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Butorphanol Increases Food-Reinforced Operant Responding in Satiated Rats

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RUDSKI, J. M., C. J. BILLINGTON AND A. S. LEVINE. *Butorphanol increases food-reinforced operant responding in satiated rats.* PHARMACOL BIOCHEM BEHAV **49**(4) 843-847, 1994.—In the present series of studies we examined the effect of butorphanol tartrate on food-reinforced operant responding in satiated rats. In the first experiment, 8.0 mg/kg butorphanol was administered subcutaneously, once per day for 4 days, to satiated rats responding under an fixed ratio 10 (FR 10) reinforcement schedule. In the second experiment, butorphanol (0, 0.3, 1.0, 3.0, 10.0 mg/kg) was administered to satiated rats responding under an FR 80 (first pellet) FR 3 (subsequent pellets) reinforcement schedule for 4 consecutive days. Repeated butorphanol administration increased total amount of food consumed over sessions in both experiments. Under the FR 80 schedule component, butorphanol initially increased latency to acquire the first pellet, an effect attenuated by repeated administration. Whereas vehicle administration was associated with consumption of relatively large quantities of food within the first 10 min of receiving the first pellet, butorphanol was associated with continued feeding as the session progressed. These data suggest that butorphanol-induced food intake is associated with maintenance rather than initiation of feeding.

Butorphanol Opiates Feeding Operant Reinforcement

ADMINISTRATION of mu-, kappa-, and delta-opioid receptor agonists produces reliable increases in free feeding in satiated rats [for review see (3)]. Whereas specific receptor agonists all produce reliable increases in short-term free feeding (6), the synthetic morphinan butorphanol, a mixed mu and kappa receptor agonist, stimulates dramatically more ingestion (14,20).

Butorphanol can be classified as a partial, or low-efficacy, agonist at both mu and kappa receptor subtypes. Mu agonist characterization is based upon findings that butorphanol substitutes for mu agonists in drug discrimination procedures in a variety of species (23,24,29), is self-administered when substituted for codeine or morphine (30,35), and that pretreatment with the mu antagonist beta-funaltrexamine attenuates butorphanol-induced analgesia (36). However, butorphanol precipitates withdrawal in morphine-dependent animals (5,9,25,34) and antagonizes methadone's antinociceptive effects in a shock titration procedure with monkeys (4), suggesting low efficacy. Butorphanol increases urinary output in rats (8,13), which is a kappa agonist effect, yet it decreases urinary output and antinocoception produced by high-efficacy kappa agonists (4,13,25), again suggesting low efficacy. Butorphanol's effects on food intake appear to be opioid in nature, as they are antagonized by 1 nmol norbinaltorphimine (kappa antagonist) or 50 nmol beta-funaltrexamine (mu antagonist) (19).

In contrast to increases observed in free feeding following administration of mu or kappa agonists, decreases have been reported following administration of these agonists in food-maintained operant responding under a wide variety of reinforcement schedules (2,23,31-33,35). Studies that have examined butorphanol's effects on food reinforcement have reported similar dose-related decreases (7,22,24).

In operant studies that reported decreased response rates following butorphanol, the animals were maintained at 80-85% of their free-feeding weights. Studies reporting butorphanol-induced free feeding typically use nondeprived animals. In the current studies, we evaluated whether butorphanol would increase food-reinforced lever pressing when rats were not food deprived.

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METHOD

Subjects

Experimentally naive, male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) were housed individually in wire hanging cages in a colony room maintained on a 12 L : 12 D cycle (lights on 0700 h). Each rat had unlimited access to food (Teklad Laboratory Chow) and water.

Apparatus

Experimental sessions were conducted in six commercially available small-animal operant chambers (Model E10-10TC, Coulbourn Instruments, Inc.). Each chamber was enclosed in an isolation cubicle (Model E10-20, Coulbourn Instruments, Inc.) to attenuate outside noise, and were equipped with an exhaust fan to supply ventilation. Chambers were equipped with two operant levers on opposite sides of the front panel of the chamber. The house light, located in a top-central position, was illuminated throughout the experimental sessions. Dustless precision pellets (45 mg, Bioserv Holton Industries, Frenchtown, NJ) could be delivered to a pellet trough between the levers. When a pellet was delivered, a 4-W light above the pellet trough was illuminated for 3 s. A Zenith computer (Zenith Computer systems) immediately adjacent to the chambers controlled experimental conditions and recorded data.

Procedure

Butorphanol's effects on FR 10 responding. In Experiment 1, 12 rats (starting weights 270–300 g, ending weights 282–318 g) were trained to press the left lever under an FR 10 reinforcement schedule. Rats were separated into two groups matched on the number of pellets consumed over 1-h sessions on the final 3 days of training. The first group received SC 8.0 mg/kg butorphanol tartrate [a dose that produces robust feeding in free-feeding studies (14)] dissolved in 3.3 M citric acid, 6.4 M citric citrate, and 6.4 M sodium chloride, and the second received vehicle 1 h before sessions for 4 consecutive days. Doses are expressed as the salt. A 1-h pretreatment time was chosen because studies examining butorphanol's effects on free feeding indicate maximal effects in the second hour following injections [e.g., (19)]. The number of pellets obtained over the session was recorded, and uneaten pellets were subtracted to provide a measure of amount consumed. On the fifth day, the two groups were crossed over, with the butorphanol group receiving vehicle and the vehicle group receiving 8.0 mg/kg butorphanol for the next 4 days. Sessions lasted 1 h. Treatment and day effects both before and after the crossover were assessed using a one-factor between (treatment)- and one-factor within (day)-subject design. Post hoc analyses were determined with Fishers Least Squared Significant Differences.

Butorphanol's effects on the initiation and maintenance of food intake. In Experiment 2, 24 rats (starting weights 265–300g, ending weights 310–355g) were trained to press the left lever under an FR 80 reinforcement schedule for the first pellet and FR 3 for each subsequent pellet. This reinforcement schedule allows for examination of butorphanol's effects on both the initiation (FR 80 component) and maintenance (FR 3) of food intake. Sessions lasted 1 h. Butorphanol (0, 0.3, 1.0, 3.0 10.0 mg/kg) was delivered SC 1 h before sessions. Order of doses was randomized. Each dose was given for 4 consecutive days, followed by 3 days in which rats were run but no drugs were administered. Time to acquire the first pellet (consisting of the initial response latency and the subsequent FR 80 completion time) and total number of pellets consumed (see above) over the session were recorded. Rats not responding during the session were assigned an initial response latency of 3600 s and were not included in the analysis of subsequent FR 80 completion time. A two-factor RMANOVA analyzed the effect of dose and day effects for total time to acquire the first pellet, initial response latency, subsequent FR 80 completion time, and total number of pellets consumed. Post hoc analyses were determined with Fishers Least Squared Significant Differences.

To examine responding over the session, number of pellets consumed after the first reinforcer delivery was accumulated into 10-min bins. If latency to acquire the first pellet precluded access to all bins, they were left empty and not included in the data analysis (e.g., with session length being 60 min, a rat that required 30 min to complete the FR 80 only had data entered in the first three 10-min bins, and the remaining bins remained empty for that subject). Data were analyzed using a two-factor RMANOVA (dose × day) for each bin. Post hoc analyses were determined with Fishers Least Squared Significant Differences.

Following completion of butorphanol's dose–response curve, naloxone (0.3, 1.0, 3.0 mg/kg presented in a random order) or vehicle was administered to the rats receiving the butorphanol doses that produced significant increases in food intake (i.e., rats receiving 1.0, 3.0, and 10.0 mg/kg butorphanol continued to receive injections) 0.5 h before sessions. Intake data were collected as described above, analyzed by RMANOVA, and means were compared by Fishers Least Squared Significant Differences.

In both experiments, food was removed from the home cages immediately following drug administration and was replaced immediately after sessions. Sessions occurred between 0900 and 1300 h (2–6 h following lights-on).

RESULTS

Butorphanol's Effects on Food Intake

Repeated administration of 8.0 mg/kg butorphanol increased intake of food presented under an FR 10 reinforcement schedule both before, $F(1, 10) = 5.8$, $p < 0.05$, and after $F(1, 10) = 13.68$, $p < 0.05$, the crossover (Fig. 1). The effect of repeated dosing was statistically significant both before and after the crossover [$F(3, 30) = 11.79$, $p < 0.05$ and

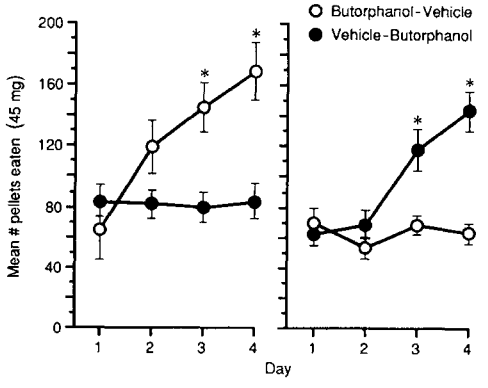

FIG. 1. Effect of butorphanol's (8 mg/kg) on food intake (mean ± SEM) produced by responding under an FR 10 reinforcement schedule (crossover indicated by the vertical line). *$p < 0.05$ Fishers LSSD.

$F(3, 30) = 12.89, p < 0.05$, respectively]. A significant interaction of treatment and day also was observed at both times $F(3, 30) = 12.46$ and $F(3, 30) = 11.17$, respectively, $p < 0.05$].

In Experiment 2, rats were trained to press the left lever under an FR 80 reinforcement schedule for the first pellet followed by an FR 3 for each subsequent pellet. Under these conditions, butorphanol produced a dose-dependent increase in food intake, $F(4, 104) = 2.49, p < 0.05$), that increased with repeated administration, $F(3, 78) = 33.08, p < 0.001$. Furthermore, there was a significant dose by day interaction, $F(12, 312) = 5.36, p < 0.0001$ (Fig. 2). Significant dose effects were observed on day 1, $F(4, 92) = 2.52, p < 0.05$, day 3, $F(4, 92) = 2.65, p < 0.05$, and day 4, $F(4, 92) = 6.26, p < 0.05$.

Butorphanol altered the temporal distribution of responding over the sessions (Fig. 3). Butorphanol decreased absolute intake in the 0-10-min bin, $F(4, 52) = 8.30, p < 0.05$, and increased intake in the 20-30-min, $F(4, 44) = 15.61, p < 0.05$, 30-40-min, $F(4, 32) = 10.56, p < 0.05$, 40-50-min, $F(4, 24) = 13.76, p < 0.05$, and 50-60-min, $F(4, 24) = 14.79, p < 0.05$, bins. It is interesting to note that certain treatments that did not increase responding (e.g., 0.3 mg/kg each day and 10 mg/kg on the first 2 days) produced similar changes in temporal pattern as those produced by doses that did increase intake.

Butorphanol's Effects on Latency to Begin Responding and Completion of the FR 80

Butorphanol increased the overall time to obtain the first pellet, $F(4, 92) = 4.71$, an effect attenuated by repeated butorphanol administration, $F(3, 69) = 16.54, p < 0.05$. Furthermore, a significant treatment × day interaction was observed, $F(12, 276) = 2.74, p < 0.05$ (Fig. 4).

Time to acquire the first pellet can be divided into two separate measures: latency of the first response (i.e., initiation) and time required to complete the remaining 79 responses in the initial ratio. Initiation of responding is less sensitive to butorphanol's effects than is the FR completion time. A significant main effect for treatment was observed for FR completion time, $F(4, 76) = 3.28, p < 0.05$, but not for initial response latency, $F(4, 92) = 2.20, p > 0.05$. Initial response latency and FR completion time both decreased with repeated administration [$F(3, 69) = 3.44, p < 0.05$ and $F(3, 57) = 10.24, p < 0.05$, respectively]. Finally, a significant day × dose interaction was also observed for initial response latency, $F(12, 276) = 2.08, p < 0.05$.

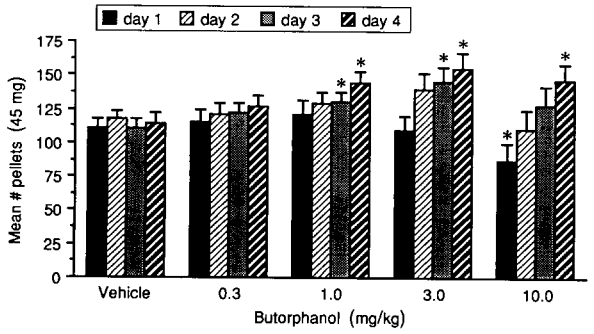

FIG. 2. Effect of repeated administration of butorphanol (1 and 3 mg/kg) on food intake (mean ± SEM). *$p < 0.05$ Fisher LSSD.

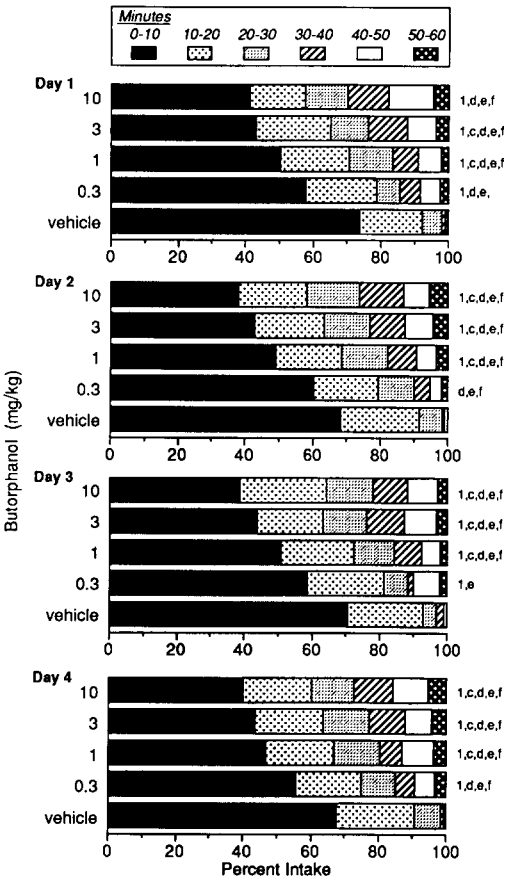

FIG. 3. Profile of food intake accumulated into 10-min bins over the session. 1, $p < 0.05$ decrease relative to saline (Fishers LSSD). b, c, d, e, f, $p < 0.05$ increase relative to saline for the 10-20-, 20-30-, 30-40-, 40-50-, and 50-60-min bins, respectively.

The Effect of Naloxone on Feeding Induced by Butorphanol

Naloxone decreased intake (assessed by a RMANOVA) induced by 1.0 mg/kg, $F(3, 9) = 11.95, p < 0.01$, 3.0 mg/kg, $F(3, 12) = 11.95, p < 0.01$, and 10.0 mg/kg, $F(3, 12) =$

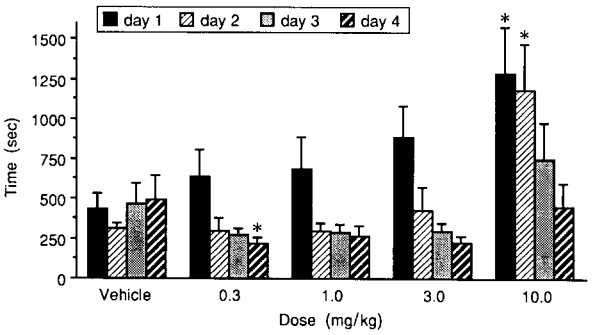

FIG. 4. Butorphanol's effect on time to acquire the first pellet (mean ± SEM). Butorphanol increased overall latency following the first three injections [$F(4, 23) = 2.85, F = 7.48, F = 2.81, p < 0.05$ on days 1, 2, and 3, respectively], but not the fourth. *$p < 0.05$ Fishers LSSD.

11.95, $p < 0.01$. All doses of naloxone (3, 1, 0.3 mg/kg) decreased pellet consumption following 1 mg/kg butorphanol (41%, 46%, 56% of vehicle-injected control intake, $p < 0.05$). Higher doses of naloxone were needed to antagonize higher doses of butorphanol. When 3 mg/kg of butorphanol was injected, only the 1- and 3-mg/kg doses of naloxone significantly ($p < 0.05$) decreased pellet consumption (3 mg/kg, 39%; 1 mg/kg, 69%; and 0.3 mg/kg, 80% of vehicle-injected control). After 10 mg/kg of butorphanol was administered, only 3 mg/kg of naloxone significantly ($p < 0.05$) decreased pellet consumption (3 mg/kg, 55%; 1 mg/kg, 77.3%; and 0.3 mg/kg, 82.4% of vehicle-injected control).

DISCUSSION

The present studies attempted to reconcile seemingly paradoxical reports of increased short-term free feeding and decreased food-reinforced operant responding following butorphanol administration. Previous research examining butorphanol effects on food-maintained operant responding reported dose-dependent decreases [e.g., (7,22,24)]. Unlike previous studies, we found that butorphanol administration increased food intake contingent upon lever pressing. There are several possible explanations for this discrepancy. First, we did not utilize food-deprived rats, as is commonly done in most operant studies. Several reports have indicated that deprivation state can alter the way opiates affect behavior (1,28). For example, morphine increases food intake in satiated rats but decreases it in food-deprived animals (28). Second, we injected butorphanol repeatedly. Opiate-induced increases in short-term free feeding typically require repeated administration. It has been suggested that repeated opiate administration allows for development of tolerance to sedative effects that may interfere with feeding behavior (16,27). In the current study, repeated administration decreased both initial response latency and FR 80 completion time, findings consistent with the hypothesis of tolerance development. Decreased intake in the first 10-min bin, an effect greatest following the highest butorphanol doses, may have also been due to sedative effects associated with butorphanol in rats (25). However, it should be noted that tolerance to sedation might not account for all the effects of repeated administration. Neuropeptide Y, a potent orexigenic agent that does not produce marked sedation, potently stimulates free feeding (15,21) and operant responding (10), producing greater effects in the operant environment with repeated administration.

Contrary to butorphanol effects in the current study, previous attempts in this laboratory to increase satiated rats lever pressing-dependent food intake with the primarily mu agonists methadone (27), morphine (unpublished observation), or the selective kappa agonist U50,488H (unpublished observation) were not successful. Whereas these agents are receptor specific, butorphanol's receptor profile is unclear and is probably not specific. Depending upon the assay, butorphanol has been classified as a mixed agonist/antagonist, exerting its effects on several different opioid receptor subtypes (24). It seems that "mixed" receptor profile produces the greatest orexigenic effects. Investigations examining specific opioid receptor agonists have identified increased feeding following administration of mu, kappa, and delta agonists (6), yet amount of food eaten following their administration is much less than that induced by butorphanol (14,20). A further report suggesting that some "mixture" of opioid receptors is involved in regulation of food intake is that the nonselective opioid antagonist LY255582 produces a more marked decrease in deprivation-induced feeding than the receptor-specific antagonists beta-FNA or nor-BNI (18).

The present investigation examined butorphanol effects on both the initiation and the maintenance of feeding behavior. Results suggest that butorphanol affects maintenance to a greater extent than initiation. Butorphanol altered the temporal pattern of food intake over the 60-min session, resulting in relatively more food being consumed later on in the session (as indicated by the 10-min bins) compared to control. Indeed, absolute intake during the first 10 min of sessions was decreased by butorphanol. It has been suggested that the opioid system is involved in the maintenance, but not the initiation, of feeding (17). For example, naloxone, the prototypical mu receptor antagonist, has no effect on running speed or percent correct choice on runway tasks (initiation), but does decrease the size of the meal once the goal box has been reached (maintenance) (11,12). That butorphanol increased food intake at doses that had no effect on time to complete the initial requirements of obtaining food, and that increases in food intake were due to feeding occurring later on in the experimental session supports this interpretation.

In conclusion, the present findings indicate that butorphanol produces increases in food intake contingent upon lever pressing. Differences between previous studies reporting decreased food-maintained responding following butorphanol administration and the present study may be due to the deprivation state of the subjects, or to issues relating to chronic vs. acute administration.

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