

Lack of Analgesic Efficacy in Female Rats of the Commonly Recommended Oral Dose of Buprenorphine

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Previous work in our laboratory showed that the recommended oral dose of buprenorphine (0.5 mg/kg) was not as effective as the standard therapeutic subcutaneous dose for postoperative analgesia in male Long-Evans (hooded) and Sprague-Dawley (albino) rats. The aim of the current study was to extend this analysis to female rats. We measured the pain threshold in adult female rats in diestrus or proestrus before and 30 and 60 min after oral buprenorphine (0.5 mg/kg), the standard subcutaneous dose of buprenorphine (0.05 mg/kg), or vehicle only (1 ml/kg each orally and subcutaneously). Female rats showed an increased pain threshold (analgesia) after subcutaneous buprenorphine but no change in pain threshold after either oral buprenorphine or vehicle only. Estrous cycle stage (proestrus versus diestrus) did not affect the analgesic effects of buprenorphine, but rats in proestrus showed significantly lower pain thresholds (less tolerance to pain) than did those in diestrus. These results show that the oral dose of buprenorphine recommended for postoperative analgesic care does not induce significant analgesia in female rats and therefore is not as effective as the standard subcutaneous dose.

Abbreviations: SC, subcutaneously; PO, orally

To reduce the need for handling and highly skilled technicians to accomplish postoperative treatment, an oral dosing regime for administration of buprenorphine for postoperative analgesia in rodents has been proposed.^{5,12} However, previous research in our laboratory has shown that the recommended oral (PO) dose (voluntarily ingested) of buprenorphine (0.5 mg/kg administered in flavored gelatin) was not comparable in analgesic efficacy to the standard subcutaneous (SC) dosage (0.05 mg/kg) and did not produce a detectable level of analgesia in our assay. An oral dose approximately 10 times higher (5.0 mg/kg PO) than that recommended was necessary to induce a level of analgesia similar to that of the therapeutic 'gold standard' (0.05 mg/kg SC).^{10,15} However, the rats would not consume the effective PO dose (5.0 mg/kg) dissolved in flavored gelatin, presumably due to its extremely bitter taste.¹⁰ We also showed that PO buprenorphine was associated with more gastrointestinal distress than was SC administration.¹⁵ These studies suggested to us that postoperative treatment with voluntarily consumed PO buprenorphine would not yield analgesia and consequent reduction of stress. We also conducted tests that ruled out the possibility that the lack of effectiveness of PO buprenorphine in our original study¹⁰ was due to strain differences or method of buprenorphine preparation.¹⁵ However, the studies in our 2 previous papers were conducted in male rats, and increasing evidence suggests that there are significant sex differences in sensitivity to the analgesic action of some opiate analgesics.² Therefore, we conducted the present study to determine whether the conclusion drawn from our studies in

male rats (that is, that voluntarily ingested PO buprenorphine is ineffective as an analgesic) can be generalized to female rats. Moreover, because sensitivity to pain and analgesia varies as function of estrous-cycle stage, we tested females in either diestrus or proestrus, the 2 stages of the cycle that show the greatest differences in pain sensitivity.³ We compared the analgesic efficacy of the recommended PO dose of buprenorphine (0.5 mg/kg)^{5,12} to the recommended SC dose (0.05 mg/kg) in female rats in diestrus and proestrus.

Materials and Methods

Subjects. The study sample comprised 36 female Long-Evans (hooded) rats (250 to 315 g) from an in-house breeding colony and were first-generation offspring of Harlan Blue Spruce stock (Harlan Sprague Dawley, Indianapolis, IN). The rats were housed in polycarbonate cages (46 × 25 × 21 cm) containing aspen hardwood shavings (Northeastern Products, New York, NY). The temperature, humidity, ventilation, and lighting were maintained at 22 ± 2 °C, 50% to 60%, 14 air changes/h, and a 14:10-h light:dark cycle (lights on at 0600), respectively. Rats were fed standard rodent chow (Teklad Rodent Diet 8640, Harlan Teklad, Madison, WI) and tap water ad libitum, except as noted. The estrus cycle was monitored in each rat by daily vaginal smears, starting 2 wk prior to and continuing until the day of testing. All rats exhibited normal 4- to 5-d cycles during the period of habituation and testing, except as noted in the Results. Testing was conducted between 1100 and 1300 to control for circadian changes in opioid sensitivity.⁹ The colony was monitored semiannually for microbiologic agents by serologic examination for antibodies to bacterial and viral agents by use of sentinel rats exposed to dirty bedding. In addition, cellophane-tape tests and gross postmortem examinations were performed to examine sentinels for parasites. Sentinel rats were found to be free of CAR bacillus, Kilham rat virus, H-1 virus, *Mycoplasma*

Received: 8 Feb 2006. Revision requested: 22 May 2006. Accepted: 22 May 2006.

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pulmonis, pneumonia virus of mice, sialodacryoadenitis virus, Sendai virus, lymphocytic choriomeningitis virus, reovirus, pinworms, and fur mites throughout the period during which this study was conducted.

The experiment was approved by the guidelines established by the Institutional Animal Care and Use Committee of the University at Buffalo. The animal facilities at the University at Buffalo are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. No rats had served in any previous study, and all rats were euthanized by inhalation of CO₂ at the completion of this study.

Drugs. Buprenorphine was prepared from powdered buprenorphine hydrochloride (RBI/Sigma, Natick, MA). Buprenorphine was mixed in deionized water to make a stock solution of 5.0 mg/ml. To ensure drug dissolution, the solution was vigorously vortexed for 2 min and sonicated for 20 min (at less than 35 °C for the last 5 min). Experimental doses were obtained by serial dilution of this stock to concentrations of 0.5 mg/ml for PO administration and 0.05 mg/ml for SC administration. Drug was prepared on each testing day, mixed in glass serum bottles, maintained in a dark cabinet until injection or infusion, and injected or infused with the aid of a 1-cc plastic syringe (BD Tuberculin Syringes, VWR, Rochester, NY).

Orogastric infusion. Orogastric infusion (gavage) was achieved by intubation by use of PE160 tubing (length, 11 cm) attached to a 1-ml, plastic tuberculin syringe fitted with an 18-gauge needle. A 2.5-cm length of a plastic, 1-ml tuberculin syringe was used as a mouth speculum to prevent the rat from biting the tubing during infusion. One experimenter held the rat and inserted the speculum while another inserted the tube, handled the syringe, and infused the drug. All rats were habituated to this procedure by 4 sham exposures (intubation without infusion) during the week before the rats were tested.

Pain-threshold assay. Pain threshold was measured by use of a standard hot-water tail-flick assay⁷ before (baseline) and 30 and 60 min after drug administration. The dependent variable was the latency (in s) 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. Rats were wrapped in a breathable cloth cone, and the distal third of the rat's tail was immersed in the bath. The time required for the rat to remove its tail was measured by use of a stopwatch, and the tail-flick latency score was calculated as the mean of the last 2 of 3 trials (the 1st trial was eliminated because of the variable novelty effect). Trials were separated by 30-s intervals. Between trials, the rat's tail was dried with a tissue (one swipe beginning at the mid-tail region). To avoid tissue damage, each trial was terminated at 30 s if no withdrawal response occurred; no tissue damage was observed in this study. Tail-withdrawal latency at baseline (untreated rats) ranged from 2.5 to 4.0 s. A statistically significant increase in pain threshold from the baseline pain threshold was interpreted as induction of analgesia. 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 daily exposure for 3 d during the week preceding the experiment.

Testing procedure. To control for stomach contents during gavage, food was removed from cages 2 h before the baseline tail-flick latency test and was withheld for the remainder of the test (approximately 1 additional hour). Immediately after the baseline tail-flick test, rats were returned to their home cages, where each rat was both infused and injected, to control for the possibly different levels of stress produced by injection and

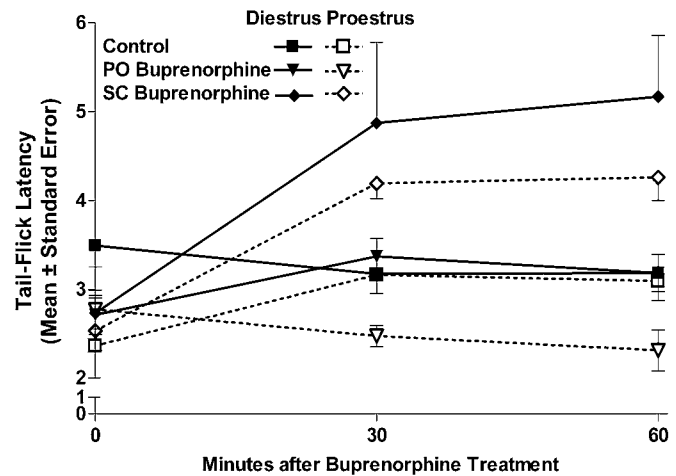


Figure 1. The effect of oral (0.5 mg/kg) or subcutaneous (0.05 mg/kg) buprenorphine on pain threshold (measured by tail-flick latency [s, mean \pm standard error of the mean]) in female rats in diestrus or proestrus. Subcutaneous buprenorphine was significantly ($P < 0.05$) more effective than either oral buprenorphine or vehicle only. In addition, only subcutaneous buprenorphine significantly ($P < 0.05$) increased the pain threshold above baseline levels.

gavage. Group 1 (experimental group) rats each received an infusion of buprenorphine (0.5 mg/kg, PO) and were injected with vehicle (water, 1 ml/kg SC); Group 2 (positive control group) rats each underwent infusion with vehicle (1 ml/kg, PO) and injection with buprenorphine (0.05 mg/kg, SC); and Group 3 (negative control group) rats each received both PO infusion and SC injection of vehicle. Procedurally, therefore, every rat received both an injection treatment (drug or vehicle) and an intubation treatment (drug or vehicle). Post-treatment pain threshold tests were performed at 30 and 60 min.

Data analysis. The study was a $3 \times 2 \times 3$ factorial design (drug [PO buprenorphine, SC buprenorphine, control] \times cycle stage [diestrus, proestrus] \times time [baseline, 30 min post-drug, 60 min post-drug]) with repeated measures on the time variable. The dependent variable at each time was mean tail-flick latency (pain threshold). We used 3-way analysis of variance with repeated measures to compare the effect of drug \times cycle stage on pain threshold. Significant interactions were probed by use of simple effects tests and Student-Neuman-Keuls posthoc pairwise comparisons. All statistical tests were conducted using SPSS v. 14 for Windows (SPSS, Inc, Chicago IL). Statistical significance was defined as $P < 0.05$.

Results

Data from 2 rats were excluded due to incomplete intubation (1 from the positive control group) or poor estrous cyclicity (1 from the negative control group). Data from the remaining 34 rats were subjected to the planned 3-way analysis of variance (drug \times cycle stage \times time) with repeated measures on the time variable. A significant drug \times time interaction ($F[4,56] = 7.09$, $P < 0.0001$) and main effects of cycle stage ($F[1, 28] = 7.07$, $P = 0.013$), drug ($F[2,28] = 11.68$, $P < 0.0001$), and time ($F[2,56] = 10.69$, $P < 0.0001$) were found (Figure 1). The 3-way interaction and the 2-way interactions involving cycle stage were not significant: drug \times cycle stage \times time, $F(4, 56) < 1$; drug \times cycle stage, $F(2, 28) = 1.98$, $P = 0.16$; cycle stage \times time, $F(2, 56) < 1$.

Statistical probes of the significant drug \times time interaction indicated that there were no group differences at baseline ($F[2, 80] < 1$). However, there were significant group differences at 30

and 60 min after the administration of buprenorphine ($F[2, 80] = 7.99, P = 0.002$; $F[2, 80] = 14.3, P < 0.0001$, respectively). At both 30 and 60 min, rats that had received SC buprenorphine had significantly ($P < 0.05$) higher pain thresholds than did controls and rats that received PO buprenorphine. Rats that received PO buprenorphine did not differ significantly from controls. Consistent with this finding, Group 2 (SC buprenorphine, PO vehicle) also was the only group to show any significant elevation in pain threshold over time (Group 1 [SC vehicle, PO buprenorphine], $F[2, 56] = 3.05, P = 0.06$; Group 2: $F[2, 56] = 11.43, P < 0.0001$; Group 3 [SC and PO vehicle], $F[2, 56] < 1$).

We also noted a main effect of cycle stage on pain threshold latencies regardless of buprenorphine treatment, in which proestrus was associated with significantly shorter tail-flick latencies (lower pain thresholds, more sensitivity) than was diestrus (proestrus: 3.02 ± 0.12 s; diestrus: 3.55 ± 0.18 s; collapsed across time and drug variables, $F[1, 28] = 7.07, P = 0.013$).

Discussion

The results of the current study show that PO buprenorphine at the commonly recommended dose of 0.5 mg/kg^{4,5,12} is significantly less effective (in fact, it seems to be ineffective) as an analgesic than is the standard SC dose (0.05 mg/kg) in female rats. Therefore, if the analgesia produced by the SC dose is considered to provide adequate analgesia for relief of postoperative pain, then the level produced by this PO dose is insufficient. Because this finding is similar to what we observed earlier in male Long-Evans and Sprague-Dawley rats,^{10,15} we can now conclude that sex differences do not have a significant impact on the effects of buprenorphine in the treatment of postoperative pain. Therefore, the sex of the subjects does not account for the difference between results obtained using algometric tests and those obtained with alternative behavioral measures, which have largely been conducted in female rats. In our previous report,¹⁰ we showed that a 10-fold higher dose of PO buprenorphine is necessary to induce a level of analgesia comparable to that produced by the standard therapeutic SC dose (0.05 mg/kg). A similar increase may be necessary for female rats as well but remains to be determined. The problems with using a 10-fold higher PO dose are that either (a) the concentration of buprenorphine is too high to be palatable, or if diluted, the volume is too large to be practical, and (b) the higher PO dose induces greater gastrointestinal distress than does the 0.05 mg/kg SC dose.¹⁵

Overall, the findings from the current study and previous research^{10,15} do not support conclusions drawn from data obtained using indirect behavioral measurements, which showed that buprenorphine at approximately 0.5 mg/kg PO is an effective treatment for pain. Importantly, our work suggests that 0.5 mg/kg buprenorphine (voluntarily ingested) does not produce detectable analgesia in algometric tests. There may be tests or circumstances in which 0.5 mg/kg PO buprenorphine causes a detectable level of analgesia—perhaps in those circumstances during which there is significant elevation of endogenous opioids—but we haven't found one, and we haven't searched systematically. What we are sure of is that no test or circumstance will emerge in which 0.5 mg/kg PO buprenorphine produces a level of analgesia comparable to that of a dose of 0.05 mg/kg SC, the commonly accepted 'gold standard.' This comparison is necessarily at the heart of our experiments, to provide an objective reference point. Perhaps the standard SC dose of 0.05 mg/kg is too high regardless of assay or technical

details, but that remains to be demonstrated empirically. The standardization of effective doses is still forthcoming because results from algometric assays may not apply directly to animals in pain (that is, do not directly correspond to drug doses required in the presence of activity of the endogenous antinociception systems). Further, analysis of pain behavior in animals is subjective and unreliable. For example, increases in body weight and eating may be masked by pica¹⁵ or may be the direct result of the effect of opioids on appetite;¹³ and different species may respond differently (that is, developed different behavioral coping strategies) when in pain.

Finally, we noted a significant effect of estrous cycle on pain threshold: rats in proestrus had significantly lower pain thresholds than did rats in diestrus both before and after buprenorphine. This result is consistent with many previous studies^{6,8,17} but not all.^{11,14,16,18} Numerous laboratories have reported variations in pain threshold over the course of the estrous (or menstrual) cycle, but there is less consensus on the precise timing of this variation.¹⁶ Although pain sensitivity was greater during proestrus, the characteristic important to the hypothesis tested in the current study is that the stage of the estrous cycle had no effect on the magnitude of the analgesic effect of buprenorphine: the buprenorphine-induced percentage increase in pain threshold was the same regardless of estrous cycle stage. This finding is consistent with previous studies on the interaction of opioids, including buprenorphine, and stage of the estrous cycle.^{14,16,17}

Acknowledgment

This research was supported by funding from the Dean's Office, College of Arts and Sciences (University at Buffalo, NY), to MBK. We thank Kim Doldan for her assistance in the collection of data.

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