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BRIEF COMMUNICATION

The Effect of Centrally Administered Naloxone on Deprivation and Drug-Induced Feeding

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LEVINE, A. S., M. GRACE AND C. J. BILLINGTON. *The effect of centrally administered naloxone on deprivation and drug-induced feeding*. PHARMACOL BIOCHEM BEHAV **36**(2) 409–412, 1990.—In the present study we evaluated the effect of intracerebroventricular (ICV) administration of naloxone on feeding induced by food deprivation, norepinephrine (NE), muscimol and neuropeptide Y (NPY). Naloxone (200, 100 and 50 μg ICV) decreased deprivation-induced feeding. In contrast, only the 200 μg dose of naloxone decreased NE-induced feeding and the 200 and 100 μg doses decreased muscimol-induced feeding. Eating stimulated by central administration of NPY was potently decreased by doses of naloxone ranging from 10–200 μg.

Naloxone Food intake Food deprivation Muscimol Neuropeptide Y Norepinephrine

THE opioid antagonist, naloxone, can decrease short-term food intake which has been provoked by a wide variety of stimuli (1, 9, 13). Among those stimuli are food deprivation, stress, electrical stimulation, and social conflict (1, 9, 13). In addition, several drugs induce feeding that can be blocked by peripheral administration of naloxone: adenosine, benzodiazepines, 2-deoxyglucose, muscimol, norepinephrine, and neuropeptide Y (1, 2, 9, 10, 13).

Those investigations which demonstrate that naloxone can decrease feeding induced by a variety of stimuli have two drawbacks. First, different doses and experimental designs have been utilized. Second, most studies have used relatively large doses of naloxone which have been administered peripherally. For example, it has been reported that feeding induced by muscimol (11) and norepinephrine (12) was decreased by doses of naloxone ranging from 5–10 mg/kg; and feeding induced by neuropeptide Y (8) by doses ranging from 1–10 mg/kg. These peripheral doses are far in excess of effective central doses (3, 9, 16). Since naloxone is thought to act centrally (3,9), it seems preferable to use a central route of administration to evaluate the effect of naloxone on deprivation and drug-induced feeding. In the present study we compared the dose of centrally administered naloxone required to decrease feeding induced by the natural stimulus of food deprivation to the naloxone dose required to decrease feeding induced by prominent centrally acting neuropeptides. Using this design, we feel more confident in suggesting the role that opioids might play in regulating these feeding paradigms.

METHOD

One hundred male Sprague-Dawley rats (BioLab, St. Paul, MN) weighing between 225–250 g at the time of arrival were housed in a controlled temperature (21–23°C) and light (12 hr dark/12 hr light; lights on 0700) vivarium. Animals were anesthetized with nembutal and cannulas were placed 0.5 mm posterior and 1.5 mm lateral to bregma and extended 3.5–3.75 mm below the outer surface of the skull (right lateral ventricle). Following surgery, these rats were placed into individual cages and allowed a seven day recovery period. None of the animals received the same dose of any one orexigenic agent more than once.

Food intake was stimulated by food deprivation (24 hours) or intracerebroventricular (ICV) injection of neuropeptide Y (NPY; 5 μg/5 μl), norepinephrine (NE; 20 μg/5 μl) or muscimol (500 ng/5 μl). NPY was purchased from Peninsula Laboratories (Belmont, CA) and NE and muscimol were purchased from Sigma Chemical Company (St. Louis, MO). Naloxone (10, 50, 100 and 200 μg/5 μl, RBI; Natick, MA) or vehicle was injected ICV immediately before injection of the orexigenic agents. Preweighed food was

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TABLE 1

EFFECT OF NALOXONE ON CUMULATIVE FOOD INTAKE INDUCED BY FOOD DEPRIVATION OR DRUG ADMINISTRATION

	Cumulative Food Intake (g)							
	FD		NE		Muscimol		NPY	
	0–1 hr	0–2 hr	0–1 hr	0–2 hr	0–1 hr	0–2 hr	0–1 hr	0–2 hr
Vehicle*	—	—	0.6 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	1.0 ± 0.3	0.1 ± 0.1	0.1 ± 0.1
Naloxone (0 μg)	5.9 ± 0.5	6.7 ± 0.7	2.1 ± 0.4†	2.2 ± 0.4†	3.3 ± 1.0	4.0 ± 1.0†	4.9 ± 0.4†	4.9 ± 0.4†
Naloxone (10 μg)	4.6 ± 0.5	6.4 ± 0.5	2.6 ± 0.3	3.0 ± 0.3	2.3 ± 0.8	2.4 ± 0.8	2.4 ± 0.4‡	2.4 ± 0.4‡
Naloxone (50 μg)	3.9 ± 0.4‡	5.1 ± 0.5	1.5 ± 0.3	1.7 ± 0.3	2.1 ± 1.0	3.0 ± 1.1	3.0 ± 1.1‡	1.1 ± 0.1‡
Naloxone (100 μg)	2.8 ± 0.5‡	4.0 ± 0.7‡	1.7 ± 0.3	2.0 ± 0.3	1.7 ± 0.6	1.9 ± 0.6‡	1.9 ± 0.6‡	0.9 ± 0.2‡
Naloxone (200 μg)	3.1 ± 0.5‡	4.5 ± 0.7‡	0.5 ± 0.1‡	0.6 ± 0.1‡	0.9 ± 0.4	1.2 ± 0.4‡	1.2 ± 0.4‡	0.7 ± 0.2‡
Rats/group	10–11		15–17		8–11		10	

*Vehicle refers to the baseline intake in the absence of any drug administration
†$p<0.05$ compared to vehicle; ‡$p<0.05$ compared to naloxone (0 μg).

then placed at the bottom of each cage and quantified for the ensuing 2 hours (at intervals of 0–1 and 1–2 hours). Food spillage was collected on the paper under each rat cage and included in the calculation of intake. Water was available ad lib during each experiment.

The results of the first series of experiments indicated that naloxone decreased NPY-induced feeding more effectively than feeding induced by other treatments. To evaluate the specificity of naloxone's effect on NPY-induced feeding we injected both the active (−) and inactive (+) enantiomers of naloxone. Food intake was quantified as described above.

All food intake data are expressed as cumulative values and are represented as the mean ± SEM. The effect of naloxone on drug or food deprivation-induced feeding was evaluated by means of a one- or two-way factorial ANOVA and group means were compared using the least significant difference test.

RESULTS

In the first study animals which were food deprived for 24 hours ingested about 6 g of food during the first hour (Table 1). During the first and second hours of the study, there was a significant main effect of naloxone on food intake [Hour 1: $F(4,51)=6.74$, $p=0.0002$; Hour 2: $F=3.45$, $p=0.0145$]. One hour after intracerebroventricular (ICV) injection of naloxone at doses of 200, 100 and 50 μg there was a significant decrease in food intake compared to the control group (Table 1). Food intake was decreased by 47%, 53% and 35% respectively after injection of 200, 100 and 50 μg of naloxone. We found similar effects on cumulative food intake two hours after injection of naloxone; however, the 50 μg dose no longer was effective (Table 1).

In the next study food intake was stimulated by injection of norepinephrine into the right lateral ventricle. NE increased food intake significantly above the control group (Table 1). There was a significant main effect of drug in this study one hour and two hours after drug administration [Hour 1: $F(5,97)=7.22$, $p=0.0001$; Hour 2: $F=9.21$, $p=0.0001$] (Table 1). However, only the 200 μg dose of naloxone significantly decreased feeding compared with the NE plus saline group (Table 1). In this case food intake was decreased by 72% during the first hour of the study.

We next tested the ability of naloxone to decrease muscimol-induced feeding. Muscimol increased food significantly above the intake of saline injected rats (Table 1). There was a main effect of drug in this study during the second hour, but not during the first hour [Hour 1: $F(5,54)=2.208$, $p=0.068$; Hour 2: $F=2.552$, $p=0.04$]. ICV administration of 200 and 100 μg of naloxone decreased muscimol-induced feeding, whereas the 50 and 10 μg doses had no significant effect (Table 1). Food intake was decreased by 74% in the 200 μg group and 48% in the 100 μg group (hours 0–2).

NPY induced food intake above the vehicle control during the first and second hours of the study (Table 1). All doses of naloxone suppressed NPY-induced feeding [Hour 1: $F(5,59)=38.43$, $p=0.0001$; Hour 2: $F=30.57$, $p=0.0001$]. Feeding was decreased between 85% to 50% over the dose range of naloxone. We tested the stereospecificity of naloxone by injecting both (−) and (+) naloxone into the right lateral ventricle just prior to injection of NPY. As expected, only the active isomer of naloxone (−) significantly decreased NPY-induced feeding (Table 2).

We compared the ability of naloxone to decrease feeding induced by food deprivation or administration of orexigenic agents by expressing the data as a percent of control. A two-way ANOVA demonstrated that there was a main effect of both naloxone [Hour 1: $F(3,166)=7.55$, $p=0.0001$; Hour 2: $F=9.25$, $p=0.0001$] and the manipulations used to induce feeding (food deprivation or NE, muscimol and NPY) during the first and second hours of the study [Hour 1: $F(3,166)=11.95$, $p=0.001$; Hour 2: $F=11.62$, $p=0.0001$]. The interaction after 1 hour of food intake was close to significant and during the second hour was significant [Hour 1: $F(3,9)=1.82$, $p=0.067$; Hour 2: $F=2.57$, $p=0.009$]. NPY was most potently affected by naloxone and NE the least (Fig. 1). We

TABLE 2

EFFECT OF (−) AND (+) NALOXONE ON NPY-INDUCED FEEDING

	Cumulative Food Intake (g)	
	0–1 Hour	0–2 Hours
Vehicle + Vehicle	0.8 ± 0.4	1.6 ± 0.5
NPY (5 μg) + Vehicle	3.1 ± 0.9*	5.2 ± 1.6*
NPY (5 μg) + (−) Naloxone (100 μg)	0.9 ± 0.4†	1.2 ± 0.5†
NPY (5 μg) + (+) Naloxone (100 μg)	2.3 ± 0.8	4.0 ± 1.1

*$p<0.05$ compared to vehicle; †$p<0.05$ compared to NPY (5 μg) + Vehicle.

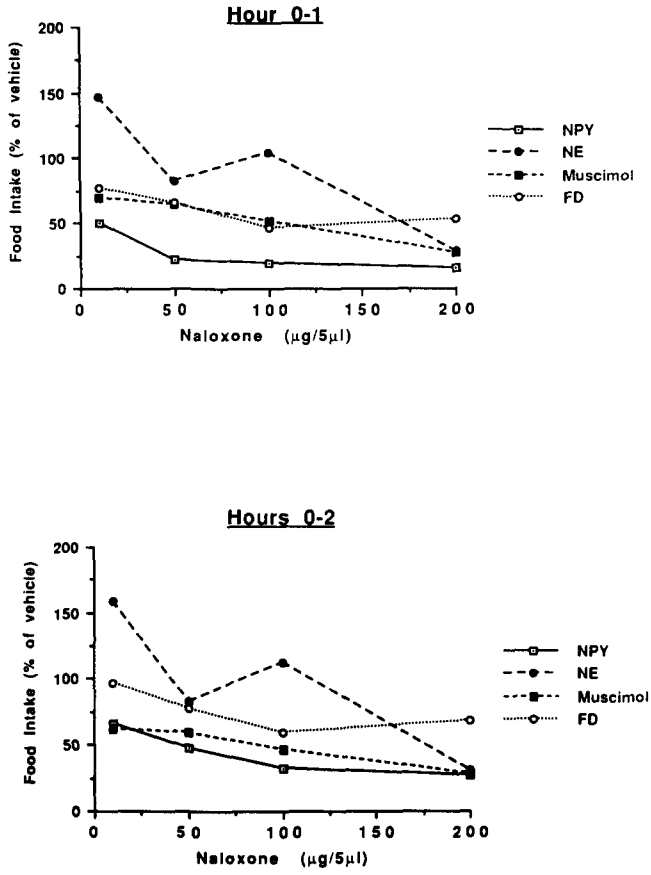

FIG. 1. Effect of central administration of naloxone on NPY, NE, muscimol and food deprivation (FD)-induced feeding. Data are derived from data presented in Table 1.

estimated ED_{50} values from equations derived from a log (abscissa)/linear (ordinate) plot of the data presented in Fig. 1. The doses of naloxone which reduced feeding by 50% in the NPY, muscimol, food deprivation and NE groups respectively were 7.9, 72.2, 173.8 and 186.2 μg during the first hour of the study and 33.9, 52.5, 549.5 and 204.2 μg during the first two hours of the study.

DISCUSSION

Holtzman (2) first demonstrated that naloxone decreased food and water intake in rats. Since that initial report, investigators have shown that naloxone decreases feeding in a variety of species under a variety of conditions (1, 9, 13). This opioid antagonist appears to reduce the size and duration of initial meal following injection as well as the first postmeal interval (5). Naloxone's effect on feeding does not seem to be due to a decrease in motor activity or illness (4,7). Recently Kirkham and Cooper (6) have demonstrated that (−) naloxone attenuates sham feeding, suggesting that opioids may be involved in orosensory reward. This observation might explain why naloxone is capable of decreasing the effect of a wide variety of drugs or manipulations which increase food intake.

We previously reported (11,12) that subcutaneous naloxone at doses ranging from 5–10 mg/kg decreased NE-induced feeding (20 μg ICV) and muscimol-induced feeding (500 ng ICV). NPY-induced feeding (5 μg ICV) was decreased one hour after subcutaneous administration of naloxone at doses of 10, 5 and 1 mg/kg by 60–75% (8). Since naloxone is thought to act centrally (3, 9, 16), we evaluated its effects on deprivation and drug-induced feeding after central administration. We found that intraventricular administration of naloxone decreased food intake induced by food deprivation or by administration of NE, muscimol and NPY. During the first hour of our study the ED_{50} for naloxone ranged from about 8 μg to 186 μg, depending on the means of stimulating food intake. NPY-induced feeding was the most sensitive to naloxone's suppressive effect on food intake and NE the least. The 10 μg dose significantly decreased feeding in the NPY group, but was not capable of decreasing food intake significantly in any of the other groups. Food deprivation induced feeding (hour 0–1) was only decreased by 44% or 2.0 g after a dose of 50 μg naloxone, whereas at an equivalent dose NPY-induced feeding was decreased by 88% or 3.8 g. NE and muscimol-induced feeding were not altered by central injection of 50 μg of naloxone.

In conclusion, we found that central administration of naloxone reliably decreased food intake induced by NPY and food deprivation at doses which generally do not decrease feeding when given peripherally (14,15). NPY-induced feeding was virtually blocked by naloxone suggesting that such feeding is dependent upon opioids. NE and muscimol-induced feeding decreased significantly only after administration of 100 or 200 μg of naloxone, doses which can decrease feeding when given peripherally to a rat (equivalent to 0.33 to 0.67 mg/kg in 300 g rats). Thus, although naloxone has been reported to decrease feeding after a variety of manipulation which increase feeding, it may not do so with equivalent effectiveness. NPY-induced feeding is potently inhibited by intraventricular administration of naloxone, whereas NE-induced feeding is only affected by relatively large doses of naloxone.

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