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A Sucrose-Based Maintenance Diet Increases Sensitivity to Appetite Suppressant Effects of Naloxone

J. M. RUDSKI,*[1] C. J. BILLINGTON† AND A. S. LEVINE‡

*Department of Psychology, Muhlenberg College, Allentown, PA 18104
†Research and Medicine Service, Veterans Administration Medical Center, Minneapolis, MN 55417
‡Departments of Psychiatry, Food Science & Nutrition, Medicine, Surgery And Medicinal Chemistry, University of Minnesota, Minneapolis and St. Paul, MN

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RUDSKI, J. M., C. J. BILLINGTON AND A. S. LEVINE. *A sucrose-based maintenance diet increases sensitivity to appetite suppressant effects of naloxone.* PHARMACOL BIOCHEM BEHAV **58**(3) 679–682, 1997.—Rats maintained under restricted access to food (but at 100% free-feeding weights) received one of two diets in their home cages: a palatable sucrose-based diet, or regular chow (grain based diet), and could respond for either sucrose- or grain-based reinforcers under an FR 40 reinforcement schedule (crossover design). Naloxone (0, 0.1,0.3, 1.0, and 3.0 mg/kg) was more potent in reducing operant-chamber responding in rats maintained on a sucrose-based diet in their home cages than those fed a grain-based diet, regardless of the type of pellets available in the operant chambers. Whereas naloxone decreased response rate over the session, it had no effect on initiation of responding. Results support the hypothesis that opioids are involved in the maintenance, but not the initiation of consummatory behavior. Furthermore, increased potency of naloxone following chronic ingestion of palatable food is similar to that observed following chronic opiate administration, suggesting a relationship between palatability and opioids. © 1997 Elsevier Science Inc.

Naloxone Opiates Feeding Operant Reinforcement Incentive

MANY reports indicate that the opioid system is involved in regulating food intake. Mu, kappa, and delta receptor agonists increase short-term free feeding. Naloxone, a primarily mu receptor antagonist, reliably decreases feeding induced by many different procedures across many species. One common suggestion accounting for these findings is that opioids are involved in modulating the incentive value of food: opioid agonists increase incentive value, whereas opioid antagonists decrease it [for reviews, see (7,24)].

Several lines of evidence illustrate the relationship between incentive value and opioid effects. Consumption of palatable food alters opioid binding and beta-endorphin levels in rat hypothalamus (10,27). Ingestion of palatable foods results in naloxone-reversible increases in nociceptive thresholds (1) and alters morphine's analgesic potency (12,17). Furthermore, opiate administration increases intake of preferred foods relative to nonpreferred (15), whereas morphine withdrawal decreases intake of preferred and sweet solutions (26). Conversely, lower naltrexone or naloxone doses are needed to disrupt intake of more preferred foods and fluids than of less preferred foods (5,8,14,25). Finally, opioid antagonists decrease the reported pleasantness of sweet foods in humans (2,9,11,34).

Whereas decreases in free feeding following naloxone administration are reliably reported, naloxone is usually reported to be ineffective in decreasing operant lever pressing [e.g., (13,16,23,28,35)]. We have recently demonstrated that naloxone's ability to suppress operant responding is inversely related to level of a rat's deprivation between sessions: naloxone is not effective in disrupting responding when rats are maintained under conditions of chronic food deprivation, but does disrupt responding at doses as low as 0.1 mg/kg when rats are not deprived (31). In the current study, we examined whether naloxone's ability to disrupt operant responding in nondeprived (but schedule fed) rats is affected by the rein-

[1]To whom requests for reprints should be addressed.

forcer's palatability, as is observed in home cage studies. We also evaluated whether rats maintained on a palatable or regular chow diets outside of the operant chambers show differential sensitivity to naloxone's rate-suppressant effects.

METHOD

Subjects

Forty-eight naive male Sprague–Dawley rats (Harlan–Sprague–Dawley, Madison WI), starting weights 290–320 g, were housed in conventional individual wire hanging cages with a 12-h light/12-h dark photoperiod (lights on at 0700 h) in a temperature-controlled vivarium (21–22°C). Food availability was restricted to 2 h access in their home cages between 1400 and 1600 h, and a 40-min session in operant chambers each morning. This feeding regimen resulted in rats maintained at 100–110% of their free-feeding weights. Tap water was available ad lib.

Materials

Experimental sessions were conducted in 12 commercially available small animal operant chambers (Model E10-10TC, Coulbourn Instruments, Inc., Lehigh Valley, PA), each enclosed in an isolation cubicle (Model E10-20, Coulbourn Instruments, Inc.) to attenuate outside noise. Chambers were equipped with two operant levers on opposite sides of the chambers' front panel. The house-light, located in a top-central position, was illuminated during experimental sessions. Forty-five migrogram dustless precision pellets (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 1 s. A Zeos 486 computer, located in the same room as the chambers, controlled experimental conditions and recorded data.

Diet Composition

Sucrose-based diets were composed of casein, sucrose, dextrin, fiber, corn oil, d1-methionine, l-Cystine, a salt mixture, vitamin mixture, corn syrup, and tableting binders. The caloric profile (Kcal/g) consisted of protein (0.79), fat (0.48), and carbohydrate (2.39). There were a total of 3.66 Kcal/g.

Grain-based diets were composed of grain base mix, sucrose, dextrose, casein, glutamic acid, tableting binders, a mineral, and vitamin mix, d1-methionine, choline chloride, and sodium proportionate. The caloric profile (Kcal/g) consisted of protein (0.93), fat (0.30), and carbohydrate (2.18). There were a total of 3.40 Kcal/g.

Procedure

Rats were trained to respond on the left lever by method of successive approximations. Once lever-pressing was acquired, the ratio was stretched over 3 days to a fixed ratio 40 (i.e., FR 40) reinforcement schedule. Forty-minute training sessions were conducted on 21 consecutive days. Rats were fed either sucrose- or grain-based food (Bioserv, Frenchtown NJ) in home cages, and responded for either sucrose- or grain-based pellets (45 mg) in operant chambers (i.e., crossover design). This resulted in four groups ($n = 12$): home cage sucrose/operant-chamber sucrose (sucrose-sucrose), home cage sucrose/operant-chamber grain (sucrose-grain), home cage grain/operant-chamber grain (grain-grain), and home cage grain/operant-chamber sucrose (grain-sucrose). Training sessions were all conducted with grain pellets. All rats received grain-based diets in home cages until the final 6 days of training, at which time half of the rats (the sucrose-sucrose and sucrose-grain groups) were switched over to the sucrose-based diet, which remained in effect throughout the naloxone dose–response.

Naloxone hydrochloride (0, 0.1, 0.3, 1.0, 3.0 mg/kg dissolved in saline and injected in volumes of 1 ml/kg) was administered subcutaneously 20 min before sessions. Doses were administered over 5 consecutive days. Total amount of pellets consumed during the session and the amount of time from the start of sessions needed to acquire the first pellet were recorded. Results were analyzed with a one-factor between–one-factor within RMANOVA. Scheffe's comparisons were used to assess post hoc differences.

It was assumed before beginning the experiment that the sucrose-based diet was more palatable than the grain-based diet. Upon completion of the experiment, rats were given concurrent access to the grain-based and sucrose-based diets in their home cages for the 2-h feeding period for 3 consecutive days. Intakes of each diet were compared on the third day with a one-factor between one-factor within RMANOVA, allowing us to assess whether the sucrose diet was, in fact, preferred to the grain diet.

RESULTS

When given a concurrent choice, rats preferred the sucrose-based diet (17.8 g $\pm$ 0.74) over the grain-based diet (4.2 g $\pm$ 0.63), $F(1, 44) = 102.2$, $p < 0.0001$. There was no significant interaction between preference and experimental group, $F(3, 44) = 0.206$, $p > 0.05$. Thus, it can be inferred that the sucrose diet was found to be more palatable than the grain-based diet.

Naloxone decreased food-reinforced responding in a dose-related manner, $F(4, 176) = 28.18$, $p < 0.0001$. Naloxone's potency depended more upon the diet available in home cages than the type of pellet available in operant chambers (Fig. 1). Responding in rats fed sucrose in their home cages was decreased following lower naloxone doses than that of rats fed grain in their home cages (i.e., 0.3 and 3.0 mg/kg, respectively). A post hoc RMANOVA comparing percent saline responding following naloxone showed significant main effects for home cage diet, $F(1, 44) = 5.42$, $p = 0.025$, but not for type of reinforcer available in operant chambers ($F = 0.55$, $p = 0.463$).

Naloxone decreased feeding more potently in the groups that had the lowest response rate under saline. Specifically, naloxones' effects were strongest in the sucrose-grain group. This group of rats consumed the fewest number of pellets in the operant chambers after saline injection (sucrose-grain: 44 $\pm$ 6 pellets; sucrose-sucrose: 68 $\pm$ 9; grain-grain: 81 $\pm$ 7; grain-sucrose: 88 $\pm$ 6). While there was a significant main effect of group for saline baseline levels, $F(3, 44) = 18.94$, $p < 0.0001$, post hoc analyses revealed that the only significant differences were between the sucrose-grain group and each of the other three groups. Thus, control values for the sucrose-sucrose group are not significantly lower than those of the two grain groups, yet naloxone's potency was still enhanced in the sucrose-sucrose group.

Different home cage operant-chamber diet combinations did not significantly alter the amount of weight gained during the experiment, $F(3, 44) = 0.55$, $p = 0.65$. The sucrose-sucrose group had the greatest mean weight gain (104 g), followed by the grain-sucrose (101 g), sucrose-grain (100 g), and grain-grain (97 g) groups.

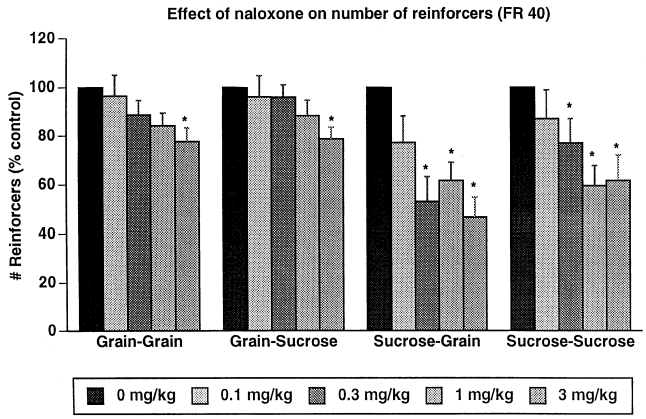

FIG. 1. Effect of naloxone (mg/kg) on the number of reinforcers (% control) received by rats in the operant chamber. *Indicates $p < 0.05$ when compared to responding following vehicle.

DISCUSSION

In the current study, we examined the relationship between home cage diet, reinforcer palatability, and naloxone on food-reinforced lever pressing. Rats maintained on either sucrose-based (i.e., palatable) or regular chow (plain) diets responded for either palatable or plain reinforcers in operant chambers. Because naloxone's anorectic effect on free feeding is reported to be proportional to a food's palatability (14,25), we expected naloxone to disrupt responding for sucrose pellets at lower doses than responding for grain pellets. It was also anticipated that naloxone's effects on sucrose-based reinforcers would be greater in rats maintained on regular chow home cage diets than rats maintained on sucrose-based diets, as the sucrose reinforcers might acquire more relative value in the latter situation. Naloxone did decrease responding differentially, but its relative potency in the various experimental conditions was not as we predicted. Increased potency was more closely related to the food available in home cages than the type of food available in operant chambers.

These results extend a similar finding reported by Kirkham (20). In his study, rats sham feeding 30% sucrose showed enhanced anorectic potency of naloxone. In the current study, responding in rats maintained on sucrose-based diets was disrupted by naloxone to a greater extent than rats maintained on grain-based diets, regardless of the type of reinforcer available in operant chambers. Thus, it appears as if chronic exposure to a sucrose diet alters naloxone's anorectic potency.

Whereas naloxone typically does not decrease operant responding [e.g., (13,16,23,28,35)], it can be quite potent in disrupting food-maintained behavior in rats with prior histories of opiate administration [e.g. (29,30)]. Increased potency of naloxone following chronic sucrose ingestion in the current experiment suggests an interesting parallel between palatable food consumption and a history of opiate administration. Several lines of evidence suggest that ingestion of palatable food is related to activity in the endogenous opioid system. Eating palatable food releases beta-endorphin in the hypothalamus (10), increases opioid receptor binding affinity (27), produces naloxone-reversible increases in nociceptive thresholds (1), and alters responsiveness to morphine-induced analgesia (12,17). In sum, chronic ingestion of sucrose alters the endogenous opioid system in some way. This may influence naloxone's potency much in the same way that chronic opiate administration does.

Whereas naloxone decreased responding (and hence consumption) in the current study, it did not delay the acquisition of the initial reinforcer in experimental sessions. Naloxone has been proposed to have less of an effect on initiation than on maintenance of consummatory behavior. Kirkham and Blundell (21,22) demonstrated that naloxone has no effects on speed of traversing a runway to acquire food, yet it decreased the amount of food consumed once rats were in the goal box. Similarly, naloxone decreases drinking of water (6) or sucrose solutions (20) without any effect on latency to begin to drink. Finally, Rudski et al. (31), using an FR 80 (first pellet) FR 3 (subsequent pellets) reported that naloxone decreased operant responding for food under the FR 3 reinforcement schedule without significantly affecting the initial FR 80 component.

It is well established that amount of food intake can influence the behavioral pharmacological properties of drugs. Rate-suppressant effects of drugs on operant behavior are often observed following lower doses when animals are maintained under conditions of low deprivation than under conditions of more severe deprivation (4,19,30–33). Drug self-administration often increases with food restriction [see (3) for review]. Results in the current study indicate that amount is not the only dietary factor that can affect drug effects on operant responding; type of diet can also influence drug effects.

Naloxone is most potent in the current study in rats receiving a sucrose-based diet in their home cages. These two groups also show the lowest amount of food ingested during operant sessions. It is possible that naloxone's potency is inversely related to rate of ingestion in the control condition, and not to any other factors. Several lines of evidence suggest that the current results are not an artifact of control response rate. First, control values for the sucrose-sucrose group are not significantly lower than those of the two grain groups, yet naloxone's potency is enhanced in the sucrose-sucrose group. Also, previous work in home cages indicates that much lower doses of naloxone decrease intake of a palatable food when compared with a grain-based diet, even though the control levels (i.e., following saline) of intake for palatable foods were higher than those of regular chow (14,25).

The rats maintained on grain diets clearly pressed for more food in operant chambers than did sucrose maintained rats (44 and 68 pellets vs. 81 and 88 pellets after vehicle, respectively), suggesting that the grain-grain and grain-sucrose rats may be effectively deprived relative to the sucrose-sucrose and sucrose-grain groups. Rudski et al. (31) demonstrated that naloxone's anorectic effect on operant responding is inversely related to deprivation level. Thus, naloxone's potentiated effect in the sucrose diet-maintained groups may have resulted from the rats being less food deprived than those maintained on grain diets. However, examination of weight gain over the course of the experiment indicates no differences between groups. If the grain-maintained rats were food deprived relative to the sucrose-maintained rats, they should have gained less weight. In fact, the group with the second greatest mean weight gain was the grain-sucrose group.

The two diets did not only differ in significantly in terms of the amount of carbohydrates, but in fat as well. Thus, the potentiation of naloxone by the sucrose diet may also be due to its fat content. This poses no threat to the argument of naloxone being potentiated by palatability; rats find fat palatable. However, this does raise concerns regarding the relative levels of deprivation existing between the groups (see previous para-

graph). As previously mentioned, comparisons of the groups' weight gains through the experiment suggests that relative deprivation was not an important factor in naloxone's effects.

In sum, naloxone's potency in disrupting food-reinforced operant responding is enhanced by chronic exposure to palatable diets in much the same way that it is enhanced by chronic exposure to opiates. The results further support the theory that the opioid system is involved in palatability. Finally, the current study illustrates that type of diet may influence drug effects on operant responding.

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