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Involvement of Opioid Peptides in the Inhibition of Oxytocin Release by Heat Stress in Lactating Mice[1] (41227)

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Abstract. Acute heat stress inhibited suckling-induced milk yield in lactating mice but did not decrease milk yield obtained by suckling following oxytocin administration. Injections of naltrexone, 2.5 and 5 mg/kg, prior to heat exposure significantly reduced the inhibition of milk yield during suckling. These results suggest that endogenous opioid peptides may be involved in the mechanism of inhibition of reflex release of oxytocin by stress.

Emotional stress reduces milk yield, in both humans and other animals. Ely and Petersen (1) demonstrated marked inhibition of milk yield in frightened cows. Newton and Newton (2) described inhibition of milk ejection due to fear, embarrassment, or anger in lactating human subjects. Cross (3, 4) did extensive studies on rabbits on the mechanisms underlying emotional inhibition of the milk-ejection reflex. He concluded that emotional stress blocks the milk-ejection reflex by activating the sympatheticoadrenal system causing the release of epinephrine and norepinephrine which act peripherally as vasoconstrictors. However, he emphasized that the central block of oxytocin release from the posterior pituitary gland is the major factor in stress-induced inhibition of oxytocin release and epinephrine might be involved in this central inhibition. Chaudhury *et al.* (5), working with guinea pigs, came to a different conclusion with regard to the involvement of epinephrine in the central inhibition of oxytocin release during stress. They observed that neither dibenamine, in a dose which blocked pressor responses to epinephrine, nor dichloroisoproterenol, in a dose which blocked the inhibitory action of epinephrine, prevented stress-induced inhibition of oxytocin release. They suggested that stress-induced inhibition of oxytocin release is probably due to nervous block and not mediated by epinephrine.

Although the physiological roles of the newly discovered opioid peptides remain obscure, there is some evidence indicating that stress may increase the level of opioid peptides in brain (6) and plasma (7). In addition, stress-induced increase in plasma prolactin level in rats can be partially blocked by the narcotic antagonist, naloxone (8).

Based on milk yield data, Haldar and Sawyer (9) reported that morphine inhibits oxytocin release in lactating mice. Later Haldar *et al.* (10) demonstrated that oxytocin release due to intraventricularly administered acetylcholine could be inhibited by morphine and β-endorphin in lactating rats. Clarke *et al.* (11) confirmed this work and showed in addition that opioid peptides inhibit suckling-induced oxytocin release also.

We wish to report here experiments on lactating mice designed to test the hypothesis that endogenous opioid peptides may participate in the inhibition of the milk-ejection reflex by heat stress.

Materials and Methods. All experiments were performed on Swiss Webster mice following the method described before (9). In brief, after a 3-hr separation period pups were allowed to suckle on their mother for two 30-min periods. Oxytocin (0.25 U) was injected ip into the mother before the second nursing period. Pups were weighed before and at the end of each suckling period. The weight gain by the pups during the first suckling period

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was designated as "initial milk yield" (S_1) and during the second suckling period as "residual milk yield" (S_2). Since the total milk yield ($S_1 + S_2$) was quite variable, the results are expressed as percentage of the total milk yield for each litter.

After the animals had gone through this schedule for 2 days the stress experiments were conducted using the following protocol. The litters were divided into two major groups, I and II. Twenty minutes before the end of the separation period mothers in group I were injected ip with 0.9% saline and mothers in group II with naltrexone (Endo Laboratories, Garden City, N.Y.) in doses of 2.5, 5, 10, and 20 mg/kg. Ten minutes before the end of the separation period half of the saline-treated mothers and half of the naltrexone-treated mothers were subjected to 10 min of thermal stress by placing them in a 40° oven. Immediately after the stress, mothers were allowed to nurse their pups, and the same protocol as described above was followed including the injection of 0.25 U of oxytocin into the mother before the second nursing period. The remaining mothers in groups I and II were used for suckling experiments following the regular protocol but were also subjected to stress on the next day. Thus, each group of mice was subjected to stress once only, except for one group which was deliberately subjected to stress in 2 consecutive days to determine the effect of repeated stress.

Statistical comparison between groups was made with an unpaired Student's t test. All values are presented as mean ± SE.

Results. Application of acute thermal stress did not cause the mothers to reject their pups, and nursing appeared to occur in the usual manner. However, the acute stress resulted in an 80 ± 10.5% (n = 8) reduction of milk yield in the S_1 period (Fig. 1A). Interestingly, the total milk yields of ($S_1 + S_2$) were not significantly different ($P < 0.21$) between control and stressed groups which were 4.23 ± 0.23 (n = 39) and 4.75 ± 0.90 (n = 8) g/100 g body wt, respectively (Fig. 1B).

Effect of naltrexone on stress-induced inhibition of oxytocin release. Figure 2 demonstrates that stress-induced inhibition

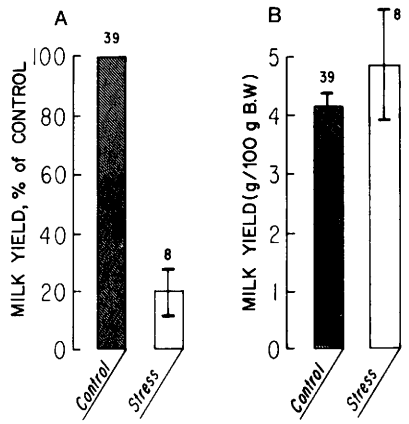

FIG. 1. Effect of acute heat stress on initial (A) and total (B) milk yield in lactating mice. Number above the histograms represents n for that group.

of oxytocin release was partially reduced by prior administration of naltrexone. Compared to 20 ± 1.0% (n = 8) milk yield in group of mice which were subjected to stress only (B), milk yield was 67 ± 14% (n = 7) when mice were injected with 2.5 mg/kg of naltrexone prior to the exposure of stress (C). Thus naltrexone administration caused a significant ($P < 0.03$) increase in milk yield even when mice were exposed to the same degree of stress. A further increase in milk yield was obtained with a prior injection of 5 mg/kg of naltrexone (D). This increase although significantly ($P <$

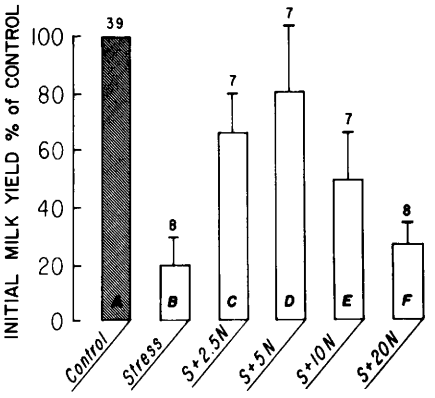

FIG. 2. Effect of naltrexone (N) on stress-induced inhibition of initial milk yield. Naltrexone in doses of 2.5, 5, 10, and 20 mg/kg was injected ip 10 min prior to the exposure to stress. Number above the histograms represents n for that group.

0.04) more when compared to that of group B was not when compared to that obtained in group C. When the dose of naltrexone was increased to 10 and 20 mg/kg, no further increase in milk yield was noticed. Instead, inhibition was observed (E and F). This inhibition most likely was due to naltrexone itself, since mice treated with naltrexone only without any exposure to stress also showed dose-related inhibition (Fig. 3). Although the inhibition caused by 5 mg/kg was not significantly different ($P < 0.17$) from control, inhibition caused by 10 and 20 mg/kg of naltrexone was highly significant ($P < 0.02$ and 0.001, respectively). However, the total milk yield ($S_1 + S_2$) in these groups was not different from control which were 4.30 ± 0.83 ($n = 9$), 4.66 ± 0.33 ($n = 9$), and 4.45 ± 0.63 ($n = 9$) g/100 g body wt for groups N5, N10, and N20, respectively.

Effect of repeated stress on oxytocin release. Figure 4 demonstrates that when the same group of mice was exposed to thermal stress in 2 consecutive days there was no inhibition on the second day. The milk yield was 20 ± 1.0% on the first day of stress and 105 ± 27.6% on the second day of stress.

Discussion. The essential role of oxytocin in causing successful milk-ejection reflex is long established. Thus, the method used in this investigation to demonstrate oxytocin release, although indirect, is a valid one.

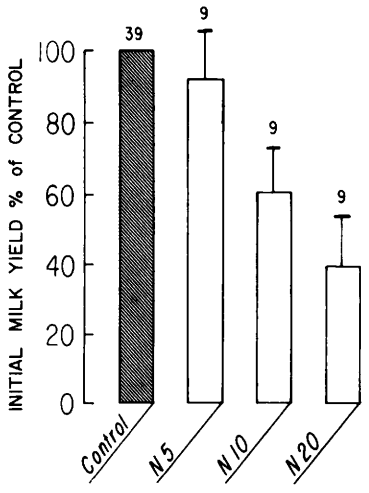

FIG. 3. Effect of naltrexone alone in doses of 5, 10, and 20 mg/kg on initial milk yield.

The present results demonstrate that acute heat stress inhibits suckling-induced milk-ejection reflex in lactating mice. Since the total milk yield ($S_1 + S_2$) was not significantly different in control and stressed groups (Fig. 1) the inhibition of the milk yield in the stressed group was due to lack of oxytocin release in response to suckling and not to lack of milk in the gland or unresponsiveness of the mammary gland itself to oxytocin.

It has been reported previously that restraint stress inhibits the milk-ejection reflex in rabbits (3, 4) and guinea pigs (5). The present experiments demonstrate that a different type of stress (heat) is also effective in causing inhibition of oxytocin release in mice. Although we did not measure plasma opioid peptides levels following stress, the partial prevention of the inhibition of oxytocin release by prior administration of naltrexone (Fig. 2) suggests that endogenous opioid peptides might be involved in acute heat stress-induced inhibition of oxytocin release. Furthermore, it is known that repeated exposures to the same stressor result in the adaptation of the opiate mediated analgesic response (6). Thus the demonstration of the adaptation of mice exposed to the same stress in 2 consecutive days (Fig. 4) further strengthened the involvement of opioid peptides in inhibition of oxytocin release by acute heat stress.

A high dose range of naltrexone causes inhibition of milk yield (Fig. 3) which suggests that at a higher range the antagonist may act like an agonist. Since the total milk yield ($S_1 + S_2$) for these groups was not different from that of controls it is likely that the inhibitory action of naltrexone at a high dose was due to inhibition of oxytocin release and not to a direct action of naltrexone on the myoepithelial elements. Our data do not specify which opioid peptide is involved in this inhibition, since stress is known to affect both enkephalin and β-endorphin levels in the hypothalamus (12, 13) and naltrexone is known to antagonize both these peptides.

Although it is most likely that opioid peptides from the brain or pituitary are responsible for inhibition of oxytocin release

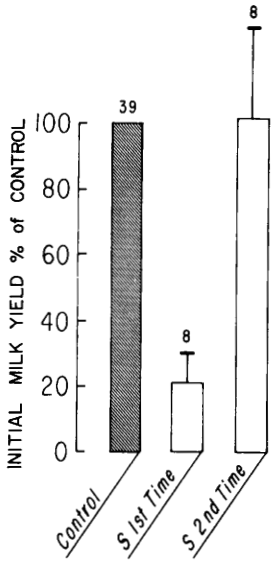

FIG. 4. Effect of repeated stress (S) on initial milk yield in lactating mice.

it is possible that circulating peptides may be involved. Recently Rossier *et al.* (14) and Yang *et al.* (15) demonstrated adrenal medullary chromaffin granules contain Leu-enkephalin and Met-enkephalin. Furthermore, Stine *et al.* (16) have demonstrated that acetylcholine (Ach) could release Met-enkephalin-like materials from adrenal medullary chromaffin cells in primary culture in a dose-dependent fashion. These data show not only that enkephalin is present in adrenal medullary cells, but that it can also be released in response to a physiological agent such as Ach. Thus it is possible that during stress both catecholamine and enkephalins are simultaneously released from the adrenal medulla and that both types of agents may exert an inhibitory action on oxytocin release. Although adrenal medullary peptides do not contain β-endorphin sequences we can not rule out the possibility that β-endorphin might have some role in stress-induced inhibition of oxytocin release since it has been shown that stress causes β-endorphin release and that β-endorphin injected into the lateral cerebral ventricles inhibits Ach-induced as well as suckling-induced (10, 11) oxytocin release in rats.

For inhibitory actions on oxytocin release opioid peptides might be acting at one or more of the following sites. First, as suggested by Clarke *et al.* (11) the site of action could be at the posterior pituitary gland where a presynaptic connection of an opioid neuron with an oxytocin-producing neuron has been postulated. A recent report by Martin and Voigt (17) favors this hypothesis since they have demonstrated that Met-enkephalin immunoreactivity is associated with nerve terminals which contain oxytocin immunoreactivity. The second possibility is that opioids released during stress may block neural transmission by a presynaptic inhibitory action on a dopaminergic terminal in a fashion similar to that which occurs in the corpus striatum (18). There are a number of reports which suggest that a dopaminergic system is involved in normal milk-ejection reflex. Clarke *et al.* (19) have showed that iv haloperidol, a dopaminergic antagonist, delays milk ejection in lactating rats. Similarly Moos and Richard (20) have demonstrated that haloperidol not only blocks suckling-induced oxytocin release but also blocks oxytocin released by vaginal and vagal stimulation. Finally, the third possibility is that opioid peptides might be acting at the hypothalamic nuclei particularly at the paraventricular nucleus (PVN), since the magnocellular paraventricular nucleus contains β-endorphin-reactive innervation (21).

It is concluded that acute thermal stress effectively inhibits suckling-induced oxytocin release in lactating mice. The results demonstrate for the first time that endogenously released opioid peptides, enkephalin and/or β-endorphin play some role in the complex phenomenon of stress-induced inhibition of oxytocin release.

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