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The Effects of ICV-CRH on Novelty-Induced Behavior[1]

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SHERMAN, J. E. AND N. H. KALIN. *The effects of ICV-CRH on novelty-induced behavior.* PHARMACOL BIOCHEM BEHAV 26(4) 699–703, 1987.—To assess whether centrally administered corticotropin-releasing hormone (CRH) modulates behavioral and antinociceptive effects of exposure to a novel environment, vehicle or 0.03, 0.3, or 3.0 μg of CRH was administered intracerebroventricularly (ICV) to rats, which were then tested under novel or familiar conditions. Novelty decreased sleeping and grooming and increased rearing, walking, and latency to respond on the hot-plate test of analgesia. CRH increased grooming and walking, decreased rearing and sleeping, and had no effect in the hot-plate test. The lowest dose was without effect on any measure; otherwise, CRH effects generally were dose-dependent. There was no evidence that CRH selectively enhanced or interfered with novelty-induced behavioral changes; it influenced behavior to the same degree in both test conditions. However, test condition selectively modulated the degree of peptide-induced self-gnawing and burrowing.

Rats CRH CRF Novelty stress Behavior Analgesia

IN 1981, Vale and his colleagues [29] identified a peptide in the hypothalamus that potently stimulates the release of corticotropin. Subsequent research demonstrated that this peptide meets the criteria for the corticotropin-releasing hormone (CRH) (see [28]), initiating the hormonal response to stress. Like other hypothalamic releasing hormones, CRH and its receptors were found to have extrahypothalamic brain distribution [19, 20, 26], raising the possibility that CRH plays a role in organizing brain systems that complement its endocrine role in the stress response.

Several lines of evidence suggest a hypothalamic and an extrahypothalamic role of CRH in the stress response. First, CRH and its receptors are found in brain stem regions associated with behavioral arousal and anxiety [8, 19, 26]. Second, CRH administered intracerebroventricularly (ICV) increases neuronal activity in these areas [30] and produces electroencephalographic changes suggestive of increased arousal [10]. Third, ICV-CRH produces physiological changes resembling stress responses. These include increases in arterial pressure, heart rate [6, 7, 11, 12], oxygen consumption [7], and plasma concentrations of ACTH, corticosterone, glucose, vasopressin, and catecholamines [7, 9, 15, 16, 31]. Lastly, ICV-CRH produces behavioral changes that may be characterized as stress-related. In rats, it elicits behavioral changes similar to those observed in the novel open-field test [3]—namely, increased grooming and decreased ingestive behavior [4, 18, 23, 25, 31]; and the anxiolytic chlordiazepoxide has been shown to attenuate anxiety-like effects of ICV-CRH in an operant conflict test [5]. ICV-CRH also potentiates the acoustic startle response [27], a reflexive response sensitive to stress or fear; and in rhesus monkeys, it evokes behaviors including vocalization, head-shaking, and struggling [16]. These data are consistent with the hypothesis that extrahypothalamic CRH brain systems play a role in integrating visceral, hormonal, and behavioral responses similar to those seen with stress.

The present study further characterizes the role of brain CRH systems in stress-related behavior by assessing the specificity of ICV-CRH's behavioral effects under conditions of environmental stress. In the rat, environmental novelty elicits a powerful stress response as measured by hormonal [2, 13, 14, 21] and behavioral indices [1,3], although research shows that the specific direction of the behavioral changes elicited by a novel environment may vary (for a review, see [1]). Unlike previous behavioral studies of ICV-CRH in which different physical environments were used for novel and familiar test environments [4,25], our study used only one environment, to which rats in the familiar treatment group were pre-exposed. The use of a single test environment for assessing the behavioral effects of novelty is desirable because it avoids confounding the stressful effects of novelty with possible effects due to physical differences in the environments [24].

Moreover, in previous studies of the effects of ICV-CRH in novel and familiar environments [4,25] the magnitudes of the behavioral effects in each environment were not directly compared, precluding an assessment of posible interactions between peptide and stressor. In the present study, the

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same behavioral measures were taken in both test environments—namely, those that we had previously found to be influenced by ICV-CRH but not by peripheral CRH [23]: sleeping, grooming, walking, rearing, self-gnawing, and burrowing. The hot-plate test of antinociception was also included because, although previous research suggests that ICV-CRH administered in a familiar environment does not evoke analgesia [5,23], novelty can elicit analgesia [22]. If brain CRH systems modulate the normal response to stress, an enhancement of novelty-induced analgesia might be obtained after ICV-CRH.

METHOD

Subjects

Experimental subjects were 87 albino Sprague Dawley-derived male rats weighing 180–200 g at time of delivery (Sasco-King Laboratories, Oregon, WI, and Omaha, NE). Rats were individually housed in standard stainless steel cages suspended above absorbent paper. Access to food and water was unrestricted in the home cages. All procedures were conducted at least one week after the rats arrived at our colony, between 0900 and 1600 of the 12-hr light component (0600–1800 hr) of the 24-hr light-dark cycle.

Apparatus and Drugs

Pre-exposure and test sessions were conducted in a separate room in the continuous presence of white noise (62 dB). Rats were individually transported to the test room in opaque plastic cages and after CRH or vehicle administration were placed in individual clear polypropylene cages 30.2 cm long, 26.2 cm wide, and 13.5 cm high, with pine-chip bedding approximately 2.5 cm deep. One milliliter of almond extract was spread on the chips. Each cage was fitted with a wire cover (Wahmann Mfg. Co., Timonium, MD), and food but no water was present. Thus the test environment had distinct olfactory, visual, auditory, and tactile characteristics, in addition to which animals were handled during repeated transfers from plastic cage to hot-plate apparatus.

Assessment of pain sensitivity was conducted with a hot-plate apparatus [23] that heated and circulated 51.3°C water under the surface of an aluminum plate. The temperature of the water during pre-exposure sessions was 22–24°C.

CRH solutions were prepared as previously described [16], using synthetic rat CRH (Bachem Co., Torrance, CA). Vehicle was 0.9% sterile saline.

Procedures

Cannula placement, drug administration, and verification of cannula placement followed procedures previously described [23]. Only data from rats in which cannulae had been accurately placed are reported.

Pre-exposure. Forty-two rats assigned to the familiar treatment were pre-exposed to the test environment five times. Each exposure consisted of a 1-hr session in which the rats were individually placed in a clean plastic cage with almond-scented pine chips. Three times during this hour, at 20-min intervals, each rat was placed on the surface of the nonheated hot plate. Rats were then returned to their home cages. Forty-five rats assigned to the novel treatment were not handled during this time.

Behavioral and hot-plate testing. One week after cannula placement, rats were transported to the testing room and infused with CRH or vehicle. In the novel-environment treatment the number of rats receiving vehicle or 0.03, 0.3, or 3.0 μg of CRH was 13, 12, 11, and 9, respectively; in the familiar environment, the number was 11, 10, 12, and 9. After infusion, each rat was tested over a 100-min period. Two-minute periods of behavioral rating were concluded 10, 20, 40, 60, 80, and 100 min after infusion. A time-sampling procedure was used for the behavioral ratings: once at the end of each 10-sec interval during the 2-min observational period, one of six behaviors was rated. Only one behavior could be rated at a time; thus, during a single 2-min observational period a total of 12 counts could be made. The behaviors rated were (1) sleeping—no movement, head down and eyes closed, (2) grooming—licking or scratching body or fur, (3) walking—locomotor behavior in which the head and front paws were above the surface of pine bedding, (4) burrowing—locomotor behavior in which the face and/or paws were below the surface of pine bedding (typically, paws were extended in front of the rat as though pushing the bedding), (5) rearing—front paws elevated off bedding (was not rated if rat groomed), and (6) self-gnawing—a stereotyped mouthing of the paws or tail.

At minutes 20, 40, 60, 80, and 100 a hot-plate test was given. In this test each rat was placed on the hot-plate surface and latency until a paw lick or jump was recorded. All rats remained on the hot plate for 60 sec. Thus there were a total of six behavioral observation periods and five hot-plate tests during the 100-min test interval. Rats were tested in squads of four. Treatment conditions and drug doses were counterbalanced for order of testing.

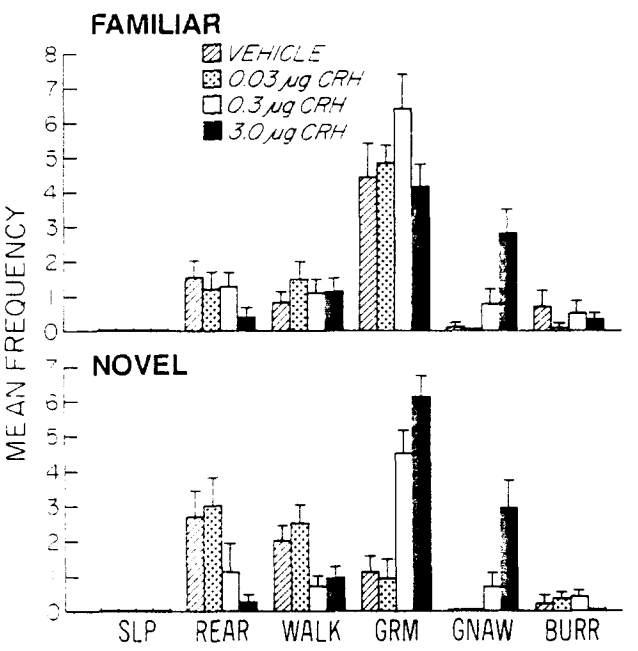

FIG. 1. Effect of test condition and CRH on the mean (±SE) frequency of sleeping (SLP), rearing (REAR), walking (WALK), grooming (GRM), self-gnawing (GNAW), and burrowing in pine chips (BURR) during the first 2-min test interval beginning 8 min after CRH administration.

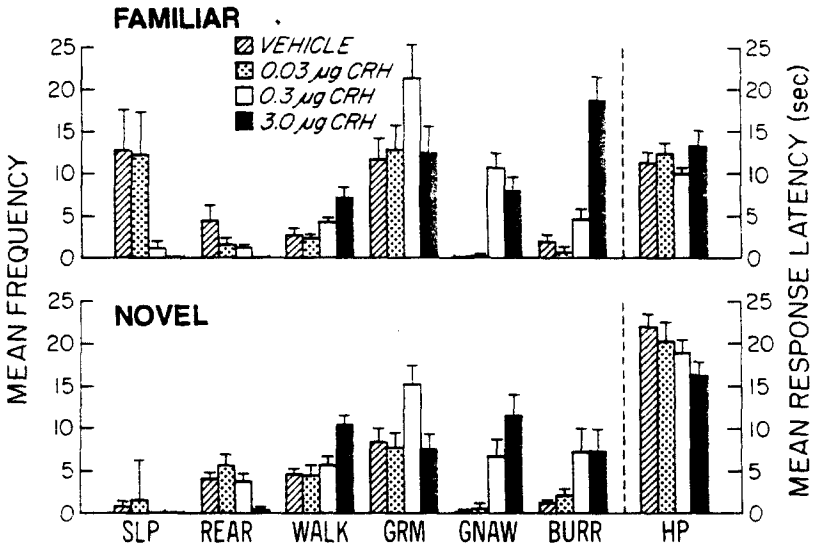

FIG. 2. Effect of test condition and CRH on the mean (±SE) frequency of sleeping, rearing walking, grooming, self-gnawing, and burrowing and mean hot-plate (HP) latencies, averaged over duration of session (excluding data reported in Fig. 1).

Statistical Analyses

Two sets of statistical analyses were conducted. One was based on results obtained from the first 2-min observational period only. The second included all five remaining behavioral observational periods and the hot-plate tests. Separate analysis of the first 2-min period maximized the possibility of detecting novelty-related behavioral responses that might habituate over the course of testing.

In both sets of analyses, each dependent measure was submitted to a factorial analysis of variance (ANOVA) of test condition (novel vs. familiar) and dose. In the second set, a within-subjects analysis was also done. The modified protected least significant difference test was used to make subsequent pair-wise comparisons of dose [17]. The criterion for statistical significance was $p<0.05$.

RESULTS

There were potent effects of both test condition and dose on behavior. Figure 1 presents the mean frequency of each of the six behavioral measures for the first 2 min of testing for each treatment condition. Figure 2 presents (1) the mean frequencies for these behavioral measures averaged across the remaining five 2-min observation periods and (2) the mean latency to respond on the hot-plate test averaged across the five tests.

Novel vs. Familiar Test Conditions

During the first 2-min test period, significant effects of test condition were found for only two behaviors (Fig. 1). The novel test condition produced more rearing, $F(1,79)=4.00$, $p=0.046$, and less grooming, $F(1,79)=8.27$, $p=0.008$, than the familiar test condition. Test condition had no effect on frequency of walking, self-gnawing, and burrowing; no sleeping was observed under either test condition.

For the remainder of the 100-min session (Fig. 2), significant effects of test condition were found for all measures except self-gnawing and burrowing. The novel test condition resulted in less sleep, $F(1,79)=11.49$, $p=0.0015$, more rearing, $F(1,79)=4.85$, $p=0.029$, more walking, $F(1,79)=12.76$, $p=0.001$, less grooming, $F(1,79)=8.77$, $p=0.004$, and longer hot-plate latencies, $F(1,79)=45.05$, $p<0.0001$. The effect of novelty did not significantly interact with repeated testing for any of the measures except sleep. Although no rat was observed to sleep at the first test, across the remaining trials there was an increasing frequency of sleeping among rats in the familiar condition but not in the novel condition.

ICV-CRH Dose

As Fig. 1 shows, effects of ICV-CRH appeared as soon as 8–9 min after administration. CRH yielded significant main effects on rearing, $F(3,79)=4.43$, $p=0.007$, walking, $F(3,79)=3.10$, $p=0.031$, grooming, $F(3,79)=5.56$, $p=0.002$, and self-gnawing, $F(3,79)=23.21$, $p=0.0001$, compared to vehicle. The lowest dose was without effect. The highest dose clearly attenuated rearing and walking and enhanced grooming and gnawing; the intermediate dose had mixed effects.

Throughout the remainder of the session, CRH produced significant changes for all measures except hot-plate responding (Fig. 2). It reduced sleeping, $F(3,79)=4.89$, $p=0.04$, and rearing, $F(3,79)=4.89$, $p=0.004$, and increased walking, $F(3,79)=12.77$, $p<0.0001$, grooming, $F(3,79)=6.12$, $p=0.001$, gnawing, $F(3,79)=33.14$, $p<0.0001$, and burrowing, $F(3,79)=20.52$, $p<0.0001$. Again, relative to vehicle, the lowest dose of CRH was without effect on any measure, and the highest dose generally produced the largest effects. For grooming, relative to vehicle the intermediate dose produced a significant increase but the highest dose was without effect. The effect of dose interacted significantly for sleeping and burrowing with repeated testing. The attenuation of sleep in treated animals became increasingly manifest as vehicle treated animals slept more. For burrowing, it appears that

the changes across testing reflected a time-dependent effect of CRH.

Interaction of Novelty and CRH

Statistical analyses of the results of the first observation period (Fig. 1) revealed an interaction of test condition and dose for grooming, $F(3,79)=4.20$, $p=0.008$. Subsequent analyses showed effects of dose on grooming in the novel test condition but not in the familiar one. Comparison of test conditions at each dose revealed significantly less grooming in the novel condition at the zero and 0.03-μg doses, whereas the frequency of grooming at the higher doses did not differ between the two test conditions ($p>0.11$). Thus the interaction of dose and test condition was due to the suppressive effects of the novel test environment on grooming, evidenced with vehicle and the lowest dose of CRH. This suppressive effect of novelty on grooming was masked by the induction of grooming at the higher doses of CRH.

Figure 2 shows the interaction of test condition with the effects of CRH for sleeping, gnawing, and burrowing, $F(3,79)=3.2$, $p=0.027$; $F(3,79)=2.98$, $p=0.036$; $F(3,79)=6.96$, $p=0.0005$, respectively. For sleeping, significant effects of dose were obtained for the familiar test condition but not the novel one. Rats in the familiar test condition slept after administration of vehicle and the lowest CRH dose, but not after the higher doses. In contrast, very little sleeping occurred in any rats in the novel test condition, making it impossible to show an effect of CRH on sleep. Consequently, this interaction reflects a difference in the amount of sleep induced by the test condition rather than a differential effect of CRH on sleep frequency.

The 0.3-μg dose yielded a greater frequency of self-gnawing than the 3.0-μg dose in the novel test condition. This relationship was reversed in the familiar test condition. For burrowing, there was a greater frequency with the 3.0-μg dose than with the 0.3-μg dose, in the familiar test condition. This difference did not occur in the novel condition.

DISCUSSION

CRH produced some effects in the same direction as novelty (for sleeping and walking) and some in the opposite direction (for grooming and rearing), and was without effect in one test (hot-plate). Clearly, these findings show that CRH does not influence all the behavioral changes elicited by the novel test condition, nor does it necessarily yield behavioral change in the same direction as that induced by novelty.

There was no clear evidence that ICV-CRH selectively enhanced or interfered with novelty-induced behavioral changes. In the two instances in which a significant test-condition by dose interaction was obtained, the interaction was attributable to differences in baseline frequencies of behavior in the two test conditions, not to differences in the potency of the peptide in the two test conditions. Thus, for those behaviors influenced by both environment and peptide the effects were generally additive.

While other investigators have examined the effects of ICV-CRH in novel and familiar test conditions [4,25], direct comparisons of the interaction of environment and CRH were not made. However, these investigators found as we did that ICV-CRH enhanced grooming above vehicle control values in both novel and familiar test environments. In addition, they found that CRH suppressed food consumption and approach in both environments. In contrast to the present study, in which ICV-CRH increased walking in both environments, Sutton *et al.* [25] reported that ICV-CRH decreased locomotion in a novel environment and increased it in the familiar environment. It is not clear whether the different environments used for the novel and familiar test conditions account for this difference in the effect of CRH between our study and theirs. Aside from this one discrepant finding, ICV-CRH has been found to produce comparable behavioral effects in novel and familiar test conditions. The present study strengthens this general conclusion by testing for interactions between CRH and test environment.

It is important to note that while the present study did not find a selective effect of ICV-CRH on behavior naturally occurring in novel and familiar test conditions, behaviors elicited by the peptide (self-gnawing and burrowing) were differentially modulated by the test conditions. Even though an explanation of these effects is not readily apparent, the findings are important because they show that the effects of CRH are sensitive to environmental input.

Lastly, the present experiments show that ICV-CRH does not influence responding on the hot-plate test of analgesia, confirming previous findings [5,23]. That an effect of novelty was found at the same time that no effect of CRH was found indicates that the lack of a peptide effect was not attributable to measurement problems. While in our experiment CRH was not found to specifically enhance novelty-induced behaviors, it is possible that other stress paradigms might yield such an effect. Our data do not preclude the possibility that endogenous CRH systems mediate some of the behavioral manifestations of stress that are not stressor specific.

REFERENCES

1. Archer, J. Tests for emotionality in rats and mice: a review. *Anim Behav* **21:** 205–235, 1973.
2. Bassett, J. R. and K. D. Cairncross. Parameters of novelty, shock predictability and response contingency in corticosterone release in the rat. *Physiol Behav* **10:** 901–907, 1973.
3. Britton, D. R. and K. T. Britton. A sensitive open field measure of anxiolytic drug activity. *Pharmacol Biochem Behav* **15:** 577–582, 1981.
4. Britton, D. R., G. F. Koob, J. Rivier and W. Vale. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci* **31:** 363–367, 1982.
5. Britton, K., J. Morgan, J. Rivier, W. Vale and G. Koob. Chlordiazepoxide attenuates CRF-induced response suppression in the conflict test. *Psychopharmacology (Berlin)* **86:** 170–174, 1985.
6. Brown, M. and L. A. Fisher. Central nervous system effects of corticotropin releasing factor in the dog. *Brain Res* **280:** 75–79, 1983.
7. Brown, M. R., L. A. Fisher, J. Rivier, J. Spiess, C. Rivier and W. Vale. Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. *Life Sci* **30:** 207–210, 1982.

8. DeSouza, E. B., M. H. Perrin, T. R. Insel, F. Rivier, W. Vale and M. Kuhar. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. *Science* **224**: 1449–1451, 1984.
9. Donald, R. A., C. Redekopp, V. Cameron, M. G. Nicholls, J. Bolton, J. Livesey, E. A. Espiner, J. Rivier and W. Vale. The hormonal actions of CRF in sheep: effect of intravenous and intracerebroventricular injection. *Endocrinology* **113**: 866–870, 1983.
10. Ehlers, C. L., S. J. Henriksen, M. Wang, J. Rivier, W. W. Vale and F. E. Bloom. Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats. *Brain Res* **278**: 332–336, 1983.
11. Fisher, L. A. and M. R. Brown. Corticotropin-releasing factor and angiotensin II: comparison of CNS actions to influence neuroendocrine and cardiovascular function. *Brain Res* **296**: 41–47, 1983.
12. Fisher, L. A., J. Rivier, C. Rivier, J. Spiess, W. Vale and M. R. Brown. Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. *Endocrinology* **110**: 2222–2224, 1982.
13. Friedman, S. B. and R. Ader. Adrenocortical response to novelty and noxious stimulation. *Neuroendocrinology* **2**: 209–212, 1967.
14. Hennessy, M. B. and S. Levine. Sensitive pituitary-adrenal responsiveness to varying intensities of psychological stimulation. *Physiol Behav* **21**: 295–297, 1978.
15. Insel, T. R., J. A. Aloi, D. Goldstein, J. H. Wood and D. C. Jimerson. Plasma cortisol and catecholamine responses to intracerebroventricular administration of CRF to rhesus monkeys. *Life Sci* **34**: 1873–1878, 1984.
16. Kalin, N. H., S. E. Shelton, G. W. Kraemer and W. T. McKinney. Corticotropin-releasing factor administered intraventricularly to rhesus monkeys. *Peptides* **4**: 217–220, 1983.
17. Keppel, G. *Design and Analysis: A Researcher's Handbook*, 2nd edition. Englewood Cliffs, NJ: Prentice-Hall, Inc., 1982, pp. 145–165.
18. Morley, J. E. and A. S. Levine. Corticotropin releasing factor, grooming and ingestive behavior. *Life Sci* **31**: 1459–1464, 1982.
19. Olschowka, J. A., T. L. O'Donohue, G. P. Mueller and D. M. Jacobowitz. Hypothalamic and extrahypothalamic distribution of CRF-like immunoreactive neurons in the rat brain. *Neuroendocrinology* **35**: 305–308, 1982.
20. Schipper, J., H. W. Steinbusch, I. Vermes and F. J. Tilders. Mapping of CRF-immunoreactive nerve fibers in the medulla oblongata and spinal cord of the rat. *Brain Res* **267**: 145–150, 1983.
21. Seggie, J. A. and G. M. Brown. Stress response patterns of plasma corticosterone, prolactin and growth hormone in the rat, following handling or exposure to novel environment. *Can J Physiol Pharmacol* **53**: 629–637, 1975.
22. Sherman, J. E. The effects of conditioning and novelty on the rat's analgesic and pyretic responses to morphine. *Learn Motiv* **10**: 383–418, 1979.
23. Sherman, J. E. and N. H. Kalin. ICV-CRH potently affects behavior without altering antinociceptive responding. *Life Sci* **39**: 433–441, 1985.
24. Stewart, W. J. Size of the environment as a determiner of effects of scopolamine. *Psychol Rep* **37**: 175–178, 1975.
25. Sutton, R. E., G. F. Koob, M. LeMoal, J. Rivier and W. W. Vale. Corticotropin-releasing factor produces behavioral activation in rats. *Nature* **297**: 331–333, 1982.
26. Swanson, L. W., P. E. Sawchenko, J. Rivier and W. W. Vale. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* **36**: 165–186, 1983.
27. Swerdlow, N. R., M. A. Geyer, W. W. Vale and G. F. Koob. Corticotropin-releasing factor potentiates acoustic startle in rats: blockade by chlordiazepoxide. *Psychopharmacology (Berlin)* **88**: 147–152, 1986.
28. Vale, W., C. Rivier, M. Brown, P. Plotsky, M. Smith, L. Bilezikjian, T. Bruhn, M. Perrin, J. Spiess and J. Rivier. Corticotropin-releasing factor. In: *Secretory Tumors of the Pituitary Gland*, edited by P. Black. New York: Raven Press, 1984, pp. 213–225.
29. Vale, W., J. Spiess, C. Rivier and J. Rivier. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* **213**: 1394–1397, 1981.
30. Valentino, R. J., S. L. Foote and G. Aston-Jones. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. *Brain Res* **270**: 363–367, 1983.
31. Veldhuis, H. D. and D. DeWied. Differential behavioral actions of corticotropin-releasing factor (CRF). *Pharmacol Biochem Behav* **21**: 707–713, 1984.