Naltrexone infusion inhibits the development of preference for a high-sucrose diet

ALLEN S. LEVINE,1,2,3,4 MARTHA K. GRACE,1 JAMES P. CLEARY,3 AND CHARLES J. BILLINGTON1,4

1Minnesota Obesity Center, Veterans Affairs Medical Center, Minneapolis 55417; and Departments of 2Psychiatry, 3Psychology, and 4Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Received 23 January 2002; accepted in final form 17 July 2002

Levine, Allen S., Martha K. Grace, James P. Cleary, and Charles J. Billington. Naltrexone infusion inhibits the development of preference for a high-sucrose diet. Am J Physiol Regul Integr Comp Physiol 283: R1149–R1154, 2002.—We hypothesized that the opioid antagonist naltrexone would inhibit the redevelopment of a preference for a high-sucrose diet after an abstention period from this diet. Rats that chose between a starch or sucrose diet for 10 days preferred the sucrose diet. Rats were then given access to the starch diet alone for another 10-day period. A miniosmotic pump containing saline or naltrexone was then implanted (70 µg/h; 1.7 mg/day) for ~10 days. During the saline infusion, 77% of the total energy came from the sucrose diet, whereas during the naltrexone infusion, 33% of the total energy came from the sucrose diet. We repeated this study in another group of rats but did not restrict the sucrose diet. In this case naltrexone failed to decrease preference for the sucrose diet. Thus naltrexone infusion inhibited redevelop-ment of a preference for a sucrose diet after a period of restriction to a starch diet for 10 days but had no effect on preference if both diets were present throughout the study.

METHODS

Subjects

The treatment of the rats in the studies described below conforms with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society (1), and these studies received local institutional animal care and use committee (IACUC) approval.

Two groups of rats weighing between 225 and 250 g were housed in single cages and given water through an auto-

Naltrexone infusion inhibits the development of preference for a high-sucrose diet

ALLEN S. LEVINE,1,2,3,4 MARTHA K. GRACE,1 JAMES P. CLEARY,3 AND CHARLES J. BILLINGTON1,4

1Minnesota Obesity Center, Veterans Affairs Medical Center, Minneapolis 55417; and Departments of 2Psychiatry, 3Psychology, and 4Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Received 23 January 2002; accepted in final form 17 July 2002

Levine, Allen S., Martha K. Grace, James P. Cleary, and Charles J. Billington. Naltrexone infusion inhibits the development of preference for a high-sucrose diet. Am J Physiol Regul Integr Comp Physiol 283: R1149–R1154, 2002.—We hypothesized that the opioid antagonist naltrexone would inhibit the redevelopment of a preference for a high-sucrose diet after an abstention period from this diet. Rats that chose between a starch or sucrose diet for 10 days preferred the sucrose diet. Rats were then given access to the starch diet alone for another 10-day period. A miniosmotic pump containing saline or naltrexone was then implanted (70 µg/h; 1.7 mg/day) for ~10 days. During the saline infusion, 77% of the total energy came from the sucrose diet, whereas during the naltrexone infusion, 33% of the total energy came from the sucrose diet. We repeated this study in another group of rats but did not restrict the sucrose diet. In this case naltrexone failed to decrease preference for the sucrose diet. Thus naltrexone infusion inhibited redevelop-ment of a preference for a sucrose diet after a period of restriction to a starch diet for 10 days but had no effect on preference if both diets were present throughout the study.

METHODS

Subjects

The treatment of the rats in the studies described below conforms with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society (1), and these studies received local institutional animal care and use committee (IACUC) approval.

Two groups of rats weighing between 225 and 250 g were housed in single cages and given water through an auto-

Naltrexone infusion inhibits the development of preference for a high-sucrose diet

ALLEN S. LEVINE,1,2,3,4 MARTHA K. GRACE,1 JAMES P. CLEARY,3 AND CHARLES J. BILLINGTON1,4

1Minnesota Obesity Center, Veterans Affairs Medical Center, Minneapolis 55417; and Departments of 2Psychiatry, 3Psychology, and 4Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Received 23 January 2002; accepted in final form 17 July 2002

Levine, Allen S., Martha K. Grace, James P. Cleary, and Charles J. Billington. Naltrexone infusion inhibits the development of preference for a high-sucrose diet. Am J Physiol Regul Integr Comp Physiol 283: R1149–R1154, 2002.—We hypothesized that the opioid antagonist naltrexone would inhibit the redevelopment of a preference for a high-sucrose diet after an abstention period from this diet. Rats that chose between a starch or sucrose diet for 10 days preferred the sucrose diet. Rats were then given access to the starch diet alone for another 10-day period. A miniosmotic pump containing saline or naltrexone was then implanted (70 µg/h; 1.7 mg/day) for ~10 days. During the saline infusion, 77% of the total energy came from the sucrose diet, whereas during the naltrexone infusion, 33% of the total energy came from the sucrose diet. We repeated this study in another group of rats but did not restrict the sucrose diet. In this case naltrexone failed to decrease preference for the sucrose diet. Thus naltrexone infusion inhibited redevelop-ment of a preference for a sucrose diet after a period of restriction to a starch diet for 10 days but had no effect on preference if both diets were present throughout the study.

METHODS

Subjects

The treatment of the rats in the studies described below conforms with the "Guiding Principles for Research Involving Animals and Human Beings" of the American Physiological Society (1), and these studies received local institutional animal care and use committee (IACUC) approval.

Two groups of rats weighing between 225 and 250 g were housed in single cages and given water through an auto-
mated system and food ad libitum. Rats were placed into a light-reversal room (lights off 9:30 AM; lights on 9:30 PM) in a temperature-regulated vivarium (−25°C). Rats were given laboratory chow ad libitum for at least 2 wk to acclimate them to the new environment, and then the experimental procedure was initiated. During this experimental period, rats were given a high-starch diet and/or a high-sucrose diet ad libitum (Table 1). Alza osmotic pumps (model 2001, Durect) were filled with naltrexone or saline, implanted subcutaneously under Metofane anesthesia, and triple antibiotic ointment was applied to the incision site.

Procedure

**Experiment 1.** The effect of naltrexone on diet selection was evaluated during four 10- to 11-day periods (baseline, starch diet only, pump, postpump). During the baseline periods, rats were given free access to isocaloric high-starch and high-sucrose diets in jars that were weighed and rotated daily. After a 10-day period, the high-sucrose diet was removed from the cage, and rats were allowed free access to the high-starch diet for another 10 days. At the end of this period, rats were implanted subcutaneously with the osmotic pumps filled with naltrexone (70 μg/h; 1.7 mg/day) or saline and given access to both diets. These pumps are manufactured to deliver drug for ~10 days. Intake of the two diets was quantified daily during each period, including the 10-day postpump period.

**Experiment 2.** The effect of naltrexone on diet selection was evaluated during six 10- to 11-day periods (baseline, 1st pump, post-1st pump, starch diet only, 2nd pump, post-2nd pump). During the baseline period, rats were given free access to isocaloric high-starch and high-sucrose diets in jars that were weighed and rotated daily. At the end of this period, rats were implanted subcutaneously with the osmotic pumps filled with naltrexone (70 μg/h; 1.7 mg/day) or saline and given access to both diets. Intake of the two diets was quantified daily during each period, including the 10-day postpump period. At the end of this time the sucrose diet was removed from the cages, and rats were given free access to the starch diet for 10 days. At the end of this period of sucrose abstinence, rats were implanted with a second pump containing either naltrexone or saline (1st pump removed). Intake was monitored during the second pump study and for a 10-day postpump period.

Statistics

Food intake was measured daily and accumulated into 10- to 11-day bins (mean daily intake ± SE) for each study period. Means were compared using a Student’s t-test. We could not use ANOVA to evaluate the main effect of the various time periods because we imposed a forced starch-only period. To account for multiple comparisons, we used a Bonferroni correction.

RESULTS

In the first study we measured food intake of the isocaloric high-starch and high-sucrose diets across four periods: 1) pre-naltrexone pump + sucrose and starch diet present, 2) starch diet/no sucrose diet, 3) naltrexone pump + sucrose and starch diet present, and 4) post-naltrexone pump + sucrose and starch diet present. As expected, total food intake differed in the control and naltrexone groups only during the period of naltrexone administration (via miniosmotic pump) (Fig. 1A). The sucrose diet was clearly the preferred diet during the prepump and postpump periods, that is, during the time in which naltrexone was not present (see Figs. 1B and 3A). Rats ingested ~85% of their energy from the high-sucrose diet during the prepump period. After a 10-day period of abstinence from the sucrose diet, the two diets were offered to rats receiving a naltrexone or saline infusion. Naltrexone decreased intake of the sucrose diet by 66% and doubled the intake of the starch diet (P < 0.05) (Fig. 1B). This shifted the preference from the high-sucrose diet to the high-starch diet. Naltrexone-infused rats ingested ~33% of their energy from the sucrose diet, whereas saline-infused rats ingested ~77% of their energy from the sucrose diet (P < 0.05) (see Fig. 3A). When the pumps were depleted of naltrexone, rats once again preferred the sucrose diet (see Figs. 1B and 3A). The reacquisition of the preference for the sucrose diet approached pre-naltrexone levels 1 day after the predicted 10-day emptying time of the pump (saline group: 75 ± 7% kcal from sucrose diet; naltrexone group: 57 ± 9% kcal from sucrose diet; P > 0.05).

In the second study we measured food intake of the high-starch and high-sucrose diets across six periods: 1) pre-naltrexone pump + sucrose and starch diet present, 2) first naltrexone pump + sucrose and starch diet present, 3) post-first naltrexone pump + sucrose and starch diet present, 4) starch diet/no sucrose diet, 5) second naltrexone pump + sucrose and starch diet present, and 6) post-second naltrexone pump. Once again we found that total food intake was decreased during the period of naltrexone infusion. The decrease in total food intake was observed during the first naltrexone pump period, which was not preceded by a period of abstinence from the sucrose diet, and during the second naltrexone pump period, which was preceded by a period of starch diet alone (P < 0.05) (Fig. 2A). The sucrose diet was clearly the preferred diet during the prepump and postpump periods (Figs. 2B and 3B). However, the sucrose diet was also highly preferred during the first naltrexone infusion (preceded by free access to both diets). While naltrexone decreased the intake of the sucrose diet, it had a relatively minor effect (P < 0.05) (25% suppression). Also, the intake of the starch diet was unaffected. There was

### Table 1. Composition of experimental diets

<table>
<thead>
<tr>
<th>Diet, % by weight</th>
<th>Cornstarch</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>63.8</td>
<td>13.8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>21.2</td>
<td>21.2</td>
</tr>
<tr>
<td>Casein</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

American Institute of Nutrition Vitamin mixture 76 and mineral mixture 76 were used in all diets. Fiber was Celuluf (U.S. Biochemical, Cleveland, OH).
no difference in the percentage of energy that was ingested from the sucrose diet in the saline vs. the naltrexone group during the first pump period (Fig. 3B). After a period of access to the starch diet only, the second infusion of naltrexone decreased intake of the sucrose diet by 44% and increased the intake of the starch diet 2.6-fold (Fig. 2B). Naltrexone significantly decreased the percentage of energy ingested from the sucrose diet from ~88% to ~60% ($P < 0.05$). When the pumps were depleted of naltrexone, rats once again highly preferred the sucrose diet (Figs. 2B and 3B).

**DISCUSSION**

In 1981 Apfelbaum and Mandenoff (2) reported that naltrexone decreased hyperphagia in rats eating a highly palatable diet. Following the latter finding, many investigators found that naltrexone and other opioid antagonists decreased intake of sweet solutions/diets and other “palatable” foods in an extremely robust manner (5a, 6, 12, 15, 17, 18, 27). Lynch (19) demonstrated that the increase in the amount of saccharin solution ingested during a 3-wk period was blunted by daily (5 days/wk) pretest naloxone injec-
Cooper and Turkish (7) reported that naltrexone decreased intake of chocolate cookies, while increasing intake of laboratory chow. However, it should be noted that the rats still preferred the chocolate cookies to the chow, consuming 1.4 g of cookies and 0.5 g of chow after 5 mg/kg of naltrexone. We found that naloxone decreased cookie consumption more effectively than chow, but chow intake was decreased more effectively than a less preferred cellulose-adulterated food (10).

Such studies suggest that opioids play a role in food preferences, either due to flavor preference or to postingestive actions of nutrients. Mehiel (20) found that conditioned preferences for sugar are attenuated by opioid receptor antagonists. However, the latter study did not differentiate between the taste and postingestive reinforcing actions of sugar. Ramirez (24) found that naloxone decreased the expression of a flavor acceptance that was conditioned by intragastric maltodextrin infusions (24).

Bodnar and Sclafani’s laboratories collaborated on a series of studies examining the involvement of opioids in conditioned flavor preferences. In the first of these studies, Yu et al. (30) studied the effect of naltrexone on acquisition and expression of a conditioned flavor preference in sham-fed rats that were given an arbitrary flavor paired with either a saccharin (less preferred) or sucrose (more preferred) solution. As expected, the flavor paired with sucrose was selected in a two-bottle test. Naltrexone decreased intake but failed to affect either the acquisition or expression of the flavor preference. In a second study, Azzara et al. (4) studied whether naltrexone would affect a flavor preference that was conditioned by in-

Fig. 2. Effect of NTX infusion on total food intake (g/day) and on intake (g/day) of the sucrose and starch diets (experiment 2). A: total food intake. B: sucrose and starch diet intake. Six diet/drug periods were studied (left to right): 1) pre-NTX pump period during which time both the high-starch and high-sucrose diets were present; 2) a period in which the NTX pump was implanted and both diets were present; 3) a period in which the NTX pump was depleted and both diets were present; 4) a period in which only the starch diet was presented; 5) a period in which the NTX pump was implanted and both diets were present; 6) a period in which the NTX pump was depleted and both diets were present. *P < 0.05, Bonferroni correction applied.
tragastric infusion of 16% sucrose or water. A flavor preference was observed for the solution paired with the intragastric infusion of sucrose. Once again naltrrexone decreased intake but failed to inhibit the acquisition or expression of this flavor preference. These studies suggest that opioids are not involved in the conditioning of a flavor preference.

Thus we found that naltrexone infused over a 10-day period had a major effect on preference for a high-sucrose diet over a high-starch diet, but only after a period of abstinence from the preferred sucrose diet. This is concordant with Lynch’s results (19) showing that daily naloxone would limit the observed rise in saccharin ingestion over a 3-wk period, presumably limiting the degree of saccharin preference that developed over the 3 wk. However, it does not agree with the studies of the laboratories of Bodnar and Sclafani (4, 30). One should note that Bodnar and Sclafani’s studies (4, 30) conditioned a taste or intragastric nutrient infusion preference with arbitrary flavors, a procedure that involves learning. In Lynch’s and our studies, the palatable substance was given to the rats directly and therefore did not involve a conditioned preference. It has been hypothesized and supported by data from animals and humans that opioids are involved in the reinforcing properties of sweet solutions but not in taste detection/recognition. Perhaps opioids are only involved when a flavor or nutrient is directly ingested, whereas a learned relationship with another substance may be imprinted by a different neural regulator. Furthermore, many studies have shown that opioids affect memory and/or learning. For example, Flood et al. (9) demonstrated that in mice, immediate posttraining administration of naloxone produces a time-dependent improvement in retention when tested 1 wk later. They also found that pretest administration of naloxone, at a dose that failed to alter acquisition, also improved test performance, suggesting that naloxone also improved recall. Several laboratories have found that opioid antagonists improved working memory-based performance in rats tested on the radial maze (5, 26). It should be noted, however, that others have reported improvement of memory with opioid administration. To further confuse the situation, naltrexone inhibits the expression, but not the acquisition, of a sucrose-reinforced place preference (8). Perhaps place preference conditioning involves different learning pathways than does conditioned taste preference.
A possible reason for the naltrexone-induced inhibition of the sucrose preference might be a drug-induced aversion. That is, rats may have associated the reintroduction of the sucrose diet with the initiation of the naltrexone infusion, a potentially aversive treatment. While Lynch (19) found that pretest treatment with naloxone reduced saccharin intake more than posttest treatment, he also found that posttest treatment reduced saccharin intake compared with saline treatment. The latter suggests that naloxone might have conditioned a taste aversion. Others have suggested that naltrexone/naloxone might be aversive; however, this effect cannot completely account for its anorectic effects (16, 22). It is important to note that in our study the rats experienced the sucrose diet early in the study, without any association with naltrexone, making it less likely for an aversion to have been conditioned after reintroduction of the sucrose diet. Also, we noted a very rapid extinction of the naltrexone effect after the emptying of the naltrexone pumps. Nevertheless, one cannot dismiss the possibility that naltrexone might decrease reacquisition of sucrose preference due to a drug-induced aversion.

Our findings suggest that naltrexone therapy for obesity might be useful if tried in concert with a period of abstention from favored foods. This would be much more complex to evaluate than the current study using rats that only had two diets available. One of the environmental factors that results in hyperphagia in humans is food variety. Nevertheless, conducting further studies evaluating the role of opioid receptors in food preference seems warranted.

This work was supported by the Department of Veterans Affairs, the National Institute of Drug Abuse (DA-03999), and the Minnesota Obesity Center (DK-50456).

REFERENCES