COMPARISON OF EFFECTS OF
6-DEHYDRO-16-METHYLENE-HYDROCORTISONE (STC 407)
AND DEXAMETHASONE ON THE SUPPRESSION OF THE
HYPOTHALAMO-PITUITARY-ADRENAL SYSTEM

By
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ABSTRACT

The suppressive effects of 6-dehydro-16-methylene-hydrocortisone (STC 407) and dexamethasone on the hypothalamo-pituitary-adrenal system were evaluated in the rat. Four µg dexamethasone or 120 µg STC 407/100 g b.w. was injected ip daily into normal rats for 16 or 31 days. These doses were of approximately equivalent antiinflammatory potencies. Both steroids decreased equally plasma and adrenal corticosterone concentrations as well as adrenal weights. However, CRF activities of the hypothalamus and ACTH contents of the pituitary glands were significantly reduced by treatment with STC 407, but not with dexamethasone. This finding may endorse the successful treatment with STC 407 of Cushing's syndrome due to adrenal hyperplasia in which the fundamental derangement appears to consist of a disturbed regulatory function of the hypothalamus.

The fundamental derangement in Cushing's syndrome with bilateral adrenal hyperplasia is thought to consist of a disturbed regulatory function of the hypothalamus (Tamm 1961), in that there is a decreased sensitivity to the inhibitory action of adrenocortical hormones and of ACTH. It is not surprising therefore, that it has not been possible to treat Cushing's syndrome effectively...
with the usual corticosteroids given in doses associated with tolerable side effects. Indeed, Jailer et al. (1953) showed that administration of cortisone to patients with Cushing's syndrome reduced 17-ketosteroid excretion but without achieving a reduction in cortisol levels. The attempt by Cope (1956) to treat Cushing's syndrome with 9α-fluorohydrocortisone was no more successful. Christy et al. (1956) using prednisone, were unable to achieve any lasting depression of elevated cortisol production. Similar results were reported by Mattingly (1964) with dexamethasone, which had been used to diagnostic advantage by Liddle (1960) in the well-known dexamethasone suppression test. Although in early therapy with some of the above steroids there was sometimes a suppression of ACTH production, this was followed after a few days by an escape of the hypophysis from the suppressive effect. It also seemed that any dosage level of the steroids capable of producing a lasting depression of ACTH release would necessarily be accompanied by excessive side effects.

Rausch-Stroomann (1967) and Rausch-Stroomann et al. (1968) first reported the successful treatment of Cushing's syndrome due to adrenal hyperplasia with 6-dehydro-16-methylene-hydrocortisone (STC 407) made by E. Merck A.G., Darmstadt, Germany. The favourable results achieved with STC 407, which has only \( \frac{1}{4} \) of the antiphlogistic action of dexamethasone, were not accompanied by undesirable side effects even after long-term administration.

In view of the therapeutic effectiveness of STC 407 in Cushing's syndrome, we decided to investigate the mechanism of this steroid. As we have recently shown in studies carried out in normal and adrenalectomized rats, one of the sites of action of STC 407 seems to be the hypothalamus, as manifested by a reduction in the corticotrophin-releasing-factor (CRF) content of the stalk median eminence (SME) of the hypothalamus (Berthold et al. 1969). If one assumes that this mechanism of action also pertains to man, the treatment of Cushing's syndrome with STC 407 would seem to meet the requirements of Tamm (1961) that for optimal therapy of Cushing's syndrome due to adrenal hyperplasia the drug should act primarily on the hypothalamus. Dexamethasone is the most effective steroid known for suppressing the hypothalamus-pituitary-adrenal function, but it cannot be used for long because of its undesirable side effects.

The present study was undertaken to compare the suppressive effects of dexamethasone and STC 407 which were given in a comparable antiinflammatory dose.

**MATERIALS AND METHODS**

Dexamethasone (9α-fluoro-16α-methyl-prednisolone) (Merck & Co. Inc.) as well as STC 407 (6-dehydro-16-methylene-hydrocortisone) obtained from E. Merck A. G.,
Darmstadt, Germany, were suspended for injection in Vehicle 100, Upjohn®. The concentration of dexamethasone was 0.02 mg/ml and the concentration of STC 407 0.6 mg/ml. The anti-inflammatory potency of 0.6 mg STC 407 is equivalent to or slightly less than that of 0.02 mg dexamethasone (Rausch-Stroemann et al. 1968).

**Animals:** Normal male rats of the Sprague-Dawley strain, obtained from Check-Jones, Texas, with an average body weight of 260 g were divided into 3 groups of 4 rats each. The first group received vehicle only; the second and third group were injected ip with 4 μg dexamethasone or 120 μg STC 407/100 g b.w/day respectively for 16 days in experiment 1 and for 31 days in experiment 2. Six hours after the last injection, the rats were decapitated when under anaesthesia with 2.5 mg Nembutal/100 g b.w. Blood was collected from the trunk and pooled for each group in heparinized tubes and the plasma separated by centrifugation. The left adrenal was dissected out, cleaned from adhering tissues, weighed, homogenized and extracted with 5 ml ethanol/saline solution (20/80). Both plasma and adrenal homogenates were stored at -25°C until used for corticosterone determinations. The anterior pituitary glands were removed, weighed on a torsion balance, pooled for each group and kept frozen at -25°C until their ACTH content was determined. Similarly, the ventral region of the hypothalamus extending from the posterior margin of the optic chiasma to the anterior boundary of the mammillary body was dissected out in a thickness of 2 mm. This region included the pituitary stalk and the median eminence (SME). The SME tissues were also stored at -25°C until used for CRF assays.

**Assay of pituitary ACTH content.** Assay rats, male, 130-160 g, were hypophysectomized transauricularly 4 h before an injection of the pituitary extracts. The extracts were prepared about 1 h before injection as follows: after thawing, the anterior pituitary glands were homogenized and then extracted with 0.9% saline solution containing 0.2% gelatine and 10% 0.1 N HCl. After centrifugation at 10 000 rpm at 4°C, the supernatant was diluted so that 0.2 ml contained 0.1 mg pituitary tissue. ACTH standard (ACTHAR, Armour) was given in doses of 0.2 and 0.4 mU/100 g b.w. Fifteen minutes after iv injection, the rats were decapitated; the blood was collected from the trunk into heparinized tubes, the plasma was separated and stored at -25°C until used for corticosterone determination.

**Assay of hypothalamic CRF activity content.** One hour before the assay the SME tissues were thawed, homogenized and extracted with 0.1 N acetic acid. After spinning at 4°C at 10 000 rpm, the supernatant was separated, diluted to an appropriate concentration and injected iv into assay rats. The assay was performed as described by Arimura et al. (1967) using the chlorpromazine-morphine-Nembutal-treated rats. The SME extracts were given to the assay rats at 2 dose levels. Fifteen minutes after iv injection of the SME extracts the rats were sacrificed, the blood was collected as above and the plasma stored at -25°C for corticosterone determination which was used as an index of CRF activity.

**Corticosterone** was determined fluorometrically (Guillemin et al. 1958) in 0.5 ml of plasma or in 4 ml of the adrenal extracts and its concentration was used as the index of adrenocortical stimulation.

**Statistical methods.** Duncan’s multiple range test was employed for comparison of the mean values of the adrenal weights, pituitary weights and body weights as well as of the corticosterone levels in plasma or in adrenal extracts (Steel & Torrie 1960).

9 1 ml contain: carboxymethylcellulose 5 mg, polysorbate 80.4 mg, sodium chloride 9 mg, benzyl alcohol NF 9 mg.
The ACTH concentration in the anterior pituitary lobes was calculated as a relative potency (Bliss 1952) from the standard values and the 95% confidence limits were determined according to Finney (1964). The CRF potencies of the experimental group were expressed as the percentage fraction of those of the controls and their 95% confidence limits were estimated by 2-dose factorial analysis according to Bliss (1952).

RESULTS

As shown in Table 1 plasma corticosterone concentration was clearly decreased after the treatment with either dexamethasone or STC 407 for 16 or 31 days. The adrenal weight also fell after administration of STC 407 for 16 days, but not after dexamethasone treatment for the same time period. After 31 days treatment, both dexamethasone and STC 407 were effective in reducing the adrenal weight significantly. At that time the adrenal weights of rats treated with STC 407 were significantly less than those of the dexamethasone-treated group.

Corticosterone concentration in the adrenal glands were reduced in both experimental groups either after 16 or 31 days of the treatment. The magnitude of reduction in adrenal corticosterone was similar for STC 407 and dexamethasone-treated groups. However, in both steroid-treated groups, the adrenal corticosterone concentration seemed to be greater after 31 days-treatment than after 16 days-treatment. The difference may be partly explained by the difference in the adrenal weights after different treatment periods.

On the other hand, the pituitary weights were significantly greater in the rats treated with these steroids for either 16 or 31 days. But again this difference could be accounted for at least partly by the decrease in body weight after treatment with these steroids. Actual pituitary weights in the control and the steroid-treated rats did not differ significantly from each other.

Fig. 1 illustrates the change in body weight of the rats during the treatment with vehicle, dexamethasone or STC 407. The rats treated with vehicle showed on the 16th and 31th day of the treatment increases in body weight of 29 and 42% respectively above the pretreatment weight. The increases in body weight in the dexamethasone-treated rats at the corresponding times were 10 % and 11 % respectively, and those in STC 407-treated animals were -4.7 % and 15 % respectively.

The ACTH concentration in the anterior pituitary gland and CRF activity of the hypothalamus after 31 days-treatment with the vehicle, dexamethasone or STC 407 are shown in Tables 2 and 3 respectively. Dexamethasone administration seemed to reduce both pituitary ACTH and hypothalamic CRF activities as compared to those of control rats. However, the reduction was not statistically significant. On the other hand, STC 407-treatment significantly reduced both ACTH and CRF activities.

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### Table 1.
Effects of treatment of normal rats with dexamethasone or STC 407.

#### Exp. 1: treatment for 16 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Corticosterone in plasma* µg/100 ml</th>
<th>Left adrenal mg/100 g b. w.</th>
<th>Duncan’s m. r. t. 5% level</th>
<th>Corticosterone in adrenal µg/100 mg</th>
<th>Duncan’s m. r. t. 5% level</th>
<th>Anterior pit. mg/100 g b. w.</th>
<th>Duncan’s m. r. t. 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vehicle</td>
<td>28.5</td>
<td>6.47 ± 0.291**</td>
<td>–</td>
<td>1.72 ± 0.351</td>
<td>–</td>
<td>2.36 ± 0.079</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>4 µg dexamethasone /100 g b. w./d</td>
<td>5.5</td>
<td>5.66 ± 0.458</td>
<td>vs. 1 NS</td>
<td>0.59 ± 0.066</td>
<td>vs. 1 S</td>
<td>2.67 ± 0.045</td>
<td>vs. 1 S</td>
</tr>
<tr>
<td>3</td>
<td>120 µg STC 407 /100 g b. w./d</td>
<td>5.0</td>
<td>4.54 ± 0.438</td>
<td>vs. 1 S vs. 2 NS</td>
<td>0.88 ± 0.114</td>
<td>vs. 1 S vs. 2 NS</td>
<td>2.81 ± 0.112</td>
<td>vs. 1 S vs. 2 NS</td>
</tr>
</tbody>
</table>

#### Exp. 2: treatment for 31 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Corticosterone in plasma* µg/100 ml</th>
<th>Left adrenal mg/100 g b. w.</th>
<th>Duncan’s m. r. t. 5% level</th>
<th>Corticosterone in adrenal µg/100 mg</th>
<th>Duncan’s m. r. t. 5% level</th>
<th>Anterior pit. mg/100 g b. w.</th>
<th>Duncan’s m. r. t. 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vehicle</td>
<td>30.0</td>
<td>5.85 ± 0.453**</td>
<td>–</td>
<td>2.32 ± 0.177</td>
<td>–</td>
<td>2.13 ± 0.092</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>4 µg dexamethasone /100 g b. w./d</td>
<td>9.0</td>
<td>4.72 ± 0.033</td>
<td>vs. 1 S</td>
<td>1.42 ± 0.092</td>
<td>vs. 1 S</td>
<td>2.64 ± 0.126</td>
<td>vs. 1 S</td>
</tr>
<tr>
<td>3</td>
<td>120 µg STC 407 /100 g b. w./d</td>
<td>13.0</td>
<td>3.77 ± 0.209</td>
<td>vs. 1 S vs. 2 S</td>
<td>1.42 ± 0.303</td>
<td>vs. 1 S vs. 2 NS</td>
<td>2.79 ± 0.080</td>
<td>vs. 1 S vs. 2 NS</td>
</tr>
</tbody>
</table>

* plasma pooled for 4 rats each group.
** standard error of the mean.
Fig. 1.
Changes in body weight of normal rats during 16 and 31 days treatment
with dexamethasone or STC 407.
vehicle: ---; dexamethasone 4 µg/100 g b. w./day: -----
STC 407 120 µg/100 g b. w./day: --.--.
* 4 rats from each group were sacrificed.

Table 2 (Exp. 2).
ACTH concentration in the anterior pituitary glands of normal rats after 31 days
treatment with dexamethasone or STC 407.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Corticosterone in plasma of assay rats* µg/100 ml</th>
<th>Duncan's multiple range test for 1% level</th>
<th>ACTH mU/mg pit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vehicle</td>
<td>8.4 ± 2.18**</td>
<td>–</td>
<td>2.24 (1.70–3.85)**</td>
</tr>
<tr>
<td>2</td>
<td>4 µg dexamethasone /100 g b. w./d</td>
<td>27.3 ± 0.43</td>
<td>vs. 1 NS</td>
<td>2.06 (1.49–2.97)</td>
</tr>
<tr>
<td>3</td>
<td>120 µg STC 407 /100 g b. w./d</td>
<td>19.0 ± 1.58</td>
<td>vs. 1 S</td>
<td>1.10 (0.85–1.64)</td>
</tr>
</tbody>
</table>

* male, Sprague-Dawley, 145 g b. w., transauricularly hypophysectomized 4 h before iv injection of extract of 0.1 mg pit./0.2 ml/100 g b. w.
** standard error of the mean.
*** 95% confidence limits.
Table 3 (Exp. 2).
CRF content in SME from normal rats after treatment with dexamethasone or STC 407 for 31 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SME /100 g b. w. of assay rats</th>
<th>Corticosterone in plasma of assay rats µg/100 ml</th>
<th>P**</th>
<th>Relative potency vs. gr 1 in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>vehicle</td>
<td>0.24</td>
<td>13.5 ± 0.35*</td>
<td>.</td>
<td>100.0</td>
</tr>
<tr>
<td>1b</td>
<td>4 µg</td>
<td>0.48</td>
<td>33.3 ± 2.86</td>
<td></td>
<td>(97.6–105.7)*</td>
</tr>
<tr>
<td>2a</td>
<td>dexamethasone</td>
<td>0.24</td>
<td>13.1 ± 1.70</td>
<td></td>
<td>97.6</td>
</tr>
<tr>
<td>2b</td>
<td>/100 g b. w./d</td>
<td>0.48</td>
<td>32.3 ± 1.11</td>
<td>NS</td>
<td>(91.4–104.2)</td>
</tr>
<tr>
<td>3a</td>
<td>120 µg STC 407</td>
<td>0.24</td>
<td>10.6 ± 0.99</td>
<td>0.01</td>
<td>79.5</td>
</tr>
<tr>
<td>3b</td>
<td>/100 g b. w./d</td>
<td>0.48</td>
<td>24.9 ± 1.01</td>
<td></td>
<td>(72.7–86.8)</td>
</tr>
</tbody>
</table>

* standard error of the mean.
** P value for difference from control value based on F-test in connection with 2-dose-factorial-analysis.
*** 95% confidence limits.

DISCUSSION

The present investigation were done to see if there are either quantitative or qualitative differences between STC 407 and dexamethasone in suppressing the activity of the hypothalamo-pituitary-adrenal system in normal rats.

The doses of STC 407 and dexamethasone used in these experiments were 120 µg and 4 µg/100 g b. w./day respectively, the ratio being 30/1. Since the ratio of antiinflammatory potencies of STC 407/dexamethasone is 1/10 (Rausch-Stroomann et al. 1968), the antiinflammatory effect of 120 µg STC 407 is slightly smaller than that of 4 µg dexamethasone.

It was found that both steroids were about equally effective in decreasing plasma corticosterone level, adrenal corticosterone concentration and adrenal weight, which may indicate that ACTH secretion by the pituitary gland was suppressed to the same extent by dexamethasone and STC 407 in similar anti-phlogistic doses.

Plasma corticosterone levels and adrenal corticosterone concentrations after the steroid treatment for 31 days appeared to be slightly greater than those after 16 days-treatment. However, it is difficult to be sure that such a small difference is significant and meaningful. Since plasma corticosterone was determined in the plasma samples pooled in groups, a standard statistical analysis...
could not be used to compare these values. Corticosterone concentration in the adrenal gland of the control rats treated with vehicle for 31 days also appeared to be greater than that after the treatment for 16 days. Therefore, the greater corticosterone concentration in the adrenal after 31 days-treatment with either dexamethasone or STC 407 may not be simply accounted for by an escape phenomena from the steroid suppression during the prolonged treatment. However, the possibility of escape phenomena cannot be absolutely excluded. It has been reported that in patients with Cushing's syndrome due to adrenal hyperplasia the treatment with STC 407 could be continued successfully for as long as 13 months (Rausch-Stroomann et al. 1968).

On the other hand, the suppressive action by STC 407 on the hypothalamus and the pituitary gland, as indicated by a considerable reduction in CRF activities of SME and ACTH concentration in the pituitary gland, was significantly greater than that by dexamethasone. Although the pathogenesis of Cushing's disease is not fully understood, it seems certain that derangement in hypothalamic function is one of its causes. Thus STC 407 appears to be a promising drug for the treatment of Cushing's syndrome due to adrenal hyperplasia. It may also be used for the treatment of the adrenogenital syndrome in which reduction of cortisol production by one of several enzymatic defects enhances ACTH secretion through the hypothalamus.

REFERENCES


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