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Prior Exposure to Palatable Solutions Enhances the Effects of Naltrexone on Food Intake in Rats

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KANAREK, R. B., W. F. MATHES, L. K. HEISLER, R. LIMA AND L. MONFARED *Prior exposure to palatable solutions enhances the effects of naltrexone on food intake in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 377–381, 1997.— Previous research has suggested that chronic intake of palatable foods and fluids enhances the activity of the endogenous opioid system. To examine this suggestion, the effect of naltrexone on food intake was examined in male Long-Evans rats with or without prior exposure to palatable solutions. In Experiment 1, rats were fed laboratory chow alone or laboratory chow and a 32% sucrose solution, and in Experiment 2, were fed chow alone, chow and a 32% Polycose solution, or chow and a 0.15% saccharin solution for three weeks. The sucrose, Polycose, and saccharin solutions were removed 18 h prior to drug administration. Rats then received injections of naltrexone hydrochloride (0.0, 0.3 or 3.0 mg/kg, sc) and chow intakes were measured during the subsequent 1, 2, 4, 6 and 24 h. Naltrexone injections had minimal effects on intakes of animals which previously had consumed only chow. In contrast, naltrexone led to significant dose-related decreases in chow intakes in rats which had previously consumed the sucrose, Polycose, or saccharin solutions. These results provide confirmation for the suggestion that chronic intake of palatable solutions alters the activity of the endogenous opioid system. © 1997 Elsevier Science Inc.

Naltrexone Food intake Carbohydrate Sucrose Polycose Saccharin Palatability Opiates Feeding behavior

OVER the past decade, a substantial body of research has accumulated to support the hypothesis that a relationship exists between endogenous opioid peptides and the pleasurable or rewarding aspects of feeding behavior. Initial evidence for this relationship came from experiments demonstrating that administration of opioid agonists and antagonists altered intake of palatable foods and fluids to a greater degree than intake of less palatable items (e.g., (1,4,5,11,16–19,21–24, 27,31,33). For example, Kirkham (16) reported that naloxone, an opioid receptor antagonist, was more potent in suppressing sham feeding of a 30% sucrose solution than sham feeding of less concentrated and less preferred sucrose solutions. Along similar lines, Levine and colleagues (19) recently observed that rats eating sweet chow were more sensitive to naloxone's anorectic actions that rats eating regular chow. Comparable effects were reported in human subjects by Yeomans and coworkers (33) who found that consumption of highly palatable foods was decreased to a greater extent after administration of the long-acting opioid antagonist, nalmefene, than intake of less palatable foods.

Further support for a relationship between endogenous opioid peptides and palatable foods comes from research demonstrating that dietary variables can (i) alter the behavioral consequences of opiate drugs; and (ii) modify the activity of the endogenous opioid peptide system. With respect to dietary modulation of the behavioral actions of opiates, chronic intake of palatable sucrose or Polycose solutions or dietary fat enhances the analgesic potency of opioid agonists (e.g., 6,7,10,14,15,26). Additionally, chronic consumption of a sucrose solution can strengthen a conditioned place preference associated with morphine administration (20).

The effects of intake of palatable substances on behavioral responses may reflect the actions of these substances on the endogenous opioid system. In support of this idea, acute intake

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of palatable sucrose-containing foods results in an increase in the amount of beta-endorphin occupying hypothalamic receptors in rats (8,9). Moreover, chronic consumption of palatable nutritive foods and fluids, such as sugar solutions and high-fat diets, increases opiate receptor binding affinity in both rats and mice (13,25).

One possibility raised by these findings is that opiate agonists and antagonists are bound more tightly to opiate receptors in animals chronically fed palatable foods. This increase in binding affinity could in part account for the fact that opiate drugs affect the intake of palatable foods to a greater extent than intake of less palatable foods. To test this possibility, in the present experiments, chow intake was measured as a function of naltrexone administration in rats which had or had not previously consumed either a sucrose solution (Experiment 1), or a Polycose or saccharin solution (Experiment 2).

EXPERIMENT 1

Method

Animals. Forty-seven male virus and antibody free Long-Evans rats (Charles River Laboratories, Portage, MI), weighing 200–250 g at the beginning of the experiment, were used. All animals were housed individually in standard stainless-steel cages in a temperature and humidity controlled room maintained on a reverse 12-12 h light/dark cycle (lights off: 0800–2000).

Drugs. Naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% sterile saline and administered subcutaneously at a volume of 1 ml/kg.

Diet Conditions. All animals were given ad lib access to tap water and ground Purina Laboratory Rodent Chow #5001 presented in Wahmann (Timonium, MD) LC-306A nonspill food cups. Twenty-three rats also had ad lib access to a 32% sucrose solution (w/v; 1.28 kcal/ml). All fluids were presented in 250 ml glass bottles with rubber stoppers and non-leaking stainless-steel drinking spouts. Food and fluid intakes and body weights were measured every other day for three weeks prior to drug administration.

Procedure. The sucrose solution was removed 18 h prior to drug administration. All animals were given chow and water during this time. Three hours after the beginning of the dark cycle, rats were given a single injection of either saline, 0.3, or 3.0 mg/kg naltrexone. Chow intakes were measured at 1, 2, 4, 6, and 24 h after the injections.

Data Analysis. Food intakes following drug injections were analyzed using analyses of variance, followed by Tukey HSD post hoc comparisons. Independent samples t-tests were used to analyze daily food intake and body weight data.

RESULTS

Pre-Drug Food Intake and Body Weight

During the three weeks prior to drug administration, rats given sucrose and chow consumed significantly more calories (mean daily caloric intake = 102.4 ± 11.8 kcals) than animals given chow alone (mean daily caloric intake = 90.7 ± 7.1 kcals) ($t = 4.15$, $p < 0.001$). Animals consuming chow and sucrose ate 53% of their calories as chow and 47% of their calories as sucrose.

Although animals eating sucrose and chow gained more weight (157 g) than animals eating chow alone (140 g), this difference was not significant.

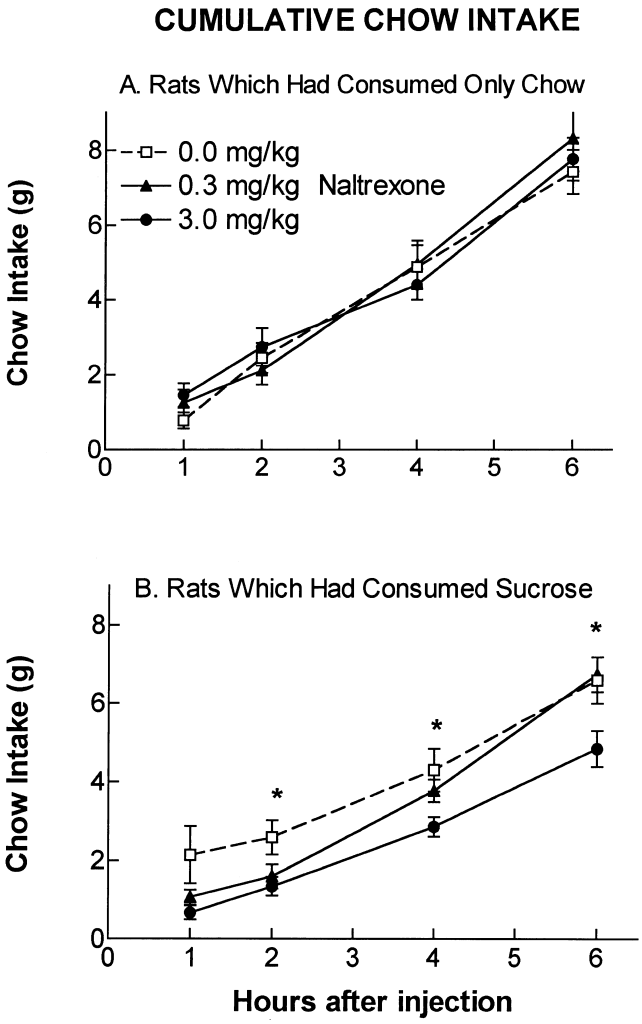

FIG. 1. Mean (± SEM) cumulative food intake following naltrexone injections for rats fed chow alone (A) or chow and a 32% sucrose solution (B) for three weeks prior to drug administration. The sucrose solution was removed 18 h prior to naltrexone injections, and all rats fed only chow. Significant differences in food intake as a function of naltrexone administration are indicated as * = $p < 0.05$.

Food Intake Following Naltrexone Administration

On the day of drug injections, at all drug doses, and at all measurement times except one hour, animals which had previously been given sucrose consumed significantly less food than animals fed only chow.

Naltrexone had no significant effects on food intake in rats which had eaten only chow prior to drug administration (Fig. 1A). In contrast, in rats which previously had consumed sucrose, naltrexone significantly decreased chow intake in a dose-related manner at 2 h, [$F(2, 19) = 3.88$, $p < 0.05$], and 4 h, [$F(2, 19) = 4.64$, $p < 0.05$] following injections (Fig. 1B). Six hours after injections, chow intake of rats given 3.0 mg/kg naltrexone was significantly less than that of rats given 0.3 mg/kg of the drug [$F(2, 19) = 4.55$, $p < 0.05$]. There was no difference in chow intake as a function of drug dose in either dietary group 24 h following injections.

EXPERIMENT 2

Results of Experiment 1 demonstrated that prior intake of a palatable solution can enhance the anorectic properties of naltrexone. These results support the hypothesis that opiate antagonists are more tightly bound to opiate receptors in rats chronically fed palatable foods and fluids (13,25). However, from the results of Experiment 1, the role of palatability on the anorectic actions of naltrexone can not be separated from that of the nutritive value of the solution. Thus, in Experiment 2, the effects of prior intake of a non-nutritive sweet saccharin solution and a nutritive non-sweet Polycose solution on naltrexone-induced anorexia were compared. Polycose is a non-sweet polysaccharide made from hydrolyzed cornstarch which easily dissolves in water. Although Polycose has been characterized as bland tasting, rats appear to find Polycose solutions palatable, and avidly consume these solutions (28–30). Previous work has shown that across a 24-h period rats drink similar amounts of a 32% sucrose solution, a 32% Polycose solution and a 0.15% saccharin solution (7, 13).

Method

Animals. Sixty-one, drug-naive, male, virus and antibody free Long–Evans rats (Charles River Laboratories, Portage, MI), weighing 200–250 g at the beginning of the experiment were used. Housing conditions were identical to those in Experiment 1.

Diet Conditions. All animals were provided with ad lib access to tap water and ground Purina Laboratory Rodent Chow #5001 presented in Wahmann LC-306A nonspill food cups. In addition, 21 rats were given ad lib access to a 32% solution of Polycose (Ross Laboratories, Columbus, OH; 1.28 kcal/ml) and 21 rats, ad lib access to a 0.15% saccharin solution (0.0 kcal/ml). The concentrations of the Polycose and saccharin solutions were chosen on the basis of previous work demonstrating that over a 24-hour period rats drank similar amounts of these solutions as of a 32% sucrose solution (7). The remaining 19 rats were maintained on chow and water alone. As in Experiment 1, food and fluid intakes were measured every other day for three weeks preceding drug administration.

Procedure. The Polycose and saccharin solutions were removed 18 h prior to drug administration. All animals were given ad lib access to chow and water during this time. Three hours after the beginning of the dark cycle, rats were given a single subcutaneous injection of either saline, 0.3 mg/kg, or 3.0 mg/kg naltrexone. Chow intakes were measured at 1, 2, 4, 6 and 24 h post-injection.

Data Analysis. Food intakes following drug injections, daily food intake, and body weight data were analyzed with between subjects analyses of variance, followed by Tukey HSD post hoc comparisons.

RESULTS

Pre-Drug Food Intake and Body Weight

During the three weeks prior to drug administration, rats fed chow and the Polycose solution had significantly greater mean daily caloric intakes (122.1 kcals/day) than rats fed either chow and the saccharin solution (95.5 kcals/day) or rats fed chow alone (95.4 kcals/day). Rats consuming chow and the Polycose solution took in 53% of their calories as chow and 47% of their calories as Polycose. Fluid intakes from the Polycose solution (46.1 ml/day) and the saccharin solution (48.2 ml/day) did not differ. Weight gain across the three-week pre-drug period differed significantly as a function of dietary conditions. Rats that drank the Polycose solution [$F(2,58) = 4.31, p < 0.05$] gained significantly more weight (132.3 g) across the three week pre-drug period than animals that drank the

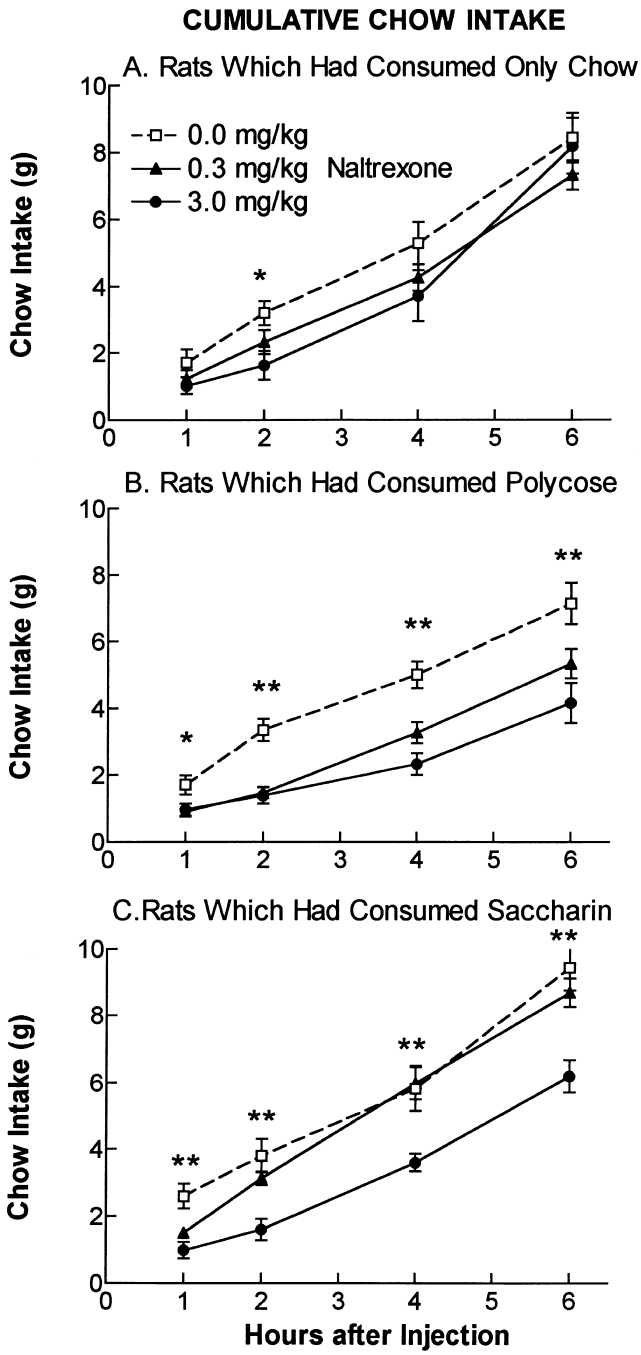

FIG. 2. Mean (± SEM) cumulative chow intake following naltrexone injections for rats fed chow alone (A), chow and a 32% Polycose solution (B) or chow and a 0.15% saccharin solution (C) for three weeks prior to drug administration. The Polycose and saccharin solutions were removed 18 h prior to naltrexone injections, and all rats fed only chow. Significant differences in food intake as a function of naltrexone administration are indicated as ** = $p < 0.01$; * = $p < 0.05$.

saccharin solution (115.6 g). Weight gain of rats given chow and water alone was intermediate between the two other groups (120.9 g).

Food Intake Following Naltrexone Administration

Naltrexone did not alter food intake in rats which had eaten only chow prior to drug administration, except at 2 hrs when 3.0 mg/kg naltrexone significantly decreased food intake relative to saline $F(2,17) = 4.10, p < 0.05$ (Fig. 2A). In comparison, in rats which had previously been drinking the Polycose solution, naltrexone significantly decreased chow intake in a dose-dependent manner at all measurement times. At 1 h [$F(2, 17) = 5.26$, $p < 0.05$], 2 h [$F(2, 17) = 18.53$, $p < 0.001$], and 4 h [$F(2, 17) = 14.82$, $p < 0.001$] both 0.3 mg/kg and 3.0 mg/kg naltrexone significantly suppressed food intake relative to saline (Fig. 2B). Additionally, for rats which had consumed Polycose, chow intake was significantly reduced at 6 h [$F(2, 17) = 7.08$, $p < 0.01$] and 24 h (saline = 19.1 g; 0.3 mg/kg = 17.6 g; 3.0 mg/kg = 14.5 g; $F(2, 17) = 4.62$, $p < 0.05$] following 3.0 mg/kg naltrexone compared to saline. Naltrexone administration also led to significant reductions in chow intake in rats which had consumed the saccharin solution (Fig. 2C). At 1 h, 0.3 and 3.0 mg/kg naltrexone led to significant decreases in chow intake compared to saline injections [$F(2, 17) = 9.40$, $p < 0.01$], while at 2 h [$F(2, 17) = 8.15$, $p < 0.01$], 4 h [$F(2, 17) = 6.17$, $p < 0.01$] and 6 h [$F(2, 17) = 8.94$, $p < 0.01$], 3.0 mg/kg naltrexone significantly decreased chow intake relative to both saline and 0.3 mg/kg naltrexone. For rats which had consumed saccharin, chow intake did not differ as a function of drug administration 24 h following injections.

DISCUSSION

Naltrexone significantly reduced food intake in rats which previously had consumed palatable solutions in addition to laboratory chow, but had only minimal effects on food intake in rats which had eaten only chow. These results are similar to those of Yeomans (32), who reported that rats given prior exposure to whole milk decreased intake of a wet mash diet to a greater degree following naloxone injections than rats not given the milk. Taken together, these studies provide evidence for the hypothesis that chronic intake of a palatable food or fluid increases the sensitivity of the endogenous opioid system (13). Previous research has demonstrated that intake of palatable nutritive substances increases opiate receptor binding affinity in whole brain of both rats and mice (13, 25). Thus, it may be that binding affinity for opioid drugs is greater in rats previously fed sucrose or milk than in those fed only chow, and that is why naltrexone and naloxone were more effective in animals which had consumed these palatable substances than in those fed chow alone.

It should be noted that in Experiment 1, rats drinking the sucrose solution and chow consumed significantly more calories, and gained more weight during the three-week predrug period than rats fed only chow. Additionally, in Experiment 2, rats drinking the Polycose solution took in significantly more calories than rats given either saccharin and chow or chow alone, and gained significantly more weight than rats consuming saccharin and chow. It is possible that the enhanced effects of naltrexone on food intake in animals drinking sucrose or Polycose solutions, in some way, is a consequence of increased caloric intake or weight gain. Although this is a possibility, it seems likely that the positive hedonic qualities associated with intake of the sucrose and Polycose solutions also contributed to the enhancement of naltrexone's effects on food intake. In support of this idea, although there were no differences in caloric intake or body weight gain between rats consuming saccharin and chow or chow alone, naltrexone was more effective in reducing food intake in rats which had consumed saccharin than in rats eating only chow. Moreover, previous work has demonstrated that, even in the absence of differences in caloric intake or weight gain, intake of sucrose and Polycose solutions can alter the analgesic effects of morphine (6), and sucrose intake can enhance the formation of a condition place preference for morphine (20). These results suggest that intake of palatable food directly affects the endogenous opioid system.

The present results suggest that the palatability rather than the nutritive value of the solution was most important in altering the effects of naltrexone on subsequent chow intake. Prior intake of both the nutritive sucrose and Polycose solutions, and the non-nutritive saccharin solution enhanced the anorectic effects of naltrexone. These results may be contrasted with the findings of previous studies demonstrating that chronic intake of palatable nutritive solutions, but not non-caloric saccharin solutions augments the analgesic potency of morphine (7). Chronic saccharin intake may alter naltrexone-induced anorexia, but not morphine-induced analgesia as a result of the different opiate receptor subtypes on which the two drugs predominantly act. Naltrexone is a general opioid receptor antagonist which blocks activity at mu, kappa and delta receptors. In comparison, although morphine has some agonist actions at kappa and delta receptors, it's primary action is at the mu receptor (12). Recent work indicates that different opioid receptor subtypes are important in mediating intake of palatable nutritive and non-nutritive solutions. Beczkowska and colleagues (2,3) reported that central administration of selective mu receptor antagonists decreased sucrose intake, and intake of a complex carbohydrate solution similar to Polycose, but did not decrease saccharin intake. In contrast, central infusions of selective delta receptor antagonists reduced intake of saccharin but not sucrose solutions. These results suggest that intake of different types of palatable solutions could result in stimulation of different receptor subtypes. If saccharin solutions stimulate activity at delta, but not mu receptors, this could explain why these non-nutritive solutions augmented naltrexone's anorectic effects, but did not alter morphine-induced analgesia.

Finally, the generality of the effects of intake of palatable foods on the actions of drugs which alter food intake should be studied. For example, one question which should be answered is does prior intake of palatable foods enhance the food stimulating effects of opioid agonists. This question is important because of its potential implication for human eating disorders. If intake of palatable food potentiates the effects of opioid agonists, it could be hypothesized that binging behavior would augment the effects of endogenous opioids on food intake in individuals suffering from bulimia.

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