EFFECT OF PROLONGED CORTISONE TREATMENT ON THE CORTICOTROPIN
RELEASING ACTIVITY OF THE RAT HYPOTHALAMUS*

By

C.D. Bagul

Department of Biochemistry, All-India Institute of Medical Sciences, New Delhi

It has been shown that ACTH output by the anterior pituitary gland can be inhibited by
the administration of corticosteroids, under basal conditions or under conditions of stress (20,
13, 18, 10). Prolonged treatment with cortisone is known to cause adrenal atrophy in dogs, rats,
and in man (11,9), due to inhibition of ACTH secretion from the anterior pituitary gland. Such
an inhibitory action has been demonstrated by Sydnor (23) who showed that the release of
ACTH from the pituitary in response to a stress is prevented if a dose of cortisol is administered
to the animal just prior to the application of stress. Farrell and Laqueur (7) observed reduction
in pituitary ACTH content after cortisone treatment to dog. The site and mechanism of blocking
action of corticosteroids is still an unsolved problem. The inhibitory effect might be exerted
at the level of the central nervous system or the anterior pituitary gland or at both these levels.

It is generally believed that ACTH secretion is regulated by a neurohumour, corticotropin
releasing factor (CRF), released from the hypothalamus (4,5, 16, 19, 17). Therefore, effect of
conditions which reduce release and content of pituitary ACTH on corticotropin releasing activity
of the hypothalamus was studied. The data are presented here.

MATERIALS AND METHODS

Male rats (about 300 g) were obtained from Hormone Assay Laboratories, Chicago,
Illinois, and were acclimatized for 1-2 weeks to the local animal house conditions. Animals
were fed Purina rat Chow and given water ad libitum. All injections were given by the subcutaneous route except where stated otherwise.

Cortisone treatment —Each rat of the experimental group received injections of 12.5 mg
cortisone acetate as 0.5 ml aqueous suspension (Upjohn, Kalamazoo, Michigan) every day,
for 24 days. After the completion of the above period, body weights were determined, the rats
decapitated and adrenal and pituitary weights measured. Control rats were given 0.5 ml of
0.9% sodium chloride solution per day, for 24 days before they were sacrificed.

Preparation of the hypothalamic extracts—The hypothalamic area around the pituitary
stalk 2 mm in anteroposterior plane, 1 mm in lateral plane and 1 mm in ventrodorsal plane
(weight about 6-8 mg) was excised and immediately crushed in a glass homogenizer containing
cold (about 4°C) 0.1 N hydrochloric acid. The hypothalamic areas from the control and treat-

*This work was carried out in the Department of Physiology, School of Medicine, Western Reserve University,
Cleveland, U.S.A.
ed group of animals were separately pooled and then homogenized. Thirty animals were used per group. The extracts thus prepared were heated to 100°C for 10 min. in order to destroy toxic substances (21). Volumes of the extracts were adjusted so that 0.5 ml of the extract represented hypothalamus from 1 animal, hereafter referred to as a hypothalamic unit (H.U.). Extracts were centrifuged for 1/2 hour at 20,000 r.p.m. in a preparative head (No. 40) of a Spinco ultracentrifuge. The corticotropin releasing activity of the supernatant was estimated by the method of Briggs and Munson (1). Male rats (120-140 g) were given pentobarbitone sodium (40 mg/kg) in 0.9% sodium chloride solution by the intraperitoneal route. Ten minutes later morphine sulfate (15 mg/kg) in 0.9% sodium chloride solution was injected by the intraperitoneal route. Ten minutes after the morphine injection, the left adrenal gland was removed via a dorsal approach, cleaned, weighed and put into centrifuge tube containing 10 ml of 4% trichloroacetic acid and a little sand and was crushed by a glass rod. This was used for ascorbic acid estimation. The left femoral vein was exposed and hypothalamic extract was infused over a period of 60 to 90 seconds. The injections of the hypothalamic extracts were made at 2 dose levels (0.25 H.U. and 1 H.U.); the volume injected was kept constant at 0.5 ml. In another series of assay rats standard ACTH (USP standard) in solution was infused at two dose levels, 0.2 mU and 0.8 mU ACTH. The ACTH solution was prepared in solution containing 0.1 N HCl, 0.1% W/V Bovine serum albumin (Nutritional Biochemicals, Cleveland, Ohio), and 0.9% sodium chloride. Bovine albumin was added to prevent sticking of micro quantities of ACTH to the wall of glass containers.

Seven to 10 assay animals were used for each dose of hypothalamic extract or of ACTH. Half an hour after the injection of the hypothalamic extract or of ACTH, the right adrenal gland was removed and processed like the left one. The ascorbic acid content of individual adrenal glands was separately estimated by the method of Sayers et al. (22).

The assay rats were also infused with either non-specific (cerebral) or specific (hypothalamic) extracts (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Ascorbic Acid Depletion (mg per 100g Adrenal wet weight)</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy</td>
<td>16 ± 7.0</td>
<td>5</td>
</tr>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy + 0.5 ml 0.1 N HCl</td>
<td>15 ± 6.0</td>
<td>15</td>
</tr>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy + 0.5 ml Cerebral Extract in 0.1 N HCl</td>
<td>8 ± 3.0</td>
<td>14</td>
</tr>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy + 0.5 ml Hypothalamic Extract in 0.1 N HCl</td>
<td>146 ± 8.0</td>
<td>6</td>
</tr>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy + 25 mU Vasopression in 0.5 ml 0.1 N HCl</td>
<td>9 ± 9.7</td>
<td>7</td>
</tr>
<tr>
<td>Laparotomy + Unilateral Adrenalectomy + 100 mU Vasopression in 0.5 ml 0.1 N HCl</td>
<td>128 ± 8.0</td>
<td>8</td>
</tr>
</tbody>
</table>
Volume 13
Number 3

Corticotropin Releasing Factor

Index of precision of the CRF-ACTH assay

Standard ACTH (obtained from the U.S.P. office, New York) was infused in 3 groups of assay rats, each containing 7 to 10 animals, at 3 dose levels 0.2 mU, 0.4 mU and 0.8 mU. A linear relationship was observed between the log dose and the response. Index of precision of the assay (λ) was calculated (22). Index of precision is the ratio of standard deviation to the slope of the fitted regression line. If the value of λ is below 0.20, the regression line is considered to be quite good to use for the bioassay. In this case λ was found to be 0.2026 (Fig. 1).

To exclude the possibility that the hypothalamic extracts could have been contaminated with ACTH, the hypothalamic extracts were tested for ACTH activity according to the ascorbic acid depletion method of Sayers et al. (22). One half ml of the extract was infused into 24 hour hypophysectomized rats and the ascorbic acid depletion caused was noted (Table 4).

TABLE 2

Effect of Prolonged Treatment with Cortisone Acetate on the Body weight, Adrenal weight and Pituitary weight of Rats

<table>
<thead>
<tr>
<th></th>
<th>Normal (Saline treated)</th>
<th>Cortisone Treated</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, in g</td>
<td>364 ± 2.00 (63)</td>
<td>209 ± 2.00 (58)</td>
<td>0.001</td>
</tr>
<tr>
<td>Combined Adrenal Weight, in mg</td>
<td>38.9 ± 0.70 (63)</td>
<td>15.5 ± 0.34 (58)</td>
<td>0.001</td>
</tr>
<tr>
<td>Interior Pituitary Weight, in mg</td>
<td>5.03 ± 0.14 (63)</td>
<td>4.80 ± 0.12 (58)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Number in the parentheses indicates number of animals in the group.

For the difference between the means.

Vasopressin in the extracts—Vasopressin is known to release ACTH. Vasopressin content of the hypothalamic extract was determined by employing the 'Rat Pressor Bioassay' technique of De Kanski (6) (Table 5).

TABLE 3

Comparison between the Corticotropin Releasing Activity of Hypothalamic Extracts Obtained from Normal and from Cortisone-Treated Rats.

<table>
<thead>
<tr>
<th></th>
<th>Ratio of Potency</th>
<th>Fiducial Limits for the Ratio of Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisone-Treated :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.59</td>
<td>0.58 to 0.60</td>
</tr>
</tbody>
</table>

For the parallelism of slopes was > 0.5
Fig. I.

Log-dose X response relationship between ACTH infused and the ascorbic acid depletion induced in
pentobarbital—morphine blocked rats.

RESULTS

The corticotropin releasing activity in the hypothalamic extracts of cortisone-treated animals was about half of that found in the hypothalamic extracts of the normal animals (Table 1). Such a comparison is valid since the slopes for the above two groups were parallel to each other (p > 0.5) (Fig. 2). Similarly, the method of assay of released ACTH might be considered valid since the slopes for cortisone-treated group and the normal controls were parallel to the slope for ACTH (p > 0.4 and p > 0.2 respectively, Fig. 2).

Cerebral extract did not release ACTH in the assay animals. Similarly, ACTH was found to be released only on infusion of hypothalamic extract or infusion of 100 mU of vasopressin (Table 1). The activity of ACTH in the hypothalamus of cortisone-treated animals in the normal controls was negligible (Table 4). Similarly, the vasopressin content in the hypothalamic extracts was too low to release ACTH and cause ascorbic acid depletion in the assay rats (Table 5).
Ascorbic acid depletion induced in pentobarbital-morphine blocked assay rats following the administration of Standard (U.S.P.) ACTH or hypothalamic extract. (The hypothalamic extract was obtained from normal or cortisone-treated rats).

**Table 4**

<table>
<thead>
<tr>
<th>Source of Hypothalamic Extract</th>
<th>Ascorbic Acid Depletion mg per 100 g</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Rats</td>
<td>-3 ± 0.22</td>
<td>6</td>
</tr>
<tr>
<td>Cortisone-Treated Rats</td>
<td>-4 ± 0.28</td>
<td>6</td>
</tr>
</tbody>
</table>

ACTH activity in the hypothalamic extracts obtained from normal and cortisone-treated rats.

The body weights of the cortisone-treated rats were significantly less than those of the saline-treated controls (Table 2). Cortisone treatment also caused adrenal atrophy in the animals. Thus the average combined weight of the two adrenals of the individual rat was 38.90 ± 0.70 mg in the normal saline treated group whereas it was 15.54 ± 0.34 mg in the cortisone-treated group (Table 2). On the other hand, anterior pituitary weights of the normal controls and those of the cortisone-treated animals were comparable (Table 2).
Table 5

<table>
<thead>
<tr>
<th>Source of Hypothalamic Extract</th>
<th>Vasopressin Content (mU)</th>
<th>Fiducial Limits of the estimate of potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Rats</td>
<td>4.4</td>
<td>82.7% to 120.9%</td>
</tr>
<tr>
<td>Cortisone Treated Rats</td>
<td>4.1</td>
<td>73.4% to 136.2%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It was found that prolonged treatment with cortisone acetate induces reduction in corticotropin releasing activity of the hypothalamus. There are no data available in the literature as to the amount of corticotropin releasing activity in the hypothalamus following treatment of animals with steroid hormones. However, while our work was in progress, a report appeared wherein an effect of a single dose of hydrocortisone on the corticotropin releasing activity of the rat median eminence is mentioned (24). According to the author, a single intravenous injection of hydrocortisone (7.5 mg/100 g) caused a significant (about 50%) fall in the corticotropin releasing activity of the rat median eminence, 4 hours later. It was also observed that stress induced an increase in the corticotropin releasing activity of the median eminence in normal but not in steroid-treated rats. One may conclude that prolonged treatment as well as single injection of corticosteroids reduces the content of the corticotropin releasing activity in the hypothalamus.

Results presented here on the effect of prolonged treatment with corticosteroid on the adrenals confirm those reported by other investigators. Ingle et al. (14) for example, have shown that the administration of adrenocortical extract leads to adrenal atrophy. Kitay et al. (15) found that cortisone acetate (5 mg/120 to 140 g/day) induced a significant decrease in adrenal weight.

Fortier (8) found that 10 days of hydrocortisone acetate treatment (3 mg/100 g/day) resulted in 40% decrease in the pituitary ACTH content in the rat. Further that the same treatment if prolonged for 24 days induced 90% decrease in pituitary ACTH content. Fortier (8) also found that 10 and 24 days of treatment with the steroid led to 50% and 70% reduction in adrenal weight, respectively.

Kitay et al. (15) found that the pituitary weights in the steroid-injected group and in the saline control group did not differ from each other although pituitary ACTH content was less by about 60% in the cortisone-treated group. The results of other workers are in agreement with this finding (8, 15). We did not find significant difference between the weights of the anterior pituitaries obtained from cortisone-treated and saline-treated rats. We did not measure pituitary ACTH content in these two groups.

Davidson and Feldman (3) feel that the hypothalamus rather than the pituitary should be regarded as the primary site of feed back inhibition of ACTH by hydrocortisone.
Corticotropin Releasing Factor

The argument is based on their observations that hydrocortisone implants in the anterolateral hypothalamus bring about abolition of compensatory adrenal hyper- 

trophy and even atrophy of the remaining adrenal; cholesterol implants were without 

effect. Also, hydrocortisone implants in other regions of the brain did not produce such 

effects. Chowers et al. (2) found that implantation of small quantities of crystalline hydrocorti-

sone acetate in the hypothalamus leads to adrenal atrophy and inhibition of adrenal ascorbic 

acid depletion, while similar implants in the pituitary are without effect. Vernikos-Danellis (24) 

has suggested that corticosteroids probably inhibit the synthesis of corticotropin releasing 

factor. At present, the identity and structure of corticotropin releasing factor is not fully known, 

though it is likely to be a polypeptide (12). If corticotropin releasing factor is a polypeptide, it 

is necessary to show that incorporation of amino acids to form corticotropin releasing factor is 

prevented in the presence of steroids, before one can draw any conclusion about the effect of 

steroids on its synthesis.

It might be argued that the low CRF content in the hypothalamus of cortisone-treated 

rats may be due to massive catabolism of proteins in the body and hence non-specific. How-

ever, the following two facts go against this possibility. Firstly, CNS is one of the organs that 

suffers least during protein catabolism. Secondly, the amount of vasopressin in the hypothala-

mus (polypeptide) in the normal as well as in the cortisone-treated rats was nearly identical in 

the experiments in here. This indicates that massive protein catabolism following cortisone 

treatment did not reduce vasopressin content. Therefore, the decrease in the content of CRF 

in the hypothalamus of cortisone-treated rats is likely to be specific.

SUMMARY

Cortisone treatment to the rats (12.5 mg/day; subcutaneously for 24 days) led to 40% 

reduction in the corticotropin releasing activity of the hypothalamic median eminence region. 

The ACTH activity and vasopressin content of this region were found to be too low to inter-


ter with the assay of corticotropin releasing activity. The results suggest that cortisone inhibits 

the synthesis of corticotropin releasing factor.

Cortisone treatment also caused a 42% and 60% fall in the body weight and the adrenal 

weight of the cortisone-treated group though pituitary weight did not differ significantly from 

those in the normal controls.

ACKNOWLEDGEMENT

The author is grateful to Professor George Sayers under whose guidance the work was 


carried out. The author is indebted to U.S.P.H.S., U.S.A. for the grant of a fellowship under 

574.

REFERENCES

1. Briggs, F.N. and P.L. Munson. Studies on the mechanism of stimulation of ACTH secre-


intrahypothalamic crystalline ste-
1, 1963.

in the inhibition of ACTH secre-
terior pituitary gland and blood
of ACTH secretion by the pituitary
.
content of ACTH by cortisone,
oids following bilateral adrena-
and adrenal weight in the rat.
Midbrain and feedback control of
64.
lesions on steroid-induced atrophy
; Hypothalamic factors releasing
54.
hebennierenrinde und 17-OH corti-
g-fristigcortisonovorbehandlung
asy of the adrenal cortex in the rat
regulation of pituitary adrenocorti-
adal cortical response to stress in
role of the supraopticohypophyseal
aluation of adrenocorticotropic hor-
31, 1959.

Hor. Res. 10:1, 1954.

20. Porter, R.W. and J.C. Jones. Effect of plasma from hypophyseal-portal vessel blood on


23. Sydnor, K.L. Blood ACTH in the stressed adrenalectomized rat after intravenous hydro-

24. Vernikos-Danellis, J. Effect of stress, adrenalectomy, hypophysectomy and hydrocortisone