HYPOTHALAMIC LESIONS AND ADRENAL FUNCTION IN THE CAT

By

Dorothy T. Krieger¹ and Irving H. Wagman²

ABSTRACT

Bilateral electrolytic lesions limited to the median eminence were produced stereotaxically in four cats which had previously shown positive responses (as measured by blood corticoid elevations) to exogenous corticotrophin (ACTH) and insulin hypoglycaemia. Following operation these animals exhibited low basal corticoid levels and an inability to respond to ACTH and an impaired response to stress. At autopsy they showed an increase in adrenal size and stainable cortical lipid. Operated controls had normal responses to both exogenous ACTH and insulin hypoglycaemia and had normal adrenal histology. Thus a lesion of the median eminence depresses adrenal cortical function by interference with release of steroid from the adrenal and not by inducing adrenal atrophy. This may be explained in part by the observation that median eminence lesions interfere with the adrenal response to exogenous ACTH.

The mechanism of hypothalamic regulation of anterior pituitary secretion has yet to be elucidated. Despite contradictory data, there is apparent agreement that median eminence lesions interfere with the release of corticotrophin (ACTH) in response to stressful stimuli (Hume & Wittenstein 1950; Laqueur et al. 1955; McCann 1955). These conclusions have been based primarily upon

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indirect or nonspecific methods of assay of adrenocortical function. There have been no determinations of ACTH levels in such preparations.

With the advent of chemical methods for the measurement of plasma 17-hydroxycorticosteroids it was felt to be of interest to further explore this problem in the cat. This species offers the advantage that electrolytic lesions can be accurately placed stereotaxically and the endocrine status evaluated by direct chemical estimation of blood corticoids.

A preliminary report of the following study has been presented (Krieger 1958).

**METHODS**

The plasma corticoids of adult cats of both sexes were determined prior to and after the administration of ACTH (Armour) (20 IU, i.m.). Three days later the same animals were similarly tested with 10 IU regular insulin. The plasma corticoids were determined in duplicate by the Peterson modification of the Sweat fluorometric method (Peterson & Wyngaarden 1955). Only those animals (a total of 11) who responded in both instances with a rise in their plasma corticoids of at least 10 µg per 100 ml were used as experimental subjects.

Bilateral discrete lesions were produced electrolytically (by means of direct current) in each of the 11 animals under nembutal anaesthesia with the aid of a stereotaxic apparatus. The active electrode (the anode) was a steel wire, 250 µ in diameter, insulated except for the last half millimeter. The stereotaxic frame served as the cathode. The two symmetrical lesions in each animal were made serially during the same operation. The size and location of the lesions are summarized below (part B under results). Nine cats survived the postoperative period longer than one week while six of these survived until sacrificed one month postoperatively. Studies similar to those of the control period were performed on these animals one week and one month postoperatively. At the end of the experiments, the animals were sacrificed either by means of insulin hypoglycaemia or nembutal overdosage. Three normal unoperated cats (2 male, 1 female) were sacrificed by nembutal overdosage, and one normal unoperated male by means of insulin hypoglycaemia. The latter four animals served as appropriate histological controls.

Thyroid glands and gonads were sectioned at 10 µ intervals and stained with haematoxylin-eosin. The adrenals were likewise sectioned and alternate sections stained with haematoxylin-eosin or Sudan Black. After formalin fixation of the brain of each of the experimental animals, a block of tissue containing the lesioned area were excised and embedded in celloidin. Serial sections were cut 50 µ in thickness, and every tenth section was stained with Weil's stain. Additional sections through the center of the lesion were stained with haematoxylin-eosin.

**RESULTS**

A. Preoperative studies

1) Chemical analyses. With the method employed (Sweat) there was a maximal variation in duplicate specimens of 15 per cent. To test the specificity of the technique employed, 150 ml of pooled cat plasma were obtained, of which 2 ml were analyzed by the Sweat method. 10 ml by the Porter-Silber
Table 1.
Effect of Median (M. E.) Lesions on plasma 17-Hydroxycorticosteroid Levels (µg/100 ml Plasma) and the Response to ACTH and Insulin Hypoglycaemia.

<table>
<thead>
<tr>
<th>Animal No. &amp; Sex</th>
<th>Types of Lesions</th>
<th>Weight in kg</th>
<th>Preoperative</th>
<th>Control</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preop.</td>
<td>Terminal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (M)</td>
<td>Bilateral</td>
<td>2.8</td>
<td>3.0</td>
<td>17.0</td>
<td>27.0</td>
</tr>
<tr>
<td>2 (M)</td>
<td>Bilateral</td>
<td>3.5</td>
<td>3.6</td>
<td>6.8</td>
<td>19.0</td>
</tr>
<tr>
<td>3 (F)*</td>
<td>Bilateral</td>
<td>3.0</td>
<td>2.8</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>4 (F)</td>
<td>Unilateral</td>
<td>2.4</td>
<td>2.5</td>
<td>4.0</td>
<td>27.0</td>
</tr>
<tr>
<td>5 (M)</td>
<td>Bilateral</td>
<td>3.7</td>
<td>3.5</td>
<td>4.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Thalamic</td>
<td></td>
<td></td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>6 (F)</td>
<td>No lesion</td>
<td>1.9</td>
<td>2.4</td>
<td>3.0</td>
<td>20.0</td>
</tr>
<tr>
<td>7 (M)</td>
<td>Bilateral</td>
<td>3.3</td>
<td>3.3</td>
<td>4.0</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Thalamic</td>
<td></td>
<td></td>
<td>3.3</td>
<td>3.4</td>
</tr>
<tr>
<td>8 (M)*</td>
<td>Bilateral</td>
<td>3.6</td>
<td>3.4</td>
<td>0.0</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Mamm. Body</td>
<td></td>
<td></td>
<td>3.6</td>
<td>3.4</td>
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<tr>
<td>9 (F)*</td>
<td>No lesion</td>
<td>2.2</td>
<td>2.5</td>
<td>20.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

method (Wallace et al. 1955) and the remainder extracted and chromatographed in the chloroform-formamide system (Burton et al. 1951). With the Sweat method, which measured essentially corticosterone and cortisol, the plasma corticoids were 14 µg/100 ml. Ultraviolet spectrophotometric determinations (peak at 240 mµ) on eluates from appropriate portions of the chromatograph gave values for corticosterone of 5µg/100 ml and 9 µg/100 ml for cortisol.

2) Basal corticoid level. The data obtained on each individual cat is presented in Table 1 and the means are summarized in Fig. 1. The mean of the basal corticoid level was 8.0 µg/100 ml with a range of 0–20 µg/100 ml. Daily control samples over a period of a week in 5 animals showed a maximum variation of 7 µg/100 ml in any given animal.
Table 1.

Postoperative

<table>
<thead>
<tr>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
<td>--------</td>
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<tr>
<td>0</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
</tr>
<tr>
<td>4.0</td>
</tr>
</tbody>
</table>

* These animals survived only one week postoperatively.
\(P^\uparrow\) = following.
† Identical results were obtained following 40 IU ACTH.

3) Responsiveness to ACTH and insulin. In the animals reported on, the average rise in the plasma corticoids over the baseline, 90 minutes after the administration of ACTH, was 14.8 µg/100 ml with a range of 10–23 µg/100 ml. The mean rise in plasma corticoids four hours after insulin administration was 19.7 µg/100 ml with a range of 12–49 µg/100 ml.

B. Postoperative studies

During the postoperative period there was no essential change in food intake, body weight or the volume of urine excretion of the operated animals.
Fig. 1.

Summary of the plasma corticoid concentrations obtained under the various conditions of the experiment. The height of each bar represents the mean level for the indicated condition. The individual determinations, summarized in Table 1, are indicated here as well, by means of the dots. The pre-operative basal levels include 2 determinations on each of the 9 cats. The points shown for the postoperative period are values obtained one month postoperatively except for those animals which survived one week (see text).

No consistent change in behaviour was noted. Four animals with lesions at various sites had Horner's syndromes which cleared within several days.

1) Location of Lesions. Histological studies demonstrated bilateral localized median eminence (M. E.) lesions in three animals. There was essentially a unilateral median eminence lesion in one animal. The lesions, which approximated spheres with a 2 mm diameter and which connected at the mid-line were
discrete enough to be considered limited to the median eminence (Fig. 2). The remaining animals which served as operative controls, consisted of one with bilateral mammillary body lesions, two with bilateral thalamic lesions, and two in whom no lesion was found in the anterior brainstem. In the latter animals, the locations of the lesions were not determined. Fig. 3 shows a lesion in an operated animal who responded normally to ACTH and insulin. Note that the hypothalamus in this area, including the mammillary bodies, the lateral hypothalamus, mammilo-thalamic tract and a small portion of the thalamus are involved.

Studies of adrenal function yielded similar responses when tested one week and one month postoperatively. Table 1 lists the corticosteroid responses on an

Fig. 2.
A typical discrete lesion involving the median eminence bilaterally (Animal No. 2). The arrows on each side point to the lesions. Note that the lesion extends across the midline. This animal did not respond to ACTH or insulin.
A bilateral lesion in a control operated animal, hat is, one which responded normally to ACTH and insulin (Animal No. 8). The mammillary bodies, the lateral hypothalamus, the mammillothalamic tract and a small portion of the thalamus are destroyed.

individual cat basis. Responses for three animals who died in the second postoperative week are also included. Fig. 1 graphs these results as averages for each series.

2) Basal Corticoid Level. In the postoperative period most of the animals, both those with M. E. lesions and those in the operated control group, showed a marked decrease in the resting corticoid level.

3) Response to Insulin. Animals with M. E. lesions showed an impaired response to the administered insulin throughout the one month period of postoperative observation. The operated control group on the other hand, exhibited a normal response to insulin administration (Table 1 and Fig. 1).

4) Response to ACTH. The most striking result observed in these experiments was the lack of response to exogenously administered ACTH in the animals with M. E. lesions. The operated control group had normal responses (Table 1 and Fig. 1). Though the same batch of ACTH was not used throughout, in all instances in which the M. E. animals were found to be unresponsive, the ACTH employed in testing these animals produced the expected responses in those animals tested pre-operatively as well as in the postoperative control
animals. This lack of response is even more striking if one excludes the animal with a unilateral M. E. lesion. This adrenocortical unresponsiveness was observed following 40 IU as well as 20 IU of ACTH (animals 1 and 2). To rule out adrenal unresponsiveness secondary to possible postoperative atrophy of the adrenal, one animal was given 20 IU ACTH for three successive days one month postoperatively without obtaining an elevation of the plasma corticoids (animal No. 1).

An attempt was made to assess other factors that might be responsible for the lack of response to ACTH. In one instance (animal No. 1, at one week postoperatively) there was no response to 20 IU ACTH when administered by the I. V. as well as by the usual I. M. route. Another animal (No. 2) was unresponsive at one week postoperatively to bovine and equine ACTH in addition to the Armour porcine ACTH that was routinely used.

5) Adrenal Size. The adrenal size (expressed as mg/kg of body weight) of animals with M. E. lesions was compared to that of normal animals and to that of the operated control group (Table 2). There was some evidence of enlargement in the group with M. E. lesions as contrasted to the other two categories.

6) Adrenal Histology. As seen in a typical section (Fig. 4) the animals with M. E. lesions showed a greater accumulation of lipid in the zona fasciculata.*

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* While it is realized that sudanophilic dyes do not identify specific steroid hormones, *from a wealth of clinical and histological evidence it seems probable that these droplets represent stores of potential precursor materials that may be converted into steroid hormones.... Histochemically the droplets in steroid producing cells are strongly sudanophilic* (Deane H. W.: »Intracellular Lipids« in Frontiers in Cytology, p. 227, New Haven, Yale Univ. Press. 1958).

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Table 2.
Average Adrenal Weight (mg/kg). †

<table>
<thead>
<tr>
<th>Normals*</th>
<th>Control operated</th>
<th>M. E. Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.2 (10)</td>
<td>71.4 (5)</td>
<td>74.1 (1)</td>
</tr>
<tr>
<td>58.4 (12)</td>
<td>56.1 (6)</td>
<td>66.2 (2)</td>
</tr>
<tr>
<td>71.4</td>
<td>58.2 (7)</td>
<td>76.1 (3)</td>
</tr>
<tr>
<td>57.4</td>
<td>59.6 (8)</td>
<td>65.4**(4)</td>
</tr>
</tbody>
</table>

Mean 58.0  60.5  70.4

† Numbers in parentheses refer to animal No. See Table 1.
* Males and females.
** Unilateral lesion.
Fig. 4.
Adrenal sections stained with Sudan Black. The three sections are pictured at the same magnification. Note that the adrenal from the animal with the bilateral median eminence lesion (Animal No. 1) shows a greater accumulation of lipid in the zona fasciculata. The control-operated adrenal is from Animal No. 8.

The adrenals of the operated control group showed no significant differences from the normal controls.

7) Hypophysis. The hypophysis was studied histologically only in two animals with M. E. lesions (Nos. 1 and 4). It was found to be normal in both. No special studies of the pituitary portal circulation were made.

8) No significant histological changes were noted in the thyroid or gonads of all animals, whether with M. E. lesions or control operated.

DISCUSSION

The data indicate that discrete bilateral median eminence (M. E.) lesions in the cat produce a depression of adrenal cortical function. This depression is characterized by an inability to respond to stress (insulin hypoglycaemia), as measured by plasma corticoid response, together with a probable increase in both adrenal size and stainable cortical lipid. There was no histological evidence of adrenal cortical atrophy such as is usually seen after pituitary failure. These findings suggest that the observed depression of adrenal function was brought about by interference with release of steroid from the
adrenal rather than by adrenal atrophy. The surprising finding was that these animals with M. E. lesions also did not respond to exogenously administered ACTH.

It has previously been postulated that animals with M. E. lesions fail to respond to stressful stimuli with ACTH release because of destruction of nervous pathways which normally mediate the influence of such stimuli to the hypophysis (Hume & Wittenstein 1950; Laqueur et al. 1955; McCann 1955). However, no studies have been done to date to determine whether or not animals with such lesions actually secrete ACTH.

It would appear from our data that perhaps part of the reason why animals with M. E. lesions do not respond to stress with an increase in plasma corticoids is due to the fact that their adrenals are not normally responsive to ACTH. McCann (1955) and Laqueur et al. (1955) in reporting a decreased responsiveness to stress in animals with M. E. lesions did not test their preparations with ACTH. Therefore, one cannot actually conclude from their data whether the decreased responsiveness was due to decreased ACTH release or decreased adrenal responsiveness to ACTH. The only studies in which positive responses to the administration of ACTH have been obtained in animals with M. E. lesions have utilized eosinophile response (Hume & Wittenstein 1950) and ascorbic acid depletion (Royce & Sayers 1958) rather than direct measurement of 17-hydroxycorticosteroids as an index of adrenal response to ACTH.

Our findings appear to be in agreement with results reported by other investigators. C. Fortier in collaboration with G. W. Harris and I. R. McDonald (pers. comm.) observed a depressed lymphopenic response to epinephrine and to exogenous ACTH in rabbits with median eminence lesions. This was only slightly corrected by the administration of ACTH for three days prior to repetition of the above tests. »These observations suggested that unresponsiveness to ACTH partly accounted for the depressed or completely prevented lymphopenic response to the adrenaline stress noted in these animals«.

Dear & Guillemain (1960) found that rats with median eminence lesions showed a decreasing response to ACTH postoperatively, which fell exponentially and disappeared completely in 60 days following lesioning. The method they used for assay of adrenal response was similar to that used in the present study. Hume & Nelson (1958) studying dogs, observed that »adrenals in many of the dogs with lesions and a decreased response to stress do not respond promptly to exogenous ACTH even in large doses. Thus in the animal with a median eminence lesion, there is a normal sized adrenal unresponsive to exogenous ACTH . . .« On the basis of these findings, Hume and Nelson further speculate; »If there is a basal ACTH secretion sufficient to maintain adrenal size and basal corticosterone output in dogs with lesions of the median eminence, it is difficult to understand why this corticotrophin fails to maintain the cortex in a state in which it will respond promptly and well to large doses
of exogenous corticotrophin. Clearly therefore the question must remain open at present.«

We concur with this latter comment on the basis of our present results. The reason for the failure of the adrenal of the M. E. lesion animal to respond to ACTH is not clear. It is a well established neurophysiological principle, that the effect of destructive nervous system lesions reflects the function of the remaining parts. Therefore, the effect produced by M. E. lesions, rather than being due entirely to local destruction, may also reflect the influence of as yet undetermined neuropituitary pathways, acting in the absence of the median eminence. If such a pathway exists one possible mode of action could be that it results in the production of a pituitary »adreno-inhibitory« hormone, and that this may account for the unresponsiveness of the animals with M. E. lesions to exogenous ACTH. In a somewhat different context, the existence of a hypothalamic inhibitory center has been postulated by others (Sayers et al. 1958). However, at present we realize that there is no evidence for the existence of such a hormone.

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REFERENCES


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