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Anterolateral Hypothalamic Deafferentation Prevents Compensatory Hypersecretion of ACTH Following Adrenalectomy in the Rat[1] (38202)

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The hypothalamus is in part autonomous and in part dependent on extra-hypothalamic neural afferents in regulating ACTH secretion. Several laboratories have shown that the nycthemeral variation in adrenocortical secretion is abolished following complete hypothalamic deafferentation (1–3). Systemic stressors which act directly on either the basal hypothalamus or anterior pituitary stimulate ACTH secretion in rats with hypothalamic deafferentation, but stresses which stimulate ACTH secretion through central nervous system pathways to the medial basal hypothalamus do not (1–2, 4–9).

In adrenalectomized rats there is an elevation of both basal and stress-induced secretion of ACTH, presumably due to reduced negative feedback by glucocorticoids (10, 11). The site of the primary controlling unit sensitive to negative feedback inhibition by glucocorticoids is uncertain. Present evidence indicates that the pituitary itself plays a role in this phenomenon since addition of glucocorticoids to the medium in which adenohypophysial tissue is incubated *in vitro* significantly reduces the secretion of ACTH induced by materials like hypothalamic extracts or vasopressin which directly stimulate secretion of this hormone by the pituitary (12–14). Other data, which have been summarized by Kendall (15), indicate that the hypothalamus or other areas of the central nervous system may be important in glucocorticoid feedback effects on ACTH secretion but the experimental design of most reports has not permitted an unambiguous interpretation.

In the present study, we have examined the effect of hypothalamic deafferentation on the increase in nonstressed (basal) ACTH secretion in the adrenalectomized rat. In addition, to test the functional integrity of the hypothalamic-pituitary unit following deafferentation, we measured plasma ACTH concentration following administration of ether, a substance which acts directly on the basal hypothalamus to stimulate ACTH secretion (20).

Materials and Methods. Data were obtained on 144 male Sprague-Dawley rats weighing 200–250 g in three separate experiments, all of which gave similar results. The animals were maintained under conditions of controlled lighting (12 hr of light beginning at 0600, 12 hr of dark) and temperature (24 ± 1°C). In all experiments jugular or trunk blood was obtained between 0900 and 0930 hours. Anterior, anterolateral, or complete deafferentation of the medial basal hypothalamus was performed in ether-anesthetized rats with a modified Halasz knife (4). The anterior cut was performed by lowering the knife in the midline to the posterior border of the optic chiasm and then rotating the knife 90° in either direction. The antero-lateral cut was performed in the same manner except that the cut was extended 1 mm posteriorly on each side. The complete isolation was a

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dome-shaped lesion extending from the posterior border of the optic chiasm to the middle of the mammillary bodies (4). In 2 experiments, 2 days after deafferentation, half of both the deafferented and nondeafferented animals were bilaterally adrenalectomized. Two weeks later, half the animals in each of the groups were anesthetized with ether vapor for 2.5 min. Two ml of blood was then withdrawn from the jugular vein. One week later, trunk blood was obtained by decapitation in all rats within 30 sec after removal from their cage.

In another experiment (Table I), the sequence of deafferentation and adrenalectomy, was reversed. Two weeks following adrenalectomy, a complete, anterolateral or sham deafferentation was performed. The sham operation consisted of passing the knife to the base of the skull and withdrawing it without rotation. Three days later the animals were subjected to 2.5 min ether anesthesia and jugular vein blood obtained. Three days later the animals were sacrificed by decapitation and trunk blood obtained.

Plasma ACTH was measured by radioimmunoassay (16) with the following modifications. The Lerner-Upton human ACTH preparation was used as the reference standard and for labelling with ^{125}I. The antibody employed was one developed in this laboratory against synthetic α^{1-24} ACTH. This antibody cross-reacts with α-MSH on a 1:1 molar basis, but does not cross-react significantly with β_p-MSH or other known pituitary hormones. The average sensitivity of the assays was 30 pg/ml. The extent of deafferentation and the absence of pituitary infarction was verified by examination of serial sections of the brains. The data from each experimental group were statistically compared using the Newman-Keuls test (17).

Results. Complete ($P < .01$) and anterolateral ($P < .05$) deafferentation prevented the rise in basal plasma ACTH in the adrenalectomized rats (Fig. 1). Basal plasma ACTH in rats with anterior deafferentation was not significantly different from that of the adrenalectomized controls ($P > .05$). Complete deafferentation in the nonadrenalectomized rats caused a nonsignificant ($P > .05$) depression of basal plasma ACTH. Similar results were obtained if deafferentation was performed 2 weeks after adrenalectomy (Table I).

Plasma ACTH following ether stress was significantly higher ($P < .05$) in all deafferented adrenalectomized groups than in the deafferented intact group (Fig. 2) and was higher, although not always at a statistically significant level, than in the intact control groups (Fig. 2, Table I).

Discussion. Our data suggest that the increase in basal ACTH secretion following bilateral adrenalectomy is dependent on extrahypothalamic influences which enter the lateral basal hypothalamus. The site

TABLE I. Effect of Hypothalamic Deafferentation Following Adrenalectomy on Basal and Ether-Stress Plasma ACTH Concentrations (mean ± SE).

	Basal		2.5 min ether stress	
Group	n	ACTH pg/ml	n	ACTH pg/ml
Intact:				
Control	5	85 ± 22[a]	4	2,212 ± 551
Adrenalectomized:				
Control	6	515 ± 189	6	16,599 ± 1,317
Sham	7	506 ± 136	8	11,767 ± 2,373
Deafferented[b]	6	73 ± 23	6	4,586 ± 1,376

[a] Mean ± SE.

[b] Data from anterolateral and complete hypothalamic deafferentations were combined since they were not statistically different ($P > .1$). Brain operations were performed 2 weeks after adrenalectomy (see text for details).

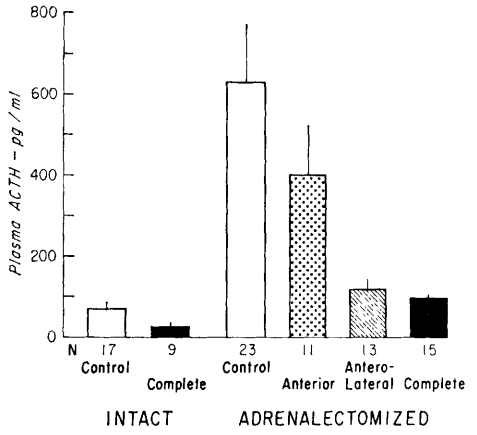

FIG. 1. Effect of hypothalamic deafferentation followed by adrenalectomy on the mean basal plasma ACTH concentration in resting nonstressed rats. Complete ($P < .01$) and anterolateral ($P < .05$) deafferentation prevented the compensatory increase in ACTH concentration observed in nondeafferented and anterior deafferented rats. The vertical line above each bar represents the standard error and the number below the number of rats per group.

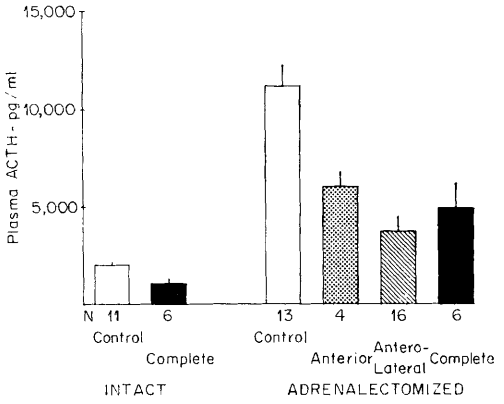

FIG. 2. Effect of hypothalamic deafferentation on the mean plasma ACTH concentration following 2.5 min of ether inhalation in intact and adrenalectomized rats. Plasma ACTH was significantly higher in all adrenalectomized than in the intact groups ($P < .05$). Note that the ordinate scale for ACTH is 20 times as large as in Fig. 1.

involved in such regulation is not known. It has been suggested that the hippocampus and septal area of the limbic system may be involved (18, 19). However, in preliminary experiments with radiofrequency lesions which destroy the septal area we have been unable to reproduce the effects herein reported.

Our data are in apparent conflict with the report of Halasz *et al.* (2) that complete hypothalamic deafferentation does not block the compensatory increase in adrenal size following unilateral adrenalectomy. We cannot definitely explain these differences but there are several possibilities. (1) Their studies extended over a longer time span than ours since unilateral adrenalectomy was performed one month after the brain operations and the second adrenal weighed one month later. It is possible some functional reorganization of transected neural tracts to the basal hypothalamus may have occurred by the end of their experiment. (2) Their data are recorded only as absolute, rather than relative, adrenal weight. Since the male rats they employed are known to gain considerable weight in 1 mo, the absolute gain in adrenal weight of approximately 50% seen in both their sham and operated groups may have been due primarily to an increase in body size. Relative adrenal weight may have remained unchanged. In our experience, compensatory adrenal hypertrophy has not proved a very reliable method of assessing ACTH secretion because of marked variation in this response even in control animals. It is unfortunate that the body weights of their rats at the time of unilateral adrenalectomy were not given. (3) The direct assay of plasma ACTH we employed is a different method of assessing corticotropin secretion and possibly more valid than theirs.

Although blood was sampled at only one time period in our studies, many laboratories have shown that hypothalamic deafferentation abolishes the normal nycthemeral pituitary-adrenal cycle in rats with either *in situ* or heterotopic pituitaries (1–4). It thus seems unlikely that contrary results would have been obtained if the blood samples had been obtained at different times.

The lesser ($P < .05$) secretion of ACTH in response to ether stress in the deafferented than in the nondeafferented groups was possibly due to: (1) traumatic destruction of some hypothalamic neurons which produce corticotropin-releasing fac-

tor (CRF), (2) loss of some positive tonic input from extrahypothalamic structures, (3) a longer delay to the peak of the plasma ACTH response to ether-stress in deafferented than in normal animals. Nevertheless, in all 3 experiments ether stress caused a greater rise in all deafferented, adrenalectomized groups than in the intact controls, although these differences were not always statistically significant (Fig. 2, Table I). This suggests that the supranormal secretion of ACTH in response to stress in chronically adrenalectomized animals may be due to decreased glucocorticoid concentration at the pituitary level rather than to tonically increased basal ACTH secretion.

Summary. Complete or anterolateral, but not anterior, basal hypothalamic deafferentation prevented the increased basal secretion of ACTH in chronically adrenalectomized rats. ACTH secretion in response to ether stress was at least as great in the deafferented, adrenalectomized rats as in intact controls. The data suggest that extrahypothalamic influences which enter the lateral basal hypothalamus are necessary to achieve a high basal ACTH secretion when plasma glucocorticoids are depressed.

1. Palka, Y., Coyer, D., and Critchlow, V., Neuroendocrinology **5**, 333 (1969).

2. Halasz, B., Slusher, M. A., and Gorski, R. A., Neuroendocrinology **2**, 43 (1967).

3. Allen, C. F., Kendall, J. W., and Greer, M. A., Endocrinology **91**, 873 (1972).

4. Greer, M. A., and Rockie, C., Endocrinology **83**, 247 (1968).

5. Makara, G. B., Stark, E., Palkovits, M., Reves, T., and Mihaly, D., J. Endocrinol. **44**, 187 (1969).

6. Feldman, S., Conforti, N., Chowers, I., and Davidson, J. M., Acta Endocrinol. (Kob.) **63**, 405 (1970).

7. Makara, G. B., Stark, E., and Palkovits, M., J. Endocrinol. **47**, 411 (1970).

8. Feldman, S., Conforti, N., and Chowers, I., Acta Endocrinol. (Kob.) **69**, 526 (1972).

9. Greer, M. A., Allen, C. F., Gibbs, F. P., and Gullickson, C., Endocrinology **86**, 1404 (1970).

10. Hodges, J. R., and Jones, M. T., J. Physiol. **173**, 190 (1964).

11. Cox, G. S., Hodges, J. R., and Vernikos, J., Endocrinology **17**, 177 (1958).

12. Saffran, M., Canad. J. Biochem. Physiol. **37**, 319 (1959).

13. Guillemin, R., Hearn, W. R., Cheek, W. R., and Householder, D. E., Endocrinology **60**, 488 (1957).

14. Fleisher, N., and Vale, W., Endocrinology **83**, 1232 (1968).

15. Kendall, J. W., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. F. Ganong, eds.), pp. 177–207. Oxford University Press, New York (1971).

16. Kendall, J. W., Allen, J. P., Jacobs, B. A., Cook, D. M., Castro, A., and McGilvra, R., *in* "Brain-Pituitary-Adrenal Interrelationships" (A. Brodish and E. S. Redgate, eds.), pp. 1–15. S. Karger, Basel (1973).

17. Winer, B. J., *in* "Statistical Principles in Experimental Design", pp. 96–104. McGraw-Hill Book Co., New York (1962).

18. McEwen, B. S., Weiss, J. M., and Schwartz, L. S., Brain Res. **17**, 471 (1970).

19. Knizley, Jr., H., Neurochemistry **19**, 2737 (1972).

20. Matsuda, K., Duyck, C., Kendall, J. W., and Greer, M. A., Endocrinology **74**, 981 (1964).

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