Pharmacology Biochemistry & Behavior, Vol. 34, pp. 517–519. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

CRF and Restraint-Stress Decrease Exploratory Behavior in Hypophysectomized Mice

CRAIG W. BERRIDGE[1] AND ADRIAN J. DUNN[2]

Department of Neuroscience, University of Florida, Gainesville, FL 32610

Received 24 February 1989

BERRIDGE, C. W. AND A. J. DUNN. *CRF and restraint-stress decrease exploratory behavior in hypophysectomized mice.* PHARMACOL BIOCHEM BEHAV **34**(3) 517–519, 1989.—Corticotropin-releasing factor (CRF) and restraint-stress both decrease exploratory behavior in rats and mice. The involvement of pituitary-adrenal hormones in eliciting these behavioral effects was examined using hypophysectomized mice. Forty minutes of restraint decreased exploratory behavior in hypophysectomized mice just as it did in intact animals. Similarly, CRF (50 ng) injected into the lateral cerebral ventricles of hypophysectomized mice decreased exploratory behavior. Therefore, the restraint- and CRF-induced decreases of exploratory behavior are apparently independent of the activation of ACTH secretion from the pituitary. It seems likely that CRF acts intracerebrally to elicit this effect of restraint, especially because a CRF antagonist can reverse the effects of restraint.

Restraint stress Corticotropin-releasing factor Exploratory behavior Hypophysectomy

CORTICOTROPIN-RELEASING factor (CRF), released from the hypothalamus, stimulates the secretion of adrenocorticotropic hormone (ACTH) and β-endorphin from the pituitary (28). This action of CRF is of critical importance in regulating peripheral responses in stress. CRF-containing neurons and high-affinity binding sites for CRF have also been identified in extrahypothalamic regions of the brain (12, 27, 30). Moreover, intracerebral administration of CRF elicits a variety of physiological (9,29), neurochemical (14), and behavioral responses (1, 5, 13, 19, 22, 26, 29). This suggests that CRF has an action in the brain distinct from its ability to activate the pituitary-adrenal axis.

The similarity between the responses elicited by CRF and those observed in stress has prompted the suggestion that this peptide might play a role in regulating behavioral and physiological responding in stress (16,20). The fact that CRF concentrations are altered in several brain regions following stress suggests that cerebral CRF may be secreted during stress (11). Consistent with this there is a preliminary report of a stress-induced increase in the concentration of CRF in the cerebrospinal fluid (6). Further support for the hypothesis that CRF is involved in the stress response has been obtained using the CRF antagonist, alpha-helical CRF_{9-41} [ahCRF; (23)]. ICV ahCRF (100 μg) inhibited the electric shock-induced inhibition of luteinizing hormone release in castrated male rats (24). It also prevented the ether stress-induced increase in plasma epinephrine (10), and attenuated the restraint-induced decrease in food consumption in 24-hr food-deprived rats without affecting eating in nonrestrained animals (21).

Similar results were obtained in studies that examined the possible involvement of CRF in the restraint-stress-induced decrease in exploratory behavior, measured by the time an animal spends investigating objects in a novel environment. In this paradigm, ICV CRF (5–100 ng) significantly decreased exploratory behavior in the absence of significant effects on locomotor activity (1). The CRF-antagonist, ahCRF, reversed the restraint-induced decrease in exploratory behavior in mice when injected ICV prior to restraint (2). These results suggest that an increased release of CRF within the brain mediates this restraint-induced change in exploratory behavior.

ACTH and the glucocorticoids have been shown to affect behavioral responding. For example, glucocorticoids and ACTH alter locomotor activity (4), and avoidance (3) and escape (25) behaviors. Thus, it is possible that restraint and CRF decrease exploratory behavior through an activation of the pituitary-adrenal axis. The following studies were designed to examine this possibility by testing the effects of these two treatments on exploratory behavior in hypophysectomized mice.

METHOD

Animals

Hypophysectomized male mice (CD-1; 24–28 g) were obtained from Charles Rivers (Wilmington, MA) and were group-housed

[1]Present address: Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

[2]Requests for reprints should be addressed to Dr. Adrian Dunn at his present address: Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

with free access to food and a 9% sucrose solution of at least 5 days prior to testing or surgery.

Surgery

In experiments requiring ICV injections, polyethylene cannulae (Clay Adams PE-50 tubing) were implanted in the lateral cerebral ventricles (17). Surgery was performed under pentobarbital anesthesia. Cannula placement was verified by visual inspection following injection of dye through the cannulae.

Procedures

Restraint was administered by allowing the mouse to enter a darkened polyethylene tube 10 cm in length and 3 cm in diameter, preventing their exit by taping the ends of the tube with laboratory labeling tape (1). Restrained animals were transferred to a room separate from both the colony and testing rooms and restrained for 40 min. Immediately following restraint, the animals were carried into the testing room and placed in the testing chamber. Unrestrained animals were transferred from the colony room to the testing room and immediately placed in the testing chamber. ICV injections consisted of a total volume of 4 μl divided equally between the two cannulae and given over a 30–45-sec period. ICV injections were performed 10 min prior to testing. Behavioral testing was conducted between 0830 and 1600.

All animals were group housed throughout the experiment. Verification of the hypophysectomy was determined after completion of the experiment by visual inspection of the sella tursica, and by measurement of plasma corticosterone concentrations using a radioimmunoassay following exposure of the animals to 30 min of restraint. Animals that either had visible tissue remnants of the pituitary and/or that had corticosterone concentrations greater than 40 ng/ml following restraint were excluded from the experiment. Using these criteria, 2 animals were excluded from each experiment.

Behavioral Testing

The experimental chamber used was as described previously (1). Briefly, this chamber consisted of nine interconnecting compartments, within each of which a wire mesh stimulus (3.0 cm sphere) was recessed in a 2.5 cm hole below the floor. The duration and frequency of a number of behaviors were recorded during a 30-min period of observation. The following behaviors were recorded: Measures of contact with the stimuli: number of contacts, duration of contacts, mean time per contact (duration of contacts/number of contacts); Measures of locomotor activity: compartment entries, rears, and inactivity; and time spent grooming.

Determination of Plasma Corticosterone Concentrations

Plasma corticosterone was determined following extraction with methylene chloride by a radioimmunoassay according to the procedure of Gwosdow-Cohen *et al.* (18).

Statistical Analysis

Statistical significance was determined using Student's *t*-test.

RESULTS

The Effects of Restraint on Exploratory Behavior in Hypophysectomized Mice

Table 1 shows the effect of restraint on stimulus-directed behavior and locomotor activity as measured by the number of rears and compartment entries in hypophysectomized mice. Restraint significantly decreased the mean time per contact with the stimuli, $t(16)=6.16$, $p<0.001$. This treatment also significantly decreased the total time of contacts, $t(16)=14.4$, $p<0.001$, but did not affect the total number of contacts with the stimuli. Restraint had no significant effects on the number of compartment entries or rears.

TABLE 1

EFFECT OF RESTRAINT ON EXPLORATORY BEHAVIOR IN HYPOPHYSECTOMIZED MICE

	Unrestrained	Restrained
Mean time per contact (sec)	3.01 ± 0.26	1.4 ± 0.09*
Total duration of contacts (sec)	527 ± 20	244 ± 5*
Total number of contacts	180 ± 18	176 ± 15
Total number of rears	196 ± 28	168 ± 16
Total number of compartment entries	376 ± 35	359 ± 29

The mean time per contact, total duration of contacts, total number of contacts with the stimuli, and the total number of rears and compartment entries (mean ± SEM) of hypophysectomized mice restrained for 40 min.

*Significantly different from unrestrained mice ($p<0.001$, Student's *t*-test).

The Effects of CRF on Exploratory Behavior in Hypophysectomized Mice

Animals were tested 3–4 days following implantation of the cannulae. A number of the hypophysectomized mice did not tolerate the surgery well (10 of a total of 24). These animals were hypoactive and lost a significant amount of weight between surgery and testing. Animals that appeared ill or that had lost more than 4 g were not tested. Mice were injected ICV with either 50 ng of CRF or saline 15 min before testing. CRF significantly decreased the mean time per contact, $t(12)=8.55$, $p<0.001$, and the total duration of contacts, $t(12)=2.48$, $p<0.05$, but did not affect the total number of contacts with the stimuli (Table 2). CRF did not significantly affect the number of compartment entries or

TABLE 2

EFFECT OF ICV CRF ON EXPLORATORY BEHAVIOR IN HYPOPHYSECTOMIZED MICE

	Saline	CRF
Mean time per contact	2.56 ± 0.15	1.19 ± 0.08†
Total duration of contacts (sec)	221 ± 36	136 ± 13*
Total number of contacts (sec)	89 ± 17	117 ± 14
Total number of rears	148 ± 24	133 ± 16
Total number of compartment entries	243 ± 25	290 ± 48

The mean time per contact, total duration of contacts, total number of contacts with the stimuli, and the total number of rears and compartment entries (mean ± SEM) of hypophysectomized mice injected with CRF (50 ng ICV).

Significantly different from saline-injected mice ($p<0.05$; †$p<0.001$, Student's *t*-test).

the number of rears (Table 2).

DISCUSSION

Our data obtained with hypophysectomized animals closely resemble those obtained previously with intact mice (1). Each of the behavioral measures we recorded was similar, although the mean stimulus-contact times were slightly higher in hypophysectomized mice. This may be because corticosterone attenuates the decreases observed in stress. A desensitization of CRF-receptors by chronic hypersecretion of CRF is unlikely because we did not observe an increased sensitivity to administered CRF. Both restraint and CRF significantly decreased exploratory behavior in hypophysectomized mice just as it did in intact animals (1).

These results suggest that the effect of stress and CRF on this behavior are not related to their ability to activate the pituitary-adrenal axis, and, thus, are not secondary to release of ACTH from the pituitary. The independence of the actions of CRF on exploratory behavior resembles that of other behavioral effects of CRF. For example, the effects of CRF on grooming and food intake (22), and on locomotor activity (15), were not attenuated by hypophysectomy. Similarly, dexamethasone injected at a dose sufficient to block CRF-induced release of ACTH did not diminish the effect of CRF on punished responding (7), grooming (7,13), or locomotor activity (7,8). Thus, these results support the hypothesis that CRF acts within the brain to affect behavioral responding. However, we do not know where in the brain CRF acts to elicit these behavioral effects, and whether CRF acts at different sites within the brain to elicit the various effects.

ACKNOWLEDGEMENTS

This research was supported by the National Institutes of Health (MH25486 and NS27283) and a fellowship to C.W.B. (MH09680).

REFERENCES

1. 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.
2. Berridge, C. W.; Dunn, A. J. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. Horm. Behav. 21:393–401; 1987.
3. Bohus, B.; Lissak, K. Adrenocortical hormones and avoidance behaviour of rats. Int. J. Neuropharmacol. 7:301–306; 1968.
4. Bohus, B.; De Kloet, E. R.; Veldhuis, H. D. Adrenal steroids and behavior adaptation: Relationship to brain corticoid receptors. In: Ganten, D.; Pfaff, D., eds. Current topics in neuroendocrinology. Berlin: Springer Verlag; 1982:107–148.
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. Britton, K. T.; Lyon, M.; Vale, W.; Koob, G. F. Stress-induced secretion of corticotropin-releasing factor immunoreactivity in rat cerebrospinal fluid. Soc. Neurosci. Abstr. 10:95; 1984.
7. Britton, D. R.; Varela, M.; Garcia, A.; Rosenthal, M. Dexamethasone suppresses pituitary-adrenal but not behavioral effects of centrally administered CRF. Life Sci. 38:211–216; 1986.
8. Britton, K. T.; Lee, G.; Dana, R.; Risch, S. C.; Koob, G. F. Activating and "anxiogenic" effects of corticotropin-releasing factor are not inhibited by blockade of the pituitary-adrenal system with dexamethasone. Life Sci. 39:1281–1286; 1986.
9. Brown, M. R.; Fisher, L. A.; Spiess, J.; Rivier, J.; Vale, W.; Rivier, C. Corticotropin-releasing factor: activities on the sympathetic nervous system and metabolism. Endocrinology 111:928–931; 1982.
10. Brown, M. R.; Fisher, L. A.; Webb, V.; Vale, W. W.; Rivier, J. E. Corticotropin-releasing factor: a physiologic regulator of adrenal epinephrine secretion. Brain Res. 328:355–357; 1985.
11. Chappell, P. B.; Smith, M. A.; Kilts, C. D.; Bissette, G.; Ritchie, J.; Anderson, C.; Nemeroff, C. B. Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. J. Neurosci. 6:2908–2914; 1986.
12. De Souza, E. B.; Perrin, M. H.; Insel, T. R.; Rivier, J.; Vale, W. W.; Kuhar, M. J. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. Science 224:1449–1451; 1984.
13. Dunn, A. J.; Berridge, C. W.; Lai, Y. I.; Yachabach, T. L. CRF-induced excessive grooming behavior in rats and mice. Peptides 8:841–844; 1987.
14. Dunn, A. J.; Berridge, C. W. Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholaminergic systems. Pharmacol. Biochem. Behav. 27:685–691; 1987.
15. Eaves, M.; Thatcher-Britton, K.; Rivier, J.; Vale, W.; Koob, G. F. Effects of corticotropin-releasing factor on locomotor activity in hypophysectomized rats. Peptides 6:923–926; 1985.
16. Gold, P. W.; Chrousos, G.; Kellner, C.; Post, R.; Roy, A.; Augerinos, P.; Schulte, H.; Oldfield, E.; Loriaux, D. L. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. Am. J. Psychiatry 141:619–627; 1984.
17. Guild, A. L.; Dunn, A. J. Dopamine involvement in ACTH-induced grooming behavior. Pharmacol. Biochem. Behav. 17:31–36; 1982.
18. Gwosdow-Cohen, A.; Chen, C. L.; Besch, E. L. Radioimmunoassay (RIA) of serum corticosterone in rats. Proc. Soc. Exp. Biol. Med. 170:29–34; 1982.
19. Kalin, N. H.; Shelton, S. E.; Kraemer, G. W.; McKinney, W. T. Corticotropin-releasing factor administered intraventricularly to rhesus monkeys. Peptides 4:217–220; 1983.
20. Koob, G. F.; Bloom, F. E. Corticotropin-releasing factor and behavior. Fed. Proc. 44:259–263; 1985.
21. 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.
22. Morley, J. E.; Levine, A. S. Corticotrophin releasing factor, grooming and ingestive behavior. Life Sci. 31:1459–1464; 1982.
23. Rivier, J.; Rivier, C.; Vale, W. Synthetic competitive antagonists of corticotropin-releasing factor: effects on ACTH secretion in the rat. Science 224:889–891; 1984.
24. Rivier, C.; Rivier, J.; Val, W. Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science 231:607–609; 1986.
25. Stone, E. A.; Egawa, M.; McEwen, B. S. Novel effect of chronic corticosterone treatment on escape behavior in rats. Behav. Neural Biol. 50:120–125; 1988.
26. Sutton, R. E.; Koob, G. F.; Le Moal, M.; Rivier, J.; Vale, W. Corticotropin releasing factor produces behavioural activation in rats. Nature 297:331–333; 1982.
27. Swanson, L. W.; Sawchenko, P. E.; Rivier, J.; Vale, W. W. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186; 1983.
28. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 213:1394–1397; 1981.
29. Veldhuis, H. D.; de Wied, D. Differential behavioral actions of corticotropin-releasing factor (CRF). Pharmacol. Biochem. Behav. 21:707–713; 1984.
30. Wynn, P. C.; Hauger, R. L.; Holmes, M. C.; Millan, M. A.; Catt, K. J.; Aguilera, G. Brain and pituitary receptors for corticotropin releasing factor: localization and differential regulation after adrenalectomy. Peptides 5:1077–1084; 1984.