

Analgesic Efficacy of Orally Administered Buprenorphine in Rats: Methodologic Considerations

Alexis C. Thompson, PhD,^{1*} Mark B. Kristal, PhD,² Abdullah Sallaj,² Ashley Acheson,² Lisa B. E. Martin, DVM,³ and Thomas Martin, BVSc, PhD³

Buprenorphine has been widely recommended for treatment of pain in rodents. We have previously documented that the recommended postoperative oral dose of buprenorphine in male Long-Evans rats, 0.5 mg/kg, is not as effective as the recommended parenteral dose of buprenorphine (0.05 mg/kg, s.c.) as an analgesic (21). In the series of experiments reported here, we compared: the analgesic effect of buprenorphine when prepared in two ways in the laboratory with that of a commercially available injectable solution of buprenorphine; the analgesic effect of buprenorphine in Long-Evans rats with that in Sprague-Dawley rats; and Long-Evans and Sprague-Dawley rats for development of pica, a commonly reported side effect of buprenorphine. We followed the pica experiment with assessment of the effectiveness of buprenorphine in establishing a conditioned flavor aversion. The results indicated that method of preparation did not result in any significant differences in the efficacy of injected buprenorphine. Strain of rat was not associated with a significant difference in the efficacy of buprenorphine. However, a significant strain difference was found in development of pica. Buprenorphine treatment was effective in inducing a conditioned flavor aversion. We concluded that the recommended oral dose of buprenorphine (0.5 mg/kg) is ineffective as an analgesic, and that this was not the result of method of preparation of the buprenorphine or strain of rat used. Furthermore, we concluded that buprenorphine treatment may induce gastrointestinal distress in both strains tested. The results reaffirm our previous conclusion that oral administration of buprenorphine at 0.5 mg/kg, despite the general recommendation, is not a reasonable treatment for postsurgical pain in rats.

In a previous report (21), we indicated that the recommended postoperative orally administered dose of buprenorphine, 0.5 mg/kg of body weight (15, 20), does not induce analgesia comparable to that of the recommended postoperative subcutaneously administered dose of buprenorphine, 0.05 mg/kg (14, 20, 25) in male Long-Evans rats. In fact, in our assay, the recommended parenteral dose of buprenorphine induced robust analgesia, whereas the recommended oral dose did not induce measurable analgesia at all. Instead, 5 mg/kg, 10 times greater than the recommended dose, given by orogastric infusion, was necessary to induce analgesia comparable to that of the subcutaneously administered dose of 0.05 mg/kg. The recommended technique for oral administration of 0.5 mg/kg was to dissolve it in flavored gelatin, then offer the rat a cube of the gelatin (2 ml/kg) containing a concentration of 0.125 mg of buprenorphine/ml (14, 25). However, concentrations of buprenorphine > 0.125 mg/ml of flavored gelatin, which yielded a volume of gelatin/buprenorphine that was still sufficiently small to be wieldy (2 ml), were too aversive (bitter) for the rats to eat voluntarily. The conclusions of our earlier study were that buprenorphine mixed in flavored gelatin

at the recommended concentration was not an effective analgesic in rats; when mixed in flavored gelatin at a concentration that would induce appreciable analgesia, the concentration was too high to be voluntarily ingested.

The objective of the study reported here was to probe some of the valid methodologic issues that arose from the report by Martin and co-workers (21). First, we examined systematically whether the effectiveness of diluted commercially available buprenorphine (Buprenex) varies from that of a solution of powdered buprenorphine HCl. We then examined the effectiveness of orally administered buprenorphine in Sprague-Dawley rats, since our earlier tests had been conducted in Long-Evans rats, to see whether strain differences could account for different results in the literature.

An incidental observation made during the experiment on the Sprague-Dawley rats was that > 80% of them (across all treatment groups) were observed to be carrying, holding, or chewing cage bedding 1 h after treatment with buprenorphine. This pica behavior, not observed in Long-Evans rats, was quite notable; in some instances, rats appeared to have bedding filling their cheek pouches or spilling out of their mouth like a large fan. Although pica resulting from buprenorphine treatment in rats has been reported (2, 8), to the authors' knowledge, strain difference had not been reported. To test this apparent strain difference in the effect of buprenorphine empirically, experiment 3 was conducted to assess the effect of buprenorphine (0.05 mg/kg, s.c.) on pica in Sprague-Dawley and Long-Evans rats.

Received: 10/03/03. Revision requested: 12/10/03. Accepted: 3/03/04.

¹Research Institute on Addictions, University at Buffalo, 1021 Main Street, Buffalo, New York 14213; ²Department of Psychology, Behavioral Neuroscience Program, University at Buffalo, Park Hall, Buffalo, New York 14260; and ³Department of Comparative Medicine and Laboratory Animal Facilities, School of Medicine and Biomedical Sciences, University at Buffalo, 116 Biomedical Education Building, 3435 Main Street, Buffalo, New York 14260.

*Corresponding author.

Pica is often regarded as resulting from gastrointestinal distress (e.g., dogs eating grass, [23]). Therefore, we conducted an additional experiment (experiment 4) to test the possibility, more directly, that buprenorphine treatment might be causing gastrointestinal distress in the rats. As a conservative test of this possibility, we used Long-Evans rats because they did not exhibit pica after buprenorphine treatment. The paradigm chosen was conditioned flavor aversion. It is well known that pairing a novel flavor with gastrointestinal illness (nausea, diarrhea, vomiting, cramps) leads to aversion to that novel flavor (1, 22). Therefore, the ease of conditioning a flavor aversion to the effects of an administered drug is taken as an index of the gastrointestinal malaise caused by that drug (11, 22, 30).

Materials and Methods

Subjects. The subjects were either male Long-Evans (hooded) rats (400 to 600 g), obtained from an in-house breeding colony (seeded from Harlan Blue Spruce, Indianapolis, Ind.: experiment 1, n = 12; experiment 3, n = 10; experiment 4, n = 30), or male Sprague-Dawley (albino) rats (350 to 600 g) obtained from a commercial vendor (Harlan Sprague Dawley, Indianapolis, Ind.: experiment 2, n = 24; experiment 3, n = 10). The colony was monitored for microbiological agents by use of serologic examination for antibodies to bacterial, and viral agents in sentinel rats. Cellophane tape tests and necropsy were performed to examine sentinels for parasites. These rats were found to be free of cilia-associated respiratory bacillus, Kilham rat virus, H-1 virus, *Mycoplasma pulmonis*, pneumonia virus of mice, sialodacryoadenitis virus, Sendai virus, lymphocytic choriomeningitis virus, reovirus, pinworms, and furmites across the period during which this study was conducted.

The rats were housed in polycarbonate cages (46 × 25 × 21 cm) containing Aspen hardwood shavings (Northeastern Products Corp., New York, N.Y.). The temperature, humidity, ventilation, and lighting were maintained at: 22° ± 2°C, 50-60% humidity, 14 air changes/h, and a 14:10 light:dark cycle (lights on at 6 a.m. EST), respectively. For experiments 1, 2 and 3, rats were fed Teklad Rodent Diet 8640 (Harlan Teklad, Madison, Wis.) and tap water ad libitum. Rats were habituated to general handling by gently handling each rat for about 2 min on each of 3 days during the week prior to testing. In experiment 4, for 2 weeks before the start of the experiment and throughout the experiment, rats were maintained on a water-restricted diet; they had access to water for only 30 min each day. A water-restricted diet was necessary to ensure that the rats would be highly motivated to drink during a finite period (during testing) and to ensure that they would drink novel-flavored substance during conditioning. Weight and hydration (tent test on a hind paw) were monitored daily throughout the study. No rat had significant weight loss (> 10%) or dehydration at any time during experiment 4. The series of experiments was approved by the guidelines established by the Institutional Animal Care and Use Committee of the University at Buffalo (UB). The UB animal facilities are fully approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). No rats served in any previous study, and during this study, no rat was used in more than one experiment. All rats were tested within the same daily time frame (8 a.m. to 1 p.m. EST) to control for circadian changes in opioid sensitivity. At completion of each experiment, rats were euthanized by inhalation of CO₂, or transferred to other laboratories to participate in other research.

Drug Preparation

Experiment 1 (method of buprenorphine preparation).

Buprenorphine was prepared from powdered buprenorphine hydrochloride (RBI/Sigma, Natick, Mass.), or from a veterinary buprenorphine hydrochloride solution (Buprenex, Reckitt & Coleman, Richmond, Va.) to a final concentration of 0.05 mg/ml. Buprenorphine solutions were prepared fresh on each day of testing. Three methods of preparation were used. For comparison, all drugs were administered subcutaneously at a dosage of 0.05 mg/kg and in a volume of 1 ml/kg. One solution was made by following the procedure of Martin and co-workers (21) for subcutaneous buprenorphine administration (SC BUP): powdered buprenorphine was mixed with sterile de-ionized water to a concentration of 0.05 mg/ml, then the solution was vortex mixed (2 min) and sonicated (20 min).

A second solution was made by following the procedure of Martin and co-workers (21) for oral buprenorphine administration (PO BUP): powdered buprenorphine was mixed with sterile de-ionized water to a concentration of 5 mg/ml, then the solution was vortex mixed (2 min) and sonicated (20 min), then was heated (< 35°C, the 5 mg/ml concentration only, to improve dissolution) during the last 5 min of sonication.

The third solution was Buprenex, which is available in a 0.3 mg/ml concentration. The PO BUP and Buprenex were diluted to a final concentration of 0.05 mg/ml with sterile de-ionized water so that the same injection volume (1 ml/kg) was used across treatment groups.

Experiment 2 (analgesic efficacy of orally administered buprenorphine in Sprague-Dawley rats).

Buprenorphine was prepared from powdered buprenorphine HCl (RBI/Sigma) at concentrations of 5, 0.5, and 0.05 mg/ml for oral and subcutaneous bolus administrations in a volume concentration of 1 ml/kg (5 and 0.5 mg/kg, and 0.05 mg/kg, respectively). The preparation of each solution was that described previously for experiment 1, corresponding exactly to the method used by Martin and co-workers (21), who conducted a similar study in Long-Evans rats.

Experiment 3 (strain difference in buprenorphine-induced pica). Buprenorphine was prepared from powdered buprenorphine HCl (RBI/Sigma) at a concentration of 0.05 mg/ml for subcutaneous bolus administration in a volume concentration of 1 ml/kg (0.05 mg/kg).

Experiment 4 (buprenorphine-induced flavor aversion conditioning). Buprenorphine HCl (RBI/Sigma) was used and prepared in the manner described for experiment 1 for a 5 mg/kg orally administered dose and a 0.05 mg/kg subcutaneously administered dose.

Pain-threshold assay (experiments 1 and 2). Pain threshold was measured before (baseline), and 1 h and 4 h after drug administration, using a standard hot-water tail-flick assay (17). 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 using 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 value for the last two of 3 trials, separated by 30-sec intervals. Tail-withdrawal latency at baseline (untreated rats) ranged from 2.5-4.0 sec. Each trial was terminated at 30 sec if no withdrawal response oc-

curred. Water at 55°C did not induce tissue damage to the tail. The experimenter conducting the tail-flick assay was blind to the experimental treatment of the rat. Rats were habituated to the procedure and equipment (but not the hot water) used in this assay by exposure once a day for three days during the week preceding the experiment. A significant increase in pain threshold from the baseline pain threshold was interpreted as induction of analgesia.

Orogastric infusion (experiments 1, 2, and 4). Orogastric infusion (gavage) was achieved, using 11 cm of PE160 tubing attached to a 1-ml, gas-tight tuberculin syringe fitted 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 inserted the speculum, while the other inserted the tube, handled the syringe, and infused the drug. All rats were habituated to this procedure by three sham exposures (intubation without infusion) during the week before they were tested.

Experimental Procedure

Experiment 1 (method of buprenorphine preparation).

Food was removed (to control for stomach contents during the gavage), and 2 h later, baseline tail-flick latency was determined for each rat. Buprenorphine was administered subcutaneously immediately thereafter. Three groups of rats received 0.05 mg of buprenorphine/kg, s.c. (in a 1-ml/kg volume). The independent variable was method of buprenorphine administration. Two-way analysis of variance (ANOVA: method of preparation [SC BUP, PO BUP, Buprenex] \times time [baseline, 1 h and 4 h after drug administration]) was used to evaluate statistically the effect of method of drug preparation on buprenorphine-induced analgesia.

Experiment 2 (analgesic efficacy of orally administered buprenorphine in Sprague-Dawley rats). Food was removed prior to the start of testing, and each rat was weighed. Two hours later, baseline tail-flick latency was determined for each rat, then the buprenorphine treatment was administered orally or subcutaneously to each rat. Rats received one of three conditions: buprenorphine (0.05 mg/kg, s.c.) and vehicle (1 ml of water/kg, p.o.); vehicle (1 ml of water/kg, s.c.), and buprenorphine (0.5 mg/kg, p.o.); or vehicle (s.c.) and buprenorphine (5 mg/kg, p.o.). Pain threshold was determined 1, 4, and 8 h after treatment. A two-way ANOVA (buprenorphine treatment [0.05 mg/kg, s.c., 0.5 mg/kg, p.o., 5 mg/kg, p.o.] \times test [baseline, 1, 4, and 8 h after drug administration]) was used for statistical evaluation of the effect of manipulating dose and route of buprenorphine administration on buprenorphine-induced analgesia.

Experiment 3 (strain difference in buprenorphine-induced pica). Food was removed prior to testing, and each rat was weighed. Two hours later, rats were given buprenorphine (0.05 mg/kg, s.c.) or vehicle at the start of testing and a few food pellets were placed in the corner of their cage. Five-minute behavioral observations were made every 15 min over the next 2 h. During the observation period, each rat was assessed for the presence or absence of bedding in its mouth. At the end of this period, the rats were killed; the stomach was removed and weighed, and its contents were inspected to see whether the mouthed shavings had actually been ingested. Strain differences in frequency of observed pica, proportion of groups with observable cage bedding in their stomach, and stomach contents dry weight, were assessed statistically using the χ^2 -test or two-way ANOVA.

Experiment 4 (buprenorphine-induced flavor aversion conditioning). After the rats became acclimated to the water-

deprivation schedule (approx. 2 weeks), a 3-day testing period was conducted. On the first of the three days (day 13: conditioning day), the regular drinking water available during the 30-min drinking period was replaced with a novel substance, grape juice (50% Welch's unsweetened white grape juice diluted with distilled H₂O). At the end of the 30-min drinking period on the conditioning day, the rats were given buprenorphine or its vehicle subcutaneously (0.05 mg/kg) or by oral gavage (5 mg/kg). To control for handling difference associated with the various routes of administration, all rats received an injection and an infusion. Buprenorphine was present in either the injection or infusion (not both), or not present at all. Thus, rats were treated in one of three groups: Bup SC (0.05 mg of buprenorphine/kg, s.c., and 1 ml of vehicle/kg, p.o.); Bup PO (1 ml of vehicle/kg, s.c., and 5 mg of buprenorphine/kg, p.o.); or control (1 ml of vehicle/kg, s.c., and 1 ml of vehicle/kg, p.o.). The next day (day 14: rest day) rats received regular tap water during the drinking period. The day after that (day 15: test day), rats were offered the grape juice-flavored water again. Fluid intake (milliliters consumed in 30 min) was measured throughout the study period. The critical test for the presence of a conditioned flavor aversion fell to the analysis of differences between the groups in fluid intake on the test day (day 15). Additional analyses were necessary to document that there were no group biases in initial grape juice intake (group differences on the conditioning day, day 13) and that there were no long-term effects of treatment on general fluid intake (group differences on the rest day, day 14). All of these comparisons were accomplished using two-way ANOVA (buprenorphine group [0.05 mg/kg, s.c., 5 mg/kg, p.o., control] \times days [baseline, conditioning day, rest day, test day]). Baseline fluid intake was defined as the average intake over the five days preceding the conditioning day (i.e., days 8-12).

Results

Experiment 1 (method of buprenorphine preparation).

The effect of buprenorphine (0.05 mg/kg, s.c.) on pain threshold did not differ by method of buprenorphine preparation ($F[4,18] < 1$ for the interaction between method of preparation and time, and $F[2,9] < 1$ for the main effect of method of preparation), although, as expected, this dose of buprenorphine (0.05 mg/kg) given subcutaneously induced an increase in pain threshold 1 h after its administration ($F[2,18] = 12.17, P < 0.01$). This result indicates that the analgesic efficacy of buprenorphine is not adversely affected by vortex mixing, sonicating, or brief heating to $< 35^\circ\text{C}$, and that the analgesic efficacy of buprenorphine powder prepared in this way is comparable to that of the commercially available Buprenex solution (Fig. 1).

Experiment 2 (analgesic efficacy of orally administered buprenorphine in Sprague-Dawley rats). One rat was removed from the study because of inadequate gavage volume. Data from the remaining 23 rats were used to compare the analgesic efficacy of the orally administered buprenorphine at the dose recommended for administration in gelatin (0.5 mg/kg), and the higher orally administered dose (5 mg/kg) recommended by Martin and co-workers (21), with the standard therapeutically administered dose of buprenorphine (0.05 mg/kg). A significant interaction between group (buprenorphine, s.c.; low-dose buprenorphine, p.o.; high-dose buprenorphine, p.o.) and test (baseline, 1, 4 and 8 h) was found ($F[6,60] = 8.63, P < 0.01$).

A probe of this interaction indicated that the high orally admin-

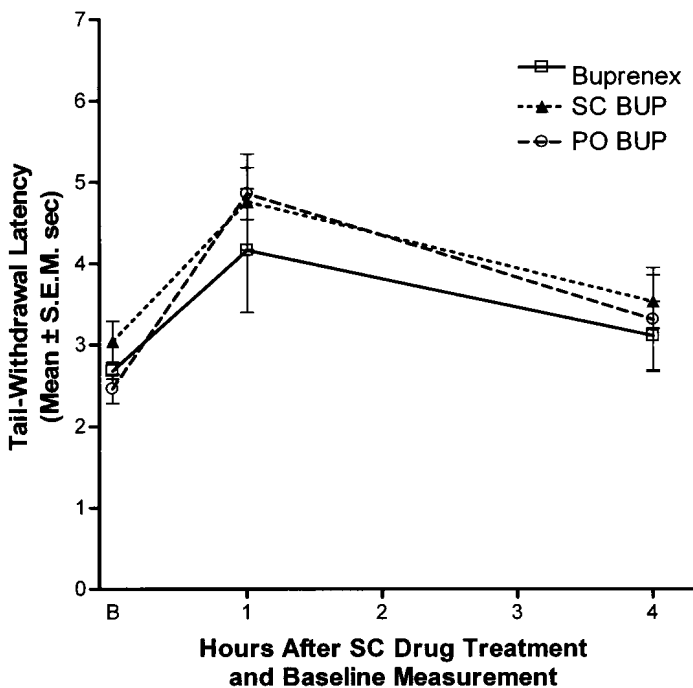


Figure 1. Analgesic efficacy of buprenorphine (0.05 mg/kg, s.c.) prepared in three ways. Buprenorphine solutions were prepared in the laboratory at a concentration of 5 mg/ml (PO BUP) or 0.05 mg/ml (SC BUP) from powder and were compared with commercially available buprenorphine (Buprenex [0.03 mg/ml]). All solutions were diluted, if necessary, to a concentration of 0.05 mg/ml prior to administration to control for volume of injection. Differences between groups (n = 4/group) were not observed; however, buprenorphine, by any method of preparation, significantly increased pain threshold at 1 h ($P < 0.05$, relative to baseline value).

istered dose (5 mg/kg) of buprenorphine ($F[3,60] = 39.65$, $P < 0.01$) and the subcutaneously administered dose ($F[3,60] = 19.44$, $P < 0.01$) resulted in significant increases in pain threshold, whereas the low orally administered dose (0.5 mg/kg) did not ($F[3,60] = 1.03$, $P > 0.05$). The 5 mg/kg, oral dose and the 0.05 mg/kg, subcutaneous dose did not differ significantly from each other at any time, but both were associated with significant increase in pain threshold, relative to baseline and at 1 h after buprenorphine administration. Pain threshold in the 5 mg/kg, oral dose group was significantly higher than that at baseline for the 4-h test period as well. In contrast, pain threshold in the 0.5 mg/kg oral dose group did not significantly change at any time after buprenorphine treatment (Fig. 2). These results paralleled the findings of Martin and co-workers (21), in Long-Evans rats and indicate that the analgesic efficacy of buprenorphine is similar in these two strains.

Experiment 3 (strain difference in buprenorphine-induced pica). Pica, mouthing or chewing of wood shavings, was only observed in Sprague-Dawley (SD) rats that received buprenorphine (Fig. 3). In 4 of 5 rats, pica was observed during at least 50% of the observation periods. Pica was not observed in Long-Evans (LE) rats given buprenorphine, and was not observed in water-treated rats of either strain. A statistical comparison of the frequency of pica by group (LE+water, LE+Bup, SD+water, and SD+Bup) was significant ($\chi^2 [3 df, n = 20] = 20.0$, $P < 0.01$). Furthermore, 3 of 4 SD rats in which pica had been observed also had discernable wood shavings in their stomach on gross inspection at the end of the 2-h observation period, indicating that the wood

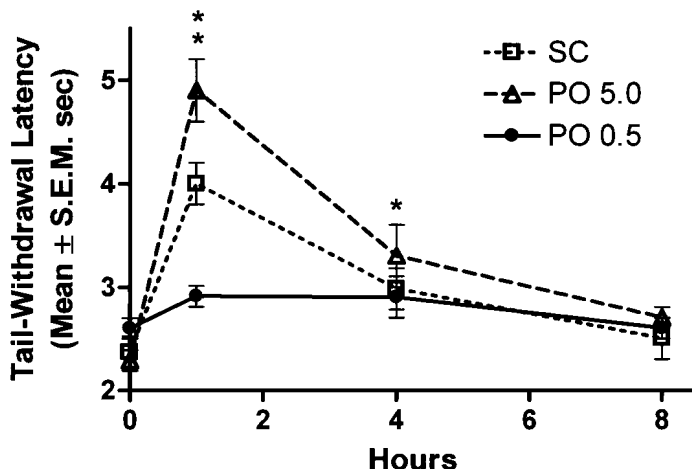


Figure 2. Analgesic efficacy of buprenorphine in Sprague-Dawley rats. Pain threshold was determined immediately before and 1, 4 and 8 h after administration of buprenorphine (0.5 mg/kg, p.o., 5 mg/kg, p.o., and 0.05 mg/kg, s.c.) in male Sprague-Dawley rats (n = 8/group). The 0.5 mg/kg orally administered dose is currently recommended for administration in gelatin and the 0.05 mg/kg subcutaneously administered dose is the standard therapeutic buprenorphine parenteral dose for postoperative analgesia. ** $P < 0.05$, 5 mg/kg, p.o., and 0.05 mg/kg, s.c., significantly greater than baseline and 0.5 mg/kg, p.o., values. * $P < 0.05$, 5 mg/kg, p.o., significantly greater than baseline value.

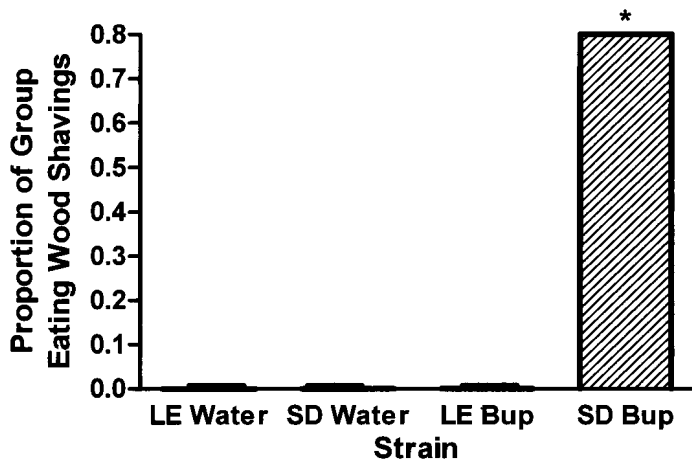


Figure 3. Strain differences in buprenorphine-induced pica. Rats were treated with buprenorphine (Bup, 0.05 mg/kg, s.c., n = 5/strain) or vehicle (water, 1 ml/kg, s.c., n = 5/strain) and were observed for 5 min every 15 min, for oral manipulation of wood shavings (chewing, mouthing). Sprague-Dawley rats had significantly more pica than did Long-Evans rats. * $P < 0.01$, compared with all other groups.

shavings being mouthed by the rats were actually being swallowed.

Wood shavings were not found in the stomach of LE rats, or SD rats treated with vehicle. Finally, buprenorphine did not significantly increase dry weight of the stomach contents of rats of either strain or by treatment group (interaction $F[1,15] < 1$, main effect of condition $F[1,15] < 1$, main effect of strain $F[1,15] = 1.7$, $P = 0.2$). The lack of difference between the dry weight of stomach contents from LE and SD rats was probably due to the difference in weight of shavings and rat chow, and the fact that LE rats were observed eating small amounts of chow during the post-treatment period.

Experiment 4 (buprenorphine-induced flavor aversion conditioning). The ability of buprenorphine to induce gastrointestinal malaise was investigated in Long-Evans rats by de-

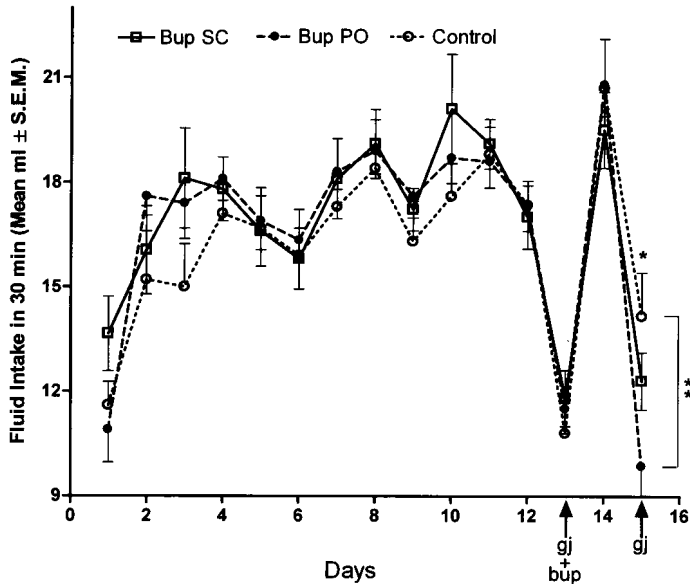


Figure 4. Buprenorphine-induced flavor aversion conditioning. Long-Evans male rats were habituated to a water-restricted diet in which water was available for only 30 min/day. On day 13 (conditioning day), water was replaced with grape juice (gj) and, after the 30-min drinking period, rats received an injection of, or gavage with buprenorphine, 0.05 mg/kg, s.c., or 5 mg/kg, p.o., respectively, or vehicle. Regular water was offered on day 14, and the second exposure (test day) to grape juice was offered on day 15 ($n = 10/\text{group}$). * $P < 0.05$ gj intake greater than that during first exposure in control rats. ** $P < 0.05$ gj intake in rats treated orally with 5 mg of buprenorphine/kg is significantly less than value for controls. Not shown: gj intake on the conditioning day (day 13) was significantly lower than baseline water intake ($P < 0.05$). Water intake on day 14 (rest day) was not different from baseline water intake ($P < 0.05$).

termining whether buprenorphine at the standard subcutaneously administered dose (0.05 mg/kg) and the equipotent orally administered dose (5 mg/kg) could induce a conditioned flavor aversion. The results (Fig. 4 and 5) indicate that the acute effects of buprenorphine can induce conditioned flavor aversion: the rats associated the novel flavor, grape, with the one-time concomitant occurrence of apparently negative gastrointestinal effects of buprenorphine.

The two-way ANOVA assessing the effect of buprenorphine treatment (SC Bup, PO Bup, or control) by day (baseline [average of days 8-12], day 13 [conditioning day], day 14 [rest day], and day 15 [test day]) on fluid intake indicated significant interaction between these two variables ($F[6,81] = 3.69$, $P < 0.003$). Simple-effect probes (set at $P < 0.05$) of this interaction indicated that: all rats drank less on the conditioning day than during the baseline period or on the rest day; fluid intake on the rest day did not differ significantly from baseline, or by treatment group; and significant group differences in fluid intake were present on the test day. Statistical probes of the group differences on the test day indicated that: control rats drank significantly more grape juice on the test day than on the conditioning day, and that fluid intake among control rats on the test day did not differ significantly from baseline water intake (indicating no aversion); rats treated orally with 5 mg of buprenorphine/kg drank significantly less grape juice than did controls on the test day and significantly less than their baseline water intake (indicating the presence of an aversion); and fluid intake among rats in the 0.05 mg/kg, s.c. group

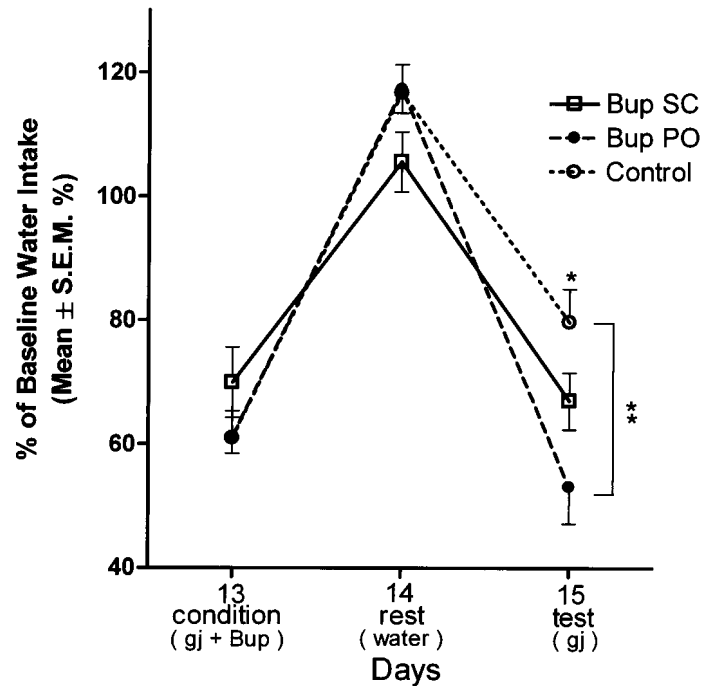


Figure 5. Fluid intake during flavor aversion conditioning as a percentage of baseline fluid intake. Long-Evans male rats treated with buprenorphine (0.05 mg/kg, s.c., or 5 mg/kg, p.o.) after their first exposure (conditioning day) to grape juice (gj) drank less grape juice on the second exposure (test day) than did control rats, and drank less fluid than they had ingested on the baseline days.

was intermediate to that of the control group and the 5 mg/kg, p.o. group, and did not differ from conditioning day intake, but was significantly lower than their baseline water intake (indicating mild conditioned aversion). These results indicate that the acute effects of orally administered buprenorphine (5 mg/kg) can be used to condition a flavor aversion, and therefore are likely to induce gastrointestinal distress. Furthermore, the results suggest that even the subcutaneous administration of buprenorphine (0.05 mg/kg) induces some malaise; grape juice intake among these rats did not show a significant increase on second exposure. The fact that conditioned rats showing an aversion still consumed some grape juice on the test day is typical in this paradigm, and was likely a result of the competing motivational state to drink that resulted from the 23.5-h water restriction schedule.

Discussion

In our earlier report (21), we stated that the recommended orally administered dose of buprenorphine for rats (0.5 mg/kg) was ineffective as an analgesic, which spawned a great deal of discussion about the generalizability and validity of our conclusion. Advocates of the buprenorphine/gelatin technique felt uncomfortable with our data, likely because of an erroneous impression that many previous studies had confirmed the effectiveness of the aforementioned dose. For example, Roughan and Flecknell (26) stated that "...other studies have clearly demonstrated the efficacy of orally administered buprenorphine" (p. 338), and cited numerous studies to support the statement. It was also suggested that our "failure" to achieve adequate analgesia with 0.5 mg of buprenorphine/kg given orally was due to: methodologic mistakes in the preparation of buprenorphine, choice of rat

strain, or choice of rat model (i.e., surgery-free rats rather than rats experiencing postoperative pain). Consideration of these possibilities led us to reconsider the literature comparing orally with parenterally administered buprenorphine dosing and to conduct the series of experiments reported here.

The studies purported to support the effectiveness of 0.5 mg of buprenorphine/kg given orally (26) can be grouped into three types: pharmacokinetic studies; studies evaluating the median effective dose (ED₅₀) of orally administered buprenorphine; and studies directly comparing the efficacy of buprenorphine administered orally with that of other routes of administration.

The first category, pharmacokinetic studies, contains one report (5). Those authors predicted that a dose of buprenorphine 10 times higher than the effective parenterally administered dose would be necessary for an orally administered dose to be effective. This conclusion was based on the bioavailability of buprenorphine in plasma, but did not address analgesic efficacy directly. This type of data supports the hypothesis that the 0.5 mg/kg orally administered dose of buprenorphine is a therapeutic postoperative analgesic that is comparable to the parenterally administered dose, but does not provide definitive evidence, as analgesic efficacy is not necessarily related to plasma drug concentration in linear manner (3).

The second category, studies assessing the ED₅₀, contains most of the literature. There are limitations to the applications of these data. First, ED₅₀ is highly technique bound, which limits the usefulness of comparisons across pain assays. Also, the ED₅₀ is necessarily much lower than a clinical analgesic dose, which presumably must be effective in all subjects, not just 50% of them. Finally, there is not necessarily a good correlation between the ED₅₀ and therapeutic dose; the ED response curves are not necessarily linear (ED₇₅ is not necessarily 150% of ED₅₀; ED₂₅ is not necessarily 50% of ED₅₀).

The third category of references, studies comparing the analgesic efficacy of different routes of administration, can actually be subdivided into two groups: those that used direct measures of analgesia (algesiometric studies) and those that used indirect measures of analgesia or well-being (e.g., feeding, locomotion). We address ourselves here only to the algesiometric studies; the studies assessing indirect indices of pain have yet to be validated and, especially with opioids, are confounded by the independent effects of opioids on feeding and locomotion (27). The algesiometric studies are summarized in Table 2 of the report by Roughan and Flecknell. That table specifically provided data on subcutaneous, intraperitoneal, and oral administrations of buprenorphine in studies using algesiometric tests. There were 36 entries based on data from 17 reports. Seventeen of the 36 table entries applied to research conducted on rats. Of those 17 entries, 5 provided information on subcutaneous administration of buprenorphine (ED₅₀: range, 0.005 to 0.4 mg/kg), 10 were about intraperitoneal administration of buprenorphine (ED₅₀: range, 0.002 to 0.2 mg/kg), and only 2 were about oral administration of buprenorphine (ED₅₀: range, 0.03 to 0.048).

Investigation of oral administration of buprenorphine was reported exclusively by Cowan and his colleagues (10, 19). In the 1977 report by Cowan and co-workers (10), the effects of orally administered buprenorphine were mentioned as a comment in the results/discussion, but the experiment was neither described in the methods nor were actual data presented in the results. The Lewis and Cowan report (19; a reference to a meeting report) is the only report that can be cited as an approximation of the test

we performed in our earlier study (21): those authors collected data on oral and subcutaneous administrations of buprenorphine using an algesiometric test, although unlike our data, their data were not collected in a manner that would allow direct statistical comparison. An experimental compound that was used (6029-M) apparently was buprenorphine (26). The ED₅₀ for the oral administration of 6029-M in a tail-pressure test was 0.05 mg/kg and the ED₅₀ for the subcutaneous administration of 6029-M in a tail-pressure test was 0.005 mg/kg. This single study, then, provides the most direct support to the idea that a 10-fold higher orally administered dose of buprenorphine (0.5 mg/kg) should be comparable in analgesic efficacy to 0.05 mg of buprenorphine/kg given subcutaneously, and stands in contrast to the report by Martin and co-workers (21).

Three major methodologic differences between their report and ours may explain the discrepancy. First, Lewis and Cowan only reported the ED₅₀ dose, and we examined and reported the therapeutic range of dose. Also, Lewis and Cowan did not discuss habituating their rats to their procedures. That report was published before researchers were widely aware of the need to habituate subjects to all the procedures used (e.g., orogastric intubation) to eliminate a confounding factor induced by acute stress and, therefore, involvement of endogenous opioids (4). Finally, Lewis and Cowan used Sprague-Dawley albino rats and we used Long-Evans hooded rats.

The numerous "other studies" indicating that 0.5 mg of buprenorphine/kg given orally is comparable in analgesic efficacy to 0.05 mg/kg given subcutaneously really amount to one 1972 report in which the contradiction to our findings actually depends on methodologic differences sufficiently great to warrant experimental clarification.

As mentioned, the methodologic problems suggested (26) as reasons for our finding that orally administered buprenorphine was not a useful analgesic at a dosage of 0.5 mg/kg included the method of preparing (solubilizing) buprenorphine solution, the strain of rat tested, and the fact that our rats were not actually experiencing postsurgical pain.

It was suggested that the method by which we prepared powdered buprenorphine, which included vortexing and heating, may have rendered the buprenorphine inactive. We tested this hypothesis directly in experiment 1 of the study reported here by comparing our method of preparing buprenorphine with commercially available buprenorphine. Results of experiment 1 indicated that the various methods for preparing buprenorphine (Buprenex diluted to 0.05 mg/kg; buprenorphine powder mixed to 0.05 mg/kg; and buprenorphine powder mixed to 5 mg/kg, then diluted to 0.05 mg/kg) did not result in differences in analgesic potency when the final buprenorphine solution was administered subcutaneously. Therefore, the method of preparation of buprenorphine (26) was not a factor in our finding that oral administration of 0.5 mg of buprenorphine/kg is ineffective as an analgesic (21).

It was suggested (26) that the lack of analgesia induced by oral administration of 0.5 mg of buprenorphine/kg, as reported by us earlier (21), may have been due to strain differences between the Long-Evans rats of our study and strains used in other studies. We had not anticipated significant strain differences when we did our original study using Long-Evans rats, since the original recommendations for oral administration of 0.5 mg/kg (14, 25) do not carry a strain caveat. In experiment 2, we tested for the possibility

Table 1. Mean percentage increase from baseline pain threshold at 1 h and 4 h after buprenorphine treatment

Rat strain	1 h	4 h
Sprague-Dawley ^a (5 mg/kg, p.o.) (0.05 mg/kg, s.c.)	214 ± 11 171 ± 12	143 ± 14 130 ± 15
Long-Evans ^b (5 mg/kg, p.o.) (0.05 mg/kg, s.c.)	160 ± 20 151 ± 16	110 ± 10 115 ± 8

Data are from different studies: ^aour experiment 2; ^bexperiment 2 from Martin and co-workers (21).

Data are expressed as mean ± SEM.

of a strain difference and found that the effect of buprenorphine in Sprague-Dawley albino rats was similar to that in Long-Evans hooded rats; buprenorphine administered by gavage at the recommended dose (0.5 mg/kg, p.o.) does not induce measurable analgesia in this algometric test, whereas the recommended parenterally administered dose (0.05 mg/kg) induces robust analgesia. Buprenorphine given orally at a dosage of 5.0 mg/kg, which is 100 times the subcutaneously administered dose, induced analgesia comparable in magnitude and duration to that induced by 0.05 mg/kg given subcutaneously. As in our previous study on Long-Evans hooded rats, the recommended orally administered dose of buprenorphine (0.5 mg/kg) was ineffective as an analgesic in Sprague-Dawley albino rats. Therefore, our finding that the recommended dose of orally administered buprenorphine was ineffective (21) was not due to the strain we chose. As reported by Morgan and co-workers (24), Sprague-Dawley rats tend to be more sensitive than Long-Evans rats to the analgesic effects of buprenorphine (our data are shown in Table 1), but although the magnitude of this difference may be physiologically relevant (24), it does not account for the difference in oral dose necessary for pharmacologic action between Sprague-Dawley and Long-Evans rats.

Martin and co-workers (21) used a standard, proven, algometric technique in which the standard recommended postsurgical dose of buprenorphine (0.05 mg/kg, s.c.; the positive control) induced reliable and robust analgesia. Since the subcutaneously administered dose recommended to be effective postsurgically was also effective in the algometric test, it is unlikely that the inadequate analgesia in rats given the recommended oral dose of buprenorphine was due to problems with the assay. However, this issue has brought a more general problem into focus. Few, if any, techniques are available for equating the effect of analgesics in subjects without underlying pain with that in subjects experiencing ongoing pain and the concomitant involvement of endogenous opioids. As for the validity of our assay (hot-water tail dip assay), it is second only to the mouse-writhing test in sensitivity, simplicity, and reproducibility (29), and one that has been widely accepted for years in experimental literature (13, 17, 18).

A large body of data suggests that the results of the thermal algometric assay in animals provide at least as good a predictor of clinical efficacy in humans (relative potency, but not actual dose) as are other pain and analgesia measures in animals (6, 12). Another advantage is that this type of animal algometric assay is least likely to lead to false-positive identification of analgesics, as verified by clinical tests in humans (29). Therefore, our method is considered accurate, reproducible, and predictable. Better tests may be possible to devise—tests that lead to no false-positive identifications and to nearly a 1:1 relationship between

dose and clinical outcome. But these “better tests” will first require identification of all neural substrates involved in the sensation and perception of pain, finding direct methods for measuring the activity in these substrates in awake behaving animals, or possibly developing a behavioral learning assay in which rats learn to provide a unique response to indicate pain or to “self-administer” analgesics. Only the last strategy is available at this time (9), and its use has resulted in substantial improvements in the assessment of pain in humans (28).

When conducting experiment 2, we noted a strain difference in response to buprenorphine treatment that was independent of analgesia: Sprague-Dawley rats manifested an appreciable degree of pica, whereas Long-Evans rats did not. This strain difference was tested systematically in experiment 3 (Fig. 3). Because pica may be a sign of gastrointestinal distress (23), we sought to verify the presence of gastrointestinal distress in experiment 4 through use of another measure that depends on gastrointestinal illness: flavor-aversion learning. The ability to condition an aversion to a novel flavor by pairing the novel flavor with the acute effects of a drug is taken as *prima facie* evidence that the drug is inducing gastrointestinal distress (1, 11, 22, 30). The use of Long-Evans rats, which do not develop pica when treated with buprenorphine, was a conservative test of the possible negative gastrointestinal effects of buprenorphine. Figures 4 and 5 clearly indicate that mild aversion to grape juice was established after only one pairing of that novel flavor with oral administration of buprenorphine. It is interesting that even parenteral administration of buprenorphine induced a measurable degree of aversion. Gastrointestinal illness, particularly nausea, in humans after narcotic and even buprenorphine treatment (7, 16) has been reported. It is possible that the degree of body weight change and amount eaten by some rat strains after buprenorphine treatment following surgery (20) may be more related to pica than to well-being.

In conclusion, the current series of experiments confirmed our previous report (21) that oral administration of buprenorphine for postoperative analgesia in the recommended dose (0.5 mg/kg), does not cause significant increase in the pain threshold in rats in our pain-threshold assay. This finding cannot be attributed to minor methodologic considerations (e.g., strain differences, buprenorphine preparation method).

It is possible that a more palatable vehicle than flavored gelatin exists that would encourage ingestion of the effective orally administered dose of 5.0 mg/kg. We tested a mixture of peanut butter and sugar. However, our data indicated that half of the rats tested could distinguish the control peanut butter and sugar from the peanut butter and sugar containing buprenorphine. Furthermore, even though the rats that ate the peanut butter and sugar laced with buprenorphine manifested the same intensity and duration of analgesia as those receiving a gavage containing 5.0 mg of buprenorphine/kg, only about 60% of the rats would voluntarily eat all of the proffered peanut butter mixture.

Oral administration of buprenorphine in rats causes more problems than it solves. At a reasonable (palatable) concentration, it is ineffective. At an effective concentration, it is unpalatable. At any dose and route likely to induce analgesia, buprenorphine likely induces gastrointestinal distress. We believe that, if buprenorphine is going to be used as a postoperative analgesic in rats, the best dose is the minimal effective subcutaneously administered dose. Whether 0.05 mg/kg buprenorphine is, in fact, the minimal effective dose remains to be seen.

Acknowledgment

This research was supported by an ACLAM Foundation grant and by funding from the College of Arts & Sciences Dean's Office awarded to MBK. Participation of personnel in the various experiments was as follows: experiment 1—ACT, MBK, LBEM, TM; experiment 2—ACT, MBK, LBEM, TM; experiment 3—AA, ACT, MBK; and experiment 4—AS, ACT, MBK.

References

1. **Barker, L. M., M. R. Best, and M. Domjan (ed.)**. 1977. Learning mechanisms in food selection. Baylor University Press, Waco, Tex.
2. **Bender, H. M.** 1998. Pica behavior associated with buprenorphine administration in the rat. *Lab. Anim. Sci.* **48**:5.
3. **Berkowitz, B. A.** 1976. The relationship of pharmacokinetics to pharmacological activity: morphine, methadone, and naloxone. *Clin Pharmacokinet.* **1**:219-230.
4. **Bodnar, R. J., D. D. Kelly, M. Brutus, and M. Glusman.** 1980. Stress-induced analgesia—neural and hormonal determinants. *Neurosci. Biobehav. Rev.* **4**:87-100.
5. **Brewster, D., M. J. Humphrey, and M. A. Mcleavy.** 1981. The systemic bioavailability of buprenorphine by various routes of administration. *J. Pharm. Pharmacol.* **33**:500-506.
6. **Campbell, I. G., E. Carstens, and L. R. Watkins.** 1991. Comparison of human pain sensation and flexion withdrawal evoked by noxious radiant heat. *Pain* **45**:259-268.
7. **Campora, E., L. Merlini, M. Pace, M. Bruzzone, M. Luzzani, A. Gottlieb, and R. Rosso.** 1991. The incidence of narcotic-induced emesis. *J. Pain Symp. Manag.* **6**:428-430.
8. **Clark, J. A., Jr., P. H. Myers, M. F. Goelz, J. E. Thigpen, and D. B. Forsythe.** 1997. Pica behavior associated with buprenorphine administration in the rat. *Lab. Anim. Sci.* **47**:300-303.
9. **Colpaert, F. C., J. P. Tarayre, M. Alliaga, L. A. Bruins Slot, N. Attal, and W. Koek.** 2001. Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. *Pain* **91**:33-45.
10. **Cowen, A., J. W. Lewis, and I. R. Macfarlane.** 1977. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br. J. Pharmacol.* **60**:537-545.
11. **Domjan, M.** 1977. Attenuation and enhancement of neophobia for edible substances, p. 151-179. *In* L. M. Barker, M. R. Best, and M. Domjan (ed.), Learning mechanisms in food selection. Baylor University Press, Waco, Tex.
12. **Dubinsky, B., S. Gebre-Mariam, R. J. Capetola, and M. E. Rosenthal.** 1987. The antialgesic drugs: human therapeutic correlates of their potency in laboratory animal models of analgesia. *Agents Actions* **20**:50-60.
13. **Fennessy, M. R. and J. R. Lee.** 1975. The assessment of and the problems involved in the experimental evaluation of narcotic analgesics, p. 73-79. *In* S. Ehrenpreis, and A. Neidle (ed.), Methods in narcotics research. Marcel Dekker, New York.
14. **Flecknell, P. A.** 1996 Laboratory animal anaesthesia, 2nd ed. Academic Press, San Diego, Calif.
15. **Flecknell P. A., J. V. Roughan, and R. Stewart.** 1999. Use of oral buprenorphine ('buprenorphine jello') for postoperative analgesia in rats—a clinical trial. *Lab. Anim.* **33**:169-174.
16. **Fullerton, T., E. G. Timm, G. B. Kolski, and J. S. Bertino, Jr.** 1991. Prolonged nausea and vomiting associated with buprenorphine. *Pharmacotherapy* **11**:90-93.
17. **Hahn, E. F.** 1985. Testing and evaluation of opioid analgesics and antagonists. *Methods Find. Exp. Clin. Pharmacol.* **7**:373-381.
18. **Janssen, P. A. J., C. J. E. Niemegeers, and J. G. H. Dony.** 1963. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneim-Forsch. Drug Res.* **13**:502-507.
19. **Lewis, J. W. and A. Cowen.** 1972. RX 6029-M, p. 514-535. *In* Report to the committee on problems of drug dependence. Thirty-fourth Annual Scientific Meeting. Committee on Problems of Drug Dependence, Ann Arbor, Mich.
20. **Liles, J. H., P. A. Flecknell, J. Roughan, I. Cruz-Madarran.** 1998. Influence of oral buprenorphine, oral naltrexone, or morphine on the effects of laparotomy in the rat. *Lab. Anim.* **32**:149-161.
21. **Martin, L. B. E., A. C. Thompson, T. Martin, and M. B. Kristal.** 2001. Analgesic efficacy of orally administered buprenorphine in rats. *Comp. Med.* **51**:43-48.
22. **Milgram, N. W., L. Krames, and T. M. Alloway.** 1977. Food aversion learning. Plenum Press, New York.
23. **Mitchell, D., M. D. Krusemark, and E. Hafner.** 1977. Pica: a species relevant behavioral assay of motion sickness in the rat. *Physiol. Behav.* **18**:125-130.
24. **Morgan, D., C. D. Cook, and M. J. Picker.** 1999. Sensitivity to the discriminative stimulus and antinociceptive effects of mu opioids: role of strain of rat, stimulus intensity, and intrinsic efficacy at the mu opioid receptors. *J. Pharmacol. Exp. Ther.* **289**:965-975.
25. **Pekow, C.** 1992. Buprenorphine Jell-O recipe for rodent analgesia. *Synapse* **25**:35.
26. **Roughan, J. V. and P. A. Flecknell.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Lab. Anim.* **36**:322-343.
27. **Roughan, J. V. and P. A. Flecknell.** 2003. Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *Eur. J. Pain* **7**:397-406.
28. **Smythe, M.** 1992. Patient-controlled analgesia: a review. *Pharmacotherapy* **12**:132-143.
29. **Taber, R. I.** 1974. Predictive value of analgesic assays in mice and rats. *Adv. Biochem. Psychopharm.* **8**:191-211.
30. **Testa, T. J. and J. W. Ternes.** 1977. Specificity of conditioning mechanisms in the modification of food preferences, p. 229-253. *In* L. M. Barker, M. R. Best, and M. Domjan (ed.), Learning mechanisms in food selection. Baylor University Press, Waco, Tex.