EFFECT OF GLYCYRRHIZIN ON THE SUPPRESSIVE ACTION OF CORTISONE ON THE PITUITARY ADRENAL AXIS

AKIRA KUMAGAI, YOSHITSUGU ASANUMA, SABURO YANO, KAZUYUKI TAKEUCHI, YASUHIKO MORIMOTO, TAIZO UEMURA AND YUICHI YAMAMURA

Third Department of Internal Medicine and Central Laboratory, Medical School of Osaka University, Osaka

SYNOPSIS

The effects of glycyrrhizin on the pituitary adrenal axis were studied. Isolated rat anterior pituitary glands were incubated with $^{14}$C-phenylalanine and biosynthetic activity of corticotropin in the pituitary was estimated. The rate of incorporation of radioactivity from $^{14}$C-phenylalanine into corticotropin fraction in the rat anterior pituitary was increased specifically by prior adrenalectomy. Cortisone treatment suppressed the increased $^{14}$C-incorporation into the corticotropin fraction induced by prior adrenalectomy. However concomitant injections of glycyrrhizin blocked the inhibitory action of cortisone on $^{14}$C-incorporation into the corticotropin fraction. Glycyrrhizin was also found to be antagonistic to the suppressive action of cortisone on compensatory adrenal hypertrophy in rats after unilateral adrenalectomy. Under condition of stress, glycyrrhizin was found to increase the inhibitory action of cortisone on adrenal ascorbic acid depletion. The possibility of distinguishing the secretory mechanism of corticotropin in the basal stage from that in the condition of stress and the mechanism of the inhibition of glycyrrhizin on the action of cortisone are discussed.

Glycyrrhizin has various biological activities, one of which is a DOC- and corticoid-like action. We have suggested that this might be due to inhibition of the metabolic degradation of steroid hormones in the liver by glycyrrhizin (Kumagai et al., 1957). It has also been claimed that glycyrrhizin improves the condition of patients with gastric ulcer (Doll et al., 1962), reduces hypercholesterolemia (Shibata, 1961) and is effective in ameliorating allergic symptoms and intoxication. The authors also reported an inhibitory effect of glycyrrhizin on the antigranulomatous action of cortisone; it was found to have no effect on the anti-inflammatory action of glucocorticoids (Kumagai et al., 1964). The mechanism of these various actions of glycyrrhizin are still not completely elucidated. In the present study, the effect of glycyrrhizin on the pituitary adrenal axis was studied with the aim of clarifying the mechanism of various actions of glycyrrhizin.

Received for publication May 16, 1966.
MATERIALS AND METHODS

Approximately 800 male Wistar rats weighing 200~250 g, unless otherwise indicated, were housed in a constant temperature room and maintained on a commercial laboratory chow diet (Oriental Kobo Co.) with tap water ad libitum for at least 1 week before use. Adrenalectomy was performed under hexobarbital anesthesia, using the dorsal approach, and 1% saline was given in place of drinking water after adrenalectomy. Daily subcutaneous injections of test substances were started on the day of adrenalectomy and continued for the following 7 days. The test substances used throughout the experiments were given in doses per 100 g body weight unless otherwise indicated. Rats were sacrificed by decapitation 20 hrs. after the last injections of cortisol acetate (Schering Co.), prednisolone acetate (Shionogi Co., Japan) and glycyrrhizin (Minophagen Co., Japan) alone or in combination. The anterior pituitary was rapidly removed intact and biosynthetic activity of corticotropin in the isolated rat anterior pituitary was estimated by the method of Wool et al. (1961).

The outline of the experimental procedures was as follows: Three anterior pituitary glands were incubated in 2 ml of medium\(^1\) containing 2\(\mu\)C of L-phenylalanine-U\(^{14}\)C (The Radiochemical Center, Amersham, England; specific activity 9.9 mc/m mole). Incubation was performed at 37°C with continuous shaking in a 95% \(O_2\)-5% \(CO_2\) atmosphere for 3 hrs. After addition of carrier anterior pituitary powder\(^2\), corticotropin was extracted with hot glacial acetic acid and purified with oxycellulose (Tennessee Eastman Co., 11~12% COOH). The eluate from the oxycellulose was lyophilized and radioactivity determined using a Nuclear Chicago gas-flow counter. The results are expressed as counts per minute per 100 mg of anterior pituitary (wet wt.).

The biosynthesis of protein in the isolated anterior pituitary was also determined by the method of Wool et al. (1961). Five pituitaries were incubated in 2 ml of medium containing 1\(\mu\)C of DL-phenylalanine-2-\(^{14}\)C (Daiichi Pure Chemical Co., Japan; specific activity 2.4 mc/m mole). The incubation time was 2 hrs. in this experiment. After incubation, the radioactivity in the trichloroacetic acid precipitate of the pituitary was measured with a Nuclear Chicago gas-flow counter. Specific activity was expressed as counts per minute per mg of protein after correction for self absorption.

The radioactive substance isolated by the oxycellulose procedure was separated by horizontal starch block electrophoresis. Sixty intact anterior pituitaries of rats which had been adrenalectomized 7 days previously were incubated with L-phenylalanine-U\(^{14}\)C and corticotropin was extracted and purified by the procedure described above. Extracted corticotropin fraction was applied to horizontal starch block electrophoresis. The conditions used for electrophoresis were as follows: the test substance was applied to the middle portion of a starch block, 3×40 cm, and subjected to electrophoresis at 600 V and 3 mA using sodium veronal buffer, pH 8.6 of 0.05 ionic strength. After 13 hrs. electrophoresis the starch block was eluted with 0.1 N HCl. On the eluate, the following estimations were performed; protein content by Lowry reaction (Lowry et al., 1951), radioactivity and bioassay of corticotropin. Bioassay of corticotropin was carried out by the adrenal ascorbic acid depletion method using steroid blocked rats, following the modified method of Hedner and Rerup (1962). In our previous experiments (Yamamura et al., 1964) it was found that a linear dose relationship existed between adrenal ascorbic acid depletion and the dose of corticotropin on a logarithmic scale in the range from 1.6 mU to 12.5 mU.

The effect of glycyrrhizin on the suppressive action of cortisol on compensatory adrenal hypertrophy after unilateral adrenalectomy was observed using male Wister rats, weighing 130~160 g. Under sodium hexobarbital anesthesia, the right adrenal was extirpated via the dorsal approach and weighed as accurately as possible after careful removal of adherent fat tissue.

\(^1\) The medium used was Krebs-Ringer bicarbonate buffer containing 200 mg% glucose.
\(^2\) Acetone-dried powder from pig anterior pituitary (Armour Co.) was used as carrier corticotropin.
Animals received daily administration of cortisone and glycyrrhizin alone or in combination subcutaneously. After 10 days treatment, the animals were sacrificed by decapitation. The left adrenal was excised and weighed.

The percent increment in the weight of the left adrenal after removal of the right adrenal was calculated as follows:

\[
\text{percent increment} = \left( \frac{\text{weight of left adrenal} - \text{weight of right adrenal}}{\text{weight of right adrenal}} \right) \times 100
\]

Rats weighing 130–160 g received 10 mg of glycyrrhizin subcutaneously once a day for 3 days. On the third day 2.5 mg of cortisone acetate was simultaneously injected. Four hrs. after the last injection, 0.5 mg of histamine chloride was injected intraperitoneally. One hour later, the animals were sacrificed by decapitation. Adrenal ascorbic acid depletion was determined by comparing ascorbic acid contents of the left adrenal of experimental animals with those of control animals. The ascorbic acid content of the adrenal was determined by the method of Roe and Kuether (1943).

RESULTS

2. Effects of adrenalectomy and prednisolone administration on incorporation of $^{14}$C-phenylalanine into corticotropin and protein

In agreement with the result of Wool et al. (1961), it was found that the incorporation of L-phenylalanine-$U-^{14}$C into the corticotropin fraction ($^{14}$C-incorporation in CFr) by isolated anterior pituitaries from rats adrenalectomized 1 week previously resulted in a 1.6-2.3 fold increase, as compared with that by pituitaries from normal rats (Table 1). However, the incorporation of DL-phenylalanine-2-$^{14}$C into total protein by the isolated anterior pituitary was not affected by prior adrenalectomy (Table 2). After daily administration of 5 mg per rat of prednisolone for 7 days, it was found that the magnitude of $^{14}$C-incorporation in CFr by isolated pituitaries was decreased to three fifths of the control level by prior treatment with prednisolone (Table 1).

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Exp. Group</th>
<th>Radioactivity Incorporated into Corticotropin Fraction Counts/Min./100 Mg Anterior Pituitary</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Normal (Saline only)</td>
<td>403±64* (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adrenalectomized (Saline)</td>
<td>643±76 (5)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Prednisolone Treated</td>
<td>233±33 (5)</td>
<td>0.05</td>
</tr>
<tr>
<td>2.</td>
<td>Normal Adrenalectomized</td>
<td>412±24 (11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>939±111 (14)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Standard error of the mean. Number of observations in parenthesis.
† Compared with normal value.
Table 2. Effect of adrenalectomy on incorporation of radioactivity from D,L-phenylalanine-2-14C into protein of isolated rat anterior pituitary gland

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>Radioactivity Incorporated into Protein Counts/Min./Mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1790±224* (5)</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>1834±184 (5)</td>
</tr>
</tbody>
</table>

* Standard error of the mean. Number of observations in parenthesis.
The difference is not significant.

2. Effect of cortisol administration on increased incorporation of 14C-phenylalanine into corticotropin fraction induced by adrenalectomy

Cortisone was injected into adrenalectomized rats at doses of 0.1 mg, 0.5 mg, 1 mg, and 5 mg. The increase of 14C-incorporation in CFr induced by prior adrenalectomy was not affected by daily administration of cortisol up to a dose of 0.5 mg, but when the dose was elevated to 1 mg, 14C-incorporation in CFr was significantly inhibited to 60% of that of the adrenalectomized group. In the group receiving 5 mg of cortisol a greater inhibition was noted. These data are illustrated in Figure 1.

![Fig. 1. Effect of cortisone on the increased incorporation of radioactivity from L-phenylalanine-U-14C into corticotropin fraction by isolated anterior pituitary of rat induced by adrenalectomy. The vertical bars represent the standard error of the mean. The number of observations is in parenthesis. * compared with group I.](image-url)
3. Effect of glycyrrhizin on the suppressive action of cortisone on $^{14}$C-incorporation in $C_{Fr}$

Daily doses of 0.1 mg, 1 mg and 10 mg of glycyrrhizin were administered to adrenalectomized rats receiving a fixed dose of 1 mg of cortisone. Another group of adrenalectomized rats was treated with glycyrrhizin at doses of 0.2 mg, 2 mg and 20 mg with a fixed dose of 2 mg of cortisone.

The increase of $^{14}$C-incorporation in $C_{Fr}$ induced by adrenalectomy was significantly blocked by cortisone treatment in each experiment (Figs. 2 and 3). The simultaneous administration of glycyrrhizin in doses of from 0.1 mg to 10 mg significantly inhibited the effect of 1 mg of cortisone in suppressing $^{14}$C-incorporation in $C_{Fr}$ (Fig. 2). Glycyrrhizin itself did not affect $^{14}$C-incorporation in $C_{Fr}$ when given alone to adrenalectomized (Fig. 2) or to intact rats (Fig. 4). In the following experiment a dose of 20 mg of glycyrrhizin when given with cortisone was significantly antagonistic to the inhibitory action of 2 mg of cortisone, but doses of 0.2 to 2 mg glycyrrhizin were insufficient to overcome the effect of 2 mg of cortisone (Fig. 3).

![Graph](image)

**Fig. 2.** Effect of glycyrrhizin on the suppressive action of cortisone on the incorporation of radioactivity from L-phenylalanine-$^{14}$C into corticotropin fraction by isolated anterior pituitary of rat. The vertical bars represent the standard error of the mean. The number of observations is in parenthesis. * compared with group I. ** compared with group II.
Fig. 3. Effect of glycyrrhizin on the suppressive action of cortisone on the incorporation of radioactivity from L-phenylalanine-U-¹⁴C into corticotropin fraction by isolated anterior pituitary of rat. The vertical bars represent the standard error of the mean. The number of observation is in parenthesis. * compared with group I. + compared with group II.

Fig. 4. Effect of glycyrrhizin on the incorporation of radioactivity from L-phenylalanine-U-¹⁴C into corticotropin fraction by isolated anterior pituitary. Vertical bars indicate the standard error of the mean. The number of observations is in parenthesis. The differences between the control value and those of the various experimental groups are not significant.
4. **Biological activity of the radioactive substance isolated to the oxycellulose procedure.**

Oxycellulose does not bind corticotropin specifically and it is quite possible that other basic peptides, for example MSH, are absorbed. Consequently, it is necessary to test whether the radioactive substance isolated by the oxycellulose procedure is really contained in the corticotropin fraction. By using horizontal starch block electrophoresis, the radioactivity was found in two fractions —1 cm and —6 cm from the starting point under the conditions of the experiments. Both fractions coincided with the peak of optical density of the Lowry reaction at 750 m\(\mu\). Much corticotropin activity was also found in these two fractions (Fig. 5).

5. **Effect of glycyrrhizin on the suppressive action of cortisone on the compensatory adrenal hypertrophy.**

Doses of 0.1 mg, 1 mg and 10 mg of glycyrrhizin were administered to unilaterally adrenalectomized rats with a fixed dosage of 1 mg of cortisone. As illustrated in Figure 6, the results indicate that there was a 50% increase in adrenal weight after unilateral adrenalectomy. (The increase was 40% since the left adrenal was 10% heavier than the right in the control group). Daily administration of 1 mg of cortisone completely blocked compensatory adrenal hypertrophy. The

---

**Fig. 5.** Horizontal starch block electrophoresis of the radioactive substance extracted by the oxycellulose procedure. The solid line indicates the protein content, expressed as the optical density at 750 m\(\mu\) determined by the Lowry reaction. The dotted line indicates the counts per minute of radioactivity determined with a Nuclear Chicago gas flow counter.
inhibition of compensatory adrenal hypertrophy by 1 mg of cortisone was partially but significantly blocked by concomitant administration of 10 mg of glycyrrhizin, doses of glycyrrhizin of from 0.1 mg to 1 mg having no effect. Glycyrrhizin alone had no effect on compensatory adrenal hypertrophy after unilateral adrenalectomy.

6. Effect of cortisone and glycyrrhizin on adrenal ascorbic acid depletion (AAAD) after histamine stress.

AAAD was adopted as an indicator of ACTH release after histamine stress. One injection of 2.5 mg of cortisone or daily injections of 10 mg of glycyrrhizin for 3 days did not affect AAAD by histamine stress significantly. However, when glycyrrhizin was concomitantly administered with cortisone, AAAD was blocked significantly after histamine stress (Fig. 7).
DISCUSSION

It was found that incorporation of $^{14}$C-phenylalanine into the corticotropin fraction of the anterior pituitary of the rat was increased specifically by prior adrenalectomy and that $^{14}$C-incorporation in CF$_R$ was lowered by prior administration of prednisolone. These results give direct evidence in support of the negative feedback control theory (Sayers and Sayers, 1948).

Using horizontal starch block electrophoresis, the extracted radioactive substance was separated into two fractions, each of which had marked corticotropin activity. This suggests that the radioactive substance exists mainly in the corticotropin fraction.

In the present experiments, glycyrrhizin was found to antagonize the actions of cortisone on the pituitary adrenal axis, i.e., the inhibitory actions of cortisone on $^{14}$C-incorporation in CF$_R$ by isolated anterior pituitary glands of rats and on compensatory adrenal hypertrophy after unilateral adrenalectomy.

From clinical studies it was also recognized that glycyrrhizin effectively prevented the suppression of the pituitary-adrenal axis by glucocorticoid (Asanuma, 1965). Oral administration of glycyrrhizin in daily doses of 150~300 mg blocked the effect of 1~2 mg of dexamethasone in lowering the amount of total urinary 17-OHCS as the result of pituitary inhibition.

Glycyrrhizin is already known to inhibit the metabolism of steroids containing the $\Delta^4$-3-ketone structure in the liver and causes retention of biologically active free steroids in circulating blood (Kumagai et al., 1957). It may be assumed that
the retention of the active steroids in the blood results in a suppression of pituitary function. In the present experiments, it was found that glycyrrhizin blocked the pituitary inhibition caused by glucocorticoids.

Glycyrrhizin might antagonize the inhibitory action of glucocorticoids on the pituitary adrenal axis as follows: glycyrrhizin might antagonize the action of glucocorticoids at the feed-back site, perhaps in the hypothalamus, and its antagonistic effect on the feed-back action of glucocorticoids might overcome the suppressive action on the pituitary caused by an increased concentration of biologically active steroids induced by glycyrrhizin as the result of inhibition of steroid metabolism in the liver (Kumagai et al., 1957).

Relatively small doses of glycyrrhizin were used in our experiment as compared with those previously used by other investigators. For example, Lawrence and Conn (1956) reported that administration of glycyrrhizin in daily oral doses of 4–6 g to man resulted in pituitary inhibition, resulting in a decrease in the amount of 17-KS excreted into the urine. The doses used in our experiments were 150–300 mg in man and 0.1–20 mg in rats. With such relatively small doses of glycyrrhizin, the inhibitory effect by glycyrrhizin on steroid metabolism in the liver may be negligible and the main effect may be blocking of the feed-back action of glucocorticoids.

In the present experiments it was found that under basal or resting conditions glycyrrhizin antagonized the feed-back action of glucocorticoids, while under histamine stress it increased the action of cortisone. It is doubtful whether the secretion of corticotropin in the stress state is under negative feed-back control. Many investigators have discussed the difference between the secretory mechanism of corticotropin in the basal or resting state and that in the stress state (Ganong and Hume, 1954; Schapiro, 1962; Vernikos-Danellis, 1963; Marks and Vernikos-Danellis, 1963). The results obtained in the present experiments offer further evidence that the secretory mechanism of corticotropin in the stress state may differ from that in the basal or resting state.

Glycyrrhizin is known to be a diglucopyranosiduronic acid of glycyrrhetic acid, which is a nonsteroidal triterpenoid resembling allopregnane stereochemically. Various effects of glycyrrhizin on the actions of glucocorticoids may be due to its structural resemblance to steroids and it may well be that the inhibition of corticoid action by glycyrrhizin is competitive.

SUMMARY

The effects of glycyrrhizin on the pituitary adrenal axis were studied. The result of Wool et al., (1961), showing that the rate of incorporation of 14C-phenylalanine into the corticotropin fraction of isolated rat pituitary was increased specifically by prior adrenalectomy, was confirmed. It was found that the extracted radioactivity could be separated into two fractions electrophoretically, each fraction having high corticotropin activity. The rate of 14C-incorporation in the corticotropin fraction was abnormally low in the pituitaries of prednisolone treated rats. Cortisone treatment suppressed the increased 14C-incorporation into the corticotropin fraction induced by prior adrenalectomy. However concomitant injections of
glycyrrhizin blocked the inhibitory action of cortisone on $^{14}$C-incorporation in the corticotropin fraction.

Glycyrrhizin was also found to be antagonistic to the suppressive action of cortisone on compensatory adrenal hypertrophy in rats after unilateral adrenalectomy.

Under conditions of stress, glycyrrhizin was found to increase the inhibitory action of cortisone on adrenal ascorbic acid depletion.

The possibility of distinguishing the secretory mechanism of corticotropin in the basal stage from that in the condition of stress is discussed and the mechanism of the inhibition of glycyrrhizin on the action of cortisone as well as the possible clinical uses of glycyrrhizin are discussed.

REFERENCES


