EFFECT OF BILATERAL ADRENALECTOMY AND CORTICOSTEROID THERAPY ON THE SECRETION OF CORTICOTROPHIN-RELEASING FACTOR ACTIVITY FROM THE HYPOTHALAMUS OF THE RAT IN VITRO

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SUMMARY

The rat hypothalamus in vitro preparation was used to investigate the effect of bilateral adrenalectomy, with and without replacement therapy, on the release of corticotrophin-releasing factor (CRF). Corticotrophin-releasing factor was estimated using 48 h basal hypothalamic lesioned assay rats and corticosterone production of excised adrenals was used as the end point.

Bilateral adrenalectomy resulted in depletion of hypothalamic CRF content within the first 2 h after the operation but this effect was prevented by replacement therapy with corticosterone. Thereafter, the hypothalamic CRF content returned to values not significantly different from the intact control level. Bilateral adrenalectomy caused an increase in both basal and acetylcholine-induced release of CRF and it is suggested that corticosteroids exert a negative feedback effect on the hypothalamus.

INTRODUCTION

Bilateral adrenalectomy results in high levels of corticotrophin (ACTH) in the circulation which can be prevented by replacement therapy with physiological doses of corticosteroids (Dallman, Jones, Vernikos-Danellis & Ganong, 1972; Dallman & Jones, 1973; Buckingham & Hodges, 1974). However, although the effects of adrenalectomy and corticosteroid therapy on ACTH secretion are well established there have been few relevant observations on corticotrophin-releasing factor secretion. The rat hypothalamus in vitro preparation, however, overcomes many of the problems presented by investigations in vivo and has provided us with an opportunity to investigate the effects of endocrine manipulation on the secretion of CRF.

The viability of the isolated hypothalamus in vitro has been extensively investigated (Bradbury, Burden, Hillhouse & Jones, 1974; Hillhouse, 1975) and the limitations of such an experimental approach have been discussed (Hillhouse, 1975; Jones, Hillhouse & Burden, 1976a). The preparation has been used to study the secretion of CRF (Bradbury et al. 1974; Hillhouse, Burden & Jones, 1975; Jones et al. 1976a, b) and the neurohypophysial hormones (Bridges, Hillhouse & Jones, 1975) and it has also been shown to secrete measurable quantities of luteinizing hormone releasing hormone (LH-RH) and thyrotrophin-releasing hormone (TRH) (S. L. Jeffcoate, E. W. Hillhouse & M. T. Jones, unpublished observations).

In this investigation the effect of adrenalectomy and corticosteroid therapy on the secretion of CRF in vitro was examined together with the effects on the tissue content of CRF.
METHODS

Experimental animals
Male Wistar-derived rats weighing 100–175 g from a specific pathogen-free colony bred at the Medical School Animal House were used in all these experiments. The animals were housed in an air-conditioned room with a controlled light cycle (lights on from 07.00 to 21.00 h). Rat chow and water, or in the case of adrenalectomized rats, 0-9 % saline solution, were available ad libitum. The operation of bilateral adrenalectomy was performed by the dorsal midline approach.

Assay of CRF
The CRF activity was assayed by intravenous injection of hypothalamic incubation fluid into 48 h basal hypothalamic-lesioned assay rats as described previously (Hillhouse et al. 1975). The index of CRF activity used was corticosterone production by excised adrenals 15 min after injection of either hypothalamic incubation fluid or a neutralized hypothalamic extract.

Removal and incubation of hypothalami
Hypothalamic tissue was obtained from unanaesthetized male rats since the oestrous cycle in females has been shown to affect the activity of the CRF neurones (Hiroshige & Wada-Okada, 1973). All the incubations were performed between 07.00 and 10.00 h to minimize any variation in the CRF content of the tissue. Rats were removed from their cages with the minimum of stress and decapitated. The skull cap was removed, the frontal lobes lifted, the optic nerves cut and the whole brain deflected backwards out onto a Petri dish. The hypothalamus was dissected out using iris scissors. The tissue was bordered rostrally by the anterior border of the optic chiasma, laterally by the hypothalamic fissures and caudally by the mammillary bodies. The ventral border of the preparation was delineated by the median eminence and the dorsal extent of the cut was at a depth of approximately 2 mm. Once the hypothalamus had been dissected out it was immediately transferred to an incubation flask containing 1 ml incubating medium. The whole procedure from time of death to commencement of incubation took less than 1 min. The flasks were incubated in a Dubnoff metabolic shaker and the incubation medium was gassed continuously with a mixture of 95 % O2: 5 % CO2. The hypothalami were incubated in groups of three and were always subjected to a pre-incubation period of 30 min. The hypothalami were incubated with 1 ml of incubating medium containing the test substance for 10 min followed by a 20 min rest period in fresh medium free of neurotransmitters; the procedure was repeated for a 2 h period. A minimum of 12 hypothalami were used in each experimental group and the medium was pooled for CRF determination in ten or more assay animals. The injection volume was 0-5 ml/assay animal unless otherwise stated.

Composition of hypothalamic incubation medium
This had a composition similar to cerebrospinal fluid as described previously (Hillhouse et al. 1975).

Extraction of CRF activity from the hypothalamus of the rat
Extracts of the hypothalami were prepared according to the method of Chan, Schaal & Saffran (1969). The hypothalamus was removed as described above and immediately placed in 100 µl 0-1 m-HCl in a tissue homogenizer. The extract was stored in a refrigerator and neutralized with sodium bicarbonate before use.

Corticosteroid therapy
Steroids were administered subcutaneously as suspensions in arachis oil 24 h before death. The injections were given in the axilla.
Adrenalectomy and CRF secretion

Hypothalamic extracts for vasopressin and oxytocin assay

For the estimation of the neurohypophysial hormone content, the rat hypothalamus was extracted by the method of Dyer, Dyball & Morris (1973).

Assay of neurohypophysial hormones

Vasopressin

Vasopressin was assayed by i.v. injection of pooled samples of hypothalamus incubation fluid into 200 g rats under ethanol anaesthesia in which constant water load was maintained and blood pressure was recorded simultaneously with urine flow (Bisset, Hilton & Poisner, 1967). All samples were assayed according to the four-point assay design (Gaddum, 1953) using a low and a high dose with a ratio of approximately 2 for standard and unknown.

Oxytocin

Oxytocin was estimated by measuring the milk-ejection pressure in lactating rats weighing between 250 and 300 g and used 3–21 days after parturition (Bisset, Clark, Harris, Lewis & Rocha é Silva, 1967). Correction was made for the intrinsic milk-ejecting activity of the vasopressin present in extracts.

Statistics

Significance levels were determined by Student's t-test.

RESULTS

Hypothalami were removed from rats at various times after adrenalectomy and the secretion of CRF in vitro was studied. The effect of adrenalectomy on the basal secretion of CRF from the rat hypothalamus in vitro is shown in Fig. 1. Hypothalami obtained from rats with intact adrenals only secreted small amounts of CRF which were just detectable when the incubation medium was injected into the assay rats in volumes of 0.5 ml/animal. The results when hypothalami were removed from 2 h adrenalectomized rats were similar. Hypothalami removed from either 4 or 24 h adrenalectomized rats showed higher basal secretion rates although neither response was statistically significant. However, hypothalami removed from 7-day adrenalectomized rats showed a considerable increase in the basal secretion of CRF, an increase which was significantly different from that seen in hypothalami removed from rats with intact adrenals (P < 0.01). The basal secretion of CRF by hypothalami in vitro then remained at this level even if the donor animals had been adrenalectomized for a period of 3 months.

Acetylcholine was used as a stimulus to CRF release and results are presented in Fig. 2; each point represents the increment of CRF released above the basal secretion rate. Hypothalami obtained from rats with intact adrenals responded to acetylcholine (3 pg/ml incubation medium) with a significant release of CRF as compared with the basal secretion (P < 0.01). However, when hypothalami obtained from rats adrenalectomized 2 h previously were incubated with acetylcholine the basal secretion rate was unaltered. As the basal secretion in the hypothalami was very close to the baseline of the assay (1.5 µg corticosterone/100 mg/h) it was difficult to determine whether the hypothalami were secreting any CRF at all (see Fig. 1). In the 4 h adrenalectomized rats the ability of the hypothalamus to secrete CRF in response to acetylcholine returned and although the response was somewhat smaller than that of hypothalami from intact rats the difference was not statistically significant. In the 1-day adrenalectomized rat there was a further increase in the ability of the hypothalamus to secrete CRF in vitro and the response was back to about the same level as that seen in the intact animal. Seven days after adrenalectomy, however, the hypothalami
Fig. 1. The basal secretion of CRF activity of hypothalami taken from rats at various times after adrenalectomy and incubated for 2 h. Each bar represents the mean ± S.E.M. of observations in 15–20 assay animals using pooled medium from incubations of 12–15 hypothalami. The broken vertical line indicates the limit of detection of the assay. ** \( P < 0.01 \) compared with hypothalami from rats with intact adrenals.

Fig. 2. Effect of acetylcholine (3 pg/ml incubation medium) on the release of CRF activity from hypothalami taken from rats at various times after adrenalectomy. Each bar represents the acetylcholine-induced increment in CRF activity above basal release and is the mean ± S.E.M. of observations in 12–21 assay animals using pooled medium from incubations of 12–15 hypothalami. *** \( P < 0.001 \) compared with hypothalami from rats with intact adrenals.
secreted considerably more CRF in vitro in response to acetylcholine than hypothalami from intact rats \( (P < 0.05) \). Fourteen days after adrenalectomy the maximal secretory capacity of the hypothalamus in vitro was reached and the release of CRF in response to acetylcholine was highly significant compared with hypothalami from rats with intact adrenals \( (P < 0.001) \). This level of response was maintained for at least 3 months which was the maximum period of adrenalectomy studied.

The specificity of the acetylcholine response was examined by measuring other hormones in the incubating medium. Acetylcholine (3 pg/ml) caused the release of both vasopressin and oxytocin (Table 3b) but had no effect upon the release of bioactive ACTH (measured by the Redox bioassay) or immunoreactive LH-RH and TRH. The CRF activity of the medium was not due to the presence of either ACTH or vasopressin since the amounts of these hormones found in the incubating medium were far below the threshold response for the assay (Table 3c). Similarly, vasopressin itself did not cause the release of CRF from the hypothalamus in vitro and neither did it potentiate the effect of CRF (Table 4).

Table 1. Corticotrophin-releasing factor (CRF) activity of hypothalami removed from intact rats, adrenalectomized (ADX) rats or ADX rats on corticosteroid therapy

<table>
<thead>
<tr>
<th>Period of adrenalectomy, and treatment</th>
<th>CRF activity ( (\mu g ) corticosterone ( / ) 100 mg adrenal/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>7.6 ± 1.3 (6)</td>
</tr>
<tr>
<td>2 h ADX</td>
<td>3.5 ± 0.7* (6)</td>
</tr>
<tr>
<td>2 h ADX + 100 ( \mu g ) corticosterone</td>
<td>6.2 ± 0.9 (6)</td>
</tr>
<tr>
<td>1 day ADX</td>
<td>6.9 ± 0.5 (6)</td>
</tr>
<tr>
<td>1 day ADX + 5 mg corticosterone</td>
<td>7.2 ± 0.8 (6)</td>
</tr>
<tr>
<td>14 day ADX</td>
<td>7.3 ± 0.7 (6)</td>
</tr>
<tr>
<td>14 day ADX + 5 mg corticosterone</td>
<td>8.9 ± 1.0 (6)</td>
</tr>
</tbody>
</table>

The CRF activity in the hypothalamus was extracted according to the method of Chan et al. (1969). The extracts were administered to the assay animal in volumes equivalent to a half hypothalamus and the CRF activity expressed in terms of \( \mu g \) corticosterone produced/100 mg excised adrenal/h. The values are means ± S.E.M. of data obtained from homogenates of 10 hypothalami. The number of observations is given in parentheses. * \( P < 0.05 \) as compared with all other values.

CRF content of the rat hypothalamus

Table 1 shows the effect of various treatments on the CRF content of the hypothalami. Two hours after adrenalectomy there was a significant decrease in the hypothalamic CRF content \( (P < 0.05) \) which was prevented by the subcutaneous injection of corticosterone \( (100 \mu g/100 \) g) immediately after adrenalectomy. This suggests that the failure of the hypothalamus to respond to acetylcholine in vitro 2 h after adrenalectomy (see Fig. 2) is due to a depletion of the CRF content of the hypothalamus.

The results in Table 2 demonstrate that the CRF content of hypothalami removed from 1-day adrenalectomized rats was not depleted after a 2 h incubation period which included 10 min periods during which the hypothalami were challenged with either acetylcholine (3 pg/ml) or 5-hydroxytryptamine (10 ng/ml). This shows that during a 2 h incubation period in which the hypothalamus was stimulated with a neurotransmitter it releases CRF into the incubation medium in greater quantities than the initial content of the tissue. The amount of CRF released from the hypothalami from adrenalectomized animals is greater than that from hypothalami from intact rats but this hypersecretion takes place without any concomitant decrease in tissue content. Measurements were also made of the hypothalamic content of various hormones which might contribute to the CRF activity of the blocks of hypothalamic tissue (Table 3a). The amounts of ACTH and vasopressin present did not
contribute significantly to the CRF activity of the blocks of hypothalamic tissue (Table 3). Also the amount of vasopressin present in the tissue did not potentiate the CRF activity (Table 4).

Table 2. Corticotrophin-releasing factor (CRF) released into hypothalamic incubation media during 2 h incubation compared with the CRF content of the tissue after incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CRF released into medium (µg corticosterone/100 mg adrenal/h)</th>
<th>Tissue CRF content (µg corticosterone/100 mg adrenal/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No incubation</td>
<td>—</td>
<td>5.0 ± 0.8 (8)</td>
</tr>
<tr>
<td>2 h incubation + 3 pg ACh/ml</td>
<td>5.8 ± 0.4** (8)</td>
<td>6.3 ± 0.8 (7)</td>
</tr>
<tr>
<td>2 h incubation + 10 ng 5-HT/ml</td>
<td>5.3 ± 0.4** (8)</td>
<td>6.2 ± 1.0 (7)</td>
</tr>
<tr>
<td>2 h incubation (basal release)</td>
<td>2.0 ± 0.8 (8)</td>
<td>4.1 ± 1.0 (10)</td>
</tr>
</tbody>
</table>

The CRF activity in either the incubation medium or the tissue extracts was estimated by the in-vitro corticosterone production of excised adrenals. The CRF activity was extracted from the hypothalami using the method of Chan et al. (1969) and injected into the assay animals in volumes equivalent to one half hypothalamus. The 2 h incubation period included four 10 min periods during which the hypothalami were stimulated with either acetylcholine (ACh) or 5-hydroxytryptamine (5-HT). The values are means ± S.E.M. and the number of observations is given in parentheses.

** *P < 0.01 compared with basal release.

Table 3.

(a) Hormone content of hypothalamic blocks of tissue from intact rats

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Content (µg/hypothalamus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>1.55 ± 0.58 µg/hypothalamus (6)</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>6.946 ± 0.946 µg/hypothalamus (9)</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>2.905 ± 0.176 µg/hypothalamus (9)</td>
</tr>
</tbody>
</table>

Hypothalami were homogenized with 0.1 M HCl for ACTH determination by the cytochemical assay method. Hypothalami were homogenized with 0.25% acetic acid for the estimation of antidiuretic and milk-ejecting activity. Number of hypothalami on which measurements were made is given in parentheses. Means ± S.E.M.

(b) Hormone present in hypothalamic incubation medium

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Basal value (µg/ml)</th>
<th>Stimulated value (µg/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>0.0-9</td>
<td>0.0-9 (1)</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>161 ± 12</td>
<td>802 ± 24 (10)</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>92 ± 10</td>
<td>415 ± 21 (10)</td>
</tr>
</tbody>
</table>

ACTH was assayed cytochemically in a pooled sample of nine hypothalami. Portions of the pooled medium were also assayed for antidiuretic and milk-ejecting activities. The number of pooled samples assayed is given in parentheses. Means ± S.E.M.

* 3 pg acetylcholine/ml.

(c) Threshold doses for ACTH and vasopressin in the basal hypothalamic-lesioned assay rat

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>Response (µg corticosterone/100 mg adrenal/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin</td>
<td>40 ± 0.3 (6)</td>
</tr>
<tr>
<td>ACTH</td>
<td>0.5 ± 0.5 (10)</td>
</tr>
<tr>
<td></td>
<td>2.5 ± 0.7 (10)</td>
</tr>
</tbody>
</table>

Vasopressin or ACTH were injected i.v. into 48 h basal hypothalamic-lesioned assay rats. Numbers in parentheses refer to number of assay animals used. Means ± S.E.M.
Effect of vasopressin added to the incubation media on the release of corticotrophin releasing factor (CRF) as compared with the basal secretion rate, and the effect of acetylcholine (ACh)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CRF activity (µg corticosterone/100 mg adrenal/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal secretion</td>
<td>2.7±0.3 (8)</td>
</tr>
<tr>
<td>Vasopressin (1 µu./ml)</td>
<td>3.0±0.7 (6)</td>
</tr>
<tr>
<td>Vasopressin (10 µu./ml)</td>
<td>2.2±0.3 (8)</td>
</tr>
<tr>
<td>ACh (3 pg/ml)</td>
<td>5.9±0.6 (10)</td>
</tr>
<tr>
<td>CRF (ACh, 3 pg/ml) + vasopressin (1 µu.)</td>
<td>4.5±0.8 (8)</td>
</tr>
<tr>
<td>CRF (ACh, 3 pg/ml) + vasopressin (10 µu.)</td>
<td>4.3±0.7 (8)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of data on pooled medium from 9 to 12 hypothalami. The numbers in parentheses refer to number of assay animals used.

Fig. 3. Effect of acetylcholine (ACh, 3 pg/ml incubation medium) on the release of CRF activity from hypothalami taken from rats adrenalectomized 1 day previously and pretreated with either a steroid or vehicle 24 h before death. Each bar represents the mean ± S.E.M. of observations in 10–15 assay rats using the pooled medium from incubations of 12–15 hypothalami. ** P < 0.01 compared with rats pretreated with corticosterone or cortisol.

Corticosteroid therapy

The results illustrated in Fig. 3 show that hypothalami removed from 1-day adrenalectomized rats treated with vehicle (arachis oil) responded to acetylcholine (3 pg/ml) with considerable increases in the release of CRF as compared with the basal value (P < 0.005). However, hypothalami obtained from 1-day adrenalectomized rats pretreated with either 5 mg corticosterone (compound B) or 5 mg cortisol (compound F) released significantly less CRF in response to acetylcholine than adrenalectomized rats pretreated with vehicle alone (P < 0.01). The secretion of CRF from the hypothalamus was not significantly different from that of hypothalami from intact rats (Fig. 2). Figure 3 shows that hypothalami obtained from rats pretreated with 11α-hydroxyprogesterone responded to acetylcholine with a release of CRF which was not significantly different from the vehicle-treated rats. Pretreatment with the steroids at the dosage and time-interval employed neither totally abolished the release of CRF nor depressed the basal secretion rates.
The CRF-like activity measured in the present experiments probably reflects corticotrophin-releasing hormone activity since the amount of arginine vasopressin and other hormones present in the hypothalamic incubation fluid has no significant CRF activity (Jones et al. 1976a) and we have shown the existence of two CRF fractions using several CRF assays following separation on Sephadex G-25 and high voltage electrophoresis (Gillham, Jones, Hillhouse & Burden, 1975). Also the purified CRH causes the release of ACTH from the hemisected anterior pituitary in vitro and has no effect on other pituitary hormones (Gillham et al. 1975).

Evidence from experiments both in vivo (Krieger, 1973) and in vitro (Jones et al. 1976a, b) indicate that acetylcholine and 5-hydroxytryptamine are the two excitatory neurotransmitters to CRF release in the rat. The CRF response, therefore, of hypothalami from intact and adrenalectomized rats to these excitatory neurotransmitters reflect changes in the dynamics of CRF function in response to the presence and absence of corticosteroids. The response to acetylcholine may be of particular significance because Hedge & Smelik (1968) found that atropine implants in the ventromedial hypothalamus block the stress response, suggesting that the stress-induced release of CRF is mediated via a cholinergic pathway.

In the present experiments the basal secretion of CRF increased after adrenalectomy, being significantly greater than normal 7 days after adrenalectomy and remaining at this high level for at least 3 months. These findings support the hypothesis that hypocorticism results in a marked increase in basal CRF secretion.

Adrenalectomy results in hypersecretion of CRF and this is consistent with findings of increased ACTH secretion in response to stress in vivo (Sydnor & Sayers, 1954; Dallman et al. 1972; Buckingham & Hodges, 1974). This implies that the corticosteroid negative-feedback mechanism influences CRF secretion under stress as well as basal conditions. During the first 2 h after adrenalectomy there is an immediate release of large amounts of CRF which results in depletion of hypothalamic stores; that this is due to adrenocortical insufficiency is shown by the fact that replacement therapy with a small dose of corticosterone prevents these changes. Whilst we have not measured the basal CRF secretion during the first 2 h in the current work, previous studies in vivo (Dallman & Jones, 1973) have shown that hypersecretion of ACTH occurs immediately following adrenalectomy which must be a reflexion of increased CRF secretion. The hypothalamus, depleted of its CRF stores, is unable to release significant quantities of the releasing hormone in response to acetylcholine. This failure to release CRF may explain why adrenalectomy, whilst causing an immediate increase of ACTH which persists for 2 h, results in low levels of ACTH in the circulation for the next few hours.

The amounts of CRF released into the medium from hypothalami stimulated by acetylcholine or 5-hydroxytryptamine greatly exceeded the initial tissue content, suggesting that these excitatory neurotransmitters must cause CRF synthesis as well as its release. Since the amount released into the medium equals the amount synthesized it is possible that there is coupling of the CRF release–synthesis mechanisms. A coupling of the two processes would account for the fact that there is no net change in tissue content despite the large amounts released in response to the neurotransmitters.

It must be emphasized that there might be changes in the hypothalamic CRF content which are difficult to detect because of the relative insensitivity of the assay system employed. However, we have observed a similar synthesis–release coupling associated with other hypothalamic neurohormones. Electrical stimulation of the isolated hypothalamus of the rat in vitro at a frequency of 30 Hz with a current strength of 100 µA and a duration of 1 ms elicited the release of large quantities of radioimmunoassayable TRH and this was not
associated with any decrease in hypothalamic content (E. W. Hillhouse, S. L. Jeffcoate & M. T. Jones, unpublished observations).

Whether the increase in CRF secretion is due to an effect primarily on synthesis or on release cannot be deduced from our data. However, we would favour a primary effect on synthesis since there is evidence that pretreatment with protein synthesis inhibitors (Arimura, Bowers, Schally, Saito & Miller, 1969) can block the delayed feedback action of corticosteroids; the mechanism studied in the present experiments clearly falls into the delayed feedback period since we are studying the effects some hours after corticosteroid administration. The fast feedback mechanism, on the other hand, acts by blocking the release of hormones by an effect on the membrane since the immediate application of corticosteroid blocks CRF release induced by acetylcholine and the tissue content increases; this effect on release is not found when the membrane is depolarized by 48 mM-K⁺ (Jones et al. 1976b).

The present data are in apparent conflict with other reports which have studied changes in the CRF activity of the median eminence after adrenalectomy. Thus Wittkowski & Bock (1972) and Brinkman & Bock (1973) found morphological evidence for an increase in both number and size of granules in the median eminence 4 weeks after adrenalectomy. Similarly, Vernikos-Danellis (1965) and Sirett & Purves (1973) found increased CRF stores in the median eminence. In the present experiments we were measuring the total tissue content and not median eminence CRF activity. R. Bock (personal communication) found an increase in the number and size of granules in the median eminence of our tissue but the effect is not as striking as in his own experiments. It is possible therefore, that some variation may be due to difference in the type of rat used in the experiments or in their treatment.

It is concluded that the study of the rat hypothalamus in vitro has provided evidence for a negative feedback action of corticosteroids at the hypothalamus. We have now shown that the CRF response of the hypothalamus to acetylcholine is altered by adrenalectomy and replacement therapy with corticosterone thus confirming our previous findings on the effect of electrical stimulation (Bradbury et al. 1974). Our data, taken in conjunction with experiments on the implantation of corticosteroids in the hypothalamus (Smelik & Sawyer, 1962; Bohus & Strashimirov, 1970), lead us to conclude that this negative feedback effect of corticosteroids on the hypothalamus is of physiological significance.

We are indebted to Drs S. L. Jeffcoate and D. L. Holland for the radioimmunoassay of TRH and LH-RH, to Dr T. E. Bridges for the assay of the neurohypophysyal hormones and to Professor R. Hodges and Dr J. Buckingham for the cytochemical assay of ACTH. The steroids used in this study were generously donated by Organon Laboratories Limited.

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