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BRIEF COMMUNICATION

Intracerebroventricular Cholecystokinin Infusions Block Beta-Endorphin-Induced Disruption of Maternal Behavior

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FELICIO, L. F., P. E. MANN AND R. S. BRIDGES. *Intracerebroventricular cholecystokinin infusions block beta-endorphin-induced disruption of maternal behavior.* PHARMACOL BIOCHEM BEHAV **39**(1) 201–204, 1991.—Recent work has shown that infusions of beta-endorphin, an endogenous opioid, into the ventricular system of lactating rats blocks normal maternal behavior. Other behavioral and biochemical studies have demonstrated that sulfated cholecystokinin-octapeptide (CCK-8) can have effects opposite those of opioids. The present study evaluated whether intracerebroventricular (ICV) administration of CCK-8 is able to antagonize the inhibitory effect of beta-endorphin on maternal behavior. The results of this study demonstrated that CCK-8 (14.5 nmol) prevented the beta-endorphin (1.45 nmol)-induced increase in latencies to retrieve the first pup, retrieve all pups, and to group and crouch over rat pups. In addition, reductions in the percentage of rats retrieving all pups and displaying full maternal behavior were prevented by CCK-8. These data suggest that CCK-8 can act as an opioid antagonist in neural systems that control maternal behavior.

Beta-endorphin Cholecystokinin Maternal behavior Neuropeptides Opioids

THERE is abundant cholecystokinin in the brain (28,32), 80% being the terminal octapeptide (CCK-8: Asp-Tyr-SO_3H-Met-Gly-Trp-Met-Asp-Phe-NH_2). The fully sulfated form of CCK-8 has a high amount of bioactivity (29,32). Under certain conditions CCK-8 has been reported to have opposite effects than those of opioids. Food intake decreases after intraperitoneal (IP) or intracerebroventricular (ICV) administration of CCK-8, whereas food consumption increases after morphine or beta-endorphin treatment by the same routes (1, 14, 16, 22). Other opioid effects such as thermoregulation (11), analgesia (6, 10, 15, 34), and even tolerance to morphine (25) are antagonized or modulated by CCK-8. In addition, opioid peptides act as antagonists of CCK-8 in peripheral tissues such as the ileum and vas deferens (34).

Reproductive behaviors, including sexual behavior (3,4) and maternal behavior (13), are also influenced by CCK-8. Recently, Linden et al. (13) reported that IP administration of CCK-8 stimulated maternal behavior in estrogen-primed, nulliparous rats. Previous studies have shown that maternal behavior is under an inhibitory influence of opiates (5, 9, 12, 17–21, 30). Infusing beta-endorphin, an endogenous opioid, into the ventricular system of lactating rats dose-dependently blocks normal maternal behavior (17). Based upon these findings, we hypothesized that CCK-8 may be able to antagonize beta-endorphin's actions on maternal behavior. We tested this hypothesis by determining whether ICV infusions of CCK-8 blocks beta-endorphin-mediated inhibition of maternal behavior.

METHOD

Animals

Nulliparous female Sprague-Dawley [Crl:CD(SD)BR (virus-free)] rats were purchased from Charles River Laboratories, Inc. (Kingston, NY). They were individually housed in polypropylene cages (45 × 25 × 20 cm) that contained approximately 1.5 liter medium grade wooden flakes. Food (Purina Rat Chow) and water were available ad lib in light (on 0500–1900 h)- and temperature (21–25°C)-controlled rooms. The females were mated to males in our colony. Day 1 of pregnancy was defined as the day sperm were observed in the vaginal smear. On days 13–15

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of gestation rats were anesthetized (chloropent) and stereotaxically implanted (Kopf Instrument) with unilateral cannulas aimed at the right lateral ventricle. The implant coordinates, derived from Pellegrino and Cushman's rat brain atlas (27), were: AP, 5.6; L, −1.6; H, 2.9. After giving birth (day 0 of lactation) females remained with their litters (culled to 6 pups on day 1 of lactation) until tested for maternal behavior. Animals used in this study were maintained in accordance with the guidelines of the committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council.

Maternal Behavior Testing

Rats were tested for maternal behavior on days 5 and 6 postpartum. Each test day pups were removed between 0900 and 1000 h. Then, 30 minutes later, mothers were administered (ICV) vehicle (day 5) or the peptides (day 6). The test for maternal responsiveness began 30 minutes after the infusions at which time pups were placed throughout each mother's home cage. Animals were observed continuously for 15 minutes, then at 15-min intervals up to 1 hour. Contact, retrieval, grouping, and crouching responses were scored during the test session. Animals were scored as fully maternal if they retrieved all 6 pups to the nest and crouched over them. If animals were not maternal by 1 h, they were spot-checked every hour until full maternal behavior was recorded. Events observed after the first 15 min of continuous observation were recorded at the time first observed, i.e., if the female was first seen to crouch over the young at 60 min, the crouching latency was scored as 60 min (or 3600 s). The same criterion was used for all other responses.

Statistical Analysis

Results were analyzed using a two-tailed Mann-Whitney U-test or Fisher's Exact Probabilities test to compare latencies and percentages, respectively, between the two experimental groups (31). The sign test or binomial test was used to compare latencies and percentages, respectively, of each group between days 5 and 6 (31). A probability of $p<0.05$ was considered significant for all comparisons made.

Treatments and Drugs

The human beta-endorphin and sulfated cholecystokinin octapeptide used in this study were purchased from Peninsula Laboratories (Torrance, CA). ICV infusions were performed over a 60-s period using a 50 μl automated microsyringe (Stoelting Co., No. 51219). The selection of the route of administration was based on the results of previous studies showing that CCK-8 is effective as a beta-endorphin antagonist on analgesia following ICV administration (10) and that beta-endorphin inhibits maternal behavior by the same route (17).

Animals were infused on day 5 with 5 μl saline and on day 6 with beta-endorphin (1.45 nmol in 5 μl) alone or beta-endorphin plus CCK-8 (1.45 nmol beta-endorphin plus 14.5 nmol CCK-8 in 5 μl volume). The selection of these doses was based on results obtained in a pilot study using a lower CCK-8 dose (7.25 nmol) which was unable to block beta-endorphin's (1.45 nmol) effects on maternal behavior.

Histology

To validate cannula placements all animals were anesthetized with chloropent and infused ICV with 2.5 μl India ink. Rats were then perfused transcardially with saline followed by 10% formalin. Brains were removed and cannula placements were confirmed.

TABLE 1

EFFECTS OF ICV CCK-8 ON BETA-ENDORPHIN-INDUCED DISRUPTION OF MATERNAL BEHAVIOR

Behavioral Responses	Day 5 Saline[a] (24)[b]	Day 6 Beta-Endorphin (11)	Day 6 Beta-Endorphin + CCK-8 (13)
Retrieve the first pup	18[c] (1–2700)	2700* (2–14400)	22 (1–10800)
Retrieve all pups	69 (29–2700)	7200* (20–14400)	120 (18–10800)
Group	98 (30–2700)	7200* (90–14400)	142 (40–10800)
Crouch	230 (80–2700)	7200† (780–14400)	300 (120–10800)

[a]The results of animals in both treatment groups were combined. On day 6 animals were treated with 1.45 nmol beta-endorphin or with the same dose of beta-endorphin plus 14.5 nmol CCK-8.

[b]Number of animals.

[c]Data represent medians latencies in seconds (ranges).

*$p<0.02$; †$p<0.002$ compared to both saline and beta-endorphin plus CCK treatments.

RESULTS

Intracerebroventricular infusions of saline on day 5 of lactation did not disrupt maternal behavior. Since there were no differences in any parameter between the two groups after saline administration on day 5, data obtained on this day are presented as a single group. Latencies in seconds to display the various components on days 5 and 6 are presented in Table 1. Beta-endorphin administration on day 6 of lactation inhibited the display of maternal behavior, confirming previous studies (5, 17–21, 30). Beta-endorphin-treated females were active, eventually contacting the pups (sniffing) even though they did not display maternal behavior. After beta-endorphin administration all behavioral latencies were longer ($p<0.02$) and the percentages of animals that retrieved all pups and displayed full maternal behavior were significantly reduced ($p<0.02$) than those present on day 5 after vehicle infusions. All beta-endorphin-treated animals eventually (by 4 hours) were maternal. In contrast, animals treated with beta-endorphin plus CCK-8 had significantly shorter latencies to retrieve the first pup ($p<0.02$), retrieve all the pups ($p<0.02$), group the pups together ($p<0.02$), and crouch over them ($p<0.002$) than those latencies observed for rats treated with beta-endorphin alone. As shown in Fig. 1, the percentages of animals retrieving all pups and displaying full maternal behavior within 60 minutes were higher in the beta-endorphin plus CCK-8-treated animals than those observed for rats treated with beta-endorphin alone ($p<0.05$). There was no significant difference between groups treated with beta-endorphin and beta-endorphin plus CCK-8 in the percentage of animals retrieving at least one pup. In addition, no significant differences were observed on any parameter of maternal behavior between rats treated with beta-endorphin plus CCK-8, on day 6 of lactation, and with saline the previous day.

DISCUSSION

These results indicate that CCK-8 is able to antagonize beta-endorphin's inhibitory action on maternal behavior in the female

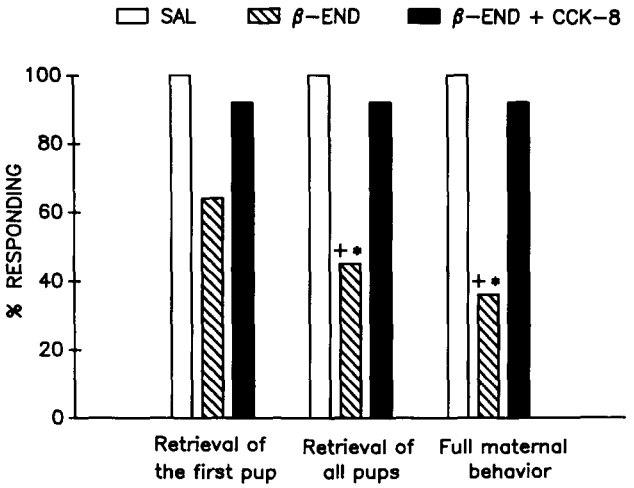

FIG. 1. Percentage of animals responding maternally following ICV treatment with saline (day 5), 1.45 nmol beta-endorphin (day 6) or with the same dose of beta-endorphin plus 14.5 nmol CCK-8 (day 6). $^{+}p<0.02$ compared to saline; $^{*}p<0.05$ compared to beta-endorphin plus CCK-8.

rat. Earlier studies had identified roles for oxytocin, CCK, and beta-endorphin in maternal care (13, 17, 26), but this study is the first to demonstrate the existence of a dual peptidergic regulation of maternal behavior. The possibility of an interactive and multiple peptidergic control of this behavior now appears likely.

The site(s) of CCK action in antagonizing beta-endorphin's inhibitory behavioral effects is unknown. Since both peptides were infused into the ventricular system, it is possible that these peptides could act at either common or different neural loci. One possible common site of peptidergic regulation is the medial preoptic area (MPOA). This region of the brain is intimately involved in regulating maternal behavior in rats (23,24), and has been identified as a site of both morphine and beta-endorphin inhibition of maternal care (20,30). Moreover, other biochemical and behavioral findings indicate that the MPOA might be a site of CCK action. Sexual behavior of both males and females can be modified by direct infusions of CCK into the hypothalamus (3,4). Direct examination of the effects of local infusions of CCK with beta-endorphin within areas such as the MPOA are needed to delineate the actual sites of peptidergic regulation of maternal behavior in order to determine whether these peptides interact directly.

CCK-8 has also been reported to attenuate beta-endorphin-induced analgesia (10). In rats the ICV dose of CCK-8 needed to prevent beta-endorphin-induced analgesia is four times greater than that of beta-endorphin [0.7 nmol beta-endorphin; 2.8 nmol CCK-8; (10)]. This dose ratio of CCK-8 to beta-endorphin does not totally block beta-endorphin action, but rather attenuates its analgesic effect. In the present study the dose of CCK used to block beta-endorphin's action on maternal behavior was 10 times higher than that of beta-endorphin. Although this ratio of doses completely blocked the actions of beta-endorphin alone, the specificity of CCK effect remains to be tested. Future experiments are needed to check if other peptides would also block this opiate effect. Interestingly, the 1.45 nmol dose of beta-endorphin did not affect all aspects of maternal responsiveness. Namely, the number of females retrieving the first pup during the 1-h test was not significantly affected by beta-endorphin administration, although their latencies to first retrieve a pup were significantly longer. Perhaps, higher doses of beta-endorphin would also affect the number of animals retrieving during the initial hour test period.

The results of the present study demonstrate a role for CCK in the regulation of ongoing maternal care. The earlier study of Linden and colleagues (13) found that IP administration of CCK-8 to estrogen-primed, nulliparous rats stimulated the onset of maternal behavior. Since peptide-based structures have limited capacity to penetrate the blood-brain barrier (8) and that most of the behavioral and endocrine effects of peripherally administrated CCK-8 are eliminated by vagotomy (2,7), it is possible that the CCK-8 given IP and that given ICV may act at different neural sites to regulate different aspects of maternal behavior. For example, the stimulation of the onset of maternal behavior by CCK-8 may be mediated through a peripheral route, while the maintenance of ongoing maternal care might be regulated by a more central pathway. It would be interesting to see whether ICV infusions of CCK-8, like IP implants (13), would stimulate the onset of maternal behavior in both steroid and nonsteroid-primed female rats or whether only ongoing maternal care is modulated by CCK-8 given ICV. These findings indicate that these peptides interact in regulating an important brain-behavioral function, maternal behavior. In the future it will be necessary to place these present studies into a physiological context. For example, does CCK release and activity in areas such as the MPOA increase around the time or parturition or during lactation and block endogenous opioid activity in this neural region when females exhibit maternal care?

In summary, the present results demonstrate that CCK-8 is able to reverse the disruptive actions of beta-endorphin on maternal behavior, and indicate that CCK-8 may play a physiological role in the maintenance of maternal behavior.

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