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Naloxone's Anorectic Effect is Dependent Upon the Relative Palatability of Food

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GIRAUDO, S. Q., M. K. GRACE, C. C. WELCH, C. J. BILLINGTON AND A. S. LEVINE. *Naloxone's anorectic effect is dependent upon the relative palatability of food.* PHARMACOL BIOCHEM BEHAV 46(4) 917–921, 1993.—It has been suggested that opioids modify food intake by enhancing palatability. In the present series of studies we evaluated the effect of naloxone on food intake of a preferred food (chocolate chip cookies), normal rat chow, and an "aversive" food (high fiber chow). We found that naloxone decreased 18- and 48-h deprivation-induced intake of chocolate chip cookies much more potently than that of chow, when these foods were presented on separate occasions. When these foods were presented concurrently, this difference in naloxone's potency was no longer apparent. When rats were offered high fiber chow, only the 10 mg/kg dose of naloxone decreased intake. In these same rats naloxone significantly decreased normal chow intake at a dose of 0.1 mg/kg. Thus, naloxone's ability to decrease food intake appears to be dependent upon the palatability of the food.

Naloxone Opiates Opioid Feeding Reward Palatability

OPIOID peptides are thought to be regulators of consummatory behaviors (5,13,21). Administration of opioid agonists increases short-term food intake and antagonists decrease feeding induced by a variety of manipulations. Although the mechanism of their action is unclear, several investigators have suggested that opioids enhance palatability, thus making food more rewarding (3,5,7,15,21). They base this argument on the following data. First, the opioid antagonist, naloxone, decreases the perceived pleasantness of a sweet solution in human subjects (15). Second, morphine increases intake of a preferred food when presented concurrent with a less preferred food (6). Third, much lower doses of naloxone decrease intake of a palatable food when compared with normal rat chow (5). Cooper and Turkish (6) reported that naloxone or naltrexone decreases the time spent eating cookies, a preferred food, while increasing the time spent eating chow. Fourth, eating a palatable food releases β-endorphin from the hypothalamus and alters opioid binding (8). Finally, satiated rats exhibit a naloxone reversible increase in nociceptive thresholds after ingesting highly palatable foods (2).

Opioids also appear to influence macronutrient selection. At least six published reports have shown that morphine and other opiates increase fat intake (16–20,22). Naloxone selectively decreases fat intake when carbohydrate and protein are also presented. However, Gosnell and associates (10) suggest that macronutrient selection is related to the natural preference a rat displays for a given macronutrient. Thus, a carbohydrate-preferring rat would not increase fat intake after morphine injection as dramatically as would a fat-preferring rat. Such data further support the idea that opioids affect palatability.

Investigators have utilized various tastants to study the role of opioids in palatability. Opioid antagonists decrease intake of sweet-tasting solutions such as sucrose, glucose, and saccharin (11). Lynch found that naloxone blocked the acquisition of saccharin preference (15). Naloxone is more effective in reducing intake of saccharin, sucrose, saline, and HCl solutions than water or quinine solutions (14). Opioid agonists increase intake of sweet and salty solutions (4).

In the current study we further examined the role of opioids in palatability. We studied whether the strength of the stimulus that initiated eating would alter the manner in which naloxone decreased intake of a preferred food. We also examined the effect of naloxone on the intake of a preferred food vs. standard rat chow when these foods were presented simultaneously. Finally, we evaluated whether naloxone would decrease intake less potently if the only food presented was "aversive."

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METHOD

Male Sprague-Dawley rats (Harlan; Madison, WI), weighing between 300–400 g, were individually housed in a controlled temperature (21–23°C) vivarium with a 12 L : 12 D photoperiod (lights on at 0700 h). Rats were given access to Purina Laboratory Certified Chow and water ad lib, except as noted below. All studies were conducted in the rats' home cages (suspended stainless steel with open grid floor). Food intake was measured by giving rats preweighed amounts of food, collecting the remainder of that food and the spillage found under the cage after a specified time limit, and reweighing; the difference thereby equaling intake.

The first series of studies was conducted to evaluate whether naloxone decreased intake of a preferred food more effectively than that of their usual diet. We confirmed that rats (n = 60) preferred chocolate chip cookies (three whole cookies placed in cage; Chips Ahoy, Nabisco) to laboratory chow by determining intake of these foods, when offered concurrently, for a 3-day period [cookies: 76 ± 2 kcal/day; laboratory chow: 29 ± 1 kcal/day; average kcal per day for 3 days, $F(1, 118) = 604.84$, $p = 0.0001$]. In the first experiment, five groups of rats (n = 12/group) were deprived of food, but not water, for 18 h to stimulate eating during the light cycle. The rats were then injected subcutaneously with naloxone hydrochloride (0.01, 0.1, 1, and 10 mg/kg, RBI; Natick, MA) or vehicle (0.9% sodium chloride). No rat received the same dose of naloxone more than once. Immediately after drug administration, we placed either two chocolate chip cookies or three pellets of Purina laboratory chow at the bottom of the cage. Food intake was determined as described above. The second experiment was identical to the first, except that the rats (n = 60, 12/group) were deprived of food for 48 h to stimulate eating. The third experiment was identical to second experiment, except that the rats (n = 60, 12/group) were given a choice of cookies or chow after drug administration.

The next experiment was conducted on a new set of rats (n = 39) to evaluate whether naloxone decreased intake of laboratory chow more effectively than of nonpreferred food. The design of this experiment was identical to that of the 48-h deprivation study above, except that the rats were given either a high-fiber diet (ground laboratory chow/powered cellulose mixed 1 : 1 by weight) or ground laboratory chow following drug administration. Diets were placed in glass jars, preweighed, and then placed inside cages. Rats failed to ingest the high-fiber diet when it was offered concurrently with laboratory chow, indicating that it was clearly nonpreferred.

All data are presented as the mean ± the standard error of the mean. To avoid problems associated with varying baseline intakes of the presented foods, data were transformed to percent of intake of vehicle-injected rats. These data were then analyzed by analysis of variance and means were compared using the least significant different test. To compare the effect of naloxone on 18-h vs. 48-h deprivation-induced feeding, we estimated the ED_{50} (effective dose that causes a 50% decrease in intake compared to control) of naloxone using regression analysis (food intake vs. log naloxone dose).

RESULTS

The 18-h deprived rats ingested 4.9 g (17.3 kcal) of chocolate chip cookies or 6.8 g (23.2 kcal) of chow 1 h after vehicle injection. During the first and second hours of the study there were significant main effects of naloxone [hour 0–1: $F(4, 110) = 26.80$, $p = 0.0001$; hour 0–2: $F = 31.79$, $p = 0.0001$] and diet [hour 0–1: $F(1, 110) = 15.58$, $p = 0.0001$; hour 0–2: $F = 12.89$, $p = 0.0005$] on food intake. The interaction between naloxone and diet was not significant at either time point. The lowest dose of naloxone (0.01 mg/kg) decreased intake of the cookies by about 55% during the first hour, whereas this dose of naloxone failed to significantly decrease intake of laboratory chow (Fig. 1). At all doses, naloxone decreased intake of cookies more effectively than that of laboratory chow. The ED_{50} for naloxone in the cookie group was 0.01 mg/kg and in the chow group 0.8 mg/kg.

The 48-h deprived rats ingested 9.4 g (33.1 kcal) of chocolate chip cookies or 8.0 g (27.3 kcal) of chow following vehicle injection. In this study, naloxone also decreased intake of the cookies more effectively than the laboratory chow (Fig. 2). Once again, during the first and second hours of the study there were significant main effects of naloxone [hour 0–1: $F(4, 110) = 39.65$, $p = 0.0001$; hour 0–2: $F = 50.96$, $p = 0.0001$] and diet [hour 0–1: $F(1, 110) = 10.77$, $p = 0.0014$; hour 0–2: $F = 8.59$, $p = 0.0041$] on food intake. The interaction between naloxone and diet was not significant at either time point. The lowest dose of naloxone (0.01 mg/kg) decreased intake of the cookies by about 30% during the first hour, whereas this dose of naloxone failed to significantly decrease intake of laboratory chow (Fig. 2). The ED_{50} for naloxone in the cookie group was 0.1 mg/kg and in the chow group 0.9 mg/kg.

In the third experiment we offered rats a choice between chow and cookies following a 48-h food deprivation. Rats injected with the vehicle ingested 6.3 g (22.2 kcal) of cookies and 2.5 g (8.5 kcal) of chow during the first hour of the study. During the first and second hours of study there was a significant main effect of naloxone [hour 0–1: $F(4, 110) = 12.18$, $p = 0.0001$; hour 0–2: $F = 8.81$, $p = 0.0001$], but not of diet [hour 0–1: $F(4, 110) = 1.01$, $p = 0.3174$; hour 0–2: $F = 0.64$, $p = 0.4266$]. The 0.1 and 1 mg/kg doses of naloxone significantly decreased cookies, but not chow intake (Fig. 3). Although the absolute decrease in cookie intake was greater than that of chow, the percent decrease in cookies and chow intake was similar.

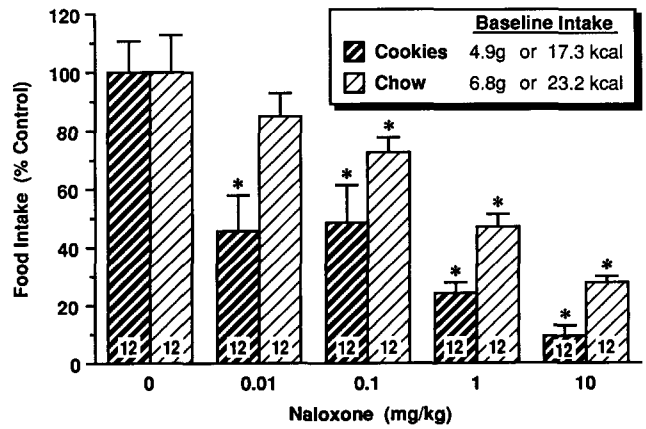

FIG. 1. Effect of naloxone on 18-h deprivation-stimulated intake (hour 0–1) of laboratory chow and chocolate chip cookies. In this study the rats were given either laboratory chow or chocolate chip cookies, but on different occasions. The amounts of chow or cookies that were consumed following a vehicle injection are shown on the legend. The number of rats studied in each group are shown on the individual bars. *$p < 0.05$ compared to vehicle injected controls.

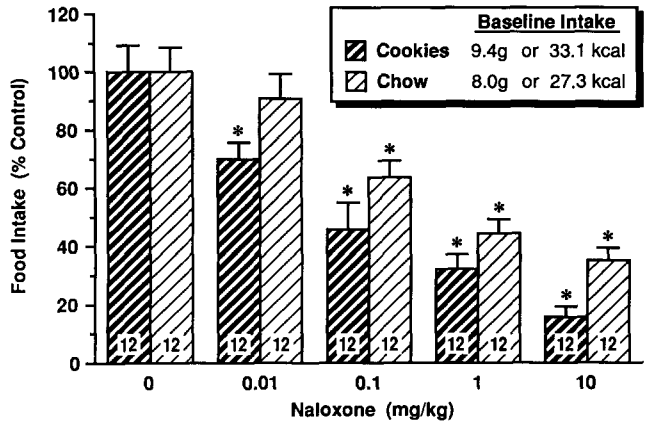

FIG. 2. Effect of naloxone on 48-h deprivation-stimulated intake (hour 0–1) of laboratory chow and chocolate chip cookies. In this study the rats were given either laboratory chow or chocolate chip cookies, but on different occasions. The amounts of chow or cookies that were consumed following a vehicle injection are shown on the legend. The number of rats studied in each group are shown on the individual bars. *$p < 0.05$ compared to vehicle injected controls.

In the last study we evaluated the effect of naloxone on intake of a nonpreferred food (high fiber chow) or normal laboratory chow, when presented on separate occasions. Following 48 h of food deprivation, the vehicle-injected rats ate 6.2 g (10.6 kcal) of the high fiber chow or 11.6 g (39.6 kcal) of normal chow. During the first and second hours of the study there were significant main effects of naloxone [hour 0–1: $F(4, 68) = 15.17$, $p = 0.0001$; hour 0–2: $F = 12.31$, $p = 0.0001$) and diet [hour 0–1: $F(1, 68) = 5.74$, $p = 0.0193$; hour 0–2: $F = 6.389$, $p = 0.0138$] on food intake. The interaction between naloxone and diet was also significant at both time points [hour 0–1: $F(4, 68) = 3.175$, $p = 0.0188$; hour 0–2: $F = 2.72$, $p = 0.0367$]. With the exception of the 0.01 mg/kg dose of naloxone, all doses decreased intake of normal chow by 42–72% (Fig. 4). In contrast, only the 10 mg/kg dose of naloxone significantly altered intake of the high fiber chow.

DISCUSSION

In this series of experiments we found that naloxone decreased intake of a preferred food much more potently than chow. In the first experiment, we had hypothesized that naloxone would more potently block feeding induced by the preferred, and, therefore, more palatable cookie diet. The estimated ED_{50} (using regression analysis) of naloxone was 0.01 mg/kg when rats were offered cookies after an 18-h fast and 0.8 mg/kg when offered laboratory chow. These data support the reports of others, indicating that opioids impact food palatability. Apfelbaum and Mandenoff (1) found that naltrexone decreased intake of a cafeteria diet during a 17-week period in rats, but failed to decrease intake of chow. Lynch (15) found that acquisition of saccharin preference in rats was blocked by pretreatment with naloxone. We found that naloxone was more effective in reducing the intake of saccharin, sucrose, saline, and HCl solutions than in reducing the intake of water or quinine solutions (14). Studies in humans have suggested that blockade of opioid receptors decreases pleasantness of certain foods, including sweet solutions, sugar/fat mixtures, and salted soup (3,7,9,23,24). It should be noted that Heatherington et al. (12) failed to observe any effect of naltrexone on preference or intake of food.

In the second experiment, we hypothesized that naloxone would block intake of the preferred cookie food more potently than chow, even when the animals were very hungry. We believed this might be so because naloxone is known to block feeding induced by deprivation and by various pharmacological feeding stimulators. We reasoned that there might be a component of preference or palatability in the food intake occurring in response to deprivation that accounts in part for the naloxone sensitivity in hungry rats. In other words, the animals might still want to eat a preferred food even when "hungry." That component of preference would, therefore,

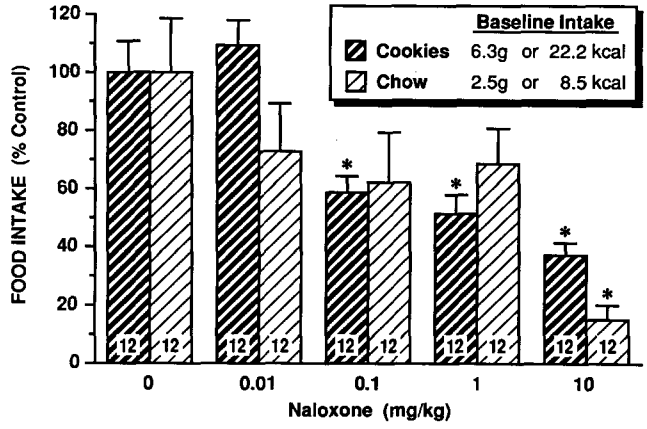

FIG. 3. Effect of naloxone on 48-h deprivation-stimulated intake (hour 0–1) of laboratory chow and chocolate chip cookies. In this study the rats were given a choice of laboratory chow or chocolate chip cookies. The amounts of chow or cookies that were consumed following a vehicle injection are shown on the legend. The number of rats studied in each group are shown on the individual bars. *$p <$ 0.05 compared to vehicle injected controls.

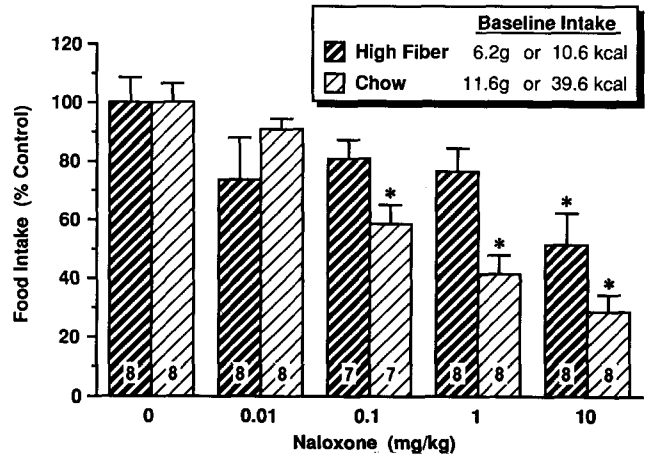

FIG. 4. Effect of naloxone on 48-h deprivation-stimulated intake (hour 0–1) of laboratory chow and high-fiber chow. In this study the rats were given either laboratory chow or high-fiber chow, but on different occasions. The amounts of chow or high-fiber chow that were consumed following a vehicle injection are shown on the legend. The number of rats studied in each group are shown on the individual bars. *$p < 0.05$ compared to vehicle injected controls.

make the animals consuming a preferred food more sensitive to naloxone. The data indicates that after a 48-h deprivation period, naloxone blocked preferred food intake more potently than chow intake. However, the difference in the ED_{50} was not as great as in the 18-h deprived rats (ED_{50} = 0.1 mg/kg in the cookie group vs. 0.9 mg/kg in the chow group). This suggests that the degree of "hunger" does, indeed, interact with the rewarding properties of food.

The third experiment is an attempt to replicate findings of Cooper and Turkish (6) in the present context. The design called for a choice between chow and preferred food after 48 h of deprivation. We found that naloxone decreased food intake of both cookies and chow. The absolute decrease was greater in the cookie group, but because the baseline intake was also higher in that group, the percent effect was similar in the two groups. Although it appeared that a higher dose of naloxone (10 mg/kg) was needed to decrease chow intake compared to the dose that decreased cookie intake (0.1 mg/kg), there was no significant main effect of diet type. In contrast, Cooper and Turkish (6) found that naltrexone in doses from 0.05 to 5 mg/kg decreased intake of chocolate cookies 44 to 79% (approximately 5.2 g cookies), while the intake of chow increased by as much as 600% (an increase of 0.5 g chow). We are uncertain why the different results were obtained.

In the fourth experiment, we attempted a corollary experiment. If enhancing the preferability or palatability of food increases the susceptibility to naloxone blockade, shouldn't reducing the palatability also reduce susceptibility to naloxone? The results are consistent with this hypothesis. As in the second experiment, the animals had been deprived for 48 h; therefore, the interaction of hunger and palatability is, again, demonstrated. Food intake occurring in the fiber group might be regarded as pure "hunger"-induced intake, while the chow intake appears to retain some palatability. This might then account for the ability of naloxone to block chow intake even in a very hungry animal.

Another perspective holds that the opioids are more related to macronutrient appetites, specifically fat intake, than to palatability. Many studies have shown that opioid agonists increase and opioid antagonists decrease high-fat diet intake (16–20,22). Naloxone has been reported to decrease carbohydrate intake as well, though not as potently as fat intake. In our study, the cookies are a high fat diet (~36% kcal from fat) relative to the chow diet (~12% kcal from fat) and the chow plus fiber diet (~12% kcal from fat, assuming that only negligible energy is derived from the cellulose). Although the chow and chow plus fiber diets both contained the same amount of fat (~12% kcal from fat), we found that naloxone more effectively reduced intake of chow. This suggests that palatability, rather than fat content per se, influences the potency of naloxone.

The results from our study further support the idea that opioids are important mediators of food palatability. Blockade of the opioid receptor decreased intake of a preferred food much more potently than normal laboratory chow. Adulteration of normal chow with cellulose decreased the potency with which naloxone decreased food intake. Thus, the potency of naloxone's anorectic effect appears to follow a "preference continuum" with high doses (10 mg/kg) needed to decrease intake of an aversive food and very low doses (0.01 mg/kg) to decrease that of a highly preferred food.

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