AN INHIBITORY EFFECT OF GLYCYRRHIZIN ON METABOLIC ACTIONS OF CORTISONE

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SYNOPSIS

It has been previously shown in our laboratory that glycyrrhizin inhibits the antigranulomatous action of cortisone without affecting the anti-inflammatory action in rats. In the present paper, the work was extended to include the effect of glycyrrhizin on metabolic actions of cortisone in the liver of adrenalectomized rats. Daily injections of cortisone induced a several fold increase of liver glycogen content, tryptophan pyrrolase activity and incorporation of [$1-^{14}\text{C}$] acetate into cholesterol in rat liver, while concomitant injections of glycyrrhizin blocked these metabolic actions of cortisone. Administration of glycyrrhizin by itself, however, had no effect on the action of cortisone.

In a previous publication (Kumagai et al., 1964), the authors presented an inhibitory effect of glycyrrhizin on the antigranulomatous action of cortisone without affect on the anti-inflammatory action of the steroid.

The present experiments were designed to observe the effect of glycyrrhizin on metabolic actions of cortisone, i.e., glycogen deposition and cholesterol biosynthesis in the liver, and hormonal induction of liver tryptophan pyrrolase.

MATERIALS AND METHODS

Wistar strain female rats maintained on a commercial laboratory chow diet and weighing approximately 150 grams were used in all experiments. Adrenalectomy was performed under Pentobarbital anesthesia using the dorsal approach and 1% saline was given in place of drinking water thereafter. Cortisone acetate (Schering Co.) and glycyrrhizin (Minophagen Co.) were administered subcutaneously alone or in combination in doses described below.

After 3 days of treatment, the animals were fasted for 10 hrs. following the last injection and sacrificed by decapitation for the assay of liver glycogen and tryptophan pyrrolase activity.

Liver glycogen was determined by the method of Carrol *et al.*, (1956). The procedure principally consists of extraction of liver glycogen by 5% trichloroacetic acid (TCA), precipitation by adding 95% ethanol to the extract and colorimetric determination at 620 m μ with anthrone reagents.

Liver tryptophan pyrrolase was determined by the method of Knox (1955). One gram of fresh rat liver was homogenized for 2 mins. in 7 ml of cold alkaline KCl by Potter-Elvehjem homogenizer. Two ml of the liver homogenate were incubated with 0.3 ml of 0.03 M L-trypto-

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phan and phosphate buffer, pH 7.0 (total volume 4 ml), at 37°C for 60 mins. and kynurenine formed during the incubation was determined at 365 m μ in a Beckman spectrophotometer.

Specific activity of the enzyme was expressed in terms of the micromoles of kynurenine formed per gram of liver dry weight per hour.

Biosynthetic activity of cholesterol in the liver was measured by incorporation of [1-¹⁴C] acetate into cholesterol. After 7 days of cortisone and/or glycyrrhizin treatment, 1 ml of isotonic saline solution containing 20 μ c of sodium [1-¹⁴C] acetate (Daiichi Pure Chemical Co., specific activity 6.7 mC/m mol) was injected intraperitoneally to the rats which had fasted for 12 hrs.

The animals were sacrificed 15 mins. later and the liver was immediately removed, weighed, homogenized for 3 mins. in a Waring blender with 25 vol. of mixed solution of chloroform-methanol (2:1) and allowed to stand at room temperature for 4 hrs. After filtration, the supernatant was washed with water following the method of Folch *et al.*, (1957).

The solvent being evaporated, the residue was dissolved in petroleum ether and phospholipid fraction was separated with acetone. After condensation and hydrolysis of the supernatant with alcoholic KOH, non-saponifiable fraction was obtained by extraction with petroleum ether. The extract was washed with water and evaporated to dryness. One per cent digitonin solution in 50% ethanol was added to the residue dissolved in acetone-ethanol (1:1). After being washed with acetone-ether (1:1) and ether, the precipitate was mounted on a planchet in methanol. Radioactivity was determined with a Nuclear Chicago gas-flow counter and specific activity was expressed as count per minute per mg of cholesterol after correction for self-absorption.

RESULTS

The liver glycogen content of adrenalectomized rats receiving 1 mg of cortisone for 3 days was about 20 times that of the untreated adrenalectomized rats (Fig. 1). When 0.1~1 mg of glycyrrhizin was administered with cortisone, liver glycogen content decreased to one-half of that of the rats treated with cortisone alone. With 10 mg of glycyrrhizin, glycogen content was reduced to one-sixth that of cortisone. However, glycyrrhizin itself was found to have no effect on glycogen content in the liver of adrenalectomized rats.

Liver tryptophan pyrrolase, an adaptative enzyme, is known to increase in activity with cortisone administration and a linear dose-activity relationship is observed in adrenalectomized rats (Fig. 2), in agreement with the report of Thomson and Mikuta (1954). Treatment with 1 mg of cortisone acetate for 3 days resulted in an increase of liver tryptophan pyrrolase activity to more than double that of untreated controls (Fig. 3). The increase of liver tryptophan pyrrolase induced by 1 mg of cortisone was inhibited by concomitant administration of glycyrrhizin in doses ranging from 0.1 to 10 mg. Administration of glycyrrhizin did not affect the activity of tryptophan pyrrolase in the liver of adrenalectomized rats.

Figure 4 shows the effects of glycyrrhizin and cortisone on the incorporation of [1-¹⁴C] acetate into cholesterol in the liver of adrenalectomized rats. It was found that the increased incorporation of ¹⁴C-acetate into cholesterol induced by cortisone was blocked by simultaneous administration of glycyrrhizin, though a single administration of glycyrrhizin even in a large dose scarcely influenced the rate of incorporation.

Fig. 1

Fig. 1. Inhibitory effect of glycyrrhizin on liver glycogen deposition by cortisone acetate. Numbers in parentheses indicate the numbers of animals used. Vertical bars represent standard error of the mean for each group.

Fig. 2

Fig. 2. Effect of cortisone acetate on the activity of adrenalectomized female rat liver tryptophan pyrrolase. Enzyme activity was assayed six hrs. after intraperitoneal injection of cortisone. Numbers in parentheses indicate the numbers of animals used. Vertical bars represent standard error of each mean.

Fig. 3

Fig. 3. Inhibitory effect of glycyrrhizin on hormonal induction of adrenalectomized rat liver tryptophan pyrrolase. Numbers in parentheses indicate the numbers of animals used. Vertical bars represent standard error of each mean.

Fig. 4

Fig. 4. Effect of glycyrrhizin and cortisone acetate on the incorporation of $[1-^{14}\text{C}]$ acetate into cholesterol in the liver of adrenalectomized rats 15 mins. after intraperitoneal injection of ^{14}C -acetate. Vertical bars represent standard error of each mean.

DISCUSSION

In the previous study, the authors demonstrated that glycyrrhizin had a blocking effect on the antigranulomatous action of cortisone, estimated by the cotton pellet method, without affecting the anti-inflammatory action with granuloma pouch assay.

The present paper shows evidence that glycyrrhizin also inhibits metabolic actions of cortisone, i.e., liver glycogen deposition in carbohydrate metabolism, induction of liver tryptophan pyrrolase in protein metabolism and increased biosynthesis of cholesterol in lipid metabolism.

The inhibitory effect of glycyrrhizin on these metabolic actions of cortisone was observed even in one-tenth dosage of cortisone acetate.

It is of great interest to note that biological actions of cortisone could be dissociated by glycyrrhizin between anti-inflammatory action and anti-granulomatous and metabolic actions.

Although the mechanism by which glycyrrhizin inhibits cortisone action remains unknown, it is suggested that glycyrrhizin works as an inhibitor to some biological actions of cortisone.

SUMMARY

1. Glycyrrhizin was found to have an inhibitory effect on depository action of liver glycogen by cortisone in adrenalectomized rats.
2. A similar inhibition was observed in hormonal (cortisone) induction of rat liver tryptophan pyrrolase when glycyrrhizin was administered to rats with cortisone.
3. Increase of cholesterol biosynthesis in rat liver by cortisone was also inhibited by concomitant administration of glycyrrhizin.
4. It was concluded from these data that glycyrrhizin inhibited metabolic actions of cortisone though the mechanism was unknown.

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