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The Effect of Opiates and Naloxone on Food Intake in Virgin and Lactating Rats

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WAGER-SRDAR, S. A., B. A. GOSNELL, J. E. MORLEY AND A. S. LEVINE. *The effect of opiates and naloxone on food intake in virgin and lactating rats.* PHARMACOL BIOCHEM BEHAV **23**(3) 345–348, 1985.—Lactation provides an excellent model of non-obese hyperphagia. There is accumulating evidence that endogenous opioids play a role in the modulation of the hormonal changes that occur during lactation. Because endogenous opioids appear also to play a role in the regulation of feeding, we studied the effects of the opiate agonist, butorphanol tartrate, and an opiate antagonist, naloxone, on food intake in virgin female rats and in rats during early, mid and late lactation and during post-weaning. It has been reported that female rats are less sensitive to the suppressant effects of nalmefene, an opioid antagonist, than male rats. Therefore, we also examined the effect of naloxone, an opioid antagonist, on spontaneous nocturnal feeding and 24 hour food deprivation-induced food intake in virgin female rats. We found that female rats were relatively insensitive to the food suppressant effects of naloxone following 24 hour food deprivation, while male rats tested under similar conditions had a decreased intake in response to naloxone. Despite the marked hyperphagia that occurred during lactation, there were minimal alterations in the response to opiate agonists and antagonists during this time period. Our data suggest that endogenous opioids may not play a pivotal role in the hyperphagia of lactation.

Opiate Naloxone Food intake Lactation Hyperphagia

DURING lactation, female rats may increase their food intake 200–300% above pre-mating levels [5, 6, 30]. Upon weaning, food consumption falls rapidly and returns to pre-mating levels by 6–9 days post-weaning [3,30]. The increase in body weight during lactation is slight and adipose tissue may actually decrease [25,30]; therefore, the lactating animal provides an excellent model to study non-obese hyperphagia. At this time, the nature of the signal(s) which mediate this hyperphagic state are unknown, although it has been postulated that decreased estradiol levels may be a contributor [5], as this steroid hormone has been shown to decrease food intake in the non-lactating animal [28,35].

Although there are a vast array of endogenous substances which have been proposed to be putative satiety factors, there are only a few substances which are effective in stimulating food intake. Numerous studies have suggested an important role for endogenous opioid peptides in stimulating feeding [17, 18, 19, 20]. Studies in our laboratory have favored a central role for the kappa opioid receptor and its endogenous ligand in initiating feeding [19]. This feeding drive appears to be modulated by a number of neurotransmitters, neuropeptides and nutrients both centrally and peripherally [16]. However, others have provided evidence favoring a role for β-endorphin [11] and also for the delta opioid receptor [34].

There have been several lines of evidence suggesting that sex hormones regulate the endogenous opioid system. Levels of β-endorphin and met-enkephalin have been found to change throughout the estrous cycle [10]. Males have higher levels of β-endorphin in the neurointermediate lobe of the pituitary [24]. The stress induced release of β-endorphin was attenuated by estradiol benzoate [24]. Morley, *et al.* found that ovariectomized rats have a decreased sensitivity to the effects of ketocyclazocine (a kappa agonist) and an increased sensitivity to naloxone (an opiate antagonist) on feeding when compared to sham operated female rats [22].

In addition to the possible role of the altered hormonal milieu on opioid activity during lactation, evidence is accumulating that suckling produces a direct alteration of opioid peptide levels. There is growing evidence that opioids regulate oxytocin release from the neurohypophysis [8]. It has been shown that naloxone can block the inhibition of LH release during hyperprolactinemia in human subjects [7,27]. These findings suggest that the opioids play a role in mediation of gonadotropins during hyperprolactinemia. Further, Sirinathsinghji and Martini [30] have provided evidence suggesting that the suckling stimulus results in an increase in endogenous opioids in the central nervous system and that this opioid surge with suckling is partially responsible for the increased prolactin and decreased LH levels seen during lactation. Thus, both suckling itself and the altered hormonal milieu during lactation could lead to increased endogenous opioid activity during lactation. In turn, this increased opioid activity may play a role in the hyperphagia of lactation.

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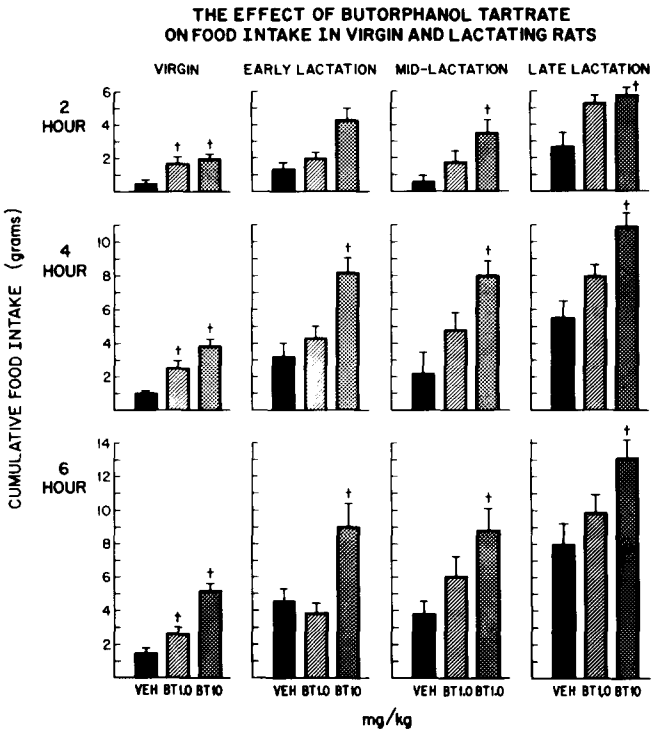

FIG. 1. The effect of butorphanol tartrate on food intake in virgin, early, mid and late lactating and post-weaning female rats. $p<0.05$. Virgin: $F(2,39)=25.22$, $p<0.0001$; Early: $F(2,31)=9.80$, $p<0.005$; Mid: $F(2,30)=11.26$, $p<0.0002$; Late: $F(2,21)=8.24$, $p<0.002$. †$p<0.05$.

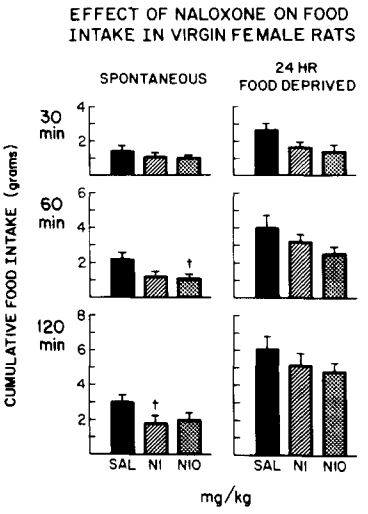

FIG. 2. The effect of naloxone on spontaneous nocturnal and 24 hour food deprivation induced food intake in virgin female rats. Spontaneous: $F(2,31)=3.66$; $p<0.05$; 24 hour deprived: $F(2,29)=2.09$, N.S. †$p<0.05$.

Paneri, *et al.* [26] have found that levels of β-endorphin and met-enkephalin are decreased in the brain of rats as lactation progresses and during periods of prolonged hyperprolactinemia following implantation of pituitary tumors. These findings suggest that opioid levels in the brain are altered during lactation, possibly via a feedback mechanism.

Butorphanol tartrate, a kappa-sigma agonist, has been reported to increase food intake in sated rats [12,29]. We examined the effect of butorphanol tartrate on food intake in virgin female rats and lactating rats during early, mid and late lactation. McLaughlin *et al.* have reported that female rats are insensitive to body weight and water intake suppression after administration of nalmefene, an opioid antagonist, while male rats had decreased body weights and decreased water intake [15]. Therefore, we examined the effect of naloxone on food intake in deprived and non-deprived virgin female rats and on the spontaneous food intake of lactating rats in three stages of lactation and during the period of post-weaning.

METHOD

Sprague-Dawley virgin females (175–225 g) and timed primiparous pregnant (pre-mating wt. 175–225 g) rats were purchased from BioLabs, St. Paul, MN. Virgin rats were housed in individual cages with 12 hour light/dark cycles (0700 hours to 1900 hours) with food and water available ad lib between experimental periods. Pregnant rats were received on the 16th day of pregnancy and housed in individual breeder boxes with a similar 12 hour light/dark cycle with food and water available ad lib between experimental periods. The day of parturition is designated day 0. Litters were all dropped within a 48 hour time span. Litters were maintained at 8±2 pups throughout the entire experimental period from day 2 of lactation through weaning by culling or foster parenting. Early lactation is defined as the first week after parturition, mid-lactation is week two and late lactation is week three. The post-weaning period is 3–7 days after weaning of the pups on the 18th or 19th day of lactation. On the 15th day of lactation, the pups were removed from the mother during the period of experimentation to eliminate food consumption by the pups. Dams were adapted to pup removal on 2 consecutive days immediately prior to the beginning of the experiment. Four groups of lactating females were used for the experiments with a total of 80 lactating rats being tested. In addition, a total of 40 virgin female rats were used as controls. All experiments were conducted on four consecutive days during each period of lactation and any one group of animals received opiate agonists only during one period of lactation. Animals were assigned to a different experimental group each day so that no animal received the same drug dosage or vehicle more than one time during the four day period. In a separate experiment, a group of 23 male rats were food deprived for 24 hours for naloxone study and tested on a single day. All other conditions for the naloxone study using male rats were the same as those for virgin female rats. All experiments were conducted in the animal's home cage. All drugs and vehicles were administered peripherally by subcutaneous injection. Food in the form of pre-weighed pellets (Purina Lab Chow) was presented 10–15 minutes after the injection. All animals had water ad lib during the experimental period. Drugs used were butorphanol tartrate (Bristol Laboratories) (1.0 and 10 mg/kg) in 0.2 ml of BT buffer (3.3 g/l citric acid, 6.4 g/l sodium citrate and 6.4 g/l sodium chloride), and naloxone (DuPont de Nemours Co., Wilmington, DE) (1.0 and 10 mg/kg) in 0.2 ml physiological saline. Experiments with BT were begun at 0800 hours and food intake was measured at 2, 4 and 6 hours post-injection. Naloxone experiments were begun at 1900 hours (lights out) and food intake was measured at 30, 60 and 120 minutes post-injection. Fasted animals were food deprived 24 hours

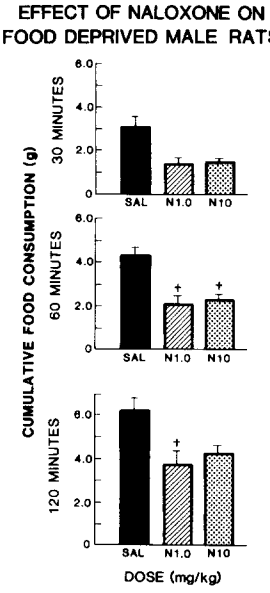

FIG. 3. The effect of naloxone on 24 hour food deprivation induced food intake in male rats. $F(2,20)=8.76$, $p<0.002$; $^{\dagger}p<0.05$.

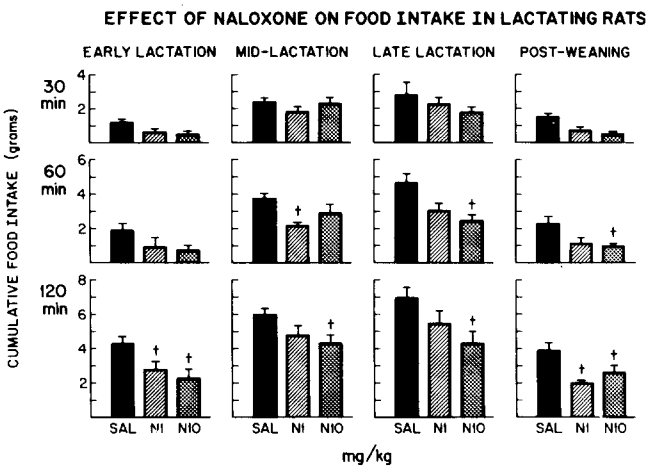

FIG. 4. The effect of naloxone on spontaneous nocturnal food intake in early, mid, late lactating and post-weaning rats. Early: $F(2,35)=9.38$, $p<0.005$; Mid: $F(2,39)=3.81$, $p<0.031$; Late: $F(2,47)=7.11$, $p<0.002$; Post-weaning: $F(2,35)=6.95$, $p<0.0029$. $^{\dagger}p<0.05$.

prior to start of each experiment and spontaneous food intake group had food removed 2 hours prior to the start of the experiment. Data was analyzed by 2-way ANOVA followed by the Least Significant Difference test.

RESULTS

In virgin female rats butorphanol tartrate (BT) enhanced feeding at all doses and all time points (Fig. 1) ($p<0.05$). During early and late lactation, BT (10 mg/kg) enhanced food intake at 4 and 6 hours and during mid lactation, BT (10 mg/kg) enhanced food intake at all time points (Fig. 1) ($p<0.05$).

Naloxone (1.0 mg/kg) suppressed spontaneous food intake in virgin female rats at 120 minutes ($p<0.05$) and naloxone (10 mg/kg) suppressed spontaneous food intake in virgin female rats at 60 minutes ($p<0.05$) (Fig. 2). In virgin female rats which had been food deprived for 24 hours, naloxone did not suppress food intake although naloxone (10 mg/kg) showed a statistical suppression on a Student's t-test. In male rats which had been food deprived for 24 hours, naloxone (1.0 mg/kg) decreased food intake at 60 and 120 minutes and naloxone (10 mg/kg) decreased food intake at 60 minutes ($p<0.05$) (Fig. 3). Naloxone (1 mg/kg) suppressed spontaneous nocturnal food intake at 60 minutes post-injection during mid lactation and at 120 minutes post-injection during early lactation and post-weaning (Fig. 4) ($p<0.05$). Naloxone (10 mg/kg) suppressed spontaneous nocturnal food intake at 60 minutes post-injection during late lactation and post-weaning and at 120 minutes post-injection in all periods of lactation and post-weaning (Fig. 4) ($p<0.05$).

DISCUSSION

Virgin and lactating rats are sensitive to the food intake enhancement effect of butorphanol tartrate and the food intake suppressant effect of naloxone. The extremely low level of estradiol present during lactation does not appear to be effective in modulating the effect of the opiate agonist, butorphanol tartrate or the opiate antagonist, naloxone. Ovariectomized animals that were not estradiol replaced failed to respond to the food intake enhancement effect of the kappa opiate agonist, ethylketocyclazocine and were very sensitive to the food intake suppressant effect of naloxone [22].

During lactation, female rats appeared to be slightly less responsive to BT's food enhancement effect compared to virgin female rats. BT (1.0 mg/kg) which was effective in increasing food intake in virgin rats had little effect during lactation, although this is only a minor difference as lactating rats overall response to BT is not different from that of virgin females. Following food deprivation in virgin female rats, there was a tendency for naloxone to suppress food intake, whereas male rats which had been food deprived responded to naloxone (1.0 mg/kg) (Fig. 3). McLaughlin *et al.* found that female rats were not as responsive to suppressant effects of nalmefene, an opioid antagonist, as male rats [15]. When the sex difference in response was analyzed, it was found that the response to naloxone of male and females was not significantly different.

During late lactation, rats are consuming an increased portion of their daily food intake during the light cycle ([33], and unpublished observations in our laboratory) and it could be expected that the opiate receptors would be occupied during this period of increased food consumption, rendering them relatively unresponsive to further induction by the administration of exogenous opiates. This does not appear to be occurring, which would indicate that some factor other than the opioid system might be modulating the hyperphagic condition of lactation.

Another possibility is that the density or affinity of the opiate receptors is altered during lactation. This might explain the decreased sensitivity during lactation to the low dose of BT. Further studies are necessary to test this possibility.

The suppression of spontaneous nocturnal food intake during lactation by naloxone indicates that the endogenous opioids are involved in food intake in the lactating rat although they do not appear to be wholly responsible for the greatly increased food intake of lactation. During spontaneous nocturnal feeding in female rats and following food dep-

rivation in male rats, naloxone (10 mg/kg) no longer suppresses food intake at 120 minutes. It has been shown that pretreatment with naloxone increases sensitivity to opiate agonists [1]. It is possible that by 120 minutes there is a compensatory rebound effect by endogenous opiates following naloxone administration. Previous studies in hyperphagic genetically obese mice have suggested an increased sensitivity to naloxone [14] and an altered sensitivity to opiate agonists [4,21]. Our results in the lactating nonobese hyperphagic model would suggest that these changes are associated either with the obesity or with the hypothalamic alterations responsible for the obesity rather than being due to the hyperphagia per se.

In conclusion, since only minimal differences in response to the opioid agonist, butorphanol tartrate, and the opioid antagonist, naloxone, are observed during lactation, it appears that the development of hyperphagia during this period may not be due to an increase in the opioid mediated feeding drive. Further studies remain to be done to determine the extent of opioid involvement in lactational hyperphagia.

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