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Evidence of Inhibitive Role of Hippocampus in Neural Regulation of ACTH Release.* (28587)

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Several studies(1-7) have examined the role of extrahypothalamic neural structures in regulation of adenohypophyseal secretion of ACTH. In rats stressed by physical immobilization(8), amygdaloid nuclei appeared to be necessary for the normal rapid release of ACTH; an inhibitive role of hippocampus was suggested by the observation that basal, non-stressed plasma corticoid levels were significantly higher after bilateral electrolytic lesions of this area. The present study was designed to investigate further the possible interrelationship between these excitatory and inhibitory areas.

Materials and methods. Bilateral electrolytic lesions (3 ma, 30 seconds) were placed by stereotaxic means in hippocampus, amygdala, habenula and midbrain reticular formation of adult Sprague-Dawley rats. The position and extent of the lesions in hippocampus and amygdala were as previously described (8). Anterior, vertical and lateral coordinates for lesions in midbrain reticular formation were: (+1.0, −2.0, ±0.6 mm) respectively accordingly to the atlas of deGroot(9); current parameters were 1.5 ma for 30 seconds. Two weeks after lesions in amygdala or reticular formation, adrenocortical response to acute ether anesthesia was tested. After 3-5 minutes of anesthesia, 1.5 ml blood was obtained by cardiac puncture. Plasma corticosterone levels, used as a measure of ACTH release and consequent adrenocortical response, were determined fluorometrically with 0.5 ml plasma aliquots(8). One-half of the lesioned animals that were unresponsive to this stressor subsequently received additional bilateral lesions in hippocampus or habenula (coordinates: +3.8, +0.7, ±0.7 mm; 1 ma for 15 seconds). The remaining were left as "controls" and their adrenocortical response to ether anesthesia tested a second time. Animals lesioned additionally in hippocampus or habenula were ether stressed again 2-4 weeks after lesioning. In one group of animals the order of lesioning was reversed; hippocampal lesions were made first, followed by lesions in reticular formations.

Results. Table I summarizes results of these experiments. Plasma corticosterone level in non-lesioned, non-stressed rats (Group I) was 13.4 μg/100 ml. Etherization and withdrawal of 1.5 ml blood by heart puncture led rapidly to a 4-fold increase in corticoid level (Group II). Bilateral lesions in amygdala or midbrain reticular formation were capable of preventing this acute response (Groups III, V). In animals with either of these lesions and unresponsive to etherization stress, placement of additional bilateral lesions in hippocampus resulted in a maximal output of adrenocorticoids when subjected to a second etherization test (Groups IV, VI). In ani-

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TABLE I. Plasma Corticosterone Response to Ether Anesthesia and Heart Puncture in Rats with Single and Combined Lesions in Limbic System and Reticular Formation.

Group	1st lesion site	1st stress response (μg/100 ml)	2nd lesion site	2nd stress response (μg/100 ml)
I	Control (non-stress)	13.4 ± 2.2 (22) †		
II	"	57.0 ± 5.0 (13)	—	51.2 ± 7.2 (13)
III	Amygdala	18.1 ± 4.3 (16)	—	16.0 ± 2.8 (12)
IV	"	21.1 ± 3.7 (18)	Hippocampus	73.7 ± 6.4 (17)
V	R.F.*	24.7 ± 4.0 (15)	—	27.1 ± 3.7 (12)
VI	"	20.3 ± 3.1 (14)	Hippocampus	69.3 ± 5.3 (12)
VII	"	18.1 ± 2.7 (16)	Habenula	22.1 ± 3.0 (15)
VIII	Hippocampus	49.7 ± 4.7 (13)	R.F.	61.4 ± 5.8 (9)

* R.F. = reticular formation.
† Mean ± standard deviation; number of animals in parentheses.

mals previously lesioned in reticular formation and exhibiting no stress response, additional lesions in habenula were without effect in altering their low corticoid response (Group VII). When hippocampal lesions were placed first (Group VIII) adrenocortical response was unimpaired; subsequent lesions in reticular formation of these rats lead to higher plasma corticoid levels after the second ether test.

Discussion. The presence of neural regulatory mechanisms for the control of adenohypophyseal secretion is well established and there is much interest in negative feedback aspects and inhibitory components of these neural regulatory mechanisms. In the present experiments etherization and heart puncture of normal animals led to activation of neural mechanisms resulting in acute release of ACTH. When bilateral lesions were placed in either amygdala or the midbrain reticular formation, the response was not evoked. This may be interpreted as indicating that the synergistic action of both amygdala and reticular formation is necessary to evoke discharge of ACTH or that a certain summation of excitatory stimuli from both sources must be reached before ACTH is released. The latter interpretation appears more likely. In animals unresponsive to this stressor because of lesions in either amygdala or midbrain reticular formation, additional destruction of a hippocampal area negates this block and permits an acute ACTH discharge. The neural alterations produced by this second hippocampal lesion is at present not clear. Inhibitive pathways from hippocampus may project directly upon amygdala or the midbrain reticular formation; alternately, pathways from hippocampus, amygdala and the midbrain reticular formation may project separately to some hypothalamic site which represents either the source of a corticotropin releasing factor (CRF) or an intermediate station where excitatory and inhibitory afferent stimuli are coordinated into a final directive signal to the cellular source of CRF. In either case, removal of the inhibitive influence of hippocampus permitted the excitatory stimulus of either amygdala or reticular formation alone to reach sufficient magnitude to cause ACTH discharge.

Summary. The pituitary-adrenal axis of normal rats, as reflected by plasma corticoid levels, was activated acutely by the stress of ether anesthesia and withdrawal of blood by heart puncture. Bilateral electrolytic lesions in portions of the midbrain reticular formation or the amygdaloid nuclei suppressed or blocked this acute response. In such lesioned, unresponsive animals, placement of an additional lesion in hippocampus negated the block created by the first lesion in reticular formation or amygdala. The results support the opinion that hippocampus contributes an inhibitory component to the neural mechanisms regulating ACTH release.

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Localization of Cholesterol Esterase and Cholesterol in Mucosal Fractions of Rat Small Intestine.* (28588)

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It has been reported that dietary cholesterol penetrates the intestinal mucosa in the free form but appears in the lymph almost entirely as ester(1). Also, it has been shown that lymph is not the site of cholesterol esterification in absorption(2). Therefore, the esterification of this cholesterol must occur between these two sites, i.e., in the intestinal mucosa. Moreover, the enzyme, cholesterol esterase, which catalyzes the synthesis of cholesterol esters and their hydrolysis has been demonstrated in the mucosa(3). The purposes of the experiments presented here were to determine, firstly, the subcellular location of the cholesterol esterase in the mucosa of the rat small intestine; secondly, to determine the levels of free and esterified cholesterol in the wall of the small intestine; and thirdly, to determine the distribution of free and esterified cholesterol in subcellular fractions of the mucosa.

Experimental. Male Carworth rats, weighing between 100 and 200 g, were fasted 18 hours before sacrifice. The intestinal mucosa was scraped from the previously washed and everted intestine (severed posterior to the common duct and at the ileo-cecal junction) and fractionated into brush border and supernatant fractions in .005 M EDTA according to the procedure of Miller and Crane(4) with modifications. Rat intestinal mucosa was used rather than hamster mucosa(4) and homogenization of the mucosa was carried out with a Potter-Elvehjem type homogenization tube fitted with a Teflon pestle by making 2 up and down passes at a pestle speed of 1200 rpm. This procedure was adequate for rupture of all the intact cells as observed by microscopic examination and at the same time left the brush border particles intact, as determined by invertase assay (Table I). It was also determined by invertase assay that repeated washing and centrifugation of the brush border did not result in its rupture (Table I). Brush border preparations which contained 90-100% of the invertase activity of the mucosal homogenate and which under microscopic examination were found free of intact cells and cell debris were considered adequate for further study. Invertase was measured by the method of Borgstrom and Dahlqvist(5).

Fractionation of the intestinal mucosa into subcellular fractions was carried out in 0.25 M sucrose, using the differential centrifugation procedure of Hogeboon and Schneider(6).

Cholesterol esterase activity was determined in the EDTA and sucrose homogenates of the intestinal mucosa and in the subfractions prepared as described above. (It should be noted that the term supernatant fraction is used to designate that fraction of the EDTA homogenate free of brush border, and that the term soluble fraction is used to designate that frac-

TABLE I. Recovery of Invertase Activity in Brush Border Preparations.

Fraction	Invertase activity* (units)	Recovery (%)
Homogenate	357	—
Brush border	343	96
First supernatant	21	6
Washed brush border	343	96

* Activity—μmoles reducing sugar/hr at 37°C.

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