Handling in Infancy: Increased Levels of the Hypothalamic Corticotropin Releasing Factor (CRF) Following Exposure to a Novel Situation[1] (36776)

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Animals which are stimulated in infancy by handling or other techniques have been shown to differ in adulthood from nondisturbed controls on a variety of behavioral and physiological parameters (1). Several investigators have suggested that a neuroendocrine mechanism may be responsible for these changes (2, 4). For example, Denenberg and Zarrow (4) have postulated that stimulation in infancy acts to release corticosterone from the adrenal cortex, and that this acts upon the brain, presumably the hypothalamus, to modify neural organization resulting in an animal which is less emotional. Since handled rats release less plasma corticosterone to the stimulus of exposure to a novel environment than do nonhandled controls (5, 6), this suggests that the neural centers controlling corticotropin releasing factor (CRF) release should be functionally different in handled and nonhandled animals. The purpose of this study was to test the above hypothesis.

Materials and Methods. Hypothalamic CRF levels were determined by measuring plasma corticosterone levels after injection of an extract of the median eminence (ME) obtained with 0.1 *N* HCl. Young adult male rats weighing between 180 and 220 g were injected with 200 μg dexamethasone phosphate/100 g body weight on the evening before a CRF assay. Fourteen hours later each rat received a second injection of 250 μg dexamethasone phosphate/100 g body weight. Later (3.5 to 4 hr) the rats were injected with 5 mg sodium pentobarbital/100 g body weight, and the median eminence extract was injected into the exposed carotid artery 15 min later. Plasma corticosterone levels were determined 15 min after the injection of median eminence extract by means of a modified fluorometric procedure (7). The plasma corticosterone response to crude median eminence extract was found to increase with increasing amounts of median eminence tissue and was used as an index of CRF levels. Neural tissue other than the hypothalamus did not produce a significant effect on plasma corticosterone. Since we had also established a dose–response curve for plasma corticosterone levels in dexamethasone-blocked rats following ACTH injection, it was possible to estimate the amount of ACTH released after injection of crude median eminence extract (Table I). From such calculations we estimate that approximately 70 μU of ACTH were released with 1 ME and 138 μU with 2 ME.

Results. In the first experiment adult male Purdue-Wistar rats were exposed to a 1-min ether stress and sacrificed by decapitation. Based on the ACTH releasing activity of the median eminence, a 15% increase in CRF levels over control occurred 1 min after exposure to the ether and a 64% increase within 5 min. A slight decrease occurred during the next hour and by 2 hr the CRF levels had fallen significantly and were back to normal by 3 hr (Table II). These findings agree with those reported by others using different techniques (8).

In the second experiment 24 litters each containing 6 male rat pups were either handled at an ambient temperature of 24° for 3 min daily during the first 5 days of life or were left undisturbed until weaning at 21 days. At 60–90 days of age the rats were

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TABLE I. Elevation of Plasma Corticosterone Following Intracarotid Injection of Median Eminence (ME) Extract.

Treatment of assay animal (ml of saline or ME extract/100 g body wt)	*N*	Plasma corticosterone (μg/100 ml $\pm$ SE)	ACTH equiv released (μU/100 g body wt)
Acid-saline control	25	6.4 $\pm$ 0.4	—
Cerebral cortex	24	7.2 $\pm$ 1.3	—
1/2 ME	13	5.9 $\pm$ 0.7	—
1 ME	16	10.1 $\pm$ 1.1	70
2 ME	15	16.6 $\pm$ 1.6	138

exposed to a novel open field for 3 min and were then returned to their home cages. Five or 15 min later they were removed from their cages and sacrificed by decapitation.

We chose 5 min since Expt. 2 indicated an increase in hypothalamic CRF levels at that time. The second group was killed 15 min later since the plasma corticosterone response to a novel stimulus is near maximal at this time in both handled and nonhandled rats (5).

The results indicate no differences in hypothalamic CRF levels 5 min after exposure to the open field. However, at 15 min the median eminence of the handled rats caused a highly significant increase in plasma corticosterone whereas the median eminence of the nonhandled rat caused only a slight increase (Fig. 1). If these values are expressed in equivalents of ACTH released, the handled rats show an increase of 50% after exposure for 15 min; whereas, in the nonhandled rats the increase is of the order of 10%. The hypothalamic CRF levels in the handled rat at 15 min is significantly greater than in nonhandled controls ($p < .05$) as determined by the *t* test.

Hypothalamic CRF levels in both handled and nonhandled rats are quite low at 5 min after open field exposure in comparison to that observed at 5 min after ether stress. These differences may be the result of differences in the stimulus intensity. Etherization is obviously a more noxious stress than exposure to a novel situation and may result in a faster rise in hypothalamic CRF levels.

Since plasma corticosterone levels are lower in handled rats 15 min after exposure to a novel situation (4, 6), it seems reasonable to

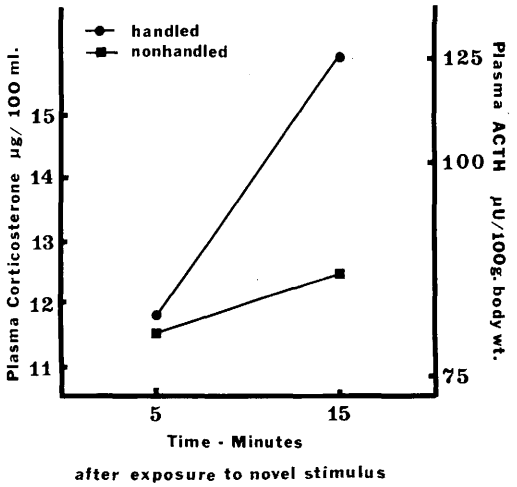

FIG. 1. Effects of handling in infancy upon CRF levels in the 60- to 90-day old rats after 3 min of exposure to the novel environment of an open field. Animals were killed 5 or 15 min after termination of the exposure and the CRF content of the median eminence of the rats was obtained by determining plasma corticosterone levels in the dexamethasone-blocked assay rats. The corticosterone levels are also equated with ACTH equivalents.

postulate that the handled animal releases less CRF. Experiment 2 demonstrated that stressful stimulation resulted in rapid accumulation of CRF in the hypothalamus while in Expt. 3 the handled animals had a greater amount of CRF at 15 min after stimulation than did the nonhandled controls. Hence, CRF synthesis may be greater than CRF release in the handled animal. If this is the case, functionally different neural control mechanisms for CRF may exist as a result of infantile stimulation.

Summary. The levels of the corticotropin

TABLE II. Hypothalamic CRF Activity After Exposure to an Ether Stress.

Minutes after 1-min exposure to ether	No. of assay animals	Plasma corticosterone (μg/100 ml $\pm$ SE)	ACTH equiv released (μU/100 g body wt)
Control	16	10.1 $\pm$ 1.1	70.0
1	10	11.7 $\pm$ 0.9	81.2
5	10	15.2 $\pm$ 1.9	115.0
15	11	14.1 $\pm$ 1.3	100.0
30	11	14.5 $\pm$ 1.6	105.0
60	11	14.2 $\pm$ 1.2	100.0
120	12	12.5 $\pm$ 1.2	87.5
180	14	10.7 $\pm$ 0.9	73.8

releasing factor (CRF) were determined in the hypothalamus of rats by injecting crude extracts of the median eminence into assay animals pretreated with dexamethasone phosphate. Exposure to ether caused a 64% increase in hypothalamic CRF levels within 5 min and return to normal by 3 hr. Hypothalamic levels of CRF were then determined in 2 groups of adult rats following exposure to a novel environment; 1 group had been handled in infancy and a second group constituted the nonhandled controls. No significant difference was noted between the two groups at 5 min but the handled group had a significantly greater amount of CRF present than the nonhandled controls at 15 min after exposure to the novel environment. This agrees with previous findings that handled animals release less corticosterone than nonhandled animals when exposed to a novel environment and suggest that a functionally different mechanism for release of CRF may exist as a result of infantile stimulation.

1. Denenberg, V. H., *in* "The Behaviour of Domestic Animals" (E. S. E. Hafez, ed.), 2nd ed. Baillière, Tindall & Cassell, London (1969).

2. Zarrow, M. X., Philpott, J. E., Denenberg, V. H., and O'Connor, W. B., Nature (London) **218**, 1264 (1968).

3. Levine, S., and Mullins, R. F., Jr., Science **152**, 1585 (1966).

4. Denenberg, V. H., and Zarrow, M. X., *in* "Early Childhood" (D. N. Walcher, and D. Peters, eds.), p. 39. Academic Press, New York (1971).

5. Denenberg, V. H., and Haltmeyer, G. C., J. Comp. Physiol. Psychol. **63**, 394 (1967).

6. Hess, J. L., Denenberg, V. H., Zarrow, M. X., and Pfeiffer, W. D., Physiol. Behav. **4**, 109 (1969).

7. Glick, D., Von Redlich, D., and Levine, S., Endocrinology **74**, 653 (1964).

8. Vernikos-Danellis, J., Endocrinology **75**, 514 (1964); and **76**, 122 (1965).

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