Pharmacology Biochemistry and Behavior, Vol. 64, No. 1, pp. 59–64, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(99)00065-9

CRF-Deficient Mice Respond Like Wild-Type Mice to Hypophagic Stimuli

ARTUR H. SWIERGIEL AND ADRIAN J. DUNN

Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130

Received 23 September 1998; Revised 18 February 1999; Accepted 18 February 1999

SWIERGIEL, A. H. AND A. J. DUNN. *CRF-deficient mice respond like wild-type mice to hypophagic stimuli.* PHARMACOL BIOCHEM BEHAV **64**(1) 59–64, 1999.—Corticotropin-releasing factor (CRF) has been implicated in physiological processes associated with stress, including changes in feeding behavior. Intracerebroventricular (ICV) administration of CRF and urocortin have been shown to depress feeding, and antagonism of CRF receptors has been reported to attenuate hypophagic responses to many treatments, suggesting that brain CRF may mediate these responses. We have now studied feeding behavior of mice lacking the CRF gene (CRFko), comparing them to wild-type (CRFwt) mice. Feeding was assessed in nondeprived mice by measuring the intake of sweetened milk in a 30-min period and the food pellet intake over 24 h. ICV administration of CRF or urocortin (1 μg, but not lower doses) depressed milk and food pellet intake in normal mice. Physical restraint for 30 min, or administration of mouse interleukin-1β (mIL-1β, 100 ng, IP), lipopolysaccharide (LPS, 1 μg, IP), or the serotonergic agonist (*d*-fenfluramine, 4 mg/kg, IP) reliably reduced milk intake. LPS also reduced food pellet intake. The responses to restraint, IL-1, LPS, and fenfluramine were indistinguishable between the CRFwt and CRFko mice. These results suggest that CRF is not essential for the reduction in sweetened milk intake that occurs following restraint, LPS, IL-1, or *d*-fenfluramine administration to mice. © 1999 Elsevier Science Inc.

Interleukin-1 LPS *d*-Fenfluramine CRF knockout Feeding

DEPRESSION of feeding is one of the most visible aspects of sickness behavior (17), and is also observed during stress or after administration of cytokines (36,41). Concurrent increases in hypothalamo–pituitary–adrenocortical (HPA) activity (9) suggests that corticotropin-releasing factor (CRF) may be involved. Intracerebral administration of CRF or urocortin produces changes in many behavioral patterns, including depression of feeding and the set-point for body weight regulation (6,8,13,35). Evidence has been presented for the involvement of CRF in the anorexic effects of exercise, estradiol, bombesin, leptin, caffeine, and increased serotonergic activity (13,27–30). CRF has been implicated in the control of energy balance, and elevated concentrations of CRF have been observed in the cerebrospinal fluid of patients with anorexia nervosa (22,29). The behavioral responses to CRF resemble those elicited by stressors (8,26). Physical stressors produce both hypophagia and release of CRF, and it is plausible that the effects of stress on feeding are linked to CRF. This is supported by findings that the hypophagia induced by physical restraint could be reversed by a CRF receptor antagonist (19,32).

Acute administration of bacterial endotoxin (lipopolysaccharide, LPS) or mouse interleukin-1β (mIL-1β) has been shown to inhibit food intake in mice (36). Both LPS and IL-1 stimulate the release of CRF (1,31). Because treatment with a CRF antibody attenuated IL-1–induced hypophagia, it was suggested that the cytokine might reduce appetite through elevated release of CRF (39). The effects of fenfluramine, a potent releaser of serotonin (5-HT), have also been suggested to involve CRF (20).

In the present experiments, we attempted to determine whether CRF participates in changes in feeding behavior evoked by physical restraint, or administration of bacterial endotoxin, IL-1, or the anorexigen, *d*-fenfluramine.

METHOD

Animals and Materials

Six-week-old, CD-1 male mice were purchased from Charles River and weighed 30–35 g at the time of the experiment. Adult CRF knockout male mice were provided by Dr. Joseph A. Majzoub from Children's Hospital in Boston, MA.

Requests for reprints should be addressed to Dr. Adrian J. Dunn, Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Wild-type controls (CRFwt) were homozygotes ($Crf^{+/+}$) and the CRF knockout mice (CRFko) were transgenic homozygotes ($crf^{-/-}$) with an inserted inactivated CRF gene produced as described previously (24). CRF deficiency was confirmed by Southern blot analysis of genomic DNA isolated from the tails (24). Body weights ranged from 24–37 g during the experiments, but there were no significant differences between the two genotypes. The animals were housed singly in an AAALAC-accredited facility under a 12L:12D cycle (lights on at 0700 h) at 22–23°C and a relative humidity of 50–55%, were weighed every morning, and had ad lib access to Teklad pelletted chow (3.99 kcal/g) and water. All procedures were approved by the Louisiana State University Medical Center Animal Care and Use Committee and were in compliance with the NIH guide for Care and Use of Laboratory Animals.

CRF and human urocortin were gifts from Dr. Jean Rivier, The Peptide Laboratory, The Salk Institute, San Diego, CA. *E. coli* endotoxin (lipopolysaccharide, LPS) was obtained from Sigma Co. (St. Louis, MO; L3755, serotype 026:B6). Recombinant mouse interleukin-1β (mIL-1β) was obtained from R&D Systems (Minneapolis, MN), and *d*-fenfluramine from Wyeth-Ayerst. Compounds were dissolved in sterile pyrogen-free isotonic saline such that the total dose for each mouse was contained in 0.1 ml and injected intraperitoneally (IP). All compounds were administered at low doses that had produced reliable reductions in milk intake by mice in a number of previous experiments.

Feeding Behavior

Feeding procedures have been described previously (36,37). In brief, the mice were habituated for at least 3 days to drink sweetened condensed milk diluted with three parts of water from 20-ml glass bottles fitted with metal spouts. The weighed bottles were placed in the cages at around 11 am for 30 min, then removed and reweighed. Only the animals that drank at least 1.5 g of milk in the session on the final day of habituation were used for the experiments. To estimate food intake two fresh and firm food pellets were weighed and left in the cage of each mouse overnight. Pellets from the previous day were removed each morning at 0900 h and weighed. Water intake was not measured. Many previous experiments had indicated that the drinking of the sweetened milk in a 30-min period did not affect food pellet intake or the body weight gain.

Surgery and Intracerebroventricular (ICV) Injection

CD-1 mice were anesthetized with modified Hypnorm (2.5 mg of Fentanyl from Abbot Laboratories, Chicago, IL, 175 mg of droperidol from American Regent Laboratories, Shirley, NY, and 125 mg of midazolam from Roche Laboratories, Nutley, NJ, made up to 145 ml of sterile saline and administered IP at a dose of 10 μl per gram of body weight) and implanted with polyethylene cannulae in the lateral ventricles as previously described (16). After surgery, each animal was placed in an individual cage and returned to the colony room. Two microliters of artificial cerebral spinal fluid (aCSF) or CRF or urocortin were slowly infused into each lateral ventricle using an adapted Hamilton syringe.

Experimental Procedure and Behavioral Observations

All milk drinking tests were conducted in the animal colony room during the light part of the cycle. Animals were restrained after removing them from their home cages, transferring them to another room, and placing them for 30 min in 50-ml centrifuge tubes as previously described (2). Immediately after removal from the tubes they were returned to their home cages and presented with milk.

Statistical Analysis

Data were analyzed using Student's *t*-test or two-way analysis of variance followed by Fisher's LSD test. Data are the mean ± standard error of the mean.

RESULTS

Effect of CRF and Urocortin on Milk Intake

CD-1 mice were implanted with ICV cannulae, and tested approximately 2 weeks later. In the experiments of Fig. 1, CRF or urocortin was administered ICV at a total dose of 1 μg, and milk intake observed starting 15 min later. Both peptides induced statistically significant reductions in milk intake ($t_{12} = 2.85$ and $t_{12} = 2.87$, $p < 0.05$, respectively). The overnight intake of food pellets was also significantly depressed (data not shown). Lower doses of either CRF (100–500 ng) or urocortin (500 ng) did not induce reductions in milk intake (data not shown).

Effect of Physical Restraint on Milk Intake

CRF wild-type (CRFwt) and CRF knockout (CRFko) mice displayed similar growth rates as indicated by body weight, and both groups developed robust and similar amounts of milk drinking within 4 days. Physical restraint for 30 min significantly, $F(1, 14) = 22$, $p < 0.001$, decreased milk intake (Fig. 2). There was no difference between the genotypes, $F(1, 14) = 3.4$, and no significant genotype × restraint interaction, $F(1, 14) = 0.5$.

Effects of LPS and IL-1 on Milk and Food Intake and Body Weight

Wild-type and CRFko mice were administered LPS (1 μg/mouse, IP) and milk intake observed 120–150 min later [previously shown to be the nadir of the response (36)]. LPS induced statistically significant reductions in milk intake, $F(1, 14) = 117$, $p < 0.001$ (Fig. 3, upper panel). There were no differences between the CRFwt and CRFko mice, $F(1, 14) = 1.9$, and no genotype × LPS interaction, $F(1, 14) = 1.2$. Food pellet intake over the next 22 h (Fig. 3, middle panel) and

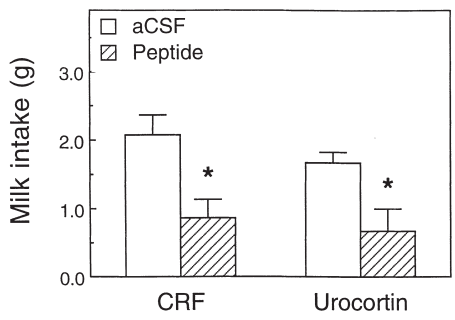

FIG. 1. Effect of CRF or urocortin on milk intake. Mice were infused with aCSF and CRF or urocortin (1 μg/mouse, ICV) and 15 min later presented with milk for 30 min. The peptides were tested on two different days. $n = 6$ for the aCSF groups, $n = 7$ for the peptide groups. Significantly different from the aCSF control group (*$p < 0.05$).

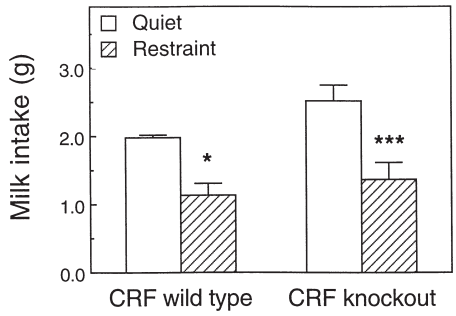

FIG. 2. Effect of restraint on milk intake. Wild-type and CRFko mice were left undisturbed (quiet) or were restrained for 30 min, and then immediately presented with milk. $n = 5$ for CRFko groups and $n = 4$ for CRFwt groups. Significantly different from the quiet control mice ($*p < 0.05$, $***p < 0.001$).

body weight (Fig. 3, lower panel) were significantly decreased by the LPS injections, $F(1, 14) = 17$, $p < 0.001$, and $F(1, 14) = 5.3$, $p < 0.05$, respectively. There were no statistically significant genotype × LPS interactions, $F(1, 14) = 0.1$; $F(1, 14) = 0.9$, food intake and body weight, respectively.

Similar results were obtained when milk was presented 90–120 min after IP administration of 100 ng/mouse of mIL-1β [previously shown to be the nadir of this response (36)]. IL-1β induced a statistically significant, $F(1, 14) = 26$, $p < 0.001$, reduction in milk intake (Fig. 4). There was no difference between the CRFwt and CRFko mice, $F(1, 14) = 0.1$, and no genotype × IL-1β interaction, $F(1, 14) = 0.0$.

Effect of d-*Fenfluramine on Milk Intake*

Wild-type and CRFko mice were administered *d*-fenfluramine (4 mg/kg, IP) and milk intake observed 15 min later. *d*-Fenfluramine induced a statistically significant, $F(1, 12) = 53$, $p < 0.001$, reduction in milk intake (Fig. 5). There was no difference between the CRFwt and CRFko mice, $F(1, 12) = 1.3$, and no genotype × fenfluramine interaction, $F(1, 12) = 0.0$.

DISCUSSION

Decreases in food intake have been observed following ICV administration of CRF (5,13,19) and urocortin (35). Our present results confirm these observations in our specific model, intake of sweetened milk in nonfood-deprived mice. However, it should be noted that both we and others (5,19) found it necessary to use relatively high doses of ICV CRF to produce these effects. The doses of CRF necessary to induce a reduction in milk intake were markedly higher than those previously shown to be behaviorally active in mice. For example, only 5 ng of CRF (a 200-fold lower amount) was sufficient to induce clear reductions of behavioral activity in the multicompartment chamber (MCC) (3) [see (8) and (13) for comprehensive reviews of behaviorally active doses of CRF]. The significance of high-dose effects is always difficult to evaluate, especially when reductions of behavior are elicited. Nevertheless, our results indicate that the drinking of sweetened milk by mice is sensitive to CRF and urocortin.

The present studies failed to reveal any significant differences between wild-type mice and CRFko mice in the intake of food pellets or sweetened milk in a 30-min daily exposure. This is consistent with the observation that both genotypes display similar growth rates (23). Furthermore, a variety of

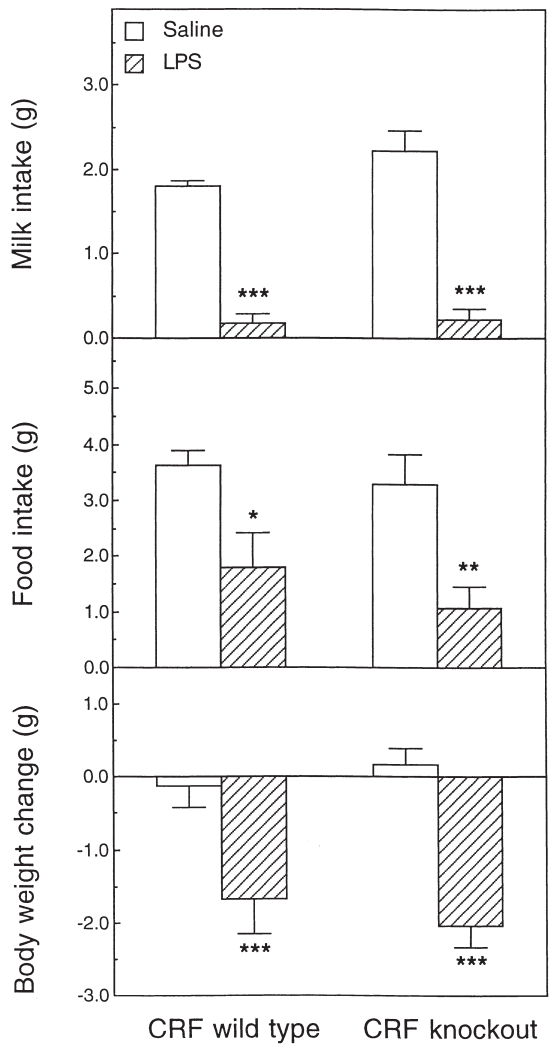

FIG. 3. Effect of LPS on milk intake (upper panel), food intake (middle panel) and body weight change (lower panel). Wild-type and CRFko mice were injected with saline or LPS (1 μg/mouse, IP) and 120 min later presented with milk. Food intake and body weight were observed over a period of 22 h following LPS administration. $n = 5$ for CRFko groups and $n = 4$ for CRFwt groups. Significantly different from the saline control mice ($*p < 0.05$, $**p < 0.01$, or $***p < 0.001$).

different treatments, physical restraint, IL-1β, LPS, and *d*-fenfluramine elicited very similar hypophagic effects in both CRFwt and CRFko mice. The results are not confined to the intake of sweetened milk, because the reduction in food pellet intake induced by LPS administration did not differ between CRFko and CRFwt mice (Fig. 3). The absence of a difference between the two mouse genotypes resembles the results of a previous study in which we failed to observe differences between the genotypes in behavior in the MCC, the elevated plus-maze, and in the open field, whether or not the animals were physically restrained (42).

Physical restraint has been shown previously to reduce food intake in rats, and this effect was attenuated by ICV treatment with the CRF antagonist, alpha-helical $CRF_{9–41}$ (ah-CRF) (19,32). IL-1 and LPS have been shown in numerous

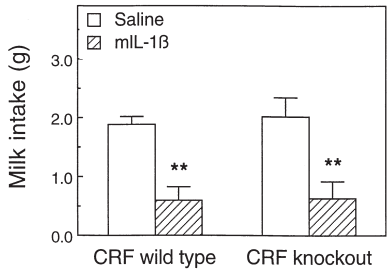

FIG. 4. Effect of mIL-1β on milk intake. Wild-type and CRFko mice were injected with saline or mIL-1β (100 ng/mouse, IP) and 90 min later presented with milk. $n = 5$ for CRFko groups and $n = 4$ for CRFwt groups. **Significantly different from the saline control mice ($p < 0.01$).

studies to reduce feeding behavior in many species (41), as well as in precisely the same paradigm used in the present study (36). Uehara et al. reported that the IL-1-induced hypophagia in fasted rats was attenuated with ICV administration of an antibody to CRF (39). However, we did not observe any tendency of ICV ahCRF to attenuate the reduction in milk drinking induced in mice by IL-1 (Swiergiel and Dunn, unpublished observations), although such injections were found to attenuate the responses of mouse behavior in the MCC to peripheral administration of IL-1 (7). The attenuation in the Uehara study was relatively small, and the conflicts could be explained by the species differences, the fact that the rats were fasted, the use of ahCRF or a CRF antibody (which probably recognized urocortin and possibly other related peptides), and the use of a different feeding paradigm.

It is well established that serotonin (5-HT) is involved in feeding behavior (11). 5-HT releasing agents, such as fenfluramine, and 5-HT reuptake blockers, such as fluoxetine are well-known anorexic agents (11,12). 5-HT has been implicated as a modulator of CRF secretion (10). Fluoxetine has been shown to stimulate hypothalamic release of CRF, and 5-HT has been strongly implicated in this response (12). It has been suggested that fenfluramine may affect food intake and energy balance through stimulating the release of hypothalamic CRF (20). ICV pretreatment with an antibody of CRF significantly attenuated the reductions in food intake observed in response to central injections of 5-HT, 5-hydroxy-

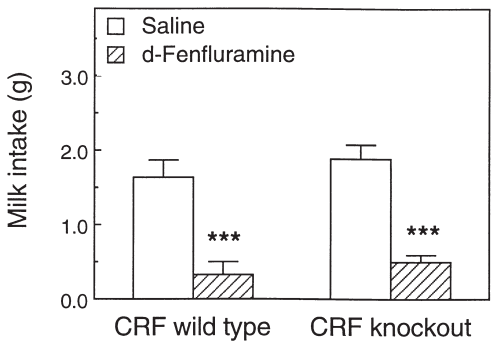

FIG. 5. Effect of *d*-fenfluramine on milk intake. Wild-type and CRFko mice were injected with saline or *d*-fenfluramine (4 mg/kg, IP), and 15 min later presented with milk. $n = 5$ for CRFko groups and $n = 4$ for CRFwt groups. Significantly different from the saline control mice (***$p < 0.001$).

tryptophan, and *d*-fenfluramine. However, the antibody did not significantly attenuate the anorexic actions of peripheral injection of fenfluramine (20). Bovetto et al. reported that ahCRF treatment did not prevent the hypophagic effects of 5-HT_{1B} (RU-24969) or 5-$HT_{2A/2C}$ (DOI) agonists on food intake (4). They noted that central injections of *d*-fenfluramine could cause anorexia by 5-HT–unrelated (stress-related) mechanisms and, thus, the finding that a CRF antibody could block the anorectic effect of 5-HT should be interpreted cautiously. They concluded that their results did not support a role for CRF in the anorectic effects of serotonin, and that the stimulation of CRF-containing neurons located in the hypothalamic paraventricular nucleus does not necessarily predict changes in food intake and energy expenditure. Our results indicate that CRFko mice responded to *d*-fenfluramine in the same way as the CRFwt mice, suggesting that CRF is not critical for the hypophagic responses to *d*-fenfluramine. Interestingly, another report suggested that the hypophagic effects of CRF are mediated by 5-HT_{2A} receptors (14).

Short-term milk drinking by animals having ad lib access to rodent chow represents nonessential consumption that is not necessary to maintain energy balance. It, thus, differs from long-term feeding behavior of animals that have been food deprived. Previous studies of the effects of CRF or urocortin on feeding have been performed testing intake of everyday diet, usually in food-deprived animals (5,19,35). It is entirely possible that had we used a different feeding paradigm, we might have observed differences between the CRFwt and CRFko mice.

It is possible, as others have suggested, that an anorexic role has been incorrectly ascribed to CRF, when that role may be played by urocortin (40). Two major subtypes of the CRF receptor have been identified, CRF_1 and CRF_2, and there are two splice variants of the CRF_2 receptor, $CRF_{2\alpha}$ and $CRF_{2\beta}$, that may have different pharmacological characteristics (15). Both CRF and urocortin are active on all three CRF receptors, albeit with different affinities (40). CRF antagonists such as ahCRF can also act on both CRF_1 and CRF_2 receptors. A study using administration of antisense oligonucleotides to the CRF receptor subtypes found that defensive withdrawal behavior was affected by antisense oligonucleotides to CRF_1, but not to CRF_2, suggesting that behaviors associated with anxiety are largely related to CRF_1 receptors (18). A similar conclusion was drawn from studies using an antagonist specific for the CRF_1 receptor, CP-154,526 (21), and CRF_1-receptor knockout mice (34,38). Conversely, the anorexia associated with ICV CRF and urocortin was attenuated by antisense oligonucleotides to CRF_2 receptors, but not by a CRF_1 antagonist (NBI27914), suggesting that feeding behavior may be associated with CRF_2 receptors (33). Unfortunately, feeding behavior studies have not yet been reported for CRF_1 receptor knockout mice, and CRF_2-specific antagonists and CRF_2-receptor knockouts are not yet available. Thus, it is possible that urocortin, which has a significantly higher affinity for the CRF_2 receptor than CRF itself, is the endogenous modulator of feeding. Alternatively, both peptides (and other, as yet unidentified, peptides) may contribute to this modulation in a redundant manner such that the absence of CRF is not evident.

The present studies and the previous ones with CRFko mice (42) indicate a clear dissociation between the role of CRF in behavioral responses, and its endocrine effects. CRFko mice have low basal plasma concentrations of ACTH and corticosterone, and exhibit very small responses to stressors (23), and the same is true for CRF_1-receptor knockouts (34, 38).

In view of the critical endocrine role of CRF, the wealth of data suggesting a role for CRF as a behavioral modulator, and the potential role of CRF in clinical disorders such as depression, anorexia, and obesity (22,25), the lack of differences in feeding responses between the genotypes in the present experiments is important. To assess the true biological role of CRF, it will be important to explore other kinds of behavior and physiological responses in CRFko mice to identify differences between the genotypes.

Our data indicate that CRF is not necessary for the expression of changes in the sweetened milk intake of mice in response to physical restraint, IL-1β, LPS, or *d*-fenfluramine—factors that are thought to contribute to the development of eating disorders. Other related peptides, such as urocortin, may assume this role, but it is just as likely that CRF is not a modulator of feeding in normal mice.

ACKNOWLEDGEMENTS

This research was supported by grants from the U.S. National Institute of Mental Health (MH46261 and MH50947) and the U.S. National Institute of Neurological Diseases and Stroke (NS25370). We thank Dr. Joseph A. Majzoub and Dr. Louis Muglia (Children's Hospital, Boston, MA) for providing the homozygous CRF knockout mice ($crf^{-/-}$) and the wild type ($Crf^{+/+}$) mice. The technical assistance of Mrs. Galina Mikhaylova is greatly appreciated.

REFERENCES

1. Berkenbosch, F.; van Oers, J.; del Rey, A.; Tilders, F.; Besedovsky, H.: Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. Science 238:524–526; 1987.
2. Berridge, C. W.; Dunn, A. J.: Corticotropin-releasing factor elicits naloxone-sensitive stress-like alterations in exploratory behavior in mice. Regul. Pept. 16:83–93; 1986.
3. Berridge, C. W.; Dunn, A. J.: Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF. J. Neurosci. 9:3513–3521; 1989.
4. Bovetto, S.; Rouillard, C.; Richard, D.: Role of CRH in the effects of 5-HT-receptor agonist on food intake and metabolic rate. Am. J. Physiol. 271:R1231–R1238; 1996.
5. Britton, D. R.; Koob, G. F.; Rivier, J.; Vale, W.: Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci. 31:363–367; 1982.
6. Cabanac, M.; Richard, D.: Acute intraventricular CRF lowers the hoarding threshold in male rats. Physiol. Behav. 57:705–710; 1995.
7. Dunn, A. J.; Antoon, M.; Chapman, Y.: Reduction of exploratory behavior by intraperitoneal injection of interleukin-1 involves brain corticotropin-releasing factor. Brain Res. Bull. 26:539–542; 1991.
8. Dunn, A. J.; Berridge, C. W.: Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress response? Brain Res. Rev. 15:71–100; 1990.
9. Dunn, A. J.; Powell, M. L.; Meitin, C.; Small, P. A.: Virus infection as a stressor: Influenza virus elevates plasma concentrations of corticosterone, and brain concentrations of MHPG and tryptophan. Physiol. Behav. 45:591–594; 1989.
10. Fuller, R. W.: The involvement of serotonin in regulation of pituitary–adrenocortical function. Front. Neuroendocrinol. 13:250–270; 1992.
11. Garattini, S.; Mennini, T.; Bendotti, C.; Invernizzi, R.; Samanin, R.: Neurochemical mechanisms of action of drugs which modify feeding via the serotonergic system. Appetite (Suppl.) 7:15–38; 1986.
12. Gibbs, D. M.; Vale, W.: Effect of the serotonin reuptake inhibitor fluoxetine on corticotropin-releasing factor and vasopressin secretion into hypophysial portal blood. Brain. Res. 280:176–179; 1983.
13. Glowa, J. R.; Barrett, J. E.; Russell, J.; Gold, P. W.: Effects of corticotropin releasing hormone on appetitive behaviors. Peptides 13:609–621; 1992.
14. Grignaschi, G.; Sironi, F.; Samanin, R.: Stimulation of 5-HT_2 receptors in the paraventricular hypothalamus attenuates neuropeptide Y-induced hyperphagia through activation of corticotropin releasing factor. Brain Res. 708:173–176; 1996.
15. Grigoriadis, D. E.; Lovenberg, T. W.; Chalmers, D. T.; Liaw, C.; DeSouza, E. B.: Characterization of corticotropin-releasing factor receptor subtypes. Ann. NY Acad. Sci. 780:60–80; 1996.
16. Guild, A. L.; Dunn, A. J.: Dopamine involvement in ACTH-induced grooming behavior. Pharmacol. Biochem. Behav. 17:31–36; 1982.
17. Hart, B. L.: Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12:123–137; 1988.
18. Heinrichs, S. C.; Lapasansky, J.; Lovenberg, T. W.; De Souza, E. B.; Chalmers, D. T.: Corticotropin-releasing factor CRF_1, but not CRF_2, receptors mediate anxiogenic-like behavior. Regul. Pept. 71:15–22; 1997.
19. Krahn, D. D.; Gosnell, B. A.; Grace, M.; Levine, A. S.: CRF antagonist partially reverses CRF- and stress-induced effects on feeding. Brain Res. Bull. 17:285–289; 1986.
20. Le Feuvre, R. A.; Aisenthal, L.; Rothwell, N. J.: Involvement of corticotrophin releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res. 555:245–250; 1991.
21. Mansbach, R. S.; Brooks, E. N.; Chen, Y. L.: Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor antagonist. Eur. J. Pharmacol. 323:21–26; 1997.
22. Morley, J. E.: Neuropeptide regulation of appetite and weight. Endocr. Rev. 8:256–287; 1987.
23. Muglia, L.; Jacobson, L.; Dikkes, P.; Majzoub, J. A.: Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature 373:427–432; 1995.
24. Muglia, L.; Jacobson, L.; Majzoub, J. A.: Production of corticotropin-releasing hormone-deficient mice by targeted mutation in embryonic stem cells. Ann. NY Acad. Sci. 780:49–59; 1996.
25. Nemeroff, C. B.: The corticotropin-releasing factor (CRF) hypothesis of depression: New findings and new directions. Mol. Psychiatry 1:336–342; 1996.
26. Owens, M. J.; Nemeroff, C. B.: Physiology and pharmacology of corticotropin-releasing factor. Pharmacol. Rev. 43:425–474; 1991.
27. Plamondon, H.; Merali, Z.: Anorectic action of bombesin requires receptor for corticotropin-releasing factor but not for oxytocin. Eur. J. Pharmacol. 340:99–109; 1997.
28. Raber, J.; Chen, S.; Mucke, L.; Feng, L.: Corticotropin-releasing factor and adrenocorticotrophic hormone as potential central mediators of OB effects. J. Biol. Chem. 272:15057–15060; 1997.
29. Richard, D.: Involvement of corticotropin-releasing factor in the control of food intake and energy expenditure. Ann. NY Acad. Sci. 697:155–172; 1993.
30. Rivest, S.; Richard, D.: Involvement of corticotropin-releasing factor in the anorexia induced by exercise. Brain Res. Bull. 25:169–172; 1990.
31. Sapolsky, R.; Rivier, C.; Yamamoto, G.; Plotsky, P.; Vale, W.: Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238:522–524; 1987.
32. Shibasaki, T.; Yamauchi, N.; Kato, Y.; Masuda, A.; Imaki, T.; Hotta, M.; Demura, H.; Oono, H.; Ling, N.; Shizume, K.: Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43:1103–1110; 1988.
33. Smagin, G. N.; Howell, L. A.; Ryan, D. H.; De Souza, E. B.; Harris, R. B. S.: The role of CRF_2 receptors in corticotropin-releasing factor- and urocortin-induced anorexia. Neuroreport 9:1601–1606; 1998.

34. Smith, G. W.; Aubry, J.-M.; Dellu, F.; Contarino, A.; Bilezikjian, L. M.; Gold, L. H.; Chen, R.; Marchuk, Y.; Hauser, C.; Bentley, C. A.; Sawchenko, P. E.; Koob, G. F.; Vale, W.; Lee, K.-F.: Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron. 20:1093–1102; 1998.
35. Spina, M.; Merlo-Pich, E.; Chan, R. K. W.; Basso, A. M.; Rivier, J.; Vale, W.; Koob, G. F.: Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. Science 273:1561–1564; 1996.
36. Swiergiel, A. H.; Smagin, G. N.; Dunn, A. J.: Influenza virus infection of mice induces anorexia: Comparison with endotoxin and interleukin-1 and the effects of indomethacin. Pharmacol. Biochem. Behav. 57:389–396; 1997.
37. Swiergiel, A. H.; Smagin, G. N.; Johnson, L. J.; Dunn, A. J.: The role of cytokines in the behavioral responses to endotoxin and influenza virus infection in mice: Effects of acute and chronic administration of the interleukin-1-receptor antagonist (IL-1ra). Brain Res. 776:96–104; 1997.
38. Timpl, P.; Spanagel, R.; Sillaber, I.; Kresse, A.; Reul, J. M. H. M.; Stalla, G. K.; Blanquet, V.; Steckler, T.; Holsboer, F.; Wurst, W.: Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. Nat. Genet. 19:162–166; 1998.
39. Uehara, A.; Sekiya, C.; Takasugi, Y.; Namiki, M.; Arimura, A.: Anorexia induced by interleukin-1: Involvement of corticotropin-releasing factor. Am. J. Physiol. 257:R613–R617; 1989.
40. Vaughan, J.; Donaldson, C.; Bittencourt, J.; Perrin, M. H.; Lewis, K.; Sutton, S.; Chan, R.; Turnbull, A. V.; Lovejoy, D.; Rivier, C.; Sawchenko, P. E.; Vale, W.: Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378:287–292; 1995.
41. Weingarten, H. P.: Cytokines and food intake: The relevance of the immune system to the student of ingestive behavior. Neurosci. Biobehav. Rev. 20:163–170; 1996.
42. Weninger, S. C.; Dunn, A. J.; Muglia, L. J.; Miczek, M. A.; Swiergiel, A. H.; Berridge, C. W.; and Majzoub, J. A.: CRH-deficient mice display normal behaviors during stress and in response to CRH. Proc. Natl Acad. Sci. US, in press.