

# Naloxone-Induced Suppression of Food Intake in Normal and Hypothalamic Obese Rats

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Received 3 October 1979

KING, B. M., F. X. CASTELLANOS, A. J. KASTIN, M. C. BERZAS, M. D. MAUK, G. A. OLSON AND R. D. OLSON. *Naloxone-induced suppression of food intake in normal and hypothalamic obese rats.* PHARMAC. BIOCHEM. BEHAV. 11(6) 729-732, 1979.—Intraperitoneal injections of naloxone hydrochloride (1, 2, 4, and 8 mg/kg) suppressed food intake in both normal and hypothalamic obese rats maintained on a 4-hr per day feeding schedule. The decrease in feeding was more pronounced in the animals with ventromedial hypothalamic lesions. Appetitively motivated feeding, i.e., the consumption of sweetened milk under nondeprived conditions, was also suppressed by naloxone, but there was no reliable difference between groups. It is concluded that opiate receptors located in the ventromedial hypothalamus are not essential for the effects of opiate agonists and antagonists on feeding behavior.

Naloxone      Ventromedial hypothalamus      Food intake

RECENT evidence has implicated opiate receptor mechanisms in the regulation of food intake. Systemic administration of naloxone, an opiate antagonist, has been reported to decrease feeding in food deprived animals [2, 5, 10, 11], confirming earlier reports of naloxone-induced decreases in responding on operant schedules for food reinforcement [4, 6, 7, 12]. Margules *et al.* [16] reported that the suppressive effects of naloxone on feeding is even more pronounced in genetically obese rats and mice. Such animals were found to have elevated levels of  $\beta$ -endorphin, an endogenous polypeptide hormone with opiate-like activity, in the blood and the pituitary. Marked increases in feeding after intraventricular administration of either morphine or  $\beta$ -endorphin has recently been reported with food-deprived normal rats [1,13].

The medial hypothalamus is one of several areas of the brain found to be high in opiate receptor sites [14,18]. Microinjection of  $\beta$ -endorphin directly into the ventromedial hypothalamus has been found to increase food intake in satiated rats, an effect which can be blocked by naltrexone [8]. If the effects of opiate agonists and antagonists on feeding behavior are mediated by receptors in the ventromedial hypothalamus, damage to which has long been known to result in marked overeating and obesity [3,9], then lesions of the VMH should attenuate the suppressive effects of naloxone on food intake. This hypothesis was tested by examining the effects of naloxone on food intake in deprived and satiated rats.

## METHOD

### Animals

Six unoperated and six VMH-lesioned adult female

Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA) were used. The animals were fed ad lib for 50 days before being placed on a schedule of food deprivation, at which time the control and VMH-lesioned rats weighed an average of 289.1 g and 666.1 g, respectively. All animals were individually caged in a temperature controlled colony (22-24°C) with a 12-hr light/dark cycle (lights on at 8:00 a.m.) throughout the course of the experiment.

### Surgery and Histology

Bilateral ventromedial hypothalamic lesions were produced under sodium pentobarbital (Nembutal) anesthesia (50 mg/kg) by passing a 1.5 mA anodal current between the 0.5 mm uninsulated tip of a teflon-insulated stainless steel electrode (No. 0 insect pin) and a rectal cathode for 20 sec. With the upper incisor bar positioned 5 mm above the interaural line, the electrodes were stereotaxically positioned 0.8 mm posterior to the bregma, 0.7 mm lateral to the midsagittal suture, and 10.0 mm below the surface of the skull.

Upon completion of the experiment, animals with VMH lesions were anesthetized and intracardially perfused with isotonic saline followed by a 10% formol saline solution. Histological analysis was performed by light microscopic examination of cresyl violet stained 50  $\mu$  coronal sections, cut on a freezing microtome. The atlas of Pellegrino and Cushman [17] was used in estimating the extent of the lesions.

### Procedure

*Part 1.* Fifty days after surgery, the hypothalamic obese (n=6) and unoperated (n=6) animals were placed on a deprivation schedule on which food was available for only four hours per day (12:00 noon-4:00 p.m.). The animals were not

deprived of water. As animals with VMH lesions are hyper-reactive to handling, all the rats received intraperitoneal injections of isotonic saline before feeding in order to habituate them to the experimental procedure. The animals received injections of naloxone hydrochloride in 0.9 percent saline (1, 2, 4, and 8 mg/ml/kg administered in ascending order) every third day beginning on the ninth day of the food deprivation schedule. Food intake after injections of naloxone was compared to that after control injections of 0.9 percent saline on the immediately preceding day. All injections were administered 5 min before feeding. Food intake (Ralston Purina Rodent Laboratory Chow) was measured hourly by subtracting spillage (collected on paper towels) and uneaten food from the premeasured supply.

*Part 2.* The second part of the experiment examined the effects of naloxone on appetitively-induced feeding behavior, i.e., the consumption of sweetened milk under nondeprived conditions. After the last injection of naloxone in Part 1, all of the animals were replaced on an ad lib diet of rat chow and allowed to consume sweetened milk (Borden's condensed sweetened milk and water 1:2) for 10 min per day in order to adapt them to the novel diet. Testing with naloxone (1, 2, 4, and 8 mg/ml/kg administered in ascending order) began 10 days after the return to ad lib feeding conditions. Naloxone was administered every fourth day and the effects on 30-min milk consumption compared to the amount of milk consumed after injections of diluent on the immediately preceding day. All injections were given 15 min before the presentation of the milk diet. Regular lab chow was available to the animals at all times, including during the test sessions. Calibrated bottles were used to measure the amount of milk consumed.

#### Statistical Analysis

The primary dependent variable for all phases of the study was the ratio of food intake after administration of naloxone relative to intake after administration of diluent, expressed as a percentage. Data for both phases of the experiment were analyzed by a mixed analysis of variance and, where appropriate, by Duncan's Multiple-Range Test.

#### RESULTS

Mean 1-hr and 4-hr food intakes (expressed as a percentage of the intake after injections of diluent) are presented in Fig. 1. Analysis of variance of the first hour food intake yielded a significant effect of the lesion,  $F(1,10)=12.04$ ,  $p<0.01$ , with VMH-lesioned animals exhibiting a greater decrease in eating after administration of naloxone than control animals. A similar analysis performed on total 4-hr intake produced no reliable differences between groups. As may be seen in Fig. 1, less suppression was found for total 4-hr intake than during the first hour after administration of naloxone, indicating a rebound in feeding by both groups in hours 2-4. Neither analysis yielded a statistically reliable interaction or effect of dose.

Mean 30-min milk consumption for the second phase of the experiment is presented in Fig. 2. Analysis of variance yielded a significant effect for dose,  $F(3,30)=5.44$ ,  $p<0.01$ , but no other reliable effects. Duncan's Multiple-Range Test revealed that 8 mg/kg naloxone produced reliably less eating than did 1 mg/kg ( $p<0.05$ ), but none of the other comparisons among the doses were significant.

Histological analysis revealed that all of the obese rats

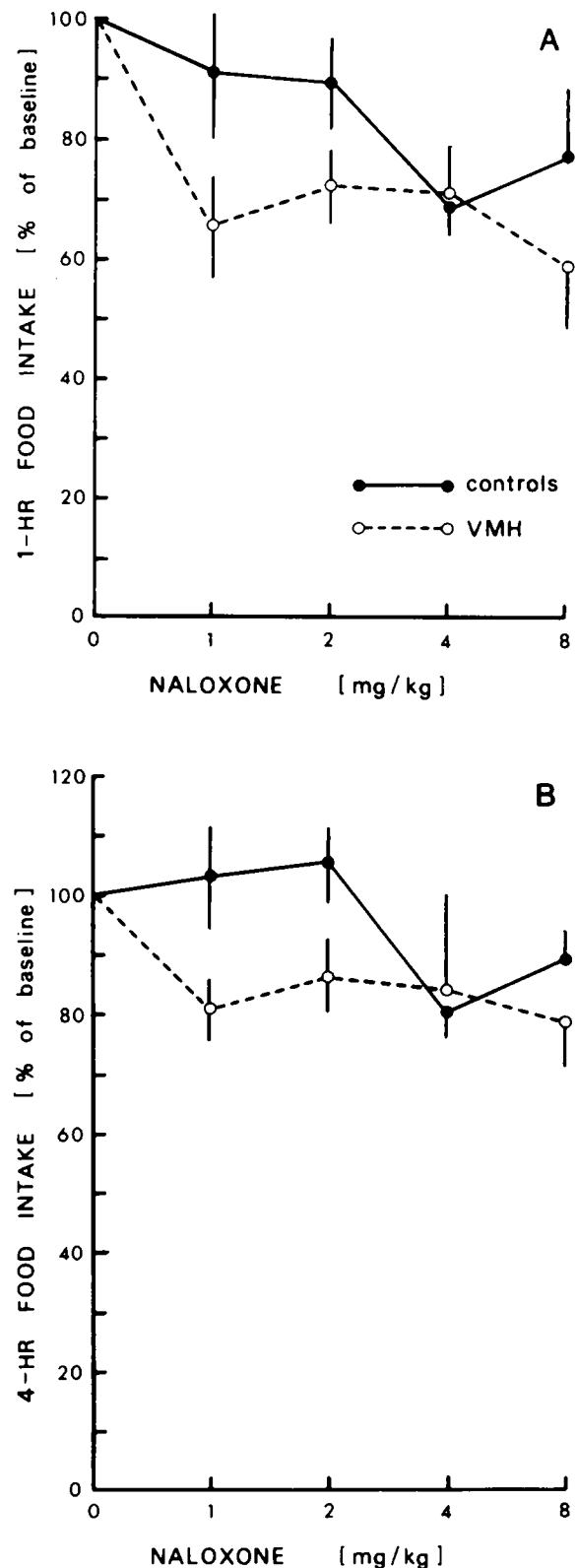


FIG. 1. Mean 1-hr (A) and 4-hr (B) food intake  $\pm$  S.E.M. (expressed as a percentage of baseline intake) after intraperitoneal injections of naloxone in normal and hypothalamic obese rats maintained on a 4-hr per day feeding schedule.

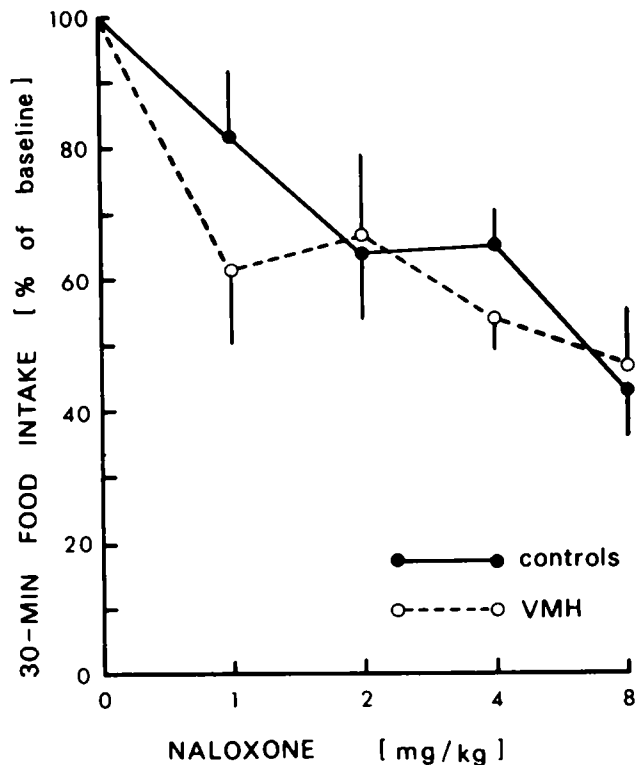


FIG. 2. Mean 30-min milk consumption  $\pm$  S.E.M. (expressed as a percentage of baseline intake) after injections of naloxone in normal and hypothalamic obese rats maintained on an ad libitum diet of lab chow.

had extensive bilateral damage to the ventromedial and arcuate nuclei of the hypothalamus. The lesions in two of the animals extended into the dorsomedial hypothalamus as well. All the lesions were bordered laterally by the fornix and rarely extended beyond the rostral or caudal borders of the ventromedial hypothalamic nuclei.

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#### DISCUSSION

Previous studies have found marked increases in feeding after intraventricular administration of morphine or  $\beta$ -endorphin [1,13]. Moderate increases in food intake in satiated animals has been reported after microinjections of  $\beta$ -endorphin directly into the ventromedial hypothalamus [8]. Several studies have reported that naloxone, which binds preferentially to opiate receptor sites [18], suppresses food intake in normal animals [2, 4, 5, 6, 7, 10, 11, 12]. Although the medial hypothalamus is known to be moderately high in opiate receptor sites [14,18], the results of the present experiment indicate that ventromedial hypothalamic opiate receptors are not essential for the effects of opiate agonists and antagonists on feeding behavior. Naloxone suppressed appetitively-motivated feeding (i.e., sweet milk) to an equal degree in both normal and hypothalamic obese animals, and the suppressive effects were more pronounced in the VMH-lesioned rats when the animals were maintained on a 4-hr per day feeding schedule.

It is possible, of course, that the enhanced suppression of food intake displayed by the hypothalamic obese animals in Part 1 was the result of their receiving greater absolute amounts of naloxone than control animals. However, as may be seen in Fig. 1A, the VMH-lesioned rats displayed considerably greater suppression of food intake in the first hour after 1 mg/kg naloxone than did the control animals after 2mg/kg, which represents a nearly equal absolute amount of naloxone because of the differences in body weight.

Systemic administration of naloxone has been reported to cause nausea [7] and supports taste aversion conditioning [5,15] at high doses (i.e., 9.6-10.0 mg/kg). Although there have been a few reports that the acquisition of taste aversions is enhanced by VMH lesions [19, 20, 21], there was no evidence that the doses of naloxone used in the present experiment resulted in taste aversions for either group. Neither the hypothalamic obese nor the control animals displayed any reduction in baseline intake over sessions in either phase of the experiment. It is unlikely, therefore, that the suppression of feeding observed after administration of naloxone was the result of illness or some other nonspecific effect.

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