

Physiologic and behavioral effects of long-acting subcutaneous and transdermal buprenorphine in rats

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OBJECTIVE

To investigate thermoregulation, thermal antinociception, food/kaolin intake, fecal output, and behavior following long-acting buprenorphine preparations in rats.

ANIMALS

8 adult male rats (*Rattus norvegicus*) were administered long-acting SC buprenorphine (SB; 0.65 mg/kg), transdermal buprenorphine (TB; 10 mg/kg), and controls in a randomized, cross-over design.

METHODS

Body temperature, self-injury, sedation, food/kaolin intake, fecal output, and thermal withdrawal latencies were measured 1, 4, 8, 12, 24, 48, and 72 hours posttreatment. Data analysis was performed with mixed linear models.

RESULTS

Self-injury was present between 1 and 12 hours and 4 and 12 hours following TB and SB, respectively; sedation was associated with TB at 12 to 24 hours. Withdrawal latencies were longer in both TB and SB groups than in the control group. Food intake decreased with time in all groups but was significantly lower 24 to 48 hours after TB and 24 to 72 hours after SB versus controls. Kaolin intake decreased from baseline 48 to 72 hours in the control group. Fecal output decreased from baseline 24 to 72 hours in all groups but was significantly lower than controls 24 hours following TB and 24 to 48 hours in SB. Body temperature increased from baseline at 1 hour, 1 to 12 hours, and 1 to 24 hours in the control, TB, and SB groups, respectively, and was significantly higher than the control group 1 to 72 hours following TB and 4 to 24 hours after SB. Transdermal buprenorphine and SB in normal rats produced antinociception, self-injurious behavior, hyperthermia, and decreased food/fecal output.

CLINICAL RELEVANCE

Although these buprenorphine preparations may produce antinociception, untoward effects such as hyperthermia, self-injurious behavior, and reduced food intake/fecal output may be seen.

Keywords: buprenorphine, rat, long-acting, physiologic, behavioral

Conventional buprenorphine is a semisynthetic, lipophilic opiate that is 25 to 40 times more potent than morphine. It is a partial mu-opioid receptor agonist, which binds avidly and dissociates slowly with a half-life of 2.1 to 3 hours.^{1,2} It is used to treat mild to moderate pain in laboratory animals as well as companion “pocket pets” including rats.^{1,3,4} The currently recommended dosing in rats is 0.05 to 0.1 mg/kg SC every 6 to 8 hours.⁵ Although recognized as a relatively safe analgesic with a “ceiling” effect,⁶⁻⁹ the relatively short duration of action of the conventional

product limits its usefulness in species that become stressed with restraint required to deliver it at appropriate dosing intervals. Thus, long-lasting buprenorphine preparations may offer advantages over conventional buprenorphine in rodents.¹⁰ However, regardless of formulation, side effects of buprenorphine may include reduced food intake, impaired gastrointestinal (GI) motility, self-injurious behavior, and pica, depending on rat strain.^{6,9,11,12} In addition, alterations in arterial blood gases including hypoxemia may be associated with both conventional and long-acting buprenorphine administration, although only one pilot investigation⁸ with small sample sizes has been published on these effects.

A novel extended-release buprenorphine preparation has recently become FDA indexed for SC use

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in rodents and ferrets (SB; Ethiqx XR; Fidelis Animal Health). Buprenorphine is bound in a lipid capsule and suspended in medium-chain fatty acid triglycerides; this formulation may provide analgesia for up to 72 hours.¹³ In comparison, an alternative long-acting preparation encapsulates buprenorphine in a liquid polymer dissolved in a biocompatible organic solvent (Buprenorphine ER-LAB).¹⁴ Each product has its distinct advantages and disadvantages,¹⁰ and both have been associated with histologically different skin lesions in some species.¹⁵⁻²²

A novel long-acting transdermal buprenorphine solution (TB; Zorbium; Elanco) has recently been FDA approved for cats and has the potential to offer unique advantages to species that would benefit from less frequent and stressful application (ie, not requiring frequent handling and injections) such as rodents. Although the pharmacokinetics and pharmacodynamics of multiple buprenorphine preparations including TB have been studied in cats,²³ the physiologic and behavioral effects of SB and TB formulations have not been fully investigated in rats. To this end, we investigated the physiologic and behavioral effects of TB and compared them with SB, specifically measuring thermal antinociception, food and kaolin intake, fecal output, self-injurious behavior and sedation, and thermoregulation.

Methods

Animals

All protocols were approved by the University of Wisconsin's IACUC and performed with standard guidelines for the care and use of laboratory animals. Eight healthy adult male Sprague-Dawley rats were housed in pairs until the start of experimentation and then housed in individual, wire-bottom cages with adequate environmental enrichment, and they had free access to a preweighed commercial rat pellet and water. Rats were allowed at least 7 days for acclimatization to their home cages before any experimentation.

Rats were administered 1 of 4 treatments in a randomized (www.randomizer.org), cross-over design: 1) SB (Ethiqx XR; Fidelis Animal Health; 0.65 mg/kg; 1.3 mg/mL; n = 8 rats); 2) TB (Zorbium; Elanco; 10 mg/kg; 20 mg/mL; n = 8 rats); 3) medium-chain fatty acid triglyceride oil SC as the carrier/control solution for SB (0.5 mL/kg; n = 4 rats); or 4) anhydrous alcohol transdermal as the carrier/control solution for TB (0.5 mL/kg; n = 4 rats). Each treatment was administered once, and the control solution volume was matched to the appropriate buprenorphine formulation volume. Since TB has not previously been studied in rats, preliminary investigations were performed before the full study using 10 mg/kg (n = 2 rats) or 20 mg/kg (n = 2 rats) doses of TB followed by physiologic and behavioral testing as defined below; 10 mg/kg TB was ultimately chosen (see Results). Transdermal application consisted of drawing up the appropriate solution volume in a pipette, separating the hair on the dorsum with the pipette tip just behind the scapulae, and dispensing the liquid directly on the skin. Rats were gently restrained

for 5 minutes until dry and closely observed for 30 minutes to ensure rats did not groom the location. At least 21 days lapsed between treatments for drug washout. Investigators performing the experiments were blinded to the treatments. Twenty-four hours before treatment and every 24 hours for 3 days post-treatment, rats were weighed. Behavior (self-injury and sedation) scores, thermal withdrawal latencies (antinociceptive testing), and body temperatures were recorded at baseline and throughout experimentation as detailed below. Food/kaolin intake and fecal output were measured every 12 hours for 48 hours before testing and throughout the study as detailed below. As part of a separate study, rats also underwent respiratory plethysmography recordings at set time points for 3 days posttreatment following nociceptive testing and scoring.

Self-injurious behavior

Similar to previously published studies,^{9,11} self-injurious behavior (self-biting and cage-biting) was quantified before treatment (time 0) and at 1, 4, 8, 12, 24, 48, and 72 hours after injection/application, assessed before any other testing. A descriptive scale adapted from previous studies^{6,24} and used in a recent investigation¹¹ of a high concentration buprenorphine preparation in rats 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 less than or equal to 30 seconds (continuous or intermittent) during a given observation period. Frequent cage-biting or self-biting was defined as occurring for more than 30 seconds during a single observation period.

Sedation scoring

A descriptive scale of sedation was adapted from a previous study²⁵ and utilized to determine the level of sedation following injection/application, where 0 = normal, awake spontaneous behavior unless sleeping; 1 = frequent spontaneous locomotor behavior during a 1-minute period with quiet behavior approximately once every 2 minutes; 2 = some spontaneous locomotor activity during a 1-minute period of observation but quiet behavior at least once every minute; 3 = no spontaneous locomotor activity during a 1-minute period of observation; and 4 = no motor response when cage was tipped 45° during a 30-second period of observation. Sedation scoring was quantified at baseline, before injection/application (time 0), and at 1, 4, 8, 12, 24, 48, and 72 hours after injection/application, assessed before any further testing.

Thermal footpad nociceptive testing

Similar to previous investigations,²⁶ nociception and antinociception were assessed by measuring

the latency of hind limb withdrawal to a radiant heat stimulus applied with a commercial testing device (Hargreaves Apparatus; Ugo Basile).²⁷ Rats were initially acclimatized to the Hargreaves apparatus by placing in each device for 45 minutes a day for 5 days before experimentation. On testing days, rats were allowed to acclimatize to the chambers for 15 minutes before testing. The intensity of the heat stimulus and the rate of heating were kept constant throughout the study to establish a target withdrawal latency range of 7 to 9 seconds for baseline measurements, with a maximum latency limit of 20 seconds to avoid thermal injury. An infrared heat stimulus was applied to a plantar surface of a randomized paw (www.randomizer.org), 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, 24, 48, and 72 ± 1 hours after injection. Each rat was tested 3 times at each time point with greater than or equal to 7 minutes between trials and the mean latency was calculated, similar to previous studies.¹¹ This pattern was chosen to minimize hyperalgesia secondary to repeated noxious stimuli.^{8,27,28} Latencies were assessed following all other data collection at each time point.

Food intake, kaolin intake, and fecal output

Rats were provided free access to a premeasured amount of commercial rat feed (150 ± 1 g; Teklad 6% fat mouse/rat diet; Envigo) in a removable wire rack and a premeasured amount of kaolin pellets (30 ± 1 g; Research Diets Inc) in a ceramic dish while they were housed singly in wire-bottomed cages beginning 48 hours before each treatment. Kaolin was provided as a nonnutritive substance to quantify pica behavior. Paper liners were placed on the cage bottom 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 ceramic dish, respectively, and any that spilled on the cage floor was measured every 12 hours from 48 hours before until 72 hours after injection/application. Spilled food and kaolin were separated before measurement. Fecal pellets were collected, counted, and weighed every 12 hours for the same time periods. Measurements for individual rats were expressed as an index of body weight (mg/kg); rats were weighed before treatment and at 24, 48, and 72 hours posttreatment. Food intake, kaolin intake, and fecal output were averaged over each 24-hour period posttreatment and combined over 48 hours for the time 0 (pretreatment) time point to assess changes in food intake, kaolin intake (pica), and fecal output.

Body temperature

Subcutaneous radiofrequency identification implants (IPTT-300 Programmable Temperature Transponders; Avidity Science) were injected dorsally between the scapulae before all experimentation for identification and SC body temperatures, required for a separate study of respiratory

plethysmography (data not presented here). Rats were scanned at baseline (time 0) and at 1, 4, 8, 12, 24, 48, and 72 hours after treatment and assessed before nociceptive testing.

Statistical analysis

Statistical analyses were conducted using SAS software (Version 9.4; SAS Institute Inc). All reported *P* values are 2 sided, and *P* ≤ .050 was used to define statistical significance. Mixed linear model (PROC MIXED) and generalized linear mixed models (GLIMMIX) were used to account for the correlation from repeated measures. Normality was assessed, and plots were visually analyzed for appropriateness of fit. For those variables normally distributed (body mass, temperature, latencies, food intake, and fecal output), the PROC MIXED model was used to assess time, treatment, and the interaction effects. Sedation and self-injury scores were represented as ordinal data; however, the ordinal model would not converge for either variable. Thus, the repeated-measures ANOVA was used for their analyses since the ANOVA is robust to nonnormality. Kaolin intake was exponentially distributed and the GLIMMIX procedure was used to assess the effects of time, treatment, and their interactions. When significance was found, the time-by-treatment slice effects were assessed followed by appropriate pairwise comparisons using the gatekeeping approach. Only comparisons between posttreatment time points (1 to 72 hours) and baseline (time 0) within a treatment group and comparisons between treatment groups within a given time point are presented. No statistical differences were found in any analyzed variable or time point between the separate control groups for SB and TB (for each group, *n* = 4 rats; all *P* > .050; data not shown), and the data were subsequently combined into a single control group (*n* = 8 rats). All data are expressed as mean ± SD (**Figures 1–6**) or differences of least squares means ± SE with 95% CIs (**Supplementary Tables S1–S14**).

Results

Preliminary investigations of TB

Preliminary investigations were performed using only 10 mg/kg (*n* = 2 rats) or 20 mg/kg (*n* = 2 rats) doses of TB. Although statistical analyses were not performed due to small sample sizes, rats receiving 10 and 20 mg/kg TB showed similar patterns of antinociception at early time points, as both increased thermal latencies at 1 and 4 hours from baseline values (approx 42% and 52%, and 43% and 35%, respectively); however, although thermal latencies following 10 mg/kg TB remained above control values for up to 72 hours, 20 mg/kg TB thermal latencies returned to near baseline by 8 hours and potentially resulted in a degree of hyperalgesia by 48 and 72 hours with withdrawal times approximately 6% and 8% faster than baseline values. Average food intake decreased from baseline but was similar from 1 to 72 hours in both the 10 and 20 mg/kg groups (15.8 and 16.2 g/kg/24 h, respectively);

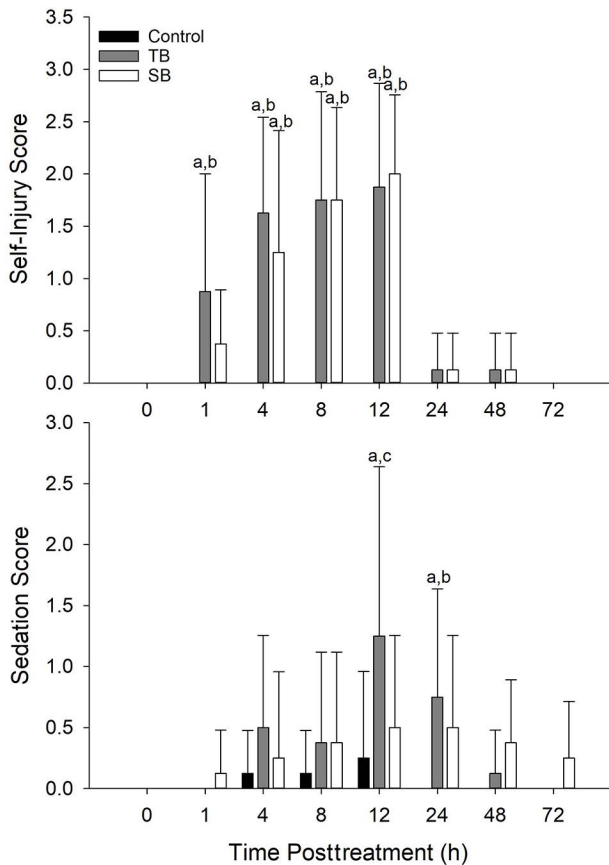


Figure 1—Mean \pm SD of self-injury scores (top) and sedation scores (bottom) for 8 adult male Sprague-Dawley rats before (time 0) and 1 to 72 hours following transdermal buprenorphine (TB; gray bars) or long-acting SC buprenorphine (SB; white bars) compared with a control group (black bars) in a randomized, crossover design with at least 21 days washout between experiments. Control scores all = 0 for self-injury and are hidden. Values of $P \leq .050$ were considered significant. ^aSignificantly increased from time 0 (baseline) values within the same treatment group. ^bSignificantly increased from the control group within the same time period. ^cSignificantly increased from both the control and SB groups within the same time period.

however, average fecal output decreased from baseline and was slightly higher in the 10 mg/kg group compared to the 20 mg/kg group (3.8 and 2.8 g/kg/24 h, respectively). Average kaolin intake increased from baseline but was lower in the 10 mg/kg group compared with the 20 mg/kg group (4.6 and 11.5 g/kg/24 h, respectively). In addition, greater self-injurious behavior was present at 20 mg/kg, with scores between 2 and 3 for up to 24 hours. Thus, 10 mg/kg TB was chosen for the full study.

Body mass

Body mass differed between treatment groups when all time points were considered ($P = .001$) but did not differ from pretreatment values at 24, 48, and 72 hours posttreatment in any treatment group ($P = .979$). Overall, the SB group (mean \pm SD: 552 \pm

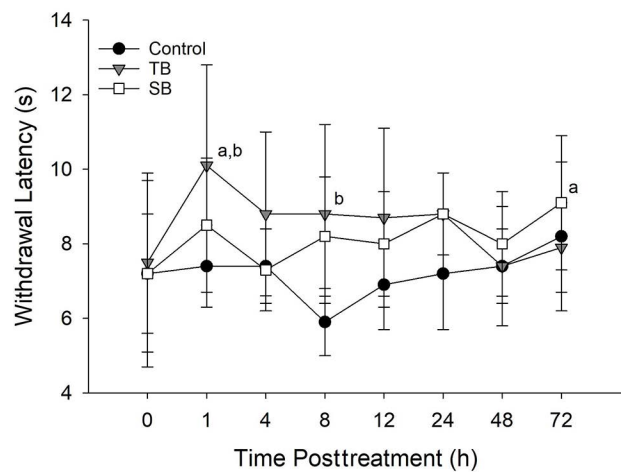


Figure 2—Mean \pm SD of thermal withdrawal latencies (s) before (time 0) and 1 to 72 hours in the control group (black circles), transdermal buprenorphine (TB; gray triangles), or long-acting SC buprenorphine (SB; white squares) as described. Values of $P \leq .050$ were considered significant. ^aSignificantly increased from time 0 (baseline) values within the same treatment group. ^bSignificantly increased from the control group within the same time period.

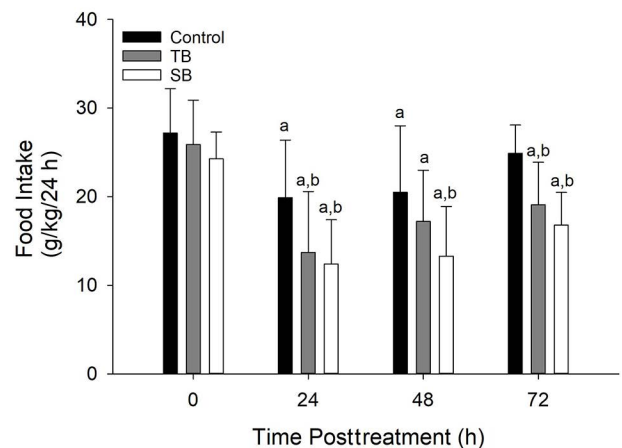


Figure 3—Mean \pm SD of food intake (g/kg/24 h) before (time 0) and 1 to 72 hours for the groups described. SB = Long-acting SC buprenorphine. TB = Transdermal buprenorphine. Values of $P \leq .050$ were considered significant. ^aSignificantly decreased from time 0 (baseline) values within the same treatment group. ^bSignificantly decreased from the control group within the same time period.

34 g) was significantly heavier when compared with the TB (mean \pm SD: 506 \pm 56 g; $P = .001$) and control (mean \pm SD: 505 \pm 71 g; $P = .001$) groups.

Self-injurious behavior and sedation scores

Self-injurious behaviors significantly differed between treatments ($P < .001$), time ($P < .001$), and their interactions ($P < .001$) with significant treatment-time slice effects for 1 to 12 hours (all $P < .005$) and the TB and SB groups (both $P < .001$; Figure 1; Supplementary Tables S1 and S2). Baseline

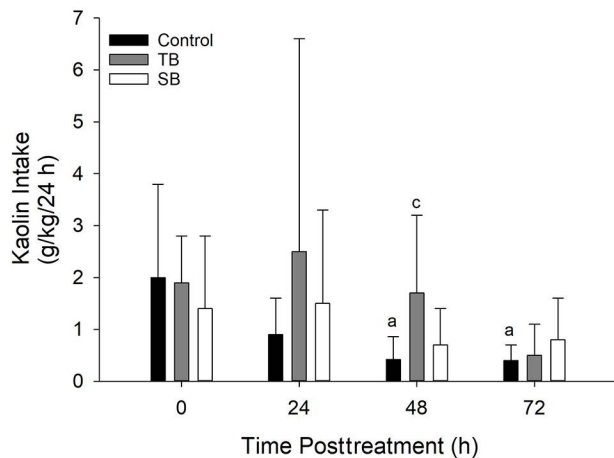


Figure 4—Mean \pm SD of kaolin intake (g/kg/24 h) before (time 0) and 1 to 72 hours for the groups described. SB = Long-acting SC buprenorphine. TB = Transdermal buprenorphine. Values of $P \leq .050$ were considered significant. ^aSignificantly decreased from time 0 (baseline) values within the same treatment group. ^cSignificantly increased from the control and SB group within the same time period.

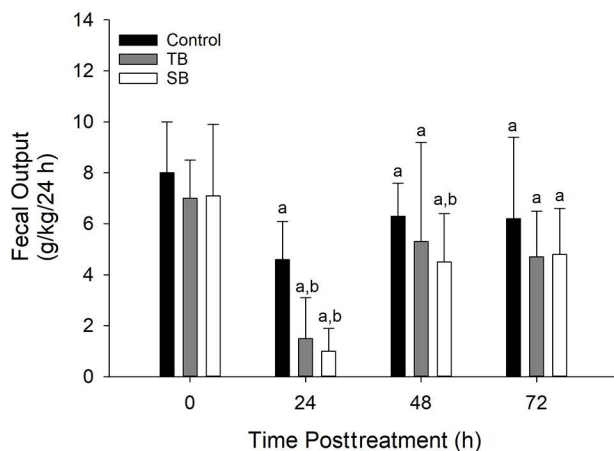


Figure 5—Mean \pm SD of fecal output (g/kg/24 h) before (time 0) and 1 to 72 hours for the groups described. SB = Long-acting SC buprenorphine. TB = Transdermal buprenorphine. Values of $P \leq .050$ were considered significant. ^aSignificantly decreased from time 0 (baseline) values within the same treatment group. ^bSignificantly decreased from the control group within the same time period.

self-injury scores did not differ between groups ($P = 1.000$). Compared with baseline (time 0), self-injury scores following TB were significantly higher at 1 ($P = .001$), 4 ($P < .001$), 8 ($P < .001$), and 12 hours ($P < .001$). Compared with baseline, self-injury scores after SB were higher at 4 ($P < .001$), 8 ($P < .001$), and 12 hours ($P < .001$). Self-injury scores were also significantly greater after TB compared with control scores at 1 ($P = .001$), 4 ($P < .001$), 8 ($P < .001$), and 12 hours ($P < .001$) and in SB compared with control at 4 ($P < .001$), 8 ($P < .001$), and 12 hours ($P < .001$). Within the control group, no time points differed, and scores did not

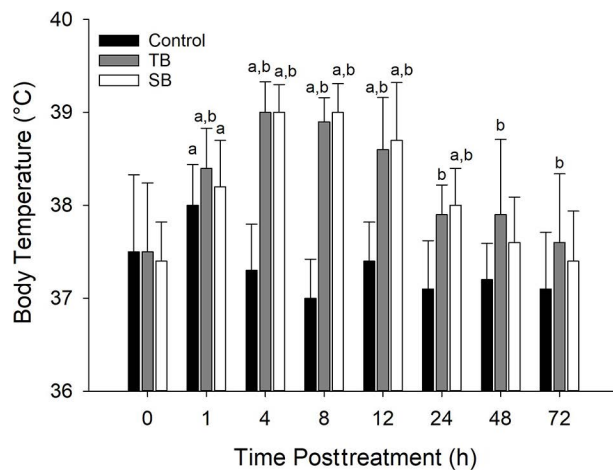


Figure 6—Mean \pm SD of SC body temperature ($^{\circ}\text{C}$) before (time 0) and 1 to 72 hours for the groups described. SB = Long-acting SC buprenorphine. TB = Transdermal buprenorphine. Values of $P \leq .050$ were considered significant. ^aSignificantly increased from time 0 (baseline) values within the same treatment group. ^bSignificantly increased from the control group within the same time period.

differ between TB and SB groups at any time point (all $P > .050$).

Sedation differed between treatments ($P = .005$) and over time ($P = .001$) with significant treatment-time slice effects for the TB group ($P < .001$), 12 and 24 hours ($P = .001$ and $P = .026$, respectively; Figure 1; Supplementary Tables S3 and S4). Baseline scores did not differ between groups ($P = 1.000$). Within the control group, no time points differed from baseline (all $P > .050$). Sedation scores were increased from baseline values (time 0) in the TB group only at 12 ($P < .001$) and 24 hours ($P = .008$). Sedation was greater following TB compared with the control group at 12 ($P < .001$) and 24 hours ($P = .008$); TB sedation scores were higher than SB scores at 12 hours ($P = .008$).

Thermal footpad antinociception

Overall, thermal withdrawal latencies differed significantly between treatments with the TB and SB groups differing from the control group ($P < .001$) with significant treatment-time effects for the TB group ($P = .024$), only at 1 and 8 hours ($P = .004$ and $P = .001$, respectively; Figure 2; Supplementary Tables S5 and S6). Baseline latencies did not differ between groups ($P = .897$). Latencies increased from baseline (time 0) at 1 hour in the TB group ($P = .002$). Compared with the control group, latencies were significantly increased in the TB group at 1 ($P = .001$) and 8 hours ($P < .001$). No other time or treatment groups differed significantly from each other (all $P > .050$).

Food intake, kaolin intake (pica), and fecal output

Food intake significantly differed between treatments and time with significant treatment-time slice effects for all groups (all $P < .002$; Figure 3,

Supplementary Tables S7 and S8). Food intake was similar across baseline for all groups ($P = .396$) but decreased from baseline (time 0) in the control group at 24 ($P < .001$) and 48 hours ($P = .002$). Compared with baseline food intake, the TB group had significantly lower values at 24 ($P < .001$), 48 ($P < .001$), and 72 hours ($P = .002$), and the SB group had lower food intake than their baseline intake at 24 ($P < .001$), 48 ($P < .001$) and 72 hours ($P = .001$). Compared with the control group, food intake was significantly decreased after TB at 24 ($P = .004$) and 72 hours ($P = .008$) and following SB at 24 ($P = .001$), 48 ($P = .001$), and 72 hours ($P < .001$).

Kaolin intake significantly differed between treatments ($P = .030$) and time ($P < .001$) with significant treatment-time slice effects for 48 hours ($P = .015$) and the TB and control groups ($P = .014$ and $P = .002$, respectively; Figure 4; Supplementary Tables S9 and S10). Kaolin intake was similar at baseline across all groups ($P = .510$) but decreased from baseline (time 0) in the control group at 48 ($P = .050$) and 72 hours ($P = .026$). Additionally, compared with the TB group, kaolin intake was significantly lower at 48 hours in the control ($P = .005$) and SB ($P = .035$) groups.

Fecal output significantly differed between treatments and time (both $P < .001$) with significant treatment-time slice effects for 24 hours ($P < .001$) and all treatments (all $P = .002$; Figure 5; Supplementary Tables S11 and S12). Fecal output was similar at baseline across all groups ($P = .443$) but significantly decreased from baseline (time 0) in the control group at 24 ($P < .001$), 48 ($P = .041$), and 72 hours ($P = .036$). Fecal output was lower than baseline following TB at 24 ($P < .001$), 48 ($P = .042$), and 72 hours ($P = .006$) and also lower than baseline following SB at 24 ($P < .001$), 48 ($P = .002$), and 72 hours ($P = .005$). Compared with the control group, fecal output was significantly lower at 24 hours in the TB group ($P < .001$) and SB group ($P < .001$) and also lower than the control group at 48 hours in the SB group ($P = .031$).

Body temperature

Body temperature differed between treatments, time, and their interactions (all $P < .001$) with significant treatment-time slice effects for 4 to 48 hours (all $P < .050$) and all groups (all $P < .003$; Figure 6; Supplementary Tables S13 and S14). Body temperature significantly increased from baseline (time 0) at 1 hour in the control group ($P = .028$) and after TB at 1 ($P < .001$), 4 ($P < .001$), 8 ($P < .001$), and 12 hours ($P < .001$). Body temperature also significantly increased from baseline following SB at 1 ($P = .002$), 4 ($P < .001$), 8 ($P < .001$), 12 ($P < .001$), and 24 hours ($P = .023$). Compared with the control group, body temperatures were significantly higher in the TB group at 1 ($P = .050$), 4 ($P < .001$), 8 ($P < .001$), 12 ($P < .001$), 24 ($P = .001$), 48 ($P = .003$), and 72 hours ($P = .026$). Body temperatures were significantly higher in the SB compared with the control group at 4 ($P < .001$), 8 ($P < .001$), 12 ($P < .001$), and 24 hours ($P = .001$). The TB and SB groups did not differ at any time point (all $P > .060$).

Discussion

The present study compared a novel transdermal formulation (TB) that has not been previously investigated in normal rats, with a commonly used long-acting subcutaneous formulation (SB) regarding antinociception, self-injurious behavior and sedation, food/kaolin intake, fecal output, and thermoregulation. While prolonged antinociception could not be demonstrated here, both formulations were associated with self-injury, reduced food intake/fecal output, and hyperthermia, whereas sedation and kaolin intake were also increased with TB. Although pharmacokinetic analyses of each preparation were not performed here and equipotent dosing cannot be confirmed, this study provides evidence that undesirable effects may be clinically relevant and expected in normal rats when using these formulations at the doses used.

The SB preparation produces significant analgesia and reduces hypersensitivity in multiple adult and neonatal rat models^{29,30}; antinociception has not previously been investigated following TB application. Our results demonstrated that overall, the TB and SB groups had greater antinociception than control rats, although significant thermal antinociception was only seen after TB at 1 and 8 hours in the present study. These results are similar to previous investigations¹¹ of other long-acting buprenorphine preparations in rats, in which antinociception was detected for only 1 hour. However, the reason for the lack of antinociception at individual time points with SB in our study is not entirely clear. One explanation could be our choice of only a thermal stimulus to assess antinociception in rats that were not previously painful. Since different types of nociceptive stimuli may differentially activate nociceptive pathways, it is likely that inflammatory pain or the use of noxious mechanical stimuli might have resulted in improved efficacy with both TB and SB preparations although thermal stimuli did not. Additionally, behavioral effects may have altered the responses to the thermal stimulus, such as the observed self-injurious behavior or sedation. A limitation of our methods was the use of a single TB dose, based on small preliminary investigations. It is possible that other doses may be more efficacious regarding antinociception and although our preliminary results suggested that 20 mg/kg TB did not increase thermal withdrawal latencies beyond the 10 mg/kg dose and may actually have produced a degree of hyperalgesia, other doses may have produced different results. In addition, pharmacokinetic effects were not studied here, which may have guided drug dosing more appropriately. Hyperalgesia, associated with repeated or prolonged dosing in rodents^{31,32} was not seen following TB at 10 mg/kg or SB, and although every effort to minimize sensitization to repeated testing was made within the study design,^{27,28} we cannot completely rule out that a degree of sensitization also did not affect the results.

Although sedation has not been quantified following TB and SB administration in rats, self-injurious behavior and sedation are associated with

other buprenorphine preparations,^{11,33} similar to our findings. However, in the present study, sedation only reached significance following TB and occurred later (12 to 24 hours) compared with self-injurious behavior (up to 12 hours). Although sedation was separately scored, our self-injury scoring system did not quantify overall activity; activity may also have been increased with TB and SB although it was not assessed in our rats. We speculate that the initial plasma buprenorphine levels associated with TB administration may have resulted in self-injurious behavior but as plasma levels decreased, sedation occurred before returning to normal behavior; plasma levels following SB administration may have been overall lower. Since plasma levels were not measured in this study, these speculations remain untested. Self-injurious behavior in humans is hypothesized to be due to alterations in opioid-mediated balances between pain and pleasure along with reduced sensory processing and sensitivity to pain³⁴; these mechanisms may also have played an underlying role in the self-injury observed here.

Body mass in the SB group was inexplicably greater (approx 50 g) than in the control and TB groups. Since food/kaolin intake and fecal output were normalized to body weight, higher body mass in the SB group may have resulted in an underestimation of values compared to values if normalization was not performed, although these underestimations likely would not have significantly altered the results. We chose to normalize food/kaolin consumption and fecal production to minimize the effects of differences in body mass and metabolic rate. Food intake and fecal output were reduced in both TB and SB groups but also in the control group when compared to baseline values. Although reduced food intake during dark periods and fecal output during light periods¹¹ are associated with other buprenorphine formulations in rats and in other laboratory species such as rabbits,³⁵ the reason these effects were seen in control animals in our study is not entirely clear. We speculate that administration of the control solutions and the subsequent testing protocols produced a degree of stress in all animals, which impacted their normal feeding behavior and fecal production. Although fecal production remained below baseline values in each group throughout our study, it was significantly lower in both the TB and SB groups compared with control at 24 hours and the SB group at 48 hours. Reduced fecal output can be attributed to reductions in food intake or to slowing of GI motility by buprenorphine.³⁶ Since food intake and fecal output were both reduced in control animals, at least part of these findings can be attributed to decreased food intake, but slowing of GI motility by other influences such as stress cannot be ruled out, even in our subchronic time frames.³⁷ Since decreases were even greater in the treated groups (TB and SB), the effects of buprenorphine on GI motility itself also likely played a role. Alternatively, changes in food and kaolin intake and fecal output in control animals may represent inherent variability over time in these rats. However, rats were randomly assigned to treatment

groups and some received control solutions early in the investigations whereas others received control solutions closer to study completion, reducing the effect of time on study outcomes.

Kaolin ingestion is used to quantify pica, an aberrant eating behavior of nonnutritive substances. Although the underlying cause of pica is not completely understood, it has been used as a marker of nausea in rats since rats cannot vomit.³⁸ Interestingly, kaolin intake decreased from baseline in the control rats by 48 and 72 hours, suggesting control rats reduced their intake overall (since food intake also decreased from baseline with time). Alternatively, rats may have lost interest in the novel alternative food source, or as rats continued to undergo experimentation over time, pica behavior was reduced through yet some unknown mechanism. However, neither SB nor TB values were significantly decreased from baseline, and kaolin ingestion was actually higher following TB compared with control or SB but only at 48 hours, suggesting that, if rats experienced any nausea associated with buprenorphine as determined by kaolin ingestion, it was likely mild.

Significant hyperthermia was seen throughout the study following both TB and SB preparations and at 1 hour in controls. Although the reason for the transient hyperthermia in the control group is not entirely clear, it may have been due to the stress of drug application/injection and quickly returned to baseline values. Hyperthermia following conventional buprenorphine is reported in multiple species including rats³⁹ and cats, particularly at higher doses.^{40,41} Hyperthermia consequent to opioid administration is at least partially attributable to resetting the hypothalamic thermoregulatory set point.⁴² Furthermore, we cannot rule out a component of hyperactivity at the early time points in our rats as self-injurious behavior, with frequent biting and chewing was also present.

This study has additional limitations. First, no pharmacokinetic data were completed. Thus, we cannot confirm equipotent doses of each agent were administered nor that therapeutic plasma buprenorphine levels were either reached or even exceeded. Only male rats from a single strain were used in this study. Although the use of single-sex, single-strain animals reduces genetic variability and variability added by differences associated with hormonal influences, especially during reproductive cycles, our data may not be completely applicable to female rats or rats of other strains. Similar to other physiologic investigations with small sample sizes,⁴³ control rats from the SB (n = 4 rats) and TB (n = 4 rats) groups were pooled into a single control group consisting of 8 rats since no statistical differences were found in any variable between the 2 separate groups. Although this increased statistical power, with larger sample sizes, differences in the 2 separate control populations may have become apparent. The concurrent plethysmography study, although a noninvasive method used to measure ventilatory capacity, may have affected multiple measured parameters, particularly food and kaolin intake. Although data

collection occurred in the same order each day with food/kaolin intake, fecal output, sedation/self-injury scoring, and body temperatures taken before plethysmography and thermal withdrawal latencies measured immediately afterward for consistency, we cannot rule out that these additional studies did not affect the data. The use of radiofrequency identification implants allowed for simple identification of individuals and quick, noninvasive SC temperatures, which may not accurately represent core body temperature. Although rectal body temperatures would likely be a better representation of core body temperature, they add handling and subsequent stress for the animals.

In summary, long-acting buprenorphine formulations pose significant benefits as well as potential, clinically relevant risks to small mammal species including rats. Long-acting SC or transdermal preparations such as those studied here offer advantages such as reduced handling and injection stress when compared to conventional buprenorphine administration. However, concerns with GI motility, hyperthermia, self-injurious behavior, and mild sedation should be considered when these agents are chosen.

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Supplementary Materials

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