DESOXYCORTICOSTERONE-MIMETIC ACTION OF AMMONIATED GLYCYRRHIZIN IN RATS* †

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A desoxycorticosterone-mimetic action of the crude watery extract of Glycyrrhiza glabra (licorice) was first observed in normal individuals by Borst (1) and his co-workers (2). The electrolyte-active constituent, glycyrrhizinic acid (2-4), is the glycoside of a polyterpene compound (glycyrrhetinic acid) which structurally resembles the phenanthrene part of the steroid series to which desoxycorticosterone belongs (3). Glycyrrhizin-induced sodium and water retention and increased potassium excretion observed in normal individuals (1, 2, 5, 6) have also been seen in patients with Addison's disease (3-5, 7-9), bilateral adrenalectomy (10), Simmonds' disease (7), and Cushing's disease (6). Glycyrrhizin and its active constituents have been used clinically in the maintenance of patients with Addison's disease (3-5, 7-9), bilateral adrenalectomy (10), as well as in the treatment of demerol addiction (11), and certain psychoneurotic states (12). A superiority of glycyrrhizin over DOCA in bilaterally adrenalectomized patients and the reduction of the cortisone maintenance dose has been observed (10).

Despite the remarkable similarity between the pharmacological actions of glycyrrhizin and desoxycorticosterone in man, glycyrrhizin has not been able to maintain adrenalectomized rats (5, 13, 14), nor to protect adrenalectomized mice from cold stress (14). This investigation was therefore undertaken to determine whether ammoniated glycyrrhizin in rats resembled desoxycorticosterone in its effect on electrolyte and water metabolism and on pituitary-adrenal inhibition.

Methods

(a) The effect of ammoniated glycyrrhizin on electrolyte and water metabolism was studied in intact and in adrenalectomized rats by means of a minero steroid assay method which consisted of a 4 hour assay of sodium and urine excretion during saline diuresis.

Minero steroid Assay Method.—Male albino rats (150 ± 15 gm.) were paired in metabolism cages and maintained on Purina chow. Intact rats received tap water ad libitum; adrenal-

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ectomized rats received saline ad libitum until 24 hours before use, when tap water was substituted. The night before the assay, food and water were removed and replaced by 10 per cent glucose ad libitum until the time of the assay. On the morning of the assay, bladders were emptied (by inhalation of ether for 5 seconds), physiological saline (5.0 cc. at 40°C.) was administered intraperitoneally, and ammoniated glycyrrhizin in saline (0.5 cc.) was administered subcutaneously. Similarly handled control animals received the vehicle without the drug. The animals were placed in pairs into metabolism cages immediately after drug administration, and urine from each pair was collected in a graduated tube. At the end of 4 hours, bladders were again emptied, and this urine pooled with the 4 hour volume. Urinary sodium (mg./4 hours) was determined with a Barclay flame photometer.

The study of intact rats consisted of 3 experiments, each composed of 3 parts, (1) a control assay, (2) an experimental period of 5 days, and (3) a period of drug withdrawal for 4 days. The control assay was performed on all animals prior to the experimental period to determine the norm. During the experimental period, 5 consecutive daily assays of the same dose were performed. This was followed by the period of drug withdrawal with assays on the 3rd and 4th days to determine whether urine volume and sodium excretion had returned to the original norm. In Experiment 1 (20 mg./rat day), as well as in Experiment 2 (40 mg./rat day), there were 6 pairs of experimental animals (30 determinations during treatment, 12 determinations after drug withdrawal) and 4 pairs of control animals (28 determinations). In Experiment 3 (80 mg./rat day), there were 3 pairs of experimental animals (15 determinations during treatment, 6 determinations after drug withdrawal) and 3 pairs of control animals (21 determinations). The urine volume and sodium excretion of the treated animals were compared with (1) their own control values and (2) those of the simultaneously assayed control animals.

The study on adrenalectomized rats consisted of 2 experiments involving a single assay of two different doses. In both experiments, 5 pairs of experimental animals and 5 pairs of control animals were used. The urine volume and sodium excretion of the treated animals were compared with those of the simultaneously assayed control animals. Experiment 1 (40 mg./rat) was performed 1 week after adrenalectomy, and Experiment 2 (80 mg./rat) was performed 3 days after adrenalectomy.

Experiments designed to determine the pituitary-adrenal inhibition with glycyrrhizin consisted of a study of the changes in adrenal ascorbic acid as a reflection of ACTH activity in response to specific stress. Male albino rats (150 ± 50 gm.), which were subjected to histamine stress (0.5 mg./100 gm. body weight, intraperitoneally) were sacrificed after 1 hour by decapitation. Both adrenals were weighed and homogenized in 3 per cent metaphosphoric acid. The ascorbic acid contents of the adrenal glands were determined according to the method of Bessey (15) in the following 5 groups of animals: (1) unstressed, untreated (10); (2) unstressed, orally administered glycyrrhizin for 1 week (10); (3) stressed, untreated (15); (4) stressed, subcutaneously administered glycyrrhizin (30 mg./100 gm. body weight) 17 to 20 hours before stress (9); (5) stressed, orally administered glycyrrhizin for 1 week (15). Glycyrrhizin was administered orally by the addition of 0.4 per cent ammoniated glycyrrhizin to the drinking water so that the average intake was 80 mg./100 gm. body weight daily.

RESULTS

Intact Rats.—The daily administration of ammoniated glycyrrhizin to intact rats produced a significant decrease in sodium and urine excretions. Sodium retention appeared after a latent period which was inversely related to size of the daily dose. No effect was apparent during the first 3 days with 20 mg./day, the first 2 days with 40 mg./day, or the first day with 80 mg./day. However,
Fig. 1. Dose response curves for glycyrrhizin-induced sodium retention in intact rats (4 hour assay). Ammoniated glycyrrhizin assayed daily for 5 days. Per cent sodium retention represents the mean responses to each dose during the following periods of latent sodium retention: 20 mg., days 4, 5; 40 mg., days 3, 4, 5; 80 mg., days 2, 3, 4, 5. Statistical significance: A, per cent difference from simultaneously assayed control animals, 20 mg. P <0.02, 40 mg. P <0.01, 80 mg. P <0.01; B, per cent difference from own control values, 20 mg. P 0.05, 40 mg. P <0.05, 80 mg. P <0.05.

Fig. 2. Cumulative response curves for glycyrrhizin-induced urine retention in intact rats (4 hour assay). Retention calculated as per cent difference from original control values.
Fig. 3. Dose response curve for glycyrrhizin-induced urine retention in intact rats (4 hour assay). Retention calculated as per cent difference from original control values. Mean responses during 5 day period of daily administration of ammoniated glycyrrhizin. Statistical significance: 20 mg. $P < 0.001$, 40 mg. $P < 0.001$, 80 mg. $P < 0.01$.

Fig. 4. Effect of withdrawal of treatment on glycyrrhizin-induced sodium and urine retention in intact rats (4 hour assay). Per cent difference from original control values. Retention on 5th day of treatment with 20, 40, and 80 mg. of ammoniated glycyrrhizin. Assays after drug withdrawal on 3rd and 4th days.
once retention was established, continued administration of the drug did not further increase the response. Sodium excretion for the entire period of response was in each case significantly lower than that of untreated animals, or that of test animals during control periods. The degree of response was directly related to dose: sodium retention (calculated both as per cent difference from control

| TABLE I |
| Response of Adrenalectomized Rats to Glycyrrhizin-Induced Sodium Retention (4 Hour Assay) |

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dose*</th>
<th>Sodium retention</th>
<th>P value</th>
</tr>
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<td>65.6</td>
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<tr>
<td>2</td>
<td>80</td>
<td>82.4</td>
<td>&lt;0.001</td>
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</table>

Experiment 1—Adrenalectomized 1 week before assay. Experiment 2—Adrenalectomized 3 days before assay.

* Ammoniated glycyrrhizin administered subcutaneously to 5 pairs of rats at each dose level.

† Sodium retention calculated as the per cent difference from the mean response of 5 pairs of simultaneously assayed untreated adrenalectomized rats.

| TABLE II |
| Effect of Ammoniated Glycyrrhizin on Urine Excretion of Adrenalectomized Rats (4 Hour Assay) |

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dose</th>
<th>N*</th>
<th>Ml/hr. pair</th>
<th>Urine retention</th>
<th>P value</th>
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<td>5</td>
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<td>82.4</td>
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</table>

Experiment 1—adrenalectomized 1 week before assay. Experiment 2—adrenalectomized 3 days before assay.

* N represents the number of pairs of adrenalectomized rats in each category.

† Urine retention calculated as the per cent difference from the mean response of 5 pairs of simultaneously assayed untreated adrenalectomized rats.

animals and as per cent difference from the control period) varied as a linear function of log dose (Fig. 1).

The antidiuretic response differed from the retention of sodium in that it appeared immediately and had a cumulative effect on continued administration of the same dose (Fig. 2). The dose-response curve established by plotting the mean response for the entire 5 day period also was a linear function of log dose (Fig. 3). There was no relationship between sodium retention and the antidiuretic response.

On the 3rd day after drug withdrawal (Fig. 4), (t) urine volume was nor-
mal, and sodium excretion was slightly above normal in animals which had received the 20 and 40 mg. doses, while (2) urine volume and sodium excretion were below normal in animals which had received 80 mg. doses. On the 4th day after drug withdrawal, urine volume was normal, and sodium output was slightly increased in all experimental groups. Thus, the normal physiological state was reestablished soon after withdrawal of the drug.

Adrenalectomized Rats.—The administration of 40 and 80 mg. doses of ammoniated glycyrrhizin to adrenalectomized rats produced significant degrees of sodium retention (Table I) with no latent period. 80 mg. produced a marked antidiuretic effect 3 days after adrenalectomy, while 1 week after adrenalectomy 40 mg. was ineffective (Table II). The degree of sodium retention was equal in adrenalectomized and intact rats (Fig. 1).

Pituitary-Adrenal Inhibition.—Oral feeding of ammoniated glycyrrhizin for 1 week had no effect on the ascorbic acid concentration of unstimulated adrenal glands (Fig. 5). However, it did decrease the response of these glands to the stress of histamine injection (Fig. 5). Ascorbic acid depletion of glycyrrhizin-treated animals was significantly lower than that of untreated stressed animals (Fig. 5). The results suggest that glycyrrhizin blocked the normal response of the pituitary gland to histamine, resulting in lower output of ACTH.
DISCUSSION

Glycyrrhizin-induced sodium and water retention observed in man has been reproduced in intact and in adrenalectomized rats. To determine more details of the mechanism, renal function studies will have to be undertaken. However, in this work the antidiuretic effect and the retention of sodium did not appear to be directly correlated; in intact rats retention of sodium followed a latent period, while the antidiuretic effect occurred immediately and was cumulative. The latent period preceding sodium retention was shortened by increasing the size of the dose. Removal of the adrenal gland eliminated the latent period entirely.

A decreased 17-ketosteroid output in man (6), and adrenal atrophy in guinea pigs (16), had suggested that glycyrrhizin may exert an inhibitory effect on the pituitary-adrenal system resembling that of other corticosteroids. The present work provides further evidence that glycyrrhizin inhibits the normal response of the pituitary-adrenal system to specific stress. Since sodium and water levels did not influence ACTH output (17), the pituitary-adrenal inhibition presumably was not secondary to changes in electrolyte and water metabolism. Since a diminished output of ACTH causes a decreased output of glucosteroid (18, 19), the retention of sodium seen in intact animals most likely appeared after reduction of circulating C11 oxysteroids.

Suppression of diuresis with glycyrrhizin is independent of posterior pituitary function (13). Removal of the adrenal gland increased the sensitivity to the antidiuretic effect of glycyrrhizin administered 3 days postoperatively. However, after 1 week, adrenalectomized rats retained urine in the absence of endogenous corticosteroids (Table II) and were insensitive to the antidiuretic effect of glycyrrhizin. The administration of 40 mg. to these animals was ineffective in increasing tubular reabsorption of water despite the fact that there was marked sodium retention.

Retention of water and sodium was not a result of permanent functional change. Despite the marked responses in the intact rats during a 5 day period of treatment, all animals returned to the normal physiological state shortly after withdrawal of the drug.

Since glycyrrhizin was not converted to identifiable steroids in vivo (8, 10), the effects on electrolyte and water metabolism, as well as the inhibition of pituitary function, remain unexplained.

SUMMARY

The desoxycorticosterone-mimetic action of ammoniated glycyrrhizin on electrolyte and water metabolism and on pituitary-adrenal function, was demonstrated in rats. Retention of sodium and water was observed in both intact and adrenalectomized rats during 4 hour mineroosteroid assays, the degree of
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retention being a function of log dose. In intact rats, the antidiuretic effect of glycyrrhizin was immediate and cumulative, while retention of sodium appeared only after a latent period and did not increase with time. Adrenalectomized rats, 3 days after the operation, showed a more marked antidiuretic effect than intact rats, but no greater retention of sodium.

Rats receiving glycyrrhizin prior to histamine stress showed a marked decrease in adrenal ascorbic acid depletion, suggesting that glycyrrhizin had suppressed the output of ACTH.

Whether glycyrrhizin acts on the same target organ as desoxycorticosterone in producing sodium and water retention is still an open question. However, the fact that it is capable of producing a pituitary-adrenal inhibition which resembles that of naturally occurring adrenal steroids is further evidence of the strong pharmacological resemblance between glycyrrhizin and desoxycorticosterone.

BIBLIOGRAPHY