

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OBJECTIVE

To evaluate effects of high-concentration buprenorphine (HCB) on self-injurious behavior, food intake, fecal output, and thermal withdrawal latencies in healthy rats.

ANIMALS

8 Sprague-Dawley rats.

PROCEDURES

Rats received 4 SC treatments (HCB at 0.075, 0.15, or 0.30 mg/kg [HCB0.075, HCB0.15, and HCB0.30, respectively] or 5% dextrose solution [0.20 mL/kg]) in a randomized, crossover-design study. Self-injurious behavior was assessed for 8 hours after injection. Food intake and fecal output were assessed for predetermined periods before and after treatment and separated into 12-hour light and dark periods for further analysis. Withdrawal latencies were assessed before (time 0) and at predetermined times after injection. Data were compared among treatments and time points.

RESULTS

Self-injurious behavior was observed up to 8 hours after injection for all HCB, but not dextrose, treatments. Preinjection food intake and fecal output amounts were similar among groups and higher during the dark period than during the light period. Food intake after all HCB treatments was higher during the light period and lower during the dark period, compared with preinjection results for the same treatments and with postinjection results for dextrose administration. Light-period fecal output was lower after HCB0.15 and HCB0.30 administration, compared with preinjection values for the same treatments and postinjection values for dextrose administration. Percentage change in withdrawal latency was significantly higher than that at time 0 (ie, 0%) for only 1 treatment (HCB0.30) at 1 time point (1 hour after injection).

CONCLUSIONS AND CLINICAL RELEVANCE

Although HCB0.30 produced a degree of thermal hypoalgesia in healthy rats, self-injurious behavior and alterations in food intake and fecal output were detected, potentially affecting clinical utility of the treatment. (*Am J Vet Res* 2018;79:154–162)

Buprenorphine hydrochloride, a semisynthetic, lipophilic opiate first synthesized in the 1960s, is 25 to 40 times as potent as morphine and is frequently used to treat moderate to severe pain in rats.[1,2] Hypoalgesia is mainly attributable to its action as a partial agonist at μ-opioid receptors, where it binds avidly and dissociates slowly[1] with a terminal half-life of 2.1 to 3.0 hours after IV bolus administration.[3] The current recommended dosage in rats is 0.05 mg/kg, SC or IV, every 6 to 12 hours.[2,4–6] These repeated injections predispose animals to signs of stress, reduced food intake and low weight gain, altered ambient locomotor activity, and sensitization (hyperalgesia),[7–10] which can negatively impact animal welfare and make pain assessment difficult.[7,8] The hypoalgesic efficacy of traditional formulations of buprenorphine at doses of 0.015 to 0.30 mg/kg[4,8,10–12] is well known, with a ceiling effect occurring at 0.1 mg/kg (beyond which no further hypoalgesic efficacy is observed).[10,12,13] However, higher doses in cats[14] and rats[15,16] may have prolonged antinociceptive effects (from 27 to 72 hours' duration). For example, a single injection of a sustained-release preparation of buprenorphine was shown to have a prolonged hypoalgesic effect of up to 72 hours in rats[15]; however, adverse effects of substantial respiratory depression and reduced volun-

ABBREVIATIONS

HCB	High-concentration buprenorphine
IQR	Interquartile (25th to 75th percentiles) range

tary activity were observed, making this formulation less desirable. In the aforementioned study, it was not determined whether adverse effects were associated with the drug dose, the drug vehicle, or both.

Similar to other opioids, adverse effects of buprenorphine include μ-receptor–facilitated changes in gastrointestinal function, pica, hyperalgesia, and opioid tolerance. For example, buprenorphine at doses of 0.01 to 10 mg/kg may prolong gastrointestinal transit times in rats.[1,17] Further, pica associated with repeated buprenorphine administration can exacerbate changes in gastrointestinal transit time, resulting in gastrointestinal obstruction, reduced weight gain, and death.[9,18,19] Pica behavior is induced in rats by stimuli that cause nausea and emesis in other species and has been evaluated as an index of emetogenic potential of other drugs.[20–24] Hyperalgesia is a less understood adverse effect of buprenorphine administration but has particularly been noted following repeated dosing, when plasma drug concentrations are low, and when used in animals without painful conditions.[8,16] Opioid tolerance occurs after prolonged exposure to buprenorphine (0.1 to 0.2 mg/kg, q 12 h for 7 to 10 days) and persists for up to 2 weeks after stopping treatment.[11,16] Opioid tolerance to morphine was also reported up to 10 days after a single dose of buprenorphine > 0.05 mg/kg, after surgery in rats.[4] Other abnormal behavior patterns such as stereotypic behaviors (ie, self-biting and cage-biting) have been reported after administration of other opioids such as morphine in rats.[10,15,25–29] However, these behaviors have not been fully investigated after buprenorphine administration in this species.

Commercially available HCB (1.8 mg/mL) that is not formulated in a sustained-release or extended-release preparation was shown to have hypoalgesic effects for up to 24 hours in cats when administered at 0.24 mg/kg; this concentration and dose are substantially higher than those for traditional buprenorphine administration in cats (0.3 mg/mL and 0.02 to 0.03 mg/kg, q 6 to 8 h, respectively).[14] Given the safety and efficacy of this dosing schedule in cats[14] and the disadvantages of repeated parenteral injection in rats (ie, handling-related stress, pica, and diminished food intake and loss of body weight), a once-daily dosing regimen for buprenorphine in rats could have distinct advantages. For example, treatment with a product that has a longer duration of action than traditional buprenorphine and minimal side effects, compared with repeated buprenorphine administration or use of the sustained-release or extended-release buprenorphine preparations, would be beneficial.

The objective of the study reported here was to assess the feasibility of HCB treatment in rats by evaluating self-injurious behavior, food intake and fecal output, and thermal withdrawal latencies following administration of the drug at 3 different doses. We hypothesized that SC administration of HCB would be associated with minimal dose-dependent behavioral alterations and would decrease food intake and fecal output immediately after treatment. In addition, we hypothesized that HCB would provide hypoalgesia in a dose-dependent manner with no evidence of hyperalgesia.

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 the Institute for Laboratory Animal Research guidelines.[30] Eight adult male Sprague-Dawley rats (*Rattus norvegicus*)[a] with a mean ± SD body weight of 307 ± 10 g at the start of the study were used. Rats were housed in pairs on corncob bedding in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited animal facility prior to the study, with a light-to-dark cycle of 12 hours of light to 12 hours of darkness and free access to commercial rat pellets and water. Rats were allowed 7 days to acclimatize to the animal holding facility at the start of the study and 48 hours to acclimatize to wire-bottom cages prior to each experiment. During each testing period, rats were housed singly in wire-bottomed cages, with free access to commercial rat pellets and water. Rats were returned to their corncob bedding for each drug washout period. The rats were free from ectromelia virus, Hantaan virus, K virus (murine pneumotropic virus), Kilham rat virus, lactic dehydrogenase elevating virus, lymphocytic virus, minute virus of mice, mouse adenovirus 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, as determined by laboratory tests provided by the commercial supplier.[a] Rats were euthanized by CO_2 inhalation at the end of the study in accordance with AVMA euthanasia guidelines.[31]

Study design

The study had a blinded, randomized, crossover design. All rats received each of 4 treatments as a single SC injection in randomized order[b] (HCB[c] administered at 0.075, 0.15, or 0.30 mg/kg [HCB0.075, HCB0.15, and HCB0.30, respectively], or the carrier [5% dextrose solution,[d] administered at 0.20 mL/kg]), with a 1-week washout period between experiments. One investigator who was blinded to the treatment (MA) performed all injections. Blinding for HCB dose was accomplished by surrounding each syringe with opaque tape so that injectate volumes were not known. The volume of dextrose solution corresponded to the highest injectate volume for HCB. In-

jections were performed immediately after the dark cycle, between 6:00 AM and 7:00 AM. Because no published doses for HCB in rats were available prior to the study, doses were extrapolated from a safety and efficacy study[14] in cats in which HCB doses ranged from 3 to 12 times the recommended dose of the standard buprenorphine hydrochloride formulation for that species.

Self-injurious behavior

Pica was not evaluated during the study because bedding was removed from the wire-bottomed cages used for experimental observations. However, self-injurious behavior (self-biting and cage-biting) was subjectively assessed when seen at any point after injection. Rats were directly observed for 2-minute periods intermittently in wire-bottom cages and between thermal sensitivity trials in the thermal sensitivity–testing chambers. Observation time points were not predetermined outside the thermal withdrawal latency test times. A descriptive scale with arbitrary units was adapted from previous studies[10,32] and recorded as follows: 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 evidence of skin trauma, or both; 3 = frequent self-biting with evidence of mild to moderate skin trauma (inflammation); and 4 = frequent self-biting with evidence of moderate to severe skin trauma (bleeding). Occasional cage-biting or self-biting was defined as occurring for ≤ 30 seconds (continuous or intermittent) during a 2-minute observation period. Frequent cage-biting or self-biting was defined as occurring for > 30 seconds during a 2-minute observation period.

Food intake and fecal output

Rats were provided free access to a premeasured amount of commercial rat food (200 ± 1 g) in a removable wire rack when they were housed singly in wire-bottomed cages at 48 hours before each treatment. Paper liners were placed on cage bottoms to absorb urine and water so that spilled food would not become saturated. The mass of food remaining in the wire rack and spilled on the floor was measured every 12 hours, with data collection starting 48 hours prior to injection and until 24 hours after injection. Similarly, fecal pellets were collected and the mass was measured every 12 hours from 48 hours before injection until 24 hours after injection. Measurements for individual rats were expressed as a fraction of body weight (mg/kg) because weights varied considerably over the course of the study (range, 286 to 407 g). The mean intake and output for four 12-hour periods before (preinjection) and two 12-hour periods after (postinjection) treatment were calculated; mean values for 12-hour light (inactive) and 12-hour dark (active) periods were also calculated to assess changes in food intake and fecal output associated with circadian rhythms.[33–37]

Withdrawal responses to thermal stimuli

Thermal hypoalgesia was assessed by measurement of the latency for hind limb withdrawal in response to a radiant heat stimulus[38] applied with a commercial thermal latency testing device.[e] Rats were allowed 15 minutes to acclimatize to the testing chambers. 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 pretreatment measurements, with a maximum latency limit of 20 seconds allowed to avoid thermal burns. An infrared heat stimulus was applied to each plantar surface, and the time to withdrawal of the hind limb in response to the heat stimulus was defined as the thermal withdrawal latency. Withdrawal latencies were measured prior to injection (at the beginning of each test week; time 0) and at 1, 4, 8, 12, and 24 hours after injection. At each time point, each rat was tested 3 times with ≥ 5 minutes between trials on alternating paws (≥ 10 minutes between trials on the same paw), and the mean latency was calculated. This pattern was chosen to minimize hyperalgesia secondary to repeated noxious stimuli.[15,38,39] Withdrawal latencies were performed on both right and left hind limbs. Data were summarized as the percentage change from preinjection values, similar to a previously published investigation.[15]

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. Thermal withdrawal latencies were compared between the left and right hind limbs with a Wilcoxon signed rank 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[40–44] in which thermal withdrawal latencies were evaluated. 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 suitable for data with a nonnormal distribution, with treatment as the independent factor. The percentage change in thermal withdrawal latency from preinjection (time 0) values and the food intake (which had nonnormal distribution) and fecal output measurements (as mg/kg/12 h) were expressed as mean ± SE and were analyzed by a 2-way repeated-measures ANOVA with time (0, 1, 4, 8, 12, and 24 hours for latency data, and preinjection or postinjection alone or segregated according to light or dark cycle for food intake and fecal output) 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. Sta-

tistical analysis was performed with commercially available software.[f]

Results

Self-injurious behavior

Self-injurious behavior (defined as a score ≥ 1/4) was present for up to 8 hours after injection for all HCB treatments. Self-injurious behavior scores were significantly ($P < 0.001$ for all comparisons) higher in rats after administration of HCB0.075 (median, 2.0 [IQR, 0.25 to 3.0]), HCB0.15 (median, 2.5 [IQR, 2.0 to 3.0]), or HCB0.30 (median, 3.0 [IQR, 2.0 to 3.0]) than after dextrose administration (median, 0.0 [IQR, 0.0 to 0.0]). The scores were higher in rats after receiving HCB0.15 or HCB0.30 than after receiving HCB0.075 ($P = 0.035$ and 0.039, respectively), but scores after administration of HCB0.15 did not differ significantly ($P = 0.71$) from scores after administration of HCB0.30. No animals were required to be removed from the study because of injury.

Food intake

No significant differences in overall food intake were found among the 4 treatments or between preinjection and postinjection measurements within treatment groups **(Table 1)**. When food intake was separated into 12-hour light (inactive) and dark (active) periods, the preinjection food intake amount was significantly ($P < 0.004$ for all comparisons) higher during the dark period than during the light period for all treatments **(Figure 1)**, with a mean ± SE difference of 124 ± 5% across all treatment groups.

No significant differences in preinjection food intake were found among treatments during the light period (Figure 1). However, the amount of postinjection food intake during the light period was significantly ($P = 0.016$, 0.003, and 0.003, respectively) higher after administration of HCB0.075, HCB0.15, and HCB0.30 than after dextrose administration (with differences of 37%, 51%, and 53%, respectively). Postinjection food intake during the light period was also significantly ($P < 0.005$ for all comparisons) higher after administration of HCB0.075, HCB0.15, or HCB0.30, compared with preinjection food intake during the light period for the same treatments (with differences of 63%, 78%, and 83%, respectively).

No significant differences in preinjection food intake were found among treatments during the dark period (Figure 1). The postinjection food intake amount during the dark period was significantly ($P < 0.003$ for all comparisons) lower after administration of HCB0.075, HCB0.15, or HCB0.30 than after dextrose administration (with differences of 27%, 36%, and 47%, respectively). Postinjection food intake during the dark period was also significantly ($P < 0.005$ for all comparisons) lower after administration of HCB0.075, HCB0.15, or HCB0.30, compared with the preinjection dark period values for the same treatments (with differences of 29%, 38%, and 47%, respectively).

During the dark period, the postinjection food intake amount was significantly ($P = 0.037$) lower after administration of HCB0.30 than after administration of HCB0.075 (a 28% difference; Figure 1). Finally, postinjection food intake during the dark period was significantly lower after administration of HCB0.15 ($P = 0.029$) or HCB0.30 ($P < 0.001$), compared with postinjection light period values for the same treatments (differences of 23% and 38%, respectively), whereas postinjection food intake during the dark period after dextrose administration was significantly ($P < 0.001$) higher than that during than the light period (an 81% difference).

Fecal output

No significant differences in preinjection fecal output were identified among the 4 treatments (Table 1). However, the postinjection fecal output amount after administration of HCB0.30 was significantly ($P = 0.004$) lower than that after administration of HCB0.075. Postinjection fecal output after administration of HCB0.30 was also significantly (P

Table 1—Mean ± SE food intake and fecal output data for 8 adult male Sprague-Dawley rats (*Rattus norvegicus*) before and after SC administration of each of 4 treatments (HCB at doses of 0.075 mg/kg, 0.15 mg/kg, or 0.30 mg/kg [HCB0.075, HCB0.15, and HCB0.30, respectively] or the drug carrier [5% dextrose solution, at a dose of 0.20 mL/kg]) in a randomized, crossover-design study with a 1-week washout period between experiments.

Variable	HCB0.075	HCB0.15	HCB0.30	Dextrose
Food intake (mg/kg/12 h)				
Preinjection	34.7 ± 1.4	35.2 ± 0.8	33.8 ± 1.3	32.8 ± 0.8
Postinjection	34.6 ± 2.8	34.4 ± 3.1	31.9 ± 4.5	36.1 ± 2.2
Fecal output (mg/kg/12 h)				
Preinjection	12.8 ± 0.6	13.6 ± 0.4	12.9 ± 0.6†	12.6 ± 0.4
Postinjection	14.2 ± 1.3*	12.5 ± 1.1	11.0 ± 1.4*†	12.9 ± 0.8

Singly housed rats had free access to food weighed (200 ± 1 g) and placed in a wire rack 48 hours prior to treatment. The remaining food was weighed and recorded at 12-hour intervals. Preinjection food intake was recorded as the mean of four 12-hour periods prior to injection, and postinjection intake was recorded as the mean of two 12-hour periods after injection. Data were reported as fractions of body weight because the weights of individual rats varied substantially during the study.

*Within a row, values are significantly different between treatments. †Within a column, for a given variable, preinjection and postinjection values are significantly different.

= 0.01) lower than preinjection fecal output for the same treatment. When separated into 12-hour light and dark periods, preinjection and postinjection fecal output was significantly ($P < 0.004$ and $P < 0.02$, respectively, for all comparisons) higher during the dark period than during the light period for all treatments **(Figure 2)**, with mean ± SE differences across all treatments of 51 ± 9% for preinjection values and 87 ± 3% for postinjection values.

Although preinjection fecal output during the light period did not differ significantly among treatments, postinjection fecal output during this period was significantly ($P = 0.02$ and 0.012, respectively) lower after administration of HCB0.15 or HCB0.30 than after dextrose administration (Figure 2), with differences of 32% and 37%, respectively. Postinjection fecal output during the light period was also significantly ($P = 0.006$ and 0.012, respectively) lower after administration of HCB0.15 or HCB0.30, compared with preinjection values during the light period for the same treatments (differences of 32% for both comparisons).

Although no differences in preinjection fecal output were found among treatments during the dark period, postinjection output after administration of HCB0.075 was significantly ($P = 0.026$ and 0.028, respectively) higher than that after dextrose or HCB0.30 administration in this period (Figure 2), with differences of 26% and 23%, respectively. Postinjection dark period fecal output after administration of HCB0.075 was also significantly ($P = 0.008$) higher than the preinjection dark period value for the same treatment (a 24% difference).

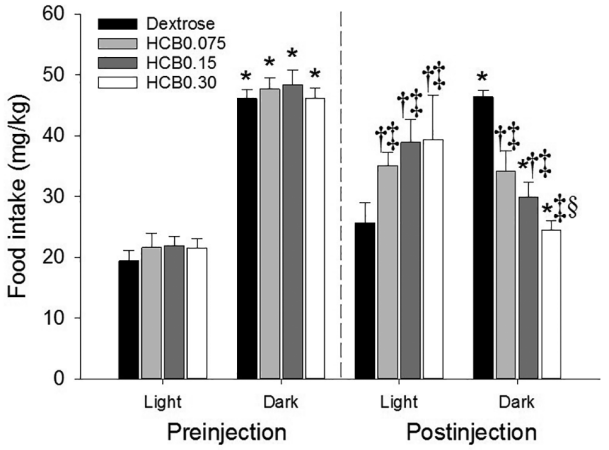

Figure 1—Mean ± SE food intake (mg/kg/12 h) for 8 adult male Sprague-Dawley rats (*Rattus norvegicus*) before (preinjection) and after (postinjection) SC administration of each of 4 treatments (HCB at doses of 0.075 mg/kg, 0.15 mg/kg, or 0.30 mg/kg [HCB0.075, HCB0.15, and HCB0.30, respectively] or the drug carrier [5% dextrose solution, at a dose of 0.20 mL/kg]) in a randomized, crossover-design study with a 1-week washout period between experiments. Data were separated into 12-hour light and dark periods for the analysis. *Significantly different from the light period value for the same treatment and measurement type (preinjection or postinjection). †Significantly different, compared with the value for dextrose treatment within a light-dark period and measurement type. ‡Significantly different from the preinjection value for the same treatment and light-dark period. §Significantly different, compared with values for dextrose and HCB0.075 treatments within a light-dark period and measurement type. Values of $P < 0.05$ were considered significant.

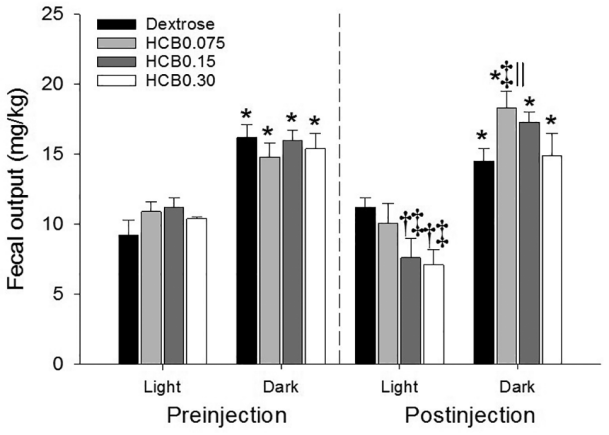

Figure 2—Mean ± SE preinjection and postinjection fecal output (mg/kg/12 h) during light and dark periods for the same rats as in Figure 1. ||Significantly different, compared with the values for dextrose and HCB0.30 treatments within a light-dark period and measurement type. ***See*** Figure 1 for remainder of key.

Thermal withdrawal latencies

No significant thermal withdrawal latency differences were found among treatments at time 0 (prior to injection) or 24 hours after injection **(Figure 3)**. The percentage change in latency from time 0 was significantly higher for HCB0.30 treatment 1 and 8 hours after injection than for dextrose ($P = 0.001$ and

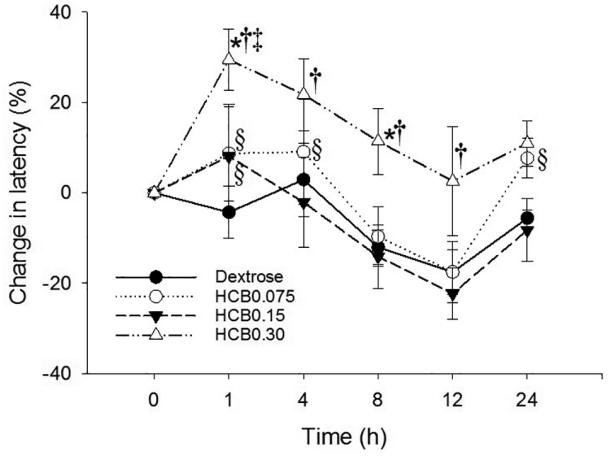

Figure 3—Mean ± SE percentage change in thermal withdrawal latencies, compared with that prior to injection (time 0) for the same rats as in Figure 1. Latencies did not differ significantly between left and right hind limbs and were combined for the analysis. *Significantly different from the values for dextrose and HCB0.075 treatments at the same time point. †Significantly different from the value for HCB0.15 treatment at the same time point. ‡Significantly different, compared with values at time 0 and 12 hours for the same treatment. §Significantly different from the value at 12 hours for the same treatment.

0.023, respectively), HCB0.075 (P = 0.020 and 0.017, respectively), and HCB0.15 (P = 0.042 and 0.022, respectively) treatments. At 4 and 12 hours after injection, the percentage change in latency from time 0 for HCB0.30 treatment was significantly (P = 0.036 and 0.027, respectively) higher than that for HCB0.15 treatment.

When compared across time, the percentage change from time 0 thermal withdrawal latency after HCB0.30 administration was significantly higher at 1 hour than at time 0 (with the time 0 value taken as 0% change; P = 0.004) and at 12 hours (P = 0.010; Figure 3). The percentage change from time 0 latency after HCB0.075 administration was significantly lower at 12 hours than at 1 (P = 0.013), 4 (P = 0.016), and 24 hours (P = 0.014). Finally, the percentage change from time 0 latency after HCB0.15 administration was significantly (P = 0.003) lower at 12 hours than at 1 hour.

Discussion

In the present study, HCB administered at 0.075, 0.15, or 0.30 mg/kg (HCB0.075, HCB0.15, and HCB0.30, respectively) was significantly associated with self-injurious behavior (cage-biting and self-biting) in rats during the first 8 hours after injection. Only 1 dose was associated with significant hypoalgesia as measured by increased thermal withdrawal latency. Withdrawal latency after administration of HCB0.30 was significantly longer than that measured after other treatments for up to 12 hours, but differed from the time 0 (preinjection) value only at the 1-hour time point. Although, to our knowledge, this was the first investigation of the feasibility of HCB administration in rats that did not undergo surgery, the finding that self-injurious behavior was significantly associated with a hypoalgesic dose suggested that its usefulness in this species may be limited in some instances.

Although mean postinjection food intake (calculated as mg/kg/12 h over a 24-hour period) did not differ among treatments, significant differences were observed in HCB-treated, but not dextrose-treated, rats when these values were divided into 12-hour light (inactive) and dark (active) periods for analysis. Mean postinjection fecal output (mg/kg/12 h over a 24-hour period) was lower after administration of HCB0.30, compared with preinjection fecal output for the same treatment, and this was mainly attributable to lower values during the 12-hour light period after treatment.

Although the HCB used in the present investigation was shown to provide postoperative hypoalgesia for up to 24 hours in cats when administered at 0.24 mg/kg, SC,[14] in our study, the drug provided only 1 hour of thermal hypoalgesia to rats as quantified by the Hargreaves (plantar test) method, and only at the highest tested dose (0.30 mg/kg). One limitation of our study was that we did not test higher doses of HCB and therefore did not observe any ceiling effect in hypoalgesia. Results of a previous investigation confirmed that the traditional buprenorphine hydrochloride formulation administered at 0.05 mg/kg produces thermal hypoalgesia in rats for 1 hour.[15] Doses up to 0.1 mg/kg produce dose-dependent increases in thermal hypoalgesia (rodent tail flick test), but higher doses only extend the duration of effect.[12,13,16] It is unclear why we did not detect significant changes in withdrawal latencies with the lower HCB doses, considering that self-injurious behavior and alterations in food intake and fecal output were clearly present in rats that received those treatments. Our study was additionally limited by the small sample size of 8 rats; it is possible that a larger sample size might have influenced these results.

The decision to assess the hypoalgesic effects of a drug by use of a noxious thermal stimulus in animals that did not undergo surgery can have implications for the conclusions drawn from test results. For example, various types of noxious stimuli produce unique responses, and HCB may have more substantial hypoalgesic effects in rats that have specific types of pain or are tested by other types of stimuli (eg, inflammatory conditions or mechanical stimulus). Moreover, the complex behavioral and pharmacodynamic effects associated with opioid receptor agonists, such as hyperalgesia, sensitization, tolerance, pica, and self-injurious behavior, may affect or even predominate over hypoalgesic responses in rats subjected to mild noxious stimuli. Although hyperalgesia has been reported after buprenorphine administration, it appears to be associated with ultralow doses, low plasma drug concentrations, or both (appearing several hours to days after acute treatment).[8,16] Although possible, we considered it less likely that hyperalgesia occurred in the present study because there were no significant postinjection reductions in withdrawal latencies, compared with the time 0 value, for any treatment at any time point. On the other hand, the percentage change in withdrawal latency at 12 hours after injection was significantly lower than that at 1 hour after injection for all HCB treatments, suggesting that a small degree of hyperalgesia may have occurred. The testing intervals for administration of noxious stimuli in this study were chosen to avoid sensitization on the basis of results of previous studies,[38,39] although this effect could not be ruled out and might have masked hypoalgesic effects of HCB.

Opioid tolerance was not assessed in the present study but could also have affected the results. Single-dose administration, randomization of treatment order, and a washout period of 1 week between treatments were used to minimize this effect. However, opioid tolerance has been reported for up to 2 weeks after cessation of repeated buprenorphine treatment[11,16] and up to 10 days after a single postsurgical dose in rats.[4] If tolerance occurred in the present study, the magnitude might have been affected by treatment order; for example, rats that received the highest dose first could have had more tolerance than

rats that received the lowest dose first. Opioid tolerance might have been better minimized by extending the washout period to 2 weeks, although this would have doubled the study duration.

Stereotypic behavior in rats (including self-injurious behavior) depends primarily on dopaminergic activation in the basal ganglia and is induced by dopamine agonists including amphetamine, methamphetamine, and apomorphine.[10,25–29] Whereas dopamine-1 receptor activation is implicated in the induction of stereotyped behavior,[10,25–29,45,46] nondopaminergic mechanisms involving noradrenergic, serotonergic, and opioid systems also influence the appearance of stereotypy.[10,45,46] For example, μ-opioid receptor activation with morphine enhances stereotypic behavior, and naloxone (μ-opioid receptor antagonist) reduces or prevents this type of behavior.[45–47] We speculate that self-injurious behavior in the present study was induced by μ-opioid receptor activation at all 3 doses of HCB tested, implicating acute treatment with HCB in the induction of stereotypic behavior in rats that did not undergo surgery. It is important to note that the rats in the present study did not have access to bedding; thus, it is possible that if bedding or another nonnutritive substrate was provided, self-injurious behavior might have been attenuated by pica. Unlike pica, self-injurious behavior has not been clearly documented in studies of traditional buprenorphine hydrochloride preparations in rats at a wide range of doses; it may be that HCB is unique in its potential to induce self-injurious behavior.

We chose to extend our assessment of food intake and fecal output data by separating these into light and dark periods because the initial assessment of these variables (as mg/kg/12 h) from a 48-hour period before and a 24-hour period after injection did not take into account the effects of circadian rhythms[33–37] and because food intake in healthy rats is greatest during the 12-hour dark period when rats are more active.[34,35] The investigation confirmed that during the dark period prior to treatments, rats consume more food than during the light period. This relationship was altered by HCB administration; during the light period, the amount of postinjection food intake after administration of HCB0.075, HCB0.15, or HCB0.30 was higher than that after dextrose administration and higher than the preinjection light-period values for the same treatments, whereas during the dark period, food intake after each of the 3 HCB treatments was lower than that after dextrose treatment and lower than preinjection dark-period values for the same treatments. It appeared that rats administered HCB had adequate food intake during the light period to offset the lower food intake during the dark period, because the overall food intake was similar before and after injections.

When separated into 12-hour light and dark periods, the amount of fecal output was lower after administration of HCB0.15 or HCB0.30, compared with that after dextrose treatment and with the corresponding preinjection light-period values, during the first 12 hours after injection (light period) but returned to preinjection, dark-period values during the next 12 hours (dark period). Given the possible inhibitory effects of buprenorphine on gastrointestinal motility,[1,17] the reduction in fecal output during the first 12 hours (light period) was not surprising. The return to pretreatment, dark-period fecal output values during the subsequent 12 hours (dark period) was less consistent with the expected 24-hour duration of HCB effects and may have been attributable to the higher food intake amounts during the first 12 hours after injection.

Buprenorphine is known to induce pica behavior in rats provided bedding, and this behavior is not significantly altered by the availability of food.[5,18,19] Pica behavior has been induced in rats by stimuli that cause nausea and emesis in other species and has therefore been evaluated as an index of emetogenic potential of other drugs.[20–24] Given the absence of bedding in the present study, it is possible that the higher food intake in HCB-treated rats during the first 12 hours (light period) after injection was attributable to food being the only available substrate for chewing. We speculate that food intake was lower during the subsequent 12 hours (dark period), when most of their time is normally spent eating,[34,35] because the daily caloric requirements had been met during the previous 12 hours (light period). Alternatively, the decrease in food intake during the dark period may have resulted from reduced gastrointestinal motility[1,17] or from cataleptic effects extending beyond the period in which rats had stereotypic behavior. Catalepsy has been associated with buprenorphine administration in rats at doses of 0.1 to 30 mg/kg, with maximal catalepsy occurring 30 minutes after a dose of 0.3 mg/kg (ie, higher and lower doses were associated with less catalepsy).[1] However, we did not assess catalepsy in this study.

Although the results of this feasibility study to assess dose responses to HCB suggested that the highest dose of HCB studied (0.30 mg/kg) resulted in a short period of thermal hypoalgesia in healthy rats 1 hour after administration, significant increases in self-injurious behavior were observed with HCB treatment, in addition to effects on food intake and fecal output patterns. Thus, these results lay the groundwork for future investigations including quantification of other stereotypic behaviors associated with various formulations of buprenorphine, including pica and self-injurious behavior. In addition, the effects of HCB in rats with painful conditions (eg, after surgery) should be investigated because these may differ from the results in healthy rats under normal conditions.

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Footnotes

a. Harlan Laboratories, Indianapolis, Ind.
b. Research Randomizer, version 4.0, Geoffrey C. Urbaniak and Scott Plous, Middletown, Conn. Available at: www.randomizer.org. Accessed May 30, 2016.
c. Simbadol (1.8 mg/mL), Zoetis Inc, Kalamazoo, Mich.
d. Baxter Healthcare, Deerfield, Ill.
e. Ugo Basile SRL, Varese, Italy.
f. SigmaPlot, version 12.0, Systat Software Inc, San Jose, Calif.

References

1. Cowan A, Lewis JW, MacFarlane IR. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br J Pharmacol* 1977;60:537–545.
2. Flecknell P. Analgesia and post-operative care. In: Flecknell P, ed. *Laboratory animal anaesthesia*. 3rd ed. Amsterdam: Elsevier, 2009;139–179.
3. Ohtani M, Kotaki H, Nishitateno K, et al. Kinetics of respiratory depression in rats induced by buprenorphine and its metabolite, norbuprenorphine. *J Pharmacol Exp Ther* 1997;281:428–433.
4. Curtin LI, Grakowsky JA, Suarez M, et al. Evaluation of buprenorphine in a postoperative pain model in rats. *Comp Med* 2009;59:60–71.
5. Schaap MWH, Uilenreef JJ, Mitsogiannis MD, et al. Optimizing the dosing interval of buprenorphine in rats. *Lab Anim* 2012;46:287–292.
6. Thompson AC, Kristal MB, Abdullah S, et al. Analgesic efficacy of orally administered buprenorphine in rats. *Comp Med* 2004;54:293–300.
7. Bourque SL, Adams MA, Nakatsu K, et al. 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* 2010;49:617–622.
8. Cooper DM, Hoffman W, Wheat N, et al. Duration of effects on clinical parameters and referred hyperalgesia in rats after abdominal surgery and multiple doses of analgesia. *Comp Med* 2005;55:344–353.
9. Raffa RB, Porreca F, Cowan A, et al. Morphine receptor dissociation constant and the stimulus-effect relation for inhibition of gastrointestinal transit in the rat. *Eur J Pharmacol* 1982;79:11–16.
10. Sharma R, Manchanda SK, Nayar U. Role of opioid receptors in self-aggression in rats. *Indian J Physiol Pharmacol* 1991;35:165–169.
11. Walker EA, Young AM. Differential tolerance to antinociceptive effects of μ opioids during repeated treatment with etonitazene, morphine, or buprenorphine in rats. *Psychopharmacology (Berl)* 2001;154:131–142.
12. Yassen A, Olofsen E, Kan J, et al. Pharmacokinetic-pharmacodynamic modeling of the effectiveness and safety of buprenorphine and fentanyl in rats. *Pharm Res* 2008;25:183–193.
13. Yassen A, Olofsen E, Dahan A, et al. Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of buprenorphine and fentanyl in rats. *J Pharmacol Exp Ther* 2005;313:1136–1149.
14. US FDA. Simbadol buprenorphine injection. Freedom of Information summary. Original new animal drug application. NADA 141–434. Available at: www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM408852.pdf. Accessed Mar 3, 2017.
15. Johnson RA. Voluntary running-wheel activity, arterial blood gases, and thermal antinociception in rats after 3 buprenorphine formulations. *J Am Assoc Lab Anim Sci* 2016;55:306–311.
16. Wala EP, Holtman JR. Buprenorphine-induced hyperalgesia in the rat. *Eur J Pharmacol* 2011;651:89–95.
17. Cowan A. Buprenorphine and gastrointestinal transit in rats: effect of naloxone on the biphasic dose-response curve. *Clin Exp Pharmacol Physiol* 1992;19:47–49.
18. Clark JA Jr, Myers PH, Goelz MF, et al. Pica behavior associated with buprenorphine administration in the rat. *Lab Anim Sci* 1997;47:300–303.
19. Jacobson C. Adverse effects on growth rates in rats caused by buprenorphine administration. *Lab Anim* 2000;34:202–206.
20. De Jonghe BC, Lawler MP, Horn CC, et al. Pica as an adaptive response: kaolin consumption helps rats recover from chemotherapy-induced illness. *Physiol Behav* 2009;97:87–90.
21. Goineau S, Castagne V. Comparison of three preclinical models for nausea and vomiting assessment. *J Pharmacol Toxicol Methods* 2016;82:45–53.
22. Shi J. Evaluating the various phases of cisplatin-induced emesis in rats. *Oncol Lett* 2014;8:2017–2022.
23. Takeda N, Hasegawa S, Masahiro M, et al. Pica in rats is analogous to emesis: an animal model in emesis research. *Pharmacol Biochem Behav* 1993;45:817–821.
24. Yamamoto K, Nakai M, Nohara K, et al. The anti-cancer drug-induced pica in rats is related to their clinical emetogenic potential. *Eur J Pharmacol* 2007;554:34–39.
25. Fog R. Behavioural effects in rats of morphine and amphetamine and a combination of the two drugs. *Psychopharmacologia* 1970;16:305–312.
26. Inbal R, Devor M, Tuchendler O, et al. Autonomy following nerve injury: genetic factors in the development of chronic pain. *Pain* 1980;9:327–337.
27. Mogilnicka E, Braestrup C. Noradrenergic influence on the stereotyped behaviour induced by amphetamine, phenethylamine and apomorphine. *J Pharm Pharmacol* 1976;28:253–255.
28. Mueller K, Saboda S, Palmour R, et al. Self-injurious behavior produced in rats by daily caffeine and continuous amphetamine. *Pharmacol Biochem Behav* 1982;17:613–617.
29. Pollock J, Kornetsky C. Evidence for the role of dopamine D_1 receptors in morphine induced stereotypic behavior. *Neurosci Lett* 1989;102:291–296.
30. Institute for Laboratory Animal Research. *Guide for the care and use of laboratory animals*. 8th ed. Washington, DC: National Academics Press, 2011.
31. AVMA. AVMA guidelines for the euthanasia of animals: 2013 edition. Available at: www.avma.org/KB/Policies/Documents/euthanasia.pdf. Accessed Mar 3, 2017.
32. Creese I, Iversen S. Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res* 1973;55:369–382.
33. Aschoff J. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol* 1960;25:11–28.
34. Siegel PS, Stuckey HL. The diurnal course of water and food intake in the normal mature rat. *J Comp Physiol Psychol* 1947;40:365–370.
35. Siegel PS. Food intake in the rat in relation to the dark-light cycle. *J Comp Physiol Psychol* 1961;54:294–301.
36. Terman M, Terman JS. Control of the rat's circadian self-stimulation rhythm by light-dark cycles. *Physiol Behav* 1975;14:781–789.
37. Zucker I. Light-dark rhythms in rat eating and drinking behavior. *Physiol Behav* 1971;6:115–126.
38. Hargreaves K, Dubner R, Brown F, et al. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988;32:77–88.
39. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev* 2001;53:597–652.
40. Meng X, Zhang Y, Lao L, et al. Spinal interleukin-17 promotes thermal hyperalgesia and NMDA NR1 phosphorylation in an inflammatory pain rat model. *Pain* 2013;154:294–305.
41. Zhang Y, Li A, Lao L, et al. Rostral ventromedial μ, but not κ, opioid receptors are involved in electroacupuncture antihyperalgesia in an inflammatory pain rat model. *Brain Res* 2011;1395:38–45.
42. Cheppudira BP. Characterization of hind paw licking and

lifting to noxious radiant heat in the rat with and without chronic inflammation. *J Neurosci Methods* 2006;155:122–125.
43. Buerkle H, Yaksh T. Comparison of the spinal actions of the μ-opioid remifentanil with alfentanil and morphine in the rat. *Anesthesiology* 1996;84:94–102.
44. Endo D, Ikeda T, Ishida Y, et al. Effect of intrathecal administration of hemokinin-1 on the withdrawal response to noxious stimulation of the rat hind paw. *Neurosci Lett* 2006;392:114–117.
45. Knapp CM, Jha SH, Kornetsky C. Increased sensitization to morphine-induced oral stereotypy in aged rats. *Pharmacol Biochem Behav* 2004;79:491–497.
46. Mori T, Ito S, Kita T, et al. Effects of μ-, δ- and κ-opioid receptor agonists on methamphetamine-induced self-injurious behavior in mice. *Eur J Pharmacol* 2006;532:81–87.
47. Horner KA, Hebbard JC, Logan AS, et al. Activation of mu opioid receptors in the striatum differentially augments methamphetamine-induced gene expression and enhances stereotypic behavior. *J Neurochem* 2012;120:779–794.