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Analgesic Efficacy of Orally Administered Buprenorphine in Rats

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The analgesic effect of orally administered buprenorphine was compared with that induced by a standard therapeutic injected dose (0.05 mg/kg of body weight, s.c.) in male Long-Evans rats. Analgesia was assessed by measuring pain threshold, using the hot-water tail-flick assay before and after administration of buprenorphine. The results suggest that a commonly used formula for oral buprenorphine in flavored gelatin, at a dose of 0.5 mg/kg, does not increase pain threshold in rats. Instead, oral buprenorphine doses of 5 and 10 mg/kg were necessary to induce significant increases in pain threshold. However, these doses had to be administered by orogastric infusion because the rats would not voluntarily eat flavored gelatin containing this much buprenorphine. The depth of analgesia induced by these infused doses was comparable to that induced by the clinically effective s.c. treatment (0.05 mg/kg).

Buprenorphine, a partial mu opioid agonist (1), is a widely used analgesic in laboratory animal medicine. It has the qualities of an ideal rodent analgesic in that it is non-sedating, long acting in many species, and is a potent pain reliever (1, 2). Buprenorphine prepared in food and water is preferable to injected buprenorphine because ingestion reduces animal handling stress and requires less technical skill (2, 3). For rodents, buprenorphine is often mixed in flavored gelatin and offered in small cubes at a dosage of 0.5 mg/kg of body weight (4, 5). It is not clear from literature how this dose was derived, but it is consistent with published reports on the bioavailability of opioids (6). The buprenorphine-in-Jell-O recipe (5) has become commonly accepted and used in laboratory animal medicine as a postoperative analgesic (4, 7), but the analgesic efficacy of this treatment has not been documented directly (3). A number of investigators have suggested that this buprenorphine-in-Jell-O recipe is effective in reducing adverse postsurgical changes in food and water intake, and in body weight (3, 8, 9). However, buprenorphine itself has been reported to stimulate activity of rats (increasing appetite and mobility) independent of its analgesic activity (7), and therefore, the reported positive postoperative effects may not necessarily be due to analgesia.

The specific aim of the study reported here was to quantify the analgesic efficacy of orally administered buprenorphine in rats, and compare it with that of a well-established parenterally administered dose (0.05 mg/kg, s.c.). A standard and humane algesiometric test, the hot-water tail-flick assay (10), was chosen to determine pain threshold in this study. The tail-flick assay is a commonly used method for assessing opioid mediated effects on pain threshold. It is easy to perform, has well-defined end points, provides reproducible data (11), and measurements can be repeated on the same animal, so each subject can serve as its own control (11).

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Materials and Methods

Subjects: Male Long-Evans (hooded) rats (300 to 400 g) were obtained from either an in-house breeding colony (seeded from Harlan Sprague Dawley stock) (n = 12, experiment 1; n = 43, experiment 2; n = 135, experiment 3) or purchased from a commercial vendor (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) (n = 32, experiment 2). The in-house breeding colony is restocked with commercially purchased males and females every two generations, and subjects are obtained by breeding purchased females or backcrossing F_1 females to purchased males, to ensure genetic variability similar those obtained from the commercial vendor. Rats were housed in polycarbonate cages (46 × 25 × 21 cm) containing Aspen hardwood shavings (Northeastern Products Corp., New York, N.Y.); purchased rats were allowed to acclimate to the facility for two to three weeks before testing. Temperature, humidity, ventilation, and lighting were maintained at: 22°C, 50 to 60%, 14 air changes/h, and 14:10-h light:dark cycle (lights on at 6 a.m. EST). Rats were fed Teklad Rodent Diet No. 8640 (Harlan Teklad, Madison, Wis.) and tap water, ad libitum. The study was conducted in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the University at Buffalo. The animal facilities are fully approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were habituated to the testing room and the experimental procedures (handling, orogastric infusion, weighing, and eating flavored gelatin) on three occasions within the week prior to testing. Habituation was expected to reduce the stress resulting from exposure to these procedures; such stress might confound interpretation of the results by causing a release of endogenous opioids (12). No rats served in any previous experiment, and in this particular study, no rats were used more than once. All rats were tested at the same time of day so that the effect of circadian rhythm on opioid sensitivity would be kept constant across all groups. At completion of each experiment, rats were humanely euthanized with CO_2, or transferred to other laboratories for research in various fields. No rats had outward signs of toxicosis at any time during the study.

Testing: Pain threshold was measured before (baseline) and af-

ter drug administration, using a standard hot-water tail-flick assay (10). The dependent variable was the latency (in seconds) for the rat to flick its tail from the hot-water bath. The water was maintained at 55°C in a constant-temperature water bath and was monitored by use of an electronic thermometer. The distal third of the rat's tail was immersed in the bath, and the time required for the rat to remove its tail was measured by use of a stopwatch. Rats were wrapped in a breathable towel and gently held for this procedure. The tail-flick latency score was calculated as the mean of the last two of three trials, separated by 30 sec. intervals. Tail withdrawal at baseline (untreated rats) occurred between 2.5 and 4.0 sec. Each trial was terminated at 30 sec. if no withdrawal response occurred. Water at 55°C did not induce tissue damage to the tail. The experimenter conducting the tail-flick assay was blind to the experimental treatments of the rats. A statistically significant increase from baseline pain-threshold measurement was interpreted as induction of analgesia.

Drugs: Buprenorphine was prepared from powdered buprenorphine hydrochloride obtained from RBI/Sigma (Natick, Mass.). For orogastric infusion, buprenorphine was mixed in sterile deionized water to make a stock solution of 5.0 mg/ml. To ensure drug dissolution, the solution was vigorously vortexed for 2 min, sonicated for 20 min, and heated during the last 5 min of sonification. Experimental doses were obtained by serial dilution of this stock solution, and all solubilized drugs were prepared fresh each day. For injection, buprenorphine was prepared similarly but at a concentration of 0.05 mg/ml.

Infusion: Orogastric infusion was achieved, using an 11-cm long piece of PE160 tubing attached to a 1-ml, gas-tight tuberculin syringe with an 18-gauge needle. A 2.5-cm portion of a plastic, 1-ml tuberculin syringe was used as a mouth speculum. One experimenter held the rat and speculum, while the other handled the syringe and infused the drug.

Flavored gelatin: In experiment 1, either of two flavors of gelatin, beef or raspberry, was offered to the rats. Both flavors were eaten enthusiastically by the rats when the gelatin was fed plain (i.e., no drug), or when it contained a concentration of 0.125 mg of buprenorphine/ml. Beef-flavored gelatin was prepared by dissolving Knox gelatin in boiling water, adding Campbell's beef broth, and cooling to 55°C before adding buprenorphine. Thorough mixing was achieved by use of a stir plate. The solution was immediately poured into plastic 2-ml ice-cube trays, and cooled in a refrigerator. Raspberry-flavored gelatin was prepared according to the directions on the package of Jell-O raspberry-flavored gelatin, but using half the recommended water to increase firmness of the cube and increase sweetness. The buprenorphine was added and cubes were prepared as indicated previously for the beef flavor. Preparation procedures followed those described by Pekow (5). Flavored gelatin cubes were weighed and fed to each animal according to body weight (2 ml of gelatin/kg).

Procedures: Three experiments were performed to: determine the analgesic effect of orally administered buprenorphine, 0.5 mg/kg, administered in flavored gelatin; determine the dose- and time-dependent effects of buprenorphine administered by orogastric infusion; and examine the effects of repeated testing on the measurement of buprenorphine analgesia.

In experiment 1, buprenorphine (the 0.5 mg/kg dose dissolved in 2-ml cubes of flavored gelatin, resulting in a concentration of 0.125 mg of buprenorphine/ml in gelatin) was fed to rats previously trained to consume flavored gelatin readily (group A, n = 4). As a negative control, rats were given flavored gelatin without drug (group B, n = 4), and as a positive control, rats were treated with buprenorphine 0.05 mg/kg, administered subcutaneously (group C, n = 4). To control for different routes of administration and to facilitate comparison between consumed and injected buprenorphine, each rat received flavored gelatin and an injection. In animals of group A, flavored gelatin contained buprenorphine and the injection contained sterile deionized water (the vehicle for the buprenorphine injection); in animals of group B, neither the flavored gelatin nor the injection contained buprenorphine; and in animals of group C, flavored gelatin without drug was administered in combination with an injection of buprenorphine. In this experiment, flavored gelatin was either beef (n = 3) or raspberry (n = 1) flavored. Pain threshold was determined prior to administration of drug (baseline), and at 30 min and 1 h after administration of drug. No differences in preference, rate of ingestion, or response, were observed for the two flavors of gelatin.

In a subsequent pilot study designed to determine an effective dose of oral buprenorphine, we attempted to feed, in flavored gelatin, a broad range of doses of buprenophine: 0.5 mg/kg (concentration of buprenorphine in gelatin of 0.125 mg/ml); 1 mg/kg (concentration of 0.25 mg/ml); 2.5 mg/kg (concentration of 0.625 mg/ml); 5 mg/kg (concentration of 1.25 mg/ml), and 10 mg/kg (concentration of 2.5 mg/ml) in 2-ml gelatin cubes. Unfortunately, the rats would not readily consume gelatin with concentrations ≥ 0.25 mg/ml of buprenorphine (equivalent to a dose of buprenorphine >1 mg/kg body weight). The higher concentrations were necessary to ensure that the final volume of the combined gelatin and buprenorphine could be readily consumed by a rat (approx. 2 ml per cube). It is not clear why rats refused to eat these higher concentrations, but buprenorphine is an alkaloid and, therefore, is probably extremely bitter tasting. Drug preparations at lower concentrations (≤ 0.125 mg/ml of gelatin) were eaten enthusiastically, but doses of 5 and 10 mg/kg in that concentration would necessitate volumes of consumed gelatin of 20 and 40 ml, respectively, which are well beyond the upper limit for practical application.

To eliminate any potentially confounding variables that resulted from the use and ingestion of flavored gelatin (i.e. duration of eating, volume eaten, buprenorphine stability during flavored gelatin preparation), and to permit testing of a wide range of doses, we chose to deliver the buprenorphine by orogastric infusion for the follow-up study. The testing times in Study 2 were also increased from 1 to 12 h to allow us to gather information on both dose-response and duration of action.

In experiment 2, seven groups of 10 rats were evaluated after orogastric infusion of buprenorphine at doses of either 0.5 mg/kg (the currently recommended oral dose), 1, 2.5, 5, or 10 mg/kg. Control groups included saline infusion (negative control group), and injection of 0.05 mg buprenorphine/kg, s.c. (positive control group). For comparison purposes, all buprenorphine-infused rats received a vehicle injection, and all buprenorphine-injected rats received a vehicle infusion. Therefore, every rat received an injection and an orogastric infusion. Pain threshold was determined prior to administration of drugs (baseline), and at 30 min, and 1, 2, 4, 8, and 12 h after administration.

In experiment 3, rats were tested to determine whether the repeated-testing design of experiment 2 compromised determination of the duration of drug action. Groups of rats were tested once before buprenorphine administration (baseline), then only once after

buprenorphine administration, at either 2, 4, 8, or 12 h.. The four groups tested were the two orogastric-infusion doses found to induce significant analgesia in experiment 2 (5 and 10 mg/kg infused, plus vehicle injected), the injection dose that was used as a positive control (vehicle infused plus 0.05 mg/kg, s.c.), and the negative control group (vehicle infused plus vehicle injected).

Statistical analysis: For all experiments, statistical comparisons were made by use of analysis of variance (ANOVA), followed by appropriate simple effect probes of the interaction and/or pairwise group comparisons, using the Newman-Keuls method (13). For experiments 1 and 2, statistical comparisons were made on pain threshold (in seconds) data, whereas in experiment 3, statistical comparisons were done on the posttreatment data expressed as a percentage of baseline. The reason for the latter transformation was to simplify the graphic representation of these data. Transformation of the data in this way did not change the statistical results of this study.

Results

Experiment 1: Analgesic effect of buprenorphine, 0.5 mg/kg, administered in flavored gelatin. Comparisons of the effects of buprenorphine on pain threshold after oral or subcutaneous administration are summarized in Fig. 1. A two-way ANOVA comparing treatment (negative control; buprenorphine at 0.5 mg/kg, p.o., in gelatin; and buprenorphine at 0.05 mg/kg, s.c.) by time (baseline; 30 min after treatment; 1 h after treatment) with repeated measures on time, revealed significant interaction [$F(4, 18) = 3.21$, $P < 0.05$]. The statistical probes of this interaction indicated that: buprenorphine administered by s.c. injection induced significant increases in pain threshold, as evidenced by a between-group difference at 30 min [$F(2,26) = 11.8$, $P < 0.01$, followed by a Newman-Keuls pairwise comparison at $P < 0.05$] and by a within-group difference at 1 h [$F(2,18) = 9.65$, $P < 0.01$]; and no change in pain threshold occurred after the buprenorphine-in-gelatin administration [$F(2,18) < 1$], or over time among negative controls [$F(2,18) < 1$]. These results clearly indicate that ingestion of 0.5 mg/kg in flavored gelatin did not induce an increase in pain threshold. Buprenorphine was not an effective analgesic at this dose-by-route of administration.

Experiment 2: Dose- and time-dependent effects of buprenorphine administered by orogastric infusion. Results of experiment 2 are summarized in Fig. 2. A two-way ANOVA comparing dose (0, 0.5, 1, 2.5, 5, and 10 mg/kg, p.o.) by time (baseline, 30 min, and 1, 2, 4, 8, and 12 h after treatment) with repeated measures on time, was conducted on the orogastric-infusion data. This analysis revealed a significant dose X time interaction [$F(8, 97) = 3.01$, $P < 0.01$, with a Greenhouse-Geisser correction for the large number of repeated measures]. Significant differences between groups were observed at 30 min and 1 h after the orogastric infusion of buprenorphine. Groups of rats receiving the highest two doses of infused buprenorphine (5 and 10 mg/kg) had significantly higher pain thresholds than did all other groups ($P < 0.05$, pairwise comparison). No significant differences between groups were observed at baseline or at 2, 4, 8, or 12 h after treatment. These results suggest that oral infusion of 5 and 10 mg of buprenorphine/kg induced analgesia that lasted 1 to 2 h. The magnitude of the response to orally infused buprenorphine was dose dependent: the 5 mg/kg, p.o., dose induced maximal increase in tail-flick latency (pain threshold) of 66% ± 18% above baseline at 30 min, and the 10 mg/kg dose induced maximal increase in tail-flick latency (pain threshold) of 122% ± 45% above baseline at 30 min.

The injected buprenorphine (0.05 mg/kg) also induced a sig-

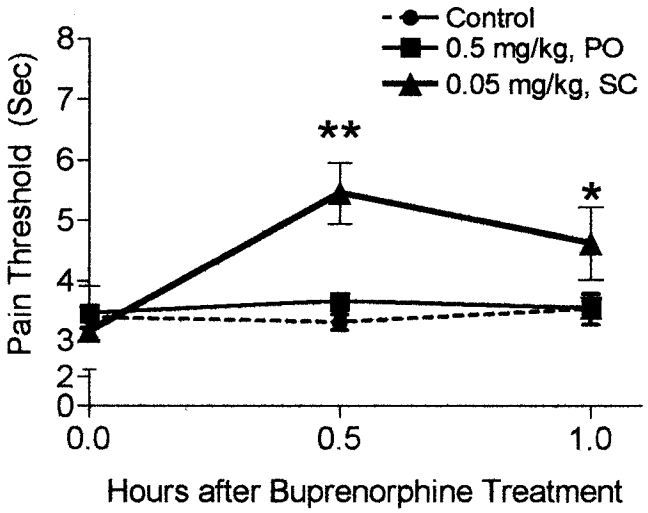

Figure 1. Comparison of the analgesic effects of buprenorphine given in currently recommended postoperative treatments. Rats were treated with buprenorphine administered orally in flavored gelatin (0.5 mg/kg of body weight, compared with injection control of 1 ml of vehicle/kg, s.c.) or administered s.c. (0.05 mg/kg, compared with ingestion control of 2 ml of gelatin/kg, p.o.) and compared with negative controls (ingestion control of 2 ml of gelatin/kg only, p.o., and injection control of 1 ml/kg vehicle, s.c.). Pain threshold was determined by use of the hot-water tail-flick assay before (baseline, time 0), and 30 min and 1 h after buprenorphine administration. *$P < 0.05$ relative to baseline, **$P < 0.05$ relative to baseline and controls.

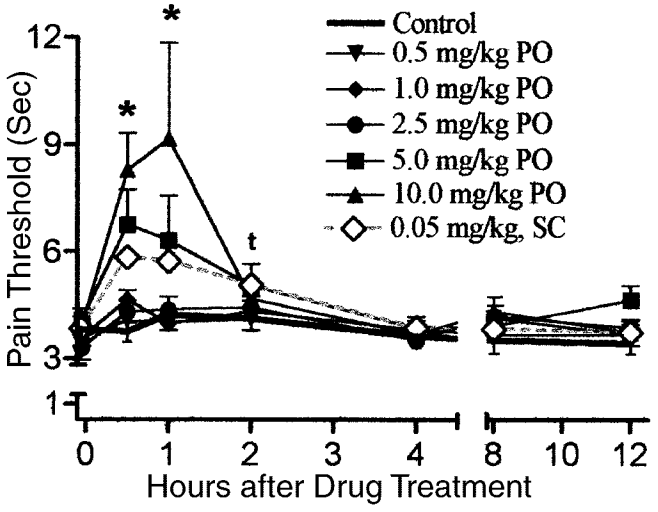

Figure 2. Dose-dependent effect of buprenorphine administered by orogastric infusion. Rats were given buprenorphine by orogastric infusion (0.5 to 10 mg/kg, p.o., compared with injection control of 1 ml of vehicle/kg, s.c.), or by s.c. administration (0.05 mg/kg, s.c., compared with ingestion control of 2 ml of water/kg, p.o.) and compared with negative controls (ingestion control of 2 ml of water/kg, p.o., compared with injection control of 1 ml of water/kg, s.c.). Pain threshold was determined by use of the hot-water tail-flick assay before (baseline, time 0) and 30 min, and 1, 2, 4, 8, and 12 h after buprenorphine administration. *$P < 0.05$, relative to baseline, to controls, and to the 0.5, 1.0, and 2.5 mg/kg p.o. dose groups. t = Ps < 0.05, relative to baseline and the controls.

nificant increase in pain threshold, similar to experiment 1. The two-way ANOVA (treatment [control versus s.c. administration] by time, [with repeated measures on time]) comparing s.c. administration of buprenorphine to s.c. administration of vehicle, yielded a significant interaction, [$F(4,79) = 6.54$, $P < 0.01$], and the probe of this interaction indicated that s.c. administration of buprenorphine significantly increased pain threshold over control values at 30 min [$F(1,58) = 24.81$, $P < 0.01$], 1 h [$F(1,58) = 12.80$, $P < 0.01$], and 2 h [$F(1,58) = 5.24$, $P < 0.05$] after treatment. No significant between-group differences were observed for tail-flick latencies at baseline, or for the 4-, 8-, or 12-h tests. The magnitude of the change in tail-flick latency in response to s.c. administration of 0.05 mg of buprenorphine/kg, was +55 ± 10%, which was similar to that induced by 5 mg/kg, given orally (+66 ± 18%).

Together, these results indicate that orally administered (orogastrically infused) buprenorphine at doses ≤ 2.5 mg/kg did not induce significant increases in the pain threshold, and therefore, did not induce analgesia. Rather, an oral dose of 5 mg/kg was necessary to increase pain threshold significantly, and to induce analgesia comparable to that induced by the standard therapeutic dose of injected buprenorphine. Durations of drug action for orogastrically infused and injected buprenorphine were 1 to 2 h, which were shorter than we expected on the basis of findings in literature. One explanation for the unexpectedly short duration may be that the rats became accustomed to the tail flick testing in experiment 2 and withdrew the tail from the heated water more quickly with each sequential procedure. In experiment 3, to determine duration of the drug more accurately, additional groups of rats were tested at baseline and only once at 2, 4, 8, or 12 h. In this way, naïve rats had only one experience with the test prior to assessment of the analgesic effect of administered buprenorphine.

Experiment 3: Test of the repeated measurement of buprenorphine analgesia. A potential problem with the repeated-measures design used in experiment 2 was that repeated exposure to the hot-water bath may have, by itself, altered the accuracy of pain threshold measurement, or altered the rat's responses due to familiarity with the paradigm, over repeated trials (i.e., practice effect). This may have confounded the interpretation of the duration of action of the drug. Therefore, in experiment 3, pain threshold was tested only twice: once at baseline and once at 2, 4, 8, or 12 h after administration of the drug. Orally administered doses of 5 and 10 mg of buprenorphine/kg were tested along with negative controls (vehicle infusion plus vehicle injection) and positive controls (vehicle infusion plus 0.05 mg of buprenorphine/kg, s.c.) at the 2-, 4-, or 8-h interval. For the 12-h interval, the positive control was omitted. Results of experiment 3 are illustrated in Fig. 3. As expected, no between-group differences in baseline pain threshold were observed (tail-flick latency range: 3.10 ± 0.18 sec. for the 4-h interval control group; to 3.74 ± 0.27 sec. for the 12-h interval control group) and pain threshold among negative controls did not change significantly over time (range: 3.56 ± 0.28 sec. for the 4-h interval control group, to 4.05 ± 0.21 sec. for the 8-h interval control group). The data for each rat were subsequently expressed as percentage of change from baseline to simplify the graphic representation in Fig. 3. Statistical analyses were carried out on the percentage of change from baseline.

At 2 h, all buprenorphine-treated groups had significant increase in pain threshold [$F(3,32) = 3.29$, $P < 0.04$, followed by pairwise comparison at $P < 0.05$]. At 4 h, significant increases in pain threshold were still evident in rats treated orally with 5 and 10 mg of buprenorphine/kg [$F(3,35) = 3.97$, $P = 0.02$, followed by pairwise comparisons at $P < 0.05$]. At 8 h, animals of the orally administered 10 mg of buprenorphine/kg group still had significantly increased pain threshold (above baseline and above values for animals of the s.c. administration group) [$F(3,28) = 5.57$, $P < 0.01$, followed by pairwise comparisons at $P < 0.05$]. At 12 h, pain threshold among rats treated orally with 10 mg of buprenorphine/kg was not different from baseline [$F(2,22) = 1.67$, $P > 0.05$]. These results indicate that, after orogastric infusion, buprenorphine at dosages of 5 and 10 mg/kg induced detectable increase in tail-flick latency (pain threshold) for more than four and less than eight hours. This is substantially longer than results indicated in experiment 2, suggesting that repeated tail-flick testing in the latter did interfere with accurate assessment of the duration of action of orogastrically infused buprenorphine. In the case of s.c. administered buprenorphine, the duration of action was estimated to be two to four hours, whether or not repeated testing was used.

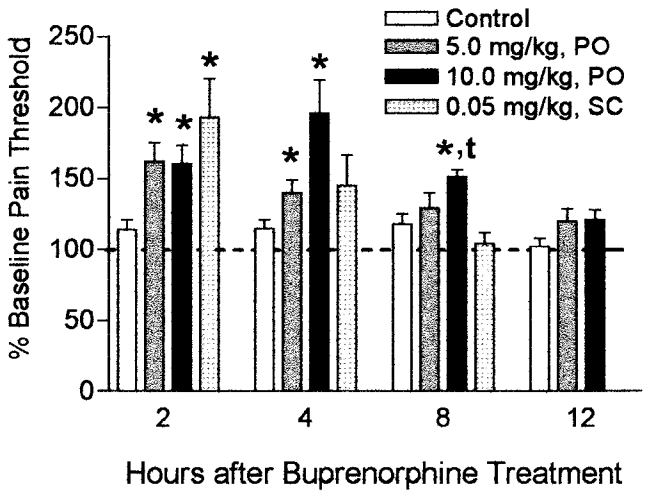

Figure 3. Duration of action of buprenorphine after orogastric or s.c. administration. Rats were treated with buprenorphine by orogastric infusion (5 mg/kg, p.o., compared with injection control of 1 ml of vehicle/kg, s.c.) or s.c. injection (0.05 mg/kg, s.c., compared with ingestion control of 2 ml of gelatin/kg, p.o.), and compared with negative controls (injection control of 1 ml of vehicle/kg, s.c., and ingestion control of 2 ml of gelatin/kg, p.o.). Pain threshold was determined by use of the hot-water tail-flick assay before (baseline, time 0) and 2, 4, 8, or 12 h after buprenorphine administration. For analysis, post-buprenorphine pain threshold was transformed to percentage of change from baseline. Dotted line represents the baseline response. *$P < 0.05$ relative to control group; t = $P < 0.05$ relative to the control groups and the s.c. group.

Discussion

The purpose of the study reported here was to determine the efficacy of buprenorphine administered orally to rats. In rodents, oral administration of buprenorphine (0.5 mg/kg) in flavored-gelatin cubes (5) is a commonly used analgesia method in laboratory animal medicine. Standard pharmacokinetic indexes suggest that oral doses of opioids should be in the order of 10 times the parenteral doses to compensate for differences in bioavailability (6). If that is true, the 0.5 mg/kg oral dose should logically be effective in relation to the established s.c. dose of 0.05 mg/kg (8). However until now, the analgesic efficacy of oral buprenorphine had not been tested empirically (3).

In experiment 1, we compared the pain threshold of buprenorphine eaten in flavored gelatin (0.5 mg/kg) with the standard therapeutic s.c. dose of buprenorphine (0.05 mg/kg), and with plain untreated flavored gelatin (negative control). The results indicated that there was no significant increase in pain threshold (no analgesia) in the group of rats eating gelatin-containing buprenorphine. The s.c. injection of buprenorphine, in contrast, induced significant increase in pain threshold, indicating significant depth of analgesia. It could be argued that the method of pain assessment (the hot-water tail-flick assay) was not sufficiently sensitive to detect the analgesic properties of the gelatin dose. However, the positive results of the s.c. dose suggest that the method of analysis was sensitive. We expected the oral gelatin dose to induce an effect that was similar to that of the s.c. dose, regardless of the method of pain assessment. We concluded that buprenorphine (0.5 mg/kg), eaten in flavored gelatin, contrary to popular belief and usage, is not an effective analgesic at this dose-by-route of administration.

Other possible explanations for the apparent ineffectiveness of the 0.5 mg/kg dose in gelatin could be: alteration of the drug during preparation, or insufficient amount of drug. It is unlikely the drug was affected by the gelatin preparation because buprenorphine is soluble in water at room temperature, stable over a wide pH range, and stable at the preparation temperature 55°C (14).

Attempts to achieve analgesia, using higher doses of buprenorphine in gelatin, were unsuccessful. When the dose of buprenorphine was increased to 5 mg/kg (buprenorphine-in-gelatin concentration of 1.25 mg/ml) it became unpalatable (i.e., bitter). Therefore, to eliminate any possible factors associated with the gelatin preparation and palatability, we subsequently delivered the drug directly into the stomach by orogastric infusion. In experiment 2, the dose range and testing time were increased, and orogastric infusion of the drug was used to determine optimal dose and duration of effect. Toxic effects were not anticipated or observed at 10 mg/kg, our highest dose; previous studies successfully involved use of orally administered doses as high as 18 mg/kg (15).

Results of experiment 2 indicated that orally infused buprenorphine at 5 and 10 mg/kg induced significant increases in pain threshold at 30 min and 1 h after infusion, but that the three lower doses (including a re-test of the commonly suggested oral dose, 0.5 mg/kg, but without the complication of gelatin preparation) did not induce significant increases at any test interval. The s.c. administered dose did induce significant increases in pain threshold at 30 min, or 1 and 2 h after administration. The duration of analgesia induced by s.c. administration of 0.05 mg of buprenorphine/kg was consistent with that reported in literature under comparable testing conditions, in that a significant reduction in analgesic effect was observed by 4 h after injection (only about 50% of group had detectable analgesia) (1). Our data suggest that the 5 and 10 mg/kg doses of orally infused buprenorphine induced analgesia that was comparable to that induced by the injected dose. The s.c. administered dose induced tail-flick latencies that were about 55% above baseline, and the orally administered 5 mg/kg dose induced latencies that were about 66% above baseline. Our data are consistent with other research of orally administered buprenorphine, which suggests that higher oral doses than previously thought may be required to induce a significant depth of analgesia. Cooper and co-workers (14) found that oral doses of 0.6, 1.6, and 2.9 mg/kg/24 h did not induce tail-flick latencies that were significantly longer than those of controls. A chronic-pain study in mice compared the effect of 0.5 mg/kg buprenorphine in flavored gelatin with that of a flavored-gelatin control, and although pain threshold was not assessed directly, indicated no differences in behavioral variables assumed to be highly correlated with analgesia, such as exploratory behavior and fur quality (16). It may be the case that the diseased condition of the animals (cancer) may have confounded the use of this indirect behavioral measure as a sole measure of analgesia. However, another interpretation of those results might now, on the basis of our observations, be that the oral dose of buprenorphine in that study was simply ineffective.

Our results lead to the conclusion that orally administered buprenorphine at a dosage of 0.5 mg/kg (and even at 1.0 and 2.5 mg/kg) does not result in a significant increase in pain threshold, and that an oral dose 100 times the common s.c. administered dose of 0.05 mg/kg is required to achieve similar depth of analgesia as that induced by the s.c. administered dose.

The duration of action for orally administered buprenorphine differed between the repeated-testing design and the non-repeated (or minimally repeated) testing design. In the repeated-testing design (experiment 2), duration for the 5 and 10 mg/kg oral doses lasted 1 to 2 h, and the s.c. dose lasted 2 to 4 h. Since we expected the analgesic effect of buprenorphine to last for 6 to 8 h, on the basis of a cursory examination of literature (11), we examined the design of our study to determine its effect on the results.

The repeated-testing design required each rat to undergo tail-flick testing seven times over the 12-h experiment. The rats may have become conditioned to the paradigm, and therefore, may have learned to flick the tail sooner in later tests; anticipation of the uncomfortable water probably caused them to escape sooner (have shorter latency) as they acquired more and more experience with the procedures. We, therefore, controlled for the repeated-testing effect in experiment 3, in that each rat was tested only twice. When repeated testing was reduced to a minimum, thereby providing more accurate appraisal of pain threshold at any one time point, the 5- and 10-mg/kg orally administered buprenorphine doses resulted in pain threshold that was still increased 8 h after drug administration. These results are consistent with those published in literature (1, 11, 17), indicating that buprenorphine is a long-acting analgesic, at least when administered in high doses.

The magnitude and duration of action of buprenorphine varies with the method of pain assessment and with model of analgesic assessment (e.g., simple pain-threshold determination versus relief of postsurgical pain). The analgesic efficacy of s.c. buprenorphine in rats is of shorter duration and lower magnitude when assessed in clinically normal non-operated rats than it is in rats after surgery or in other pain models. It is possible that the dose of buprenorphine required for alleviation of postoperative pain might be different from the dose range we found effective in non-operated rats (7). Release of endogenous opioids during postsurgical pain may contribute to analgesia, thereby decreasing the amount of exogenous analgesic required; conversely, postoperative pain, due to its severity, may result in an increased need for exogenous analgesic. Therefore, a pain model such as the formalin-paw test may be necessary for determination of the most effective oral dose of buprenorphine to give for alleviation of postoperative pain.

The importance of our findings to the clinical setting lies in the lack of effectiveness of the recommended oral dose of bupre-

norphine, relative to the effectiveness of the recommended s.c. dose. We report here that oral administration of 0.5 mg of buprenorphine/kg does not induce a pattern of pain relief similar to induced by s.c. administration of 0.05 mg of buprenorphine/kg. This s.c. dose is used commonly as a postoperative treatment in rats, and provides long-term pain relief (approx. 4 to 8 h) (5-7). However, the extent to which the dose parameters we found generalize to females and other strains of rats still remains to be determined. Previous research suggests that strain and sex may be important variables in the reaction to opioid compounds (18-21).

Long-Evans rats were chosen for the study because they are an outbred strain routinely used in research on opioid mediated analgesic mechanisms. Recently Morgan and co-workers (21) assessed strain differences in the efficacy of a large number of narcotics, including buprenorphine. Although Long-Evans rats were slightly less sensitive to buprenorphine than were Sprague Dawley rats, that difference was small in relation to the magnitude of the effects found in our study. Interestingly, the original recommendation for the buprenorphine-in-gelatin procedure was made, and is probably being used widely, without regard to rat strain or sex.

The currently recommended oral dose of buprenorphine, 0.5 mg/kg in flavored gelatin (5), is not sufficient to induce analgesia in unoperated, male, Long-Evans rats. In addition, oral administration of buprenorphine can induce a depth of analgesia comparable to that induced by s.c. administration of a conventional therapeutic dose (0.05 mg/kg [4]), but only when the oral dose (5 mg/kg) is 100 times larger than the commonly accepted injectable dose, and 10 times larger than the commonly recommended oral dose (0.5 mg/kg). At a minimum, > 2.5 mg/kg, but certainly 5 mg/kg, of orally administered buprenorphine is suitable for postoperative analgesia. However, we found that rats would not eat flavored gelatin containing concentrations of the drug that were sufficiently high to result in measurable analgesia (5 mg/kg). Therefore, further research will be necessary to determine whether a sufficiently palatable vehicle exists that will encourage rodents to consume sufficient buprenorphine to induce analgesia.

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