STUDIES OF THE CHANGES IN ADRENAL CORTICOSTERONE IN MICE TREATED WITH DEXAMETHASONE, CHLORPROMAZINE, MORPHINE AND CODEINE: DEVELOPMENT OF AN ASSAY FOR CORTICOTROPHIN-RELEASING FACTOR

By

Philip N. Cheifetz*

ABSTRACT

The concentration of corticosterone in the adrenals of mice showed the expected increase after laparotomy under pentobarbitone anaesthesia and an even greater rise using ether. Following treatment with dexamethasone, there was no change in adrenal corticosterone in response to extract of stalk-median eminence (SME) prepared from rat brain, whereas a significant increase occurred with the administration of corticotrophin (ACTH). Injections of SME extracts in mice treated with chlorpromazine and codeine resulted in significant increases of adrenal corticosterone from baseline values. Log-dose responses of adrenal corticosterone were obtained with increasing concentrations of SME extracts in chlorpromazine and codeine treated mice but no response was seen after injections of extracts of cerebral cortex or of lysine vasopressin. The measurement of corticosterone in the adrenals of mice treated with chlorpromazine and codeine may be a sensitive, specific and convenient technique for the assay of changes of CRF activity in tissue extracts.

The various techniques for measuring corticotrophin-releasing factor in extracts of median eminence are, in general, limited by a lack of specificity or sensitivity (De Wied et al. 1969). The in vivo methods, however, in which the secretion of corticotrophin (ACTH) is inhibited by pharmacological treatment prior to the systemic administration of extracts (Arimura et al. 1967), are perhaps most applicable to the study of CRF in tissues from large numbers of animals. This,

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together with the greater sensitivity possible, prompted a study of the changes of adrenal corticosterone in mice after the administration of crude extracts of median eminence tissue from rats.

MATERIALS AND METHODS

The assay mice are males weighing 25-30 g and bred at the Institute of Psychiatry. Median eminence tissue was collected from male Sprague-Dawley rats which were housed in light-proof, temperature-controlled rooms and illuminated from 07:00 to 19:00 hours.

ACTH secretion in the assay animals was depressed by pharmacological means and the effects of dexamethasone, of chlorpromazine and morphine and of chlorpromazine codeine were compared. The mice were routinely anaesthetized with pentobarbitone intraperitoneally (ip) fifteen to twenty minutes before the injection of test substances. The dose of pentobarbitone was 2.4 mg when dexamethasone was used for pretreatment and 1.2 mg when chlorpromazine and morphine or chlorpromazine and codeine were used.

Dexamethasone sodium phosphate was given both in a solution in gelatin, two per cent, subcutaneously (sc) and in saline ip. Chlorpromazine was given sc at a dose of 0.5 mg per 10 g body weight, four hours prior to assay. Morphine was used sc at a dose of 0.25 mg and codeine phosphate, sc, 1.0 mg per 10 g body weight, one hour prior to assay respectively.

Stalk-median eminence (SME) tissue was obtained from male rats decapitated immediately on removal from their cages. Fragments weighed approximately 4.0 mg and were homogenized in 0.1 N HCl to a concentration of 10 mg per ml. The homogenates were centrifuged at 2000 × g at 5°C for 60 min and the supernatant was removed and frozen for assay at a later date.

Test solutions were given in the external jugular vein of mice in a volume of 0.3 ml. The two adrenals were removed six minutes later, trimmed and ground in all glass grinding tubes in 4.5 ml of ethanol in saline (20 per cent, v/v). 4.0 ml of the homogenate was used in the determination of corticosterone by a modification of the fluorimetric procedure described by Guillemin et al. (1959).

ACTH assays of pituitary extracts from rats were performed by the method of Guillemin et al. (1958).

Lysine vasopressin was diluted in 0.02 N HCl in saline and injected intravenously (iv) in 50 mU and 10 mU dose levels per 0.3 ml.

Statistical analyses were performed according to methods outlined by Snedecor (1956). All of the values are presented as means and the standard errors of the means. Tests for significant differences using the Student’s t test were applied.

RESULTS

The concentration of corticosterone in the adrenal glands in mice subjected to ether anaesthesia and laparotomy for a period of five minutes rose significantly (Fig. 1) when compared to that seen in mice decapitated after a minimum of disturbance. The values of corticosterone in mice anaesthetized with pentobar-
Adrenal corticosterone in mice: effect of stress. 1. Numbers in brackets refer to number of mice. 2. Vertical lines indicate standard errors of the mean. 3. * P < 0.001 compared to value in intact group. 4. ** P < 0.01 compared to value in intact group.

bitone and subjected to laparotomy for five minutes prior to removing the glands were less than that in the ether-anaesthetized group but greater than that in the intact group.

The concentration of corticosterone in adrenals from mice treated with dexamethasone are shown in Table 1. The levels were reduced compared to those in mice anaesthetized with pentobarbitone when the dexamethasone was given 20 hours and again 4 hours before the adrenals were removed. Despite the depression of adrenal corticosterone following treatment with dexamethasone, no increase was seen following intravenous administration of 0.5 hypothalamic equivalent in this system. ACTH, 0.5 mU, given iv, produced a significant elevation in adrenal corticosterone in the same system (Group 4, Table 1). The concentration of adrenal corticosterone following pretreatment with dexamethasone five hours before the assay was greater than that seen in mice treated 20 and 4 hours, but again, the administration of SME extract, 0.25 equivalent was without effect in this system.

The concentration of adrenal corticosterone in mice treated with chlorpromazine and morphine and with chlorpromazine and codeine are shown in Table 2. There is a significant reduction in the morphine and the codeine groups when compared to pentobarbitone controls, respectively, (see Table 1,
### Table 1.
Corticosterone in mouse adrenal glands: Dexamethasone (Dex) treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adrenal corticosterone $\mu g/g$</th>
<th>$P$ value</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pentobarbitone -- anaesthetized controls</td>
<td>23.7 ± 1.1 (M ± SE)</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>2. Dex in gelatin, 200 $\mu g$ sc, 20 h prior to assay (PTA and 200 $\mu g$ ip (4 h PTA, aqueous)</td>
<td>12.5 ± 2.5</td>
<td>$&lt; 0.05^*$</td>
<td>6</td>
</tr>
<tr>
<td>3. Dex as in Group 2. 0.5 stalk-median eminence</td>
<td>17.5 ± 2.2</td>
<td>NS**</td>
<td>6</td>
</tr>
<tr>
<td>4. Dex as in Group 2. ACTH, 0.5 mU</td>
<td>22.3 ± 2.9</td>
<td>$&lt; 0.02^{**}$</td>
<td>5</td>
</tr>
<tr>
<td>5. Dex, 250 $\mu g$ in gelatin, 5 h PTA</td>
<td>23.8 ± 1.6</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>6. Dex, 250 $\mu g$ in gelatin, 5 h PTA, 0.25 stalk-median eminence</td>
<td>22.9 ± 1.7</td>
<td>NS***</td>
<td>6</td>
</tr>
</tbody>
</table>

$^*$ Compared to Group 1.

$^{**}$ Compared to Group 2.

$^{***}$ Compared to Group 5.

$P < 0.05$ in both cases). In the group treated with chlorpromazine and morphine, a two-fold rise in adrenal corticosterone was seen after the administration of 0.25 SME equivalent. The rise in adrenal corticosterone was highly significant when 0.4 of a SME was given in the codeine group and a yet greater concentration was obtained with the administration of 0.8 of one SME.

The results herein describe the effects of injecting extracts of SME which were weighed prior to pooling and freezing. At least 4 rats contributed to each pool.

The rise in adrenal corticosterone when extracts of 3.5 mg of SME tissue were given to mice treated with chlorpromazine and codeine was again significant as shown in Fig. 2. Furthermore, extracts of cerebral cortex at a concentration of 3.5 mg per ml did not raise the concentration of adrenal corticosterone in mice treated in this manner. Fig. 2 shows as well, that SME tissue collected from rats hypophysectomized 10 days previously was almost as potent as the equivalent weight of tissue from intact rats injected in chlorpromazine and codeine-treated mice.

To test further the effects of giving chlorpromazine and codeine and dexamethasone to mice in the assay for CRF, the ACTH in pituitary and plasma from the two groups of mice was compared. Within 1.5 to 2.0 min after laparotomy, blood was collected from the abdominal aorta from groups of 10 mice and pooled, and the anterior pituitary lobes were pooled and ground in 0.1 n
Table 2.
Corticosterone in mouse adrenal glands: Chlorpromazine-morphine and Chlorpromazine-codeine pretreatment

<table>
<thead>
<tr>
<th></th>
<th>Adrenal corticosterone ( \mu g/g )</th>
<th>( P ) value</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chlorpromazine and morphine</td>
<td>15.9 ± 1.8 (M ± se)</td>
<td>0.01*</td>
<td>10</td>
</tr>
<tr>
<td>2. Chlorpromazine and morphine</td>
<td>39.0 ± 4.8</td>
<td>0.001**</td>
<td>5</td>
</tr>
<tr>
<td>0.25 stalk-median eminence extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Chlorpromazine and codeine</td>
<td>10.5 ± 1.5</td>
<td>NS</td>
<td>7</td>
</tr>
<tr>
<td>4. Chlorpromazine and codeine</td>
<td>21.3 ± 1.1</td>
<td>0.001**</td>
<td>6</td>
</tr>
<tr>
<td>0.40 stalk-median eminence extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 0.80 stalk-median eminence extract</td>
<td>27.2 ± 2.8</td>
<td>NS</td>
<td>7</td>
</tr>
</tbody>
</table>

* Compared to Group 1.

** Compared to Group 3.

Adrenal corticosterone in mice pretreated with chlorpromazine and codeine: Effect of extracts of SME from intact hypophysectomized rats and of extract of cerebral cortex.

1. Numbers in brackets indicate number of assays. 2. \( P \) values are tests of significance against saline controls. 3. Weights under bars refer to total mass of tissue injected in each assay mouse. 4. Vertical bars represent standard errors of mean.
HCl. The results are shown in Table 3 where it is clear that the treatment with dexamethasone is associated with a depression of ACTH both in the pituitary and in plasma, at least when compared to the levels seen in mice treated with chlorpromazine and codeine.

Fig. 3 depicts a linear semi-logarithmic relationship between increasing concentrations of SME tissue and the changes of adrenal corticosterone in mice treated with chlorpromazine and codeine. The changes of adrenal corticosterone induced with extracts of SME tissue starting at 3.0 mg is expressed as a percent of control.

Lysine vasopressin was given to mice treated with chlorpromazine and codeine to study the responsiveness of this system to known hypothalamic hormones. There was no significant increase in adrenal corticosterone following injection with both 50 mU and 10 mU of lysine vasopressin (Table 4).

**DISCUSSION**

The use of the mouse in a hormonal assay has been described by *Rerup & Hedner (1963)* for the measurement of ACTH. In this study mice were treated with dexamethasone to suppress endogenous ACTH secretion and a sensitive and specific assay was described in which the concentration of corticosterone in pooled plasma was taken as the measure of injected ACTH. Suppression of ACTH secretion was achieved by giving two injections of dexamethasone, 20 hours apart, beginning approximately 24 hours prior to assay. Similar results were observed in the present work in that two doses of the steroid given over a 20 hour period was associated with levels of corticosterone in the adrenals which were closer to that seen in the intact mice, in contrast to the use of one dose of dexamethasone.

**Table 3.**

<table>
<thead>
<tr>
<th></th>
<th>ACTH in pituitary</th>
<th>ACTH in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>42.4 ± 12.5 mU/mg</td>
<td>6.3 mU/ml</td>
</tr>
<tr>
<td>Group 2</td>
<td>29.9 ± 4.3 mU/mg(5)</td>
<td>3.3 mU/ml</td>
</tr>
</tbody>
</table>

* Dexamethasone given as in Group 2, Table 1.
Fig. 3.
Corticosterone in adrenal: Pretreatment with chlorpromazine and codeine; effect of increasing concentrations of SME extract.

In the mouse treated with dexamethasone, however, there was no response to fractions of extracts of SME, equivalent to 0.5 of one SME (Table 1). This suggests that either the adrenals were insensitive to the ACTH released after stimulation with the SME extract; or, that the ACTH synthesizing and releasing mechanisms of the pituitary were unresponsive to the injected SME extracts. To test these possibilities, ACTH, 0.5 mU was given to mice treated with

Table 4.
The effect of lysine vasopressin (LVP) on the adrenal corticosterone in mice treated with chlorpromazine and codeine.

<table>
<thead>
<tr>
<th></th>
<th>Adrenal corticosterone μg/g</th>
<th>P value</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer-injected mice</td>
<td>5.4 ± 2.2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>(M ± se)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVP, 50 mU</td>
<td>6.1 ± 1.2</td>
<td>NS</td>
<td>6</td>
</tr>
<tr>
<td>LVP, 10 mU</td>
<td>4.5 ± 0.7</td>
<td>NS</td>
<td>5</td>
</tr>
</tbody>
</table>
dexamethasone. The results indicated that the adrenals in the dexamethasone-treated mice were sensitive to ACTH at this dose level and they suggest that this treatment may have resulted in almost complete suppression of ACTH-synthesizing and releasing properties of the pituitary. This confirms recent work pointing to the anterior pituitary as the site of negative feedback for corticosteroids (Russell et al. 1969). Furthermore, the results of the assay of ACTH in dexamethasone and in chlorpromazine and codeine treated mice, respectively, in which the concentrations of this hormone was found to be less with the steroid treatment, enforces further the idea that the dexamethasone is acting at the level of the ACTH synthesizing and releasing cells of the anterior pituitary.

The response of the mouse treated with chlorpromazine and morphine after the administration of extracts of SME is in keeping with reports of the effects of these drugs in other species, especially in the rat (Briggs & Munson 1955; De Wied 1967). As noted by these writers, morphine, pentobarbitone and chlorpromazine, used together restrain the secretion of ACTH in response to stress in the rat. With codeine replacing morphine in the mouse, levels of corticosterone in the adrenal were found to be less than that seen in mice anaesthetized with pentobarbitone; a logarithmic-dose response relationship was observed with extracts of SME tissue from 2.0 mg to 6.0 mg of crude, rat hypothalamus. The specificity of this assay as a measure of the ACTH-releasing potency of extracts of the SME is reflected partly in the failure of the system to respond to extracts of cerebral cortex and to pharmacological doses of lysine vasopressin.

The indirect nature of the technique as an assay for CRF need not detract from its advantages as convenient and specific system for measuring changes of CRF in extracts of hypothalamus. The sensitivity of the assay compares favourably with that described for other techniques except the intra-pituitary micro injection method (Dhariwal et al. 1969) and the in vivo – in vitro assay system of Chan et al. (1969).

ACKNOWLEDGMENTS

This work was supported by The Medical Research Council of Canada and a grant from The Ontario Mental Health Foundation.

Anthony Jenkins and John Hickman provided splendid technical assistance. I am grateful to Dr. B. T. Donovan for advice and criticism throughout the performance of this work.

REFERENCES


Received on May 21st, 1971.