Vasopressin-Evoked ACTH Release in Rats Following Forebrain Removal[1] (35476)

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(Introduced by M. Hess)

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Several recent studies have shown that rats subjected to surgical isolation of the hypothalamus retain considerable pituitary–adrenal function. Although this procedure disrupts the 24-hr rhythm in ACTH release (1, 2), it is compatible with appreciable pituitary–adrenal function in resting or "nonstress" conditions as well as significant corticosteroid responses to several different types of stress (1–4). Furthermore, rats with hypothalamic isolation show feedback suppression of resting corticosterone levels with relatively low doses of dexamethasone (2).

These findings were corroborated by our recent studies which pursued the question of hypothalamic autonomy using forebrain removal as a means of isolating the hypothalamus (5, 6). Brain removal was used to assure completeness of isolation and to preclude the possibility of surrounding brain tissue exerting an influence on the isolated hypothalamus. Twenty-four hr after surgery, rats with hypothalamic or pituitary islands had physiologic levels of plasma corticosterone in the absence of overt or acutely applied stress, and these levels were suppressed with dexamethasone (5). In addition, hypothalamic but not pituitary island preparations demonstrated corticosteroid responses to ether and immobilization stress (6).

In view of these findings, it was deemed of interest to further investigate the responsiveness of the pituitary–adrenal axis in hypothalamic and pituitary island preparations to other well-known corticotropin-releasing stimuli. The present experiments were undertaken to study the effects of lysine vasopressin (LVP) on pituitary–adrenal function 24-hr after partial or complete forebrain removal. A preliminary report of these experiments appeared previously (7).

Materials and Methods. All animals used in this study were adult (180–280 g) female (Houston, Cheek) rats, acclimated to controlled lighting (fluorescent illumination from 04:00 to 18:00) and temperature (24 ± 2°) conditions for at least 2 weeks. Purina Lab Chow and tap water were available *ad libitum*. Experimental preparations used in this study are illustrated in Fig. 1. Island preparations were accomplished by transecting the mesencephalo–diencephalic junction and removing with suction graded amounts of forebrain leaving either a hypothalamic island (HI), pituitary island (PI), or anterior pituitary island (API). In the latter two preparations, forebrain removed included median eminence, infundibular stalk and, in the case of API rats, infundibular process. The method for brain removal, subsequent hemostasis, and postoperative treatment has been discussed elsewhere (5).

To standardize experimental conditions, treatment and collection procedures were performed 24 hr after forebrain removal (09:00). Plasma concentrations of corticosterone, measured fluorometrically (8), were used as an index of pituitary–adrenal function. Correction for residual fluorescence was not made; in this laboratory, fluorescence in plasma from adrenalectomized female rats is equivalent to approximately 6 μg/100 ml of plasma. Treatments were assigned and performed according to a randomized block design. However, because of missing values, the data were analyzed according to a completely

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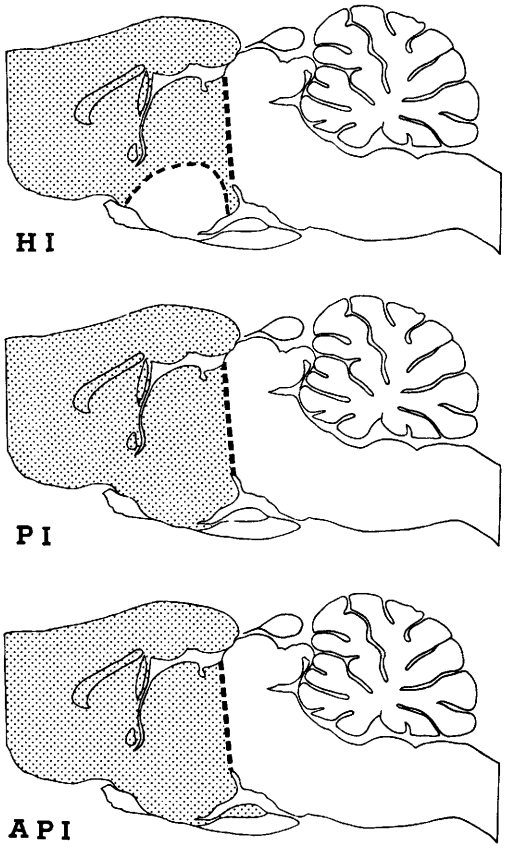

FIG. 1. Diagrams of experimental preparations. Stripped area indicates brain removed.

randomized design. Statistical probabilities were determined by analysis of variance performed by the department of Biometry. Tukey's test of means was used when more than two treatment groups were involved.

Vasopressin-evoked ACTH release. Twenty-four hr after forebrain removal, "nonstress" plasma corticosterone samples were obtained by rapidly immobilizing rats in the supine position, exposing the jugular vein following procainization of overlying skin, and removing 1.0 to 1.5 ml of blood in a heparinized syringe within 3 min of initial handling. Immediately following "nonstress" bleeding, HI and PI rats were injected intravenously with lysine vasopressin (20 mU/100 g of body wt, NIH-LVP-1) or normal saline. Fifteen min following completion of injection, rats were decapitated and trunk blood was collected in heparinized beakers. In a separate experiment, 5 API rats were subjected to a protocol similar to that described above for determining vasopressin-induced ACTH release. In all cases, blood samples were immediately centrifuged; and plasma was collected for determination of plasma corticosterone concentrations. Differences between "nonstress" and postvasopressin levels of corticosterone in plasma were calculated for individual rats and used as a measure of vasopressin-induced increments in pituitary–adrenal function.

As in previous studies (5, 6), plasma samples obtained within 3 min of initial handling and in the absence of overt stress for 18 to 24 hr are referred to as "nonstress," even though corticosterone levels may reflect responses to the stress associated with brain removal and/or food and water deprivation.

Adrenal response to ACTH. To determine whether the pituitary–adrenal response of HI rats to LVP represented a maximal response, intact and HI rats were injected with large doses of ACTH (Parke-Davis ACTH, 6 or 12 mU/100 g of body wt, iv); ACTH was administered to operated rats 24 hr after forebrain removal. ACTH was injected following rapid immobilization and exposure of the jugular vein as described above. Fifteen min following ACTH administration, animals were decapitated and trunk blood was collected for plasma corticosterone determination.

Histology. Following decapitation and collection of trunk blood, rat heads were placed in 10% formalin. After fixation, they were decalcified in a formic acid–sodium citrate solution and subsequently processed for histological examination of *in situ* brain and pituitary.

Results. Vasopressin-evoked ACTH release. The effects of LVP on plasma corticosterone concentrations in HI and PI preparations are illustrated in Fig. 2. Plasma levels of corticosterone 15 min following vasopressin injection were higher ($p<0.01$) than "nonstress" corticosterone levels in both HI and PI preparations, while steroid concentrations in saline-injected rats showed no change. As in previous studies (5, 6) "nonstress" plasma corticosterone concentrations were higher ($p<0.05$) in HI than in PI rats.

The results of the second experiment are

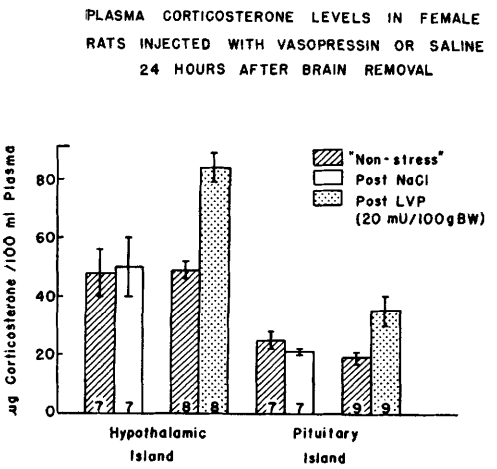

FIG. 2. Effect of lysine vasopressin (20 mU/100 g of body wt, iv) on plasma levels of corticosterone in female rats 24 hr after forebrain removal. In this and subsequent illustrations, number of animals per treatment group is indicated at the base of the columns; vertical lines indicate standard error.

summarized in Table I. API rats had basal "nonstress" corticosterone levels which were not influenced by LVP injection.

Adrenal response to ACTH. Figure 3 summarizes results of comparing adrenocortical responses to ACTH in HI rats, 24 hr after brain removal, and intact rats. Fifteen min after ACTH injection, HI rats had plasma corticosterone levels which were comparable to those of intact rats. Increasing the ACTH stimulus from 6 to 12 mU/100 g of body weight did not result in significant increases in plasma corticosterone concentrations in either intact or HI rats.

Histology. The completeness and extent of all island preparations was verified histologically. Only HI rats with islands similar to those illustrated in Fig. 1 were included in the present study. Descriptions of hypothalamic island histology have appeared elsewhere (5). However, in general, hypothalamic islands included arcuate and most of ventromedial nuclei, as well as medial preoptic area and premamillary nuclei. All PI rats included in this study had complete forebrain removal, as illustrated in Fig. 1, and pituitary infarction. The extent and size of these infarcts varied somewhat but, for the most part, necrotic tissue was centrally located leaving the caudal, dorsal, and lateral aspects of the adenohypophysis intact. Pituitaries of API rats were almost completely infarcted; only a thin outer shell of tissue adjacent to the capsule appeared viable. No infarcts were observed in pituitaries of HI rats.

TABLE I. Effect of Lysine Vasopressin (20 mU/100 g of body wt, iv) on Plasma Levels of Corticosterone in Rats with Anterior Pituitary Islands.

Corticosterone μg/100 ml of Plasma	
"Nonstress"	Post-LVP
9.6	5.6
8.9	15.2
16.6	8.2
11.2	13.8
6.8	8.2
10.6 ± 1.7[a]	10.2 ± 1.8

[a] Mean ± SE.

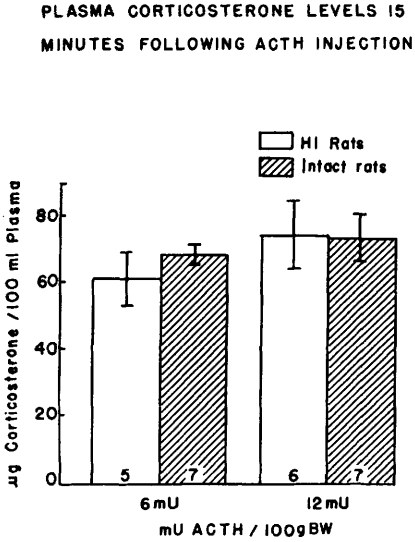

FIG. 3. Effect of ACTH on plasma levels of corticosterone in female rats 24 hr after forebrain removal.

Discussion. The results of the present study are consistent with previous observations that forebrain removal is compatable 24 hr after surgery with appreciable pituitary–adrenal function (5, 6). "Nonstress" plasma corticosterone levels in the present study were maintained within or near the physiologic range in HI and PI preparations and such levels were acutely increased with

LVP. Although it is recognized that the amount of LVP used in the present study represents a pharmacological dose for antidiuretic study, 20 mU/100 g of body weight represents a comparatively small amount of LVP for ACTH release. This relatively small dose of vasopressin appears to have induced maximal plasma corticosterone levels in HI rats since neither 6 or 12 mU/100 g of body weight doses of ACTH produced plasma corticosterone concentrations greater than those observed following vasopressin injection. In contrast, Cheifetz *et al.* (9) reported much larger doses of vasopressin necessary for maximal stimulation of plasma corticosterone concentrations in intact male rats. Sex differences may be responsible for this discrepancy. However, it is also possible that the dexamethasone–Nembutal combination used by Cheifetz and co-workers (9) suppressed the effect of vasopressin on pituitary–adrenal activity since pituitary responses to ovine corticotropin releasing factor (CRF) are reportedly depressed by dexamethasone (10).

Although there is considerable evidence that vasopressin is a strong stimulus for ACTH release, the site for effecting ACTH release remains debated. Because vasopressin may be a potent releaser of endogenous CRF (11), it has been suggested that vasopressin indirectly causes ACTH release via its action on hypothalamus and/or median eminence. However, both *in vitro* (12) and *in vivo* (13) evidence continues to appear to support a direct action on the pituitary. The data in the present study suggests that the hypothalamus and forebrain structures are not essential to vasopressin-induced ACTH secretion and that vasopressin acts, at least in part, directly on the pituitary. This is not to say, however, that additional sites are not located in the brain. In fact, the possibility that vasopressin may act on pituitary and hypothalamus is suggested by the finding that HI rats had increments in plasma corticosterone which were nearly twice those of PI preparations. On the other hand, reduced corticosterone increments in PI rats may simply reflect decreased functional pituitary tissue since all PI rats had large pituitary infarcts.

Recently Hiroshige and co-workers (14), using a microinjection technique, concluded that vasopressin is not CRF itself but elicits ACTH release through mobilization of neurohypophyseal CRF. Whether vasopressin in the present study acted directly on adenohypophyseal tissue to cause ACTH release or whether it mobilized stored neurohypophyseal CRF in PI preparations is not known. The absence of response of API rats to vasopressin does not shed light on this question since all API pituitaries contained massive infarcts and were possibly nonresponsive to stimuli for ACTH release. Although it has been reported that vasopressin may stimulate corticosteroid secretion directly through an action on adrenal cortex (15, 16), the absence of response to vasopressin in API rats indicates that the effect of vasopressin on plasma corticosterone levels in HI and PI rats was mediated by ACTH secretion.

Several studies have shown that forebrain removal is compatible with appreciable pituitary–adrenal function (5, 6, 17, 18). Twenty-four hr after surgery, rats with hypothalamic or pituitary islands had physiologic levels of plasma corticosterone in the absence of overt or acutely applied stress, and these levels were suppressed with dexamethasone (5, 18). In addition, hypothalamic but not pituitary island preparations demonstrated ACTH secretion in response to ether (6, 19) and immobilization stress (6). The present results are consistent with these earlier findings and demonstrate that hypothalamic and pituitary island preparations show ACTH secretion in response to yet another stimulus which is effective in the intact animal. Furthermore, these results suggest that the hypothalamus and other forebrain structures are not essential for vasopressin-induced ACTH secretion and offer additional *in vivo* evidence that vasopressin can act directly on the pituitary.

Summary. The effects of lysine vasopressin on plasma concentrations of corticosterone were determined in adult female rats with hypothalamic (HI), pituitary (PI) or anterior pituitary (API) islands. Twenty-four hr after partial brain removal, blood samples were obtained rapidly from the jugular vein for fluorometric determination of "nonstress," prevasopressin levels of corticos-

terone. Immediately following sampling, vasopressin (20 mU/100 g of body wt) or saline was injected intravenously; 15 min later rats were decapitated and trunk blood was collected for postvasopressin or -saline corticosterone levels. Whereas HI and PI rats responded to vasopressin with increased plasma corticosterone levels, API and saline-injected HI and PI rats showed no change in corticosterone concentrations. "Nonstress" corticosterone levels were within the physiologic range in HI and PI, but not API rats. These results suggest that the hypothalamus and other forebrain structure are not essential to vasopressin-induced ACTH secretion and that vasopressin acts, at least in part, directly on the pituitary.

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