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Buprenorphine Increases Intake of Freely Available and Operant-Contingent Food in Satiated Rats

J. M. RUDSKI, D. THOMAS, C. J. BILLINGTON AND A. S. LEVINE[1]

Research and Medicine Service, Veterans Administration Medical Center, Minneapolis, MN 55417; and Departments of Psychiatry, Food Science and Nutrition, Medicine, and Surgery, University of Minnesota, Minneapolis and St. Paul, MN 55455

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RUDSKI, J. M., D. THOMAS, C. J. BILLINGTON AND A. S. LEVINE. *Buprenorphine increases intake of freely available and operant-contingent food in satiated rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 271-276, 1995.—Opiate administration increases short-term free feeding in satiated rats. The feeding effects of the mixed opioid receptor agonist/antagonist buprenorphine were examined in both free-feeding and operant chamber paradigms. Buprenorphine (0.1 and 0.3 mg/kg) produced significant increases in short-term free feeding (i.e., 4 h), an effect enhanced by repeated administration. Buprenorphine's effects on operant responding were examined in satiated rats using a fixed ratio (FR) 80 (initial pellet) FR 3 (subsequent pellets) reinforcement schedule. Buprenorphine (0.03–0.3 mg/kg) decreased latency to begin responding for food, and increased total number of pellets consumed in a 1-h session. Increases in food intake relative to control were caused by continued responding for food as sessions progressed. Naloxone suppressed both the free-feeding and operant-contingent intake induced by buprenorphine. Thus, buprenorphine increases both freely available and lever-press contingent food intake.

Buprenorphine Opiates Feeding Operant Reinforcement Opioid

ADMINISTRATION of opioid receptor agonists increases, and of opioid antagonists decreases, short-term food intake in nondeprived rats (3,13). Examination of the feeding-inducing effects of various opiates suggests that activity at more than one opioid receptor subtype may engender the greatest orexigenic effects. For example, in a comparison of κ-agents, there is an inverse relationship between receptor specificity and the amount of food consumed (6,18–20). Further evidence for this assertion comes from the observation that the opiate that appears to produce the strongest effect on short-term feeding is butorphanol (12,15), an agent that has been characterized as a low efficacy ligand at both μ- and κ-sites, and that produces both agonist and antagonist properties depending on the behavioral assay involved (4,5,7,10,25–27).

Buprenorphine, an opiate currently enjoying popularity as a substitute for methadone in the treatment of opiate addiction (17), posesses a behavioral profile similar to butorphanol. Like butorphanol, buprenorphine often produces behaviors typical of low efficacy μ-agonists (25). Both serve as positive reinforcers when substituted for codeine, and share discriminative properties with μ- but not κ-agonists (24,34). Similarly, the analgesic effect of both agents is markedly decreased after pretreatment with the specific μ-antagonist, β-funaltrexamine, which suggests a μ-component to their antinociceptive effects (35). Morphine pretreatment in rats produces cross tolerance to response-rate disruptive effects to both butorphanol and buprenorphine (22,23,25). Furthermore, both butorphanol and buprenorphine are reported to have κ-antagonist effects (4,11,21,28).

In light of the many behavioral pharmacologic similarities between butorphanol and buprenorphine, we decided to examine whether an agent sharing many of butorphanol's behavioral effects produced similarly potent orexigenic effects.

METHOD

Subjects

Thirty-three experimentally naive, male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) weighing 250–275 g at the start of the experiment were used to assess bupren-

[1] Address reprint requests to A. S. Levine, Research Service (151), VA Medical Center, Minneapolis, MN 55417.

orphine's effects on free feeding. Six rats male rats weighing 350–375 g at the start of the experiment were used to assess buprenorphine's effects on operant responding. Rats were housed in individual hanging cages in a vivarium with a 12-h light-dark cycle (lights on at 0700 h) maintained at 23°C. Rats had unlimited access to food and water in the free-feeding study. In the operant study, rats had their food removed after injections and replaced after sessions. The rats in the operant study had previously been used to assess the effects of neuropeptide Y (NPY) on responding under a reinforcement schedule similar to that used in this study. As a result, rats had previously been implanted with guide cannulas in the right lateral ventricle and received six ICV injections of NPY. Operant rats were rested for 10 days before being used in the current study.

Drug Preparation and Administration

Buprenex (Reckitt and Colman Products, Hull, UK), a premixed solution of 0.324 mg buprenorphine hydrochloride, 50 mg anhydrous dextrose, water for injection, and HCl was dissolved in isotonic saline to give doses of 0, 0.03, 0.1, and 0.3 mg/ml, and administered in a constant volume of 1 ml/kg. Drugs were administered SC at 0900 h, a time when feeding in rats is not usually observed. Naloxone (0, 0.3, 1.0, 3.0 mg/ml) was dissolved in saline and administered in a constant volume of 1 ml/kg, and injected immediately following buprenorphine in the free-feeding study and 15 min before experimental sessions in the operant chamber studies. Animals used in operant chambers were injected SC at 0800 h, with sessions begining 75 min later. Rats were injected with the highest dose (0.3 mg/kg) of buprenophine after the last two operant training sessions so that tolerance would develop to its sedative effects.

Apparatus

Food-reinforced behavior was assessed in six standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA). Chambers were enclosed in an isolation cubicle (model E10-20; Coulbourn Instruments to attenuate outside noise, and equipped with an exhaust fan to supply ventilation. Sessions were controlled and data were recorded by a Zeos 486 computer (St Paul, MN) located in the same room as the chambers.

Procedure

Buprenorphine and free feeding. In the free-feeding study, preweighed food pellets (Purina Certified Rodent Chow, RFG Pet & Supply Co., Plymouth, MN) were placed at the base of the home cage immediately after injections. Pellets were weighed and replaced 1, 2, and 4 h after injection. Food intake was quantified by recording the differences between the initial and final weights of the pellets. Spillage was collected and included in the calculations. Experimental sessions occurred over 3 consecutive days. The orexigenic effect for each drug was assessed at each time point (1, 2, and 4 hours) using a one-factor-between (dose), one-factor-within (day) repeated measures ANOVA. Posthoc analyses were carried out by Dunnet's multiple comparison test. After a 5-day rest, the effect of naloxone on 2-h cumulative food intake induced by 0.3 mg/kg buprenorphine (the dose that produced the greatest short-term effect) was assessed. Intake was not measured at 4 h because naloxone was no longer behaviorally active at that time. Naloxone's effects were analyzed by ANOVA, and posthoc analyses were carried out by Dunnet's multiple comparison test.

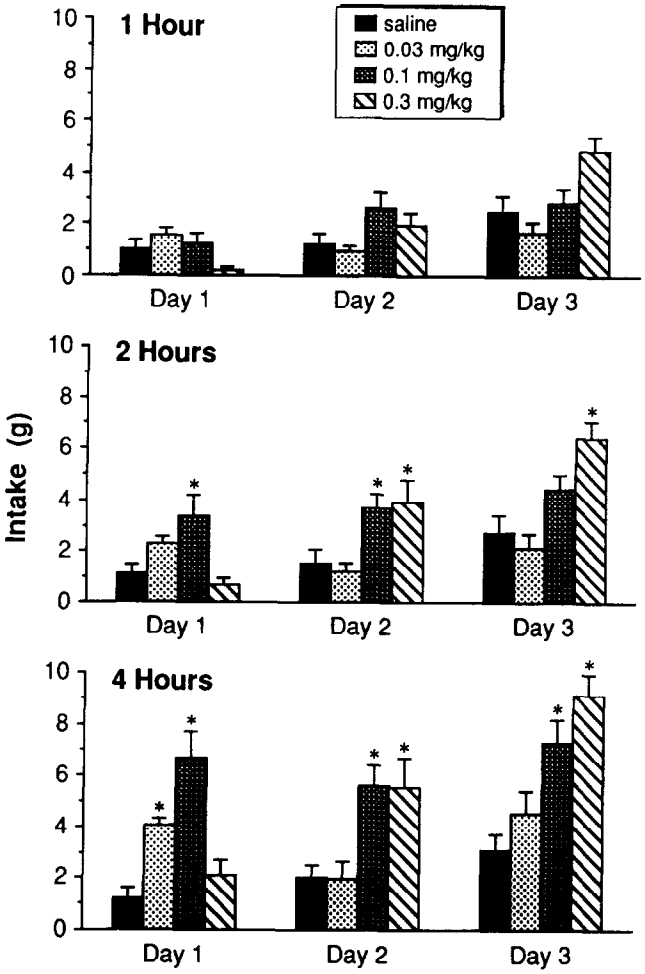

FIG. 1. Effect of buprenorphine (0.03– – 0.3 mg/kg) on cumulative food intake (mean ± SEM) at 1, 2, and 4 h after injection over 3 consecutive days. *$p < 0.05$.

Buprenorphine and operant responding. Rats had previously been trained to press the left lever under an FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule. We believe this schedule to be analogous to a runway maze, requiring substantially more work initially to obtain food than for subsequent consumption. Thus, this reinforcement schedule allows for examination of both the initiation (FR 80 component) and maintenance (FR 3 component) of feeding. Main effects for initial response latency, subsequent FR 80 completion time, and total number of pellets consumed were analyzed using RMANOVA, and posthoc analyses were determined with Dunnet's multiple comparison test.

To examine the pattern of responding over the session, the pellets consumed after the first reinforcer delivery were accumulated into bins every 10 min. Food intake was analyzed using two-factor RMANOVA (bin × dose), and posthoc analyses were determined with Dunnet's multiple comparison test.

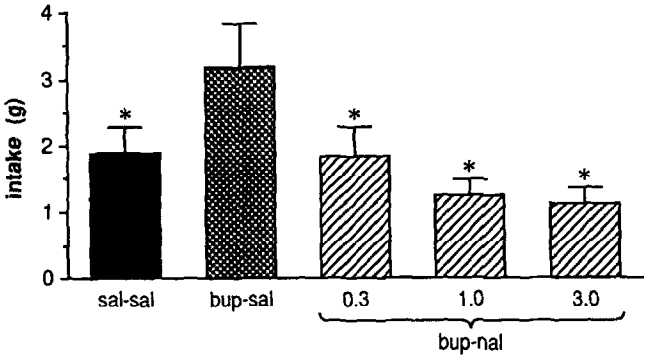

FIG. 2. Effect of naloxone (0.3–3.0 mg/kg) on feeding (mean ± SEM) induced by 0.1 mg/kg buprenorphine over 2 h. bup = buprenorphine; nal = naloxone. *$p < 0.05$ lower than 0.1 mg/kg buprenorphine to 0 mgkg naloxone.

Naloxone's (0.3, 1.0, and 3.0 mg/kg) effects on 0.1 mg/kg buprenorphine-induced operant responding (the dose producing the greatest orexigenic effect) was examined in five rats after the completion of the initial buprenorphine dose-response (the sixth rat was excluded because of illness). Mean initial response latency, subsequent FR 80 completion time, and total number of pellets consumed were analyzed using RMANOVA, and posthoc analyses were determined with Dunnet's multiple comparison test.

RESULTS

Figure 1 shows buprenorphine's effects on free-feeding. Buprenorphine administration did not produce a main effect for first hour of intake [$F(3, 29) = 2.01, p > 0.05$], although a significant day [$F(2, 58) = 37.48, p < 0.05$] and day-by-dose interaction [$F(6, 58) = 9.96, p < 0.05$] were observed. Increases in cumulative intake were evident 2 h after buprenorphine administration [$F(3, 29) = 3.29, p < 0.05$]. Repeated administration produced greater intake [$F(2, 58) = 25.77, p < 0.05$], and a significant day-by-dose interaction was also obtained [$F(6, 58) = 10.32, p < 0.05$]. Cumulative intake following buprenorphine was also elevated 4 h after injections [$F(3, 29) = 9.29, p < 0.05$], an effect that was greater after repeated injections [$F(2, 58) = 30.60, p <$

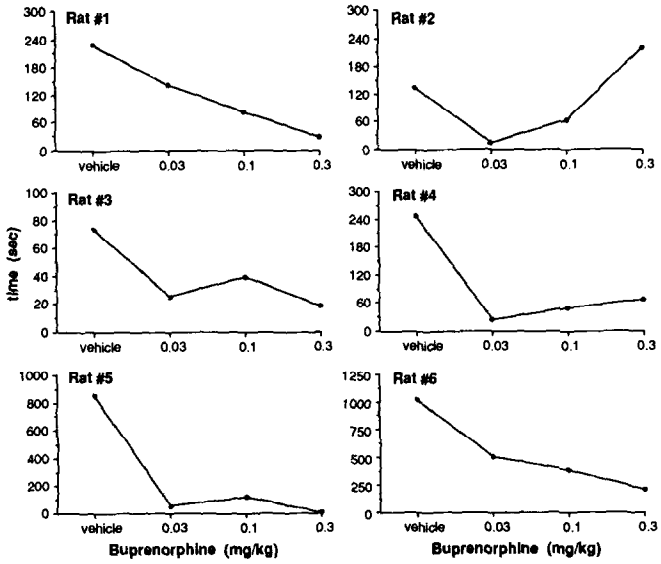

FIG. 4. Effect of buprenorphine (0.03–0.3 mg/kg) on latency (mean ± SEM) to begin responding (sec) under an FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement scheudle for individual rats.

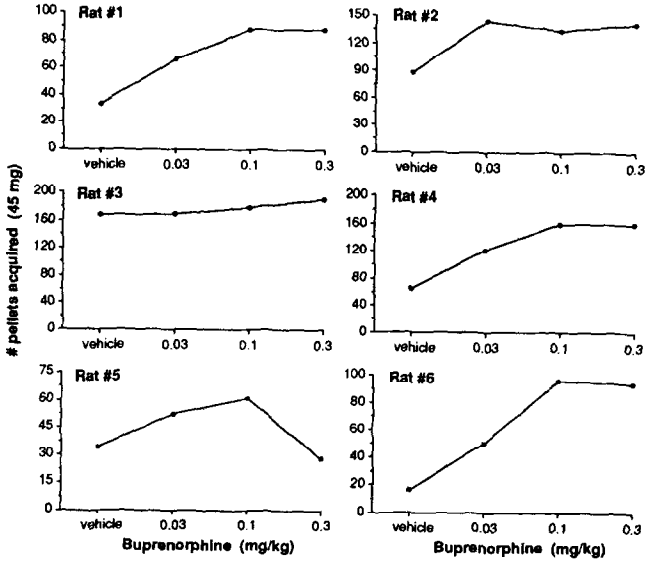

FIG. 3. Effect of buprenorphine (0.03–0.3 mg/kg) on number (mean ± SEM) of pellets (45 mg) consumed under an FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule over 60-min sessions for individual rats.

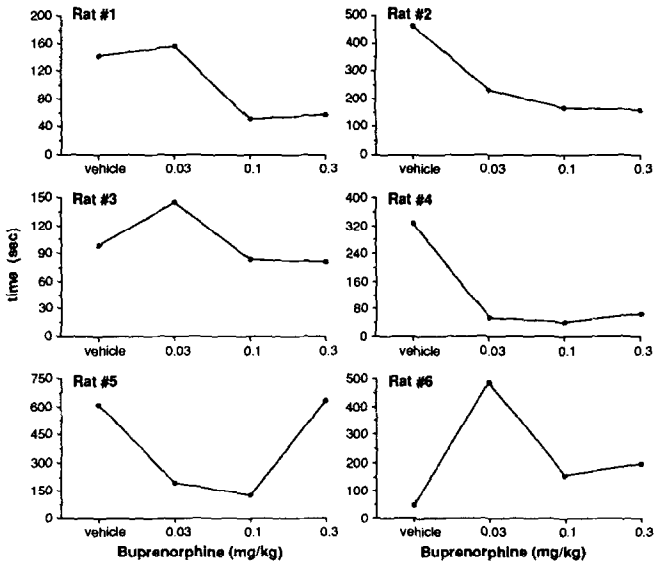

FIG. 5. Effect of buprenorphine (0.03–0.3 mg/kg) on time (mean ± SEM) required to complete the initial response requirement (i.e., FR 80) for individual rats.

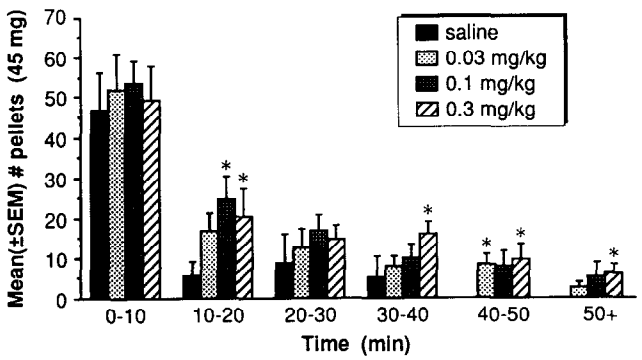

FIG. 6. Effect of buprenorphine (0.03–0.3 mg/kg) on pattern of responding over the 1-h session (mean ± SEM). *$p < 0.05$.

0.05]. Once again, a significant dose-by-day interaction was observed [$F(6, 58) = 11.88, p < 0.05$].

Naloxone adminstration (0.3–3.0 mg/kg) suppressed the orexigenic effect of 0.3 mg/kg buprenorphine [$F(4, 37) = 3.77, p < 0.05$] (Fig. 2). When food availability was contingent upon operant lever pressing, buprenorphine also increased intake (Fig. 3). Buprenorphine (0.03–0.3 mg/kg) produced significant increases in feeding at all doses examined [$F(3, 15) = 10.09, p < 0.05$]. Buprenorphine administration also decreased the latency to begin responding for food [$F(3, 15) = 4.65, p < 0.05$], although it did not reduce the time required to complete the initial FR 80 ratio once responding had commenced [$F(3, 15) = 1.357, p > 0.05$] (Figs. 4 and 5).

Figure 6 shows the distribution of feeding during the session. Buprenorphine altered the temporal distribution of feeding [$F(3, 15) = 11.11, p < 0.05$] by producing continued responding as sessions continued.

Naloxone administration decreased the total number of pellets consumed after 0.1 mg/kg buprenorphine [$F(3, 12) = 5.22, p < 0.05$], but had no effect on initial response latency [$F(3, 12) = 0.65, p > 0.05$] or FR 80 completion time [$F(3, 12) = 1.59, p > 0.05$] (Table 1).

DISCUSSION

Buprenorphine administration produced robust increases in short-term feeding in both the home-cage and operant-chamber paradigms. In the home-cage study, repeated administration of 0.1 and 0.3 mg/kg reliably produced increased feeding, and in the operant chamber all doses (0.03–0.3 mg/kg) increased intake. The magnitude of the home-cage feeding effect produced by buprenorphine (6–8 g/4 h) was similar to that produced by butorphanol, and far greater than was observed after the administration of other opiates previously examined. Buprenorphine-induced increases in feeding were suppressed by the opioid receptor antagonist naloxone in both settings.

Previous studies examining buprenorphine's effects on food-maintained responding in rats reported response rate (and hence intake) decreases at doses similar to those used in the current study (1,2,16,22),. However, many procedural differences existed between the current and previous studies. Operant procedures typically use food-deprived animals with relatively short (15–30 min) preinjection times. Furthermore, injections are typically spaced by several days. The current study used a 75-min preinjection time and food-satiated animals, with buprenorphine administered on consecutive days. All of these dimensions (time, deprivation state, dosing schedule) have been shown to influence opiate effects on free feeding. For example, in the current study, buprenorphine did not reliably increase free feeding in the first hour, indicating the importance of preinjection time. Level of food deprivation has been shown to influence morphine's effects on free feeding (32), with increases reported in satiated and decreases in deprived rats following identical doses. Finally, the effect of increased food intake after repeated opiate administration is blocked by spacing subsequent methadone injections by 4 days (31). Any or all of these procedural differences may account for the disparate results between the current and previous studies.

Before the current study, results from our laboratory suggested that butorphanol was the only agent that stimulated lever-press contingent food intake under the current deprivation and time-course procedures (29). Doses of methadone (30), morphine, and U50,488 (unpublished observation), which produce increases in free feeding, all failed to stimulate increases in lever-press contingent food intake. This further underscores the robustness of buprenorphine's effect.

The reinforcement schedule used in the present study allowed for the examination of buprenorphine's effects on both the initiation and maintenance of food intake. Results suggest that buprenorphine affects both initiation and maintenance. Buprenorphine decreased the latency to begin responding, although it did not affect time required to complete the FR 80 once responding had commenced. Buprenorphine also altered the temporal pattern of food intake over the 60-min session. Increases induced by buprenorphine as a result of feeding continuing to occur throughout the session. These findings are consistent with some and contrary to other theories regarding opioid system involvement in feeding. It has been suggested that the opioid system is involved in the maintenance but not the initiation of feeding. For example, running speed or the percentage of correct choice (initiation) in a T maze is not affected by naloxone, whereas consumption of food in the

TABLE 1

NALOXONE'S (0.3, 1.0, and 3.0 mg/kg) EFFECTS ON BUPRENORPHINE (0.1 mg/kg)-INDUCED RESPONDING

	Naloxone Dose (mg/kg)			
	0	0.3	1	3
No. of pellets	106.6 ± 15.1	67.4 ± 16.0*	82.8 ± 19.5†	47.4 ± 21.4*
Initial response latency (s)	76.7 ± 32.1	137.4 ± 75.1	55.8 ± 11.9	79.0 ± 18.3
FR 80 completion time (s)	213.9 ± 79.6	709.8 ± 494.8	93.2 ± 17.7	1525.7 ± 847.4

Values represent mean ± SEM. *$p < 0.05$ two-tailed. †$p < 0.05$ one-tailed.

goal box (maintenance) is (8,9). The current study suggests that buprenorphine affects the initiation as well as the maintenance of feeding.

Although the current study indicated that buprenorphine stimulates free feeding with a magnitude similar to that of butorphanol in home cages (i.e., 5–7 g above control over 4 h), buprenorphine's operant effects were not identical to butorphanol's. Whereas buprenorphine did not significantly affect the rate of lever pressing in the initial ratio, it did significantly decrease latency to begin responding for food. Butorphanol does not produce significant decreases for either of those measures (29). Furthermore, whereas butorphanol suppressed feeding in the first 10 min after the completion of the initial ratio, no such suppression was observed following buprenorphine.

The current data are in accordance with the hypothesis that opiates with a "mixed" receptor profile engender the greatest feeding effects. Further evidence for this assertion is provided by the observation that the nonselective opioid antagonist LY255582 suppresses feeding to a greater extent than receptor the specific antagonists β-funaltrexamine or norbinalotorphimine (14). It may be that the current classification for the analgesic and discriminative stimulus effects of opioid receptor agonists is not ideal in describing their effects on feeding.

In conclusion, the present findings indicate that buprenorphine, a mixed opioid agonist-antagonist with a behavioral profile similar to that of butorphanol, produced an extremely robust effect on feeding observable in both home-cage and operant-chamber paradigms.

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