Effects of Naloxone and Cholecystokinin on Food and Water Intake in Vasopressin-Deficient Rats (Brattleboro Strain)¹

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YIRMIYA, R. AND M. D. HOLDER. Effects of naloxone and cholecystokinin on food and water intake in vasopressin-deficient rats (Brattleboro strain). PEPTIDES 8(5) 763-767, 1987.—Opioid peptides and cholecystokinin (CCK) have been shown to play a role in regulation of feeding behavior. Another neuropeptide that has recently been suggested to be involved in feeding is vasopressin. We explored possible interactions between opiates, CCK and vasopressin in feeding regulation by studying feeding suppression produced by naloxone and CCK in Brattleboro (DI) rats, which are homozygous for diabetes insipidus and lack the ability to synthesize vasopressin. Ten DI and 15 age-matched Long Evans (LE) rats were food deprived for 14 hours on two different days and then injected with naloxone (2.5 mg/kg) on one day or saline on the other. Thirty minutes later the food was returned and food and water consumption were measured after 1, 3 and 4 hr. Naloxone suppressed the food consumption of both DI and LE rats but the suppression was greater for the DI rats. This result was specific to feeding as water consumption was suppressed in LE more than in DI rats. Two weeks later, the same rats were food deprived for 6 hours on two different days and then injected with CCK-8 (2.5 μg/kg) on one day and with saline on the other. Food was returned one minute after the injection and food and water consumption were measured 30 and 60 minutes later. Food intake was reduced equally for both DI and LE rats. Water intake was not reduced. The results suggest that the suppression of feeding by CCK does not require an intact vasopressinergic system. The greater feeding suppression by naloxone in DI rats may suggest that opiates are interacting with vasopressin in producing their effects on food intake.

Vasopressin  Feeding  Drinking  Naloxone  Cholecystokinin (CCK)  Opiates  Brattleboro
Diabetes insipidus  Paraventricular nucleus

The role of opiate peptides and cholecystokinin (CCK) in the control of food intake has been intensively investigated in the past decade (see [4] for review). Systemic injections of opiate agonists increase, and opiate antagonists decrease, food and water intake [32]. CCK has been suggested to function as a satiety peptide since its concentration in plasma markedly increases after a meal [23] and peripheral injection of CCK decreases food intake [15].

Some of the effects of both opiates and CCK on feeding are mediated through receptors in the central nervous system. Intracerebroventricular (ICV) administration of opiate agonists increases food intake [27] while ICV injection of opiate antagonists [18] or CCK [42] decreases food intake. One anatomical site that was found to be particularly sensitive to opiates and CCK administration is the paraventricular nucleus (PVN) of the hypothalamus. Direct injections of morphine or opioid peptides into the PVN increased food intake [22,28] and injections of the opiate antagonist naloxone [41] or CCK [13] decreased feeding. Lesions of the PVN, in addition to causing hyperphagia and obesity [21], attenuated or completely blocked the effects of peripherally administered morphine [36] and CCK [9].

Neurons within the PVN were shown to contain several peptides including opiate peptides [35] and CCK [5]. These peptides are sometimes colocalized with neurohypophysial hormones. For example, dynorphin and Leu-enkephalin were found in vasopressin-containing cells [39] and Met-enkephalin and CCK were found in oxytocin containing cells [29]. Interactions between opiates and vasopressin were also found in physiological studies. For example, endogenous

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opiate peptides are released together with vasopressin during dehydration [30], terminals of vasopressin-containing neurons in the neurohypophysis have opiate receptors whose activation inhibit vasopressin release [17], and morphine inhibits the firing rate of vasopressin secreting cells [8]. In contrast, naloxone injections were found to increase plasma concentrations of vasopressin [34], and potentiate vasopressin response to an injection of hypertonic saline [3], suggesting that under normal conditions endogenous opioid peptides (EOP) are tonically inhibiting vasopressin release. In contrast to opiates, no interactions between CCK and vasopressin have been found.

In addition to its well known antidiuretic and pressor effects, vasopressin has recently been suggested to be involved in feeding behavior [11]. Physiological studies showed that vasopressin is involved in carbohydrate and lipid metabolism [16], mediates the glucoprivic-feeding response after 2-deoxy-D-glucose administration [2] and excites glucose-responsive cells in the hypothalamus [20]. Anatomical studies showed that vasopressin-containing neurons project to many regions of the central nervous system, which are known to be involved in autonomic, visceral and possibly feeding regulation, e.g., central nucleus of the amygdala, parabrachial nucleus and nucleus tractus solitarius (NTS) [37]. The physiological and anatomical interactions between opiates and vasopressin may suggest that opiates interact with vasopressin in producing their effects on feeding. This possibility was studied using Brattleboro (DI) rats, which are homozygous for diabetes insipidus and lack the ability to synthesize vasopressin [38].

Koyuncuoğlu et al. [19] showed that naloxone decreases food and water intake in DI rats. However, in that study only DI rats were used, so no comparison to normal rats was possible. Additionally, Koyuncuoğlu et al. [19] used a high dose of naloxone (10 mg/kg), which could have a nonspecific effect on feeding. In the present study DI and Long Evans (LE) (the strain from which DI rats were derived) rats were injected with naloxone and CCK and the feeding suppression induced by each of these compounds in the two strains was compared. We report that DI rats showed more feeding suppression after naloxone, but not CCK, injection. These results suggest that opiates interact with vasopressin in producing their effects on food intake.

**METHOD**

**Subjects and Apparatus**

Subjects were 10 DI rats, weighing 234–378 g, and 15 age-matched LE rats, weighing 350–483 g (Blue Spruce Farms, Altamont, NY). The rats were housed individually in standard stainless steel cages located in an animal colony room. All testing was done in the animal room during the first half of the light phase of a 14:10 hr light:dark cycle. Food (Purina Rat Pellets) was available in the home cage before and during the experiments as described below. During the experiments, food was presented in stainless steel feeding cups, 5 cm in diameter and 3.5 cm deep. The cups were covered by an aluminum screen, secured by a stainless steel ring, to prevent spillage. The rats had ad lib access to tap water in all phases of the experiment. Water was available from drinking spouts in the back of the cage before and after the experiments. During the experiments, water was presented in 50 ml inverted graduated tubes, secured to the front of the cage.

**Figure 1.** Mean (±S.E.M.) food and water consumption ratios (left and right panels, respectively) of Brattleboro (DI) and Long Evans (LE) rats, injected with naloxone (Nal) on one session and with saline (Sal) on another session. A consumption ratio lower than 1 indicates naloxone-induced feeding or drinking suppression.

**Procedure**

Three days before the first injection day, the regular diet was replaced by Wayne Rodent powdered food, presented to each rat in a feeding cup. The rats were assigned to 4 groups: 2 consisting of DI and 2 of LE rats. The 2 groups within each strain were equated for mean body weight and amount of food and water consumed during a 24-hr period. The rats were food deprived for 14 hr and then one DI and one LE group (n=5 and n=8, respectively) received an injection of 2.5 mg/kg naloxone (Sigma), administered SC in saline, in a volume of 1 ml/kg. The other DI and LE groups (n=5 and n=7, respectively) received a similar injection of isotonic saline. Thirty min after the injection the powdered food was returned. The water spouts in the back of the cage were disconnected and water was presented in the graduated tubes. Food and water consumption were measured to the nearest 0.5 g and ml, respectively, after 1.2 and 4 hr. The powdered food remained in the cages for 5 days after which the same procedure was repeated with the rat to injection assignments reversed so that each rat received both naloxone and saline.

The rats were returned to their regular diet for 2 weeks and then the diet was changed again to the powdered food for 2 days. The rats were food deprived for 6 hr and then one DI group and one LE group (n=5 and n=8, respectively) received an injection of 2.5 µg/kg CCK-8 (Sigma), administered IP in saline, in a volume of 1 ml/kg. The other DI and LE groups (n=5 and n=7, respectively) received at that time a similar injection of isotonic saline. Food was returned 1 min after the injection. Water was again presented in the graduated 50 ml tubes. Food and water consumption were measured 30 and 60 min later. This procedure was repeated 2 days later with the rat to injection assignments reversed so that each rat received both CCK and saline.

**Data Analysis**

Since each rat served as its own control, consumption ratios were computed for each rat by dividing the amount
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FIG. 2. Mean (±S.E.M.) food and water consumption ratios (left and right panels, respectively) of Brattleboro (DI) and Long Evans (LE) rats, injected with CCK-8 (CCK) on one session and saline (Sal) on another session. A consumption ratio lower than 1 indicates CCK-induced feeding or drinking suppression.

consumed after the drug injection (naloxone and CCK) by the amount consumed after the saline injection. This ratio reflected the amount of consumption suppression caused by the drug. In order to determine whether the drug significantly suppressed consumption (i.e., consumption ratio significantly lower than 1) the 95% confidence interval for the mean of each consumption ratio was computed. An upper limit of this interval lower than 1 indicated a significant suppression of intake.

In order to compare the consumption ratios of the two strains, analyses of variance were performed on the food and the water consumption ratios, with the strain (DI and LE) and the drug (naloxone or CCK and saline) as the between group factors and the measurement times as a within subject, repeated measures factor.

RESULTS

The food and water consumption ratios in the naloxone experiment are presented in Fig. 1 (left and right panels, respectively). Naloxone significantly suppressed food consumption in DI rats at all measurement times (i.e., 1, 2, and 4 hr after the injection), as indicated by a lower than 1 upper limit of the 95% confidence interval at each measurement time. In the LE group, however, the suppression was significant only after 1 hr but not after 2 or 4 hr. Naloxone significantly suppressed water consumption of DI rats only 1 hr after the injection but significantly suppressed water consumption of LE rats after 1, 2 and 4 hr. The suppression of food intake was greater for the DI group, F(1,23)=4.19, p<0.05. In contrast, the water consumption ratio of the DI rats was higher than the ratio in LE rats, but this result did not quite reach the traditional level of significance, F(1,23)=3.76, p=0.07. Overall, the naloxone effect decreased with time, indicated by an increase in both food and water consumption ratios during the test period, F(2,22)=3.25, p<0.05, and F(2,22)=13.4, p<0.001, respectively.

The food and water consumption ratios in the CCK experiment are presented in Fig. 2 (left and right panels, respectively). CCK significantly suppressed food intake in both DI and LE groups after both 30 and 60 min (upper limit of 95% confidence limit lower than 1). However, there was no difference between the strains in the consumption ratios (p>0.1). CCK did not suppress water consumption in either the DI or the LE groups and no difference in the consumption ratios was obtained (p>0.1).

DISCUSSION

The results of this study indicate that DI and LE rats show suppression of food intake after both naloxone and CCK administration. Additionally, we found that the two strains differ in the magnitude of feeding suppression produced by naloxone, but not by CCK. These results support previous findings showing naloxone-induced suppression of feeding [19] and drinking [6] in DI rats, and they extend these findings by showing that in comparison to control LE rats, DI rats display less feeding suppression by naloxone. This result is specific to feeding, as naloxone suppressed drinking more in LE than DI rats though this strain effect was not statistically significant.

The suppressive effects of peripherally injected naloxone on feeding are well documented [32]. The naloxone effect is believed to occur specifically at opiate receptors in the CNS, since quaternary naloxone, which does not pass the blood brain barrier, has no effect on feeding [7]. In a mapping study of the hypothalamic sites sensitive to morphine and naloxone, injections of naloxone into the PVN significantly attenuated food intake in mildly deprived rats, suggesting the existence in this area of opiate receptors that modulate feeding [41]. Taken together, the existence of opiate receptors in the PVN, the fact that naloxone was found to increase vasopressin release [34], and the finding of greater sensitivity of DI rats to the effects of naloxone on feeding reported in the present study, may suggest that vasopressin opposes the suppressive effects of naloxone on feeding. Thus, it could be suggested that normally, vasopressin potentiates the stimulatory effects of EOP on feeding, and that this potentiation is terminated by an inhibitory feedback effect of EOP on vasopressin release. This hypothesis is in accord with the finding that vasopressin and EOP are found in the same vesicles in the pituitary [40], that both are released together by physiological stimuli [30], and that both vasopressin [2] and EOP [24] are involved in glucoprivic feeding behavior. Because both vasopressin [14] and EOP [1] are released during stress, and because stress-induced eating is mediated through endogenous opiates [31], an interaction between these two peptides in the control of feeding during stress is possible. This hypothesis is in accord with the finding that 2-deoxy-D-glucose feeding response, which may be considered as a model for stress-induced feeding [25], is significantly attenuated in DI rats [2].

In addition to their genetic impairment in vasopressin synthesis, DI rats have an altered brain opiate system. For example, they have lower immunoreactive Leu-enkephalin in the neurointermediate lobe of the pituitary [33], they have lower opiate receptor concentration in the diencephalon, but higher concentrations in the cerebral cortex [33], and they show an impaired development of tolerance to the analgesic effects of morphine [12]. These alterations may account for the altered response to naloxone in the present study. For example, if the concentration of opiate receptors in the hypothalamus, which plays a role in feeding, is lower in DI rats (as may be
suggested by the study of Rigter et al. [33], the naloxone dose given would be more effective in blocking these fewer receptors.

The results of the present study show that CCK administration suppresses feeding in both DI and LE rats. This result is consistent with previous studies showing suppression of food intake after CCK administration [15]. CCK has been suggested to modulate feeding not only peripherally, but also as a neurotransmitter that is responsible for delivering the satiety signal within the brain, especially at the NTS [10] and PVN levels [13]. CCK has also been shown to reduce norepinephrine-induced feeding [26], which is known to involve neurons within the PVN [22]. The finding that DI rats exhibit normal feeding response to paraventricular hypothalamic injection of norepinephrine [2] is consistent with the result of the present study, indicating that CCK does not interact with vasopressin in producing its effect on feeding. Further research is needed in order to examine the interactions of vasopressin with other peptides known to be involved in feeding regulation.

REFERENCES


