EFFECT OF GLYCYRRHIZIN ON ESTROGEN ACTION

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SYNOPSIS

It was demonstrated by the present study that glycyrrhizin inhibited the action of estradiol-17β on uterine weight and β-glucuronidase activity in ovariectomized mice and rats in a certain dose ratio. The inhibition was 50% or more when the dose ratio of glycyrrhizin : estradiol-17β was 1000 : 1. In any higher ratio of glycyrrhizin to estrogen, the inhibition was decreased or even reversed. Glycyrrhizin by itself had no effect on either uterine weight or β-glucuronidase activity in ovariectomized mice. The mechanism of the anti-estrogenic effect was discussed in relation to the effect of glycyrrhizin on biological actions and hepatic degradation of cortisone.

It has been reported from our laboratory that glycyrrhizin inhibited the degradation of cortisol in the liver (Kumagai et al., 1957) and that it partially interfered with some biological actions of cortisone in adrenalectomized rats (Kumagai et al., 1964 and 1966).

This paper deals with the effect of glycyrrhizin on the action of estradiol-17β on uterine weight and β-glucuronidase activity.

MATERIALS AND METHODS

Female mice of dd-strain, 28 days of age, were ovariectomized and kept on a commercial chow diet (Oriental Kobo Co.) for 2 weeks. They were then divided into several groups and treated for 3 successive days by subcutaneous injection of estradiol-17β (Teikokuzoki Co.), glycyrrhizin (Minophagen Co.) or their mixture. The uteri were removed and weighed at 46 days of age, 1 day after the last injection.

Uterine homogenates were prepared containing 2.5% (weight per volume) of uterus in ice-cold 0.1 M acetate buffer at pH 4.0. After centrifugation at 3000 rpm for 10 mins., the supernatant was used as an enzyme solution for the incubations.

Incubation mixtures (1 ml of total volume) contained 0.1 ml of 0.1 M p-nitrophenyl β-D-glucopyranosiduronic acid (Tsukamoto’s reagent), 0.2 ml of uterine enzyme solution and 0.7 ml of 0.1 M acetate buffer, pH 4.0. Each blank was prepared by replacing the substrate (Tsukamoto’s reagent) with acetate buffer. The incubations were carried out in air at 38°C for 1 hr. The reaction was stopped and color was developed by adding 1 ml of 0.1 N NaOH. After dilution of the incubation mixture with 4 ml of water, β-glucuronidase activity was determined at 400 mμ by Coleman spectrophotometer.

Female spayed rats of Sprague-Dawley strain, weighing approximately 60 g, were also used for the same purpose. Two days after ovariectomy, estradiol-17β, glycyrrhizin or a mixture of both was injected once daily for 2 days and the uteri were removed and weighed one day after the last injection.

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RESULTS

Uterine weights of spayed mice were lineally increased by 3 days injections of estradiol-17β in the dose range of 0~0.05 μg. No further response could be observed even at higher dosages (Fig. 1). To the maximal response dose of estradiol-17β, 0.1 μg, glycyrrhizin was concomitantly administered in doses of 0.01, 0.1 and 1 mg. As seen in Figure 2, concomitant administration of glycyrrhizin with estradiol-17β in dose ratio of 1000 : 1 or less decreased uterine weights, thus showing the anti-estrogenic activity of glycyrrhizin, while 1 mg of glycyrrhizin (dose ratio 10,000; 1) scarcely affected the uterine response with 0.1 μg of estradiol-17β. Injections of 1 mg of glycyrrhizin by itself did not significantly influence uterine weights.

No increase of uterine β-glucuronidase activity of spayed mice could be observed when the mice were injected 0.01 μg of estradiol-17β.

However, in higher than 0.05 μg doses, a lineal dose-response curve was obtained for
the activity of uterine $\beta$-glucuronidase (Fig. 3). As shown in Figure 4, with a fixed dose of 0.1 $\mu$g of estradiol-17$\beta$, injections of 0.01 or 0.1 mg of glycyrrhizin significantly inhibited an increase of uterine $\beta$-glucuronidase activity by estradiol-17$\beta$, but 1 mg of glycyrrhizin showed little inhibitory effect. Glycyrrhizin itself had no effect on uterine $\beta$-glucuronidase activity.

From the foregoing experiments in mice, it was found that an increase of both uterine weight and $\beta$-glucuronidase activity induced by 0.1 $\mu$g of estradiol-17$\beta$ was blocked by concomitant administration of a thousand fold amount of glycyrrhizin. The following experiments were performed to determine whether the results obtained in mice could be true in rats.

When 0.5 mg of glycyrrhizin was injected into spayed rats in combination with estradiol-17$\beta$ in various doses such as 0.1, 0.5 and 1.0 $\mu$g, an anti-estrogenic effect of glycyrrhizin was demonstrated in dose ratios of 1000: 1
and 500 : 1, although no effect was found in the ratio of 5000 : 1 (Fig. 5).

This result indicates that glycyrrhizin interfered with the action of estradiol-17β on uterine weights in the same dose ratio in rats as in mice.

With a fixed dose (2 μg) of estradiol-17β, daily doses of 0, 1, 4 and 10 mg of glycyrrhizin were injected for 2 days into spayed rats. As shown in Figure 6, a maximal inhibitory effect on uterine weights was observed when 1 mg of glycyrrhizin (ratio 500 : 1) was used, and the inhibition was decreased when a larger amount of glycyrrhizin, 4 mg, was used (2000 : 1). Moreover, 10 mg of glycyrrhizin (5000 : 1) showed even a stimulatory effect in comparison with single injection of estradiol-17β. Ten mg of glycyrrhizin without estradiol, however, had no effect on uterine weights.

DISCUSSION

Reports of anti-estrogenic substances have been accumulated until the present time. Inhibitions of estrogen action has been shown for androgen (Robson, 1938; Emmens and Bradshow, 1939; Velardo et al., 1955; Edgren and Calhoun, 1957; Dorfman et al., 1960), progesterone (Robson, 1938; Astwood, 1940; Szego and Roberts, 1948; Courrier, 1950; Mardones et al., 1954; Huggins and Jensen, 1955; Edgren and Calhoun, 1957; Dorfman et al., 1960), estrogen (de Fremery et al., 1934; Allen and Meyer, 1935; Hisaw et al., 1954) and corticoids (Robson, 1939; Szego and Roberts, 1948; Szego, 1952; Talalay et al., 1952; Roberts and Szego, 1953; Beyler and Szego, 1954; Huggins and Jensen 1955; Velardo, 1955; Velardo and Sturgis, 1955; Velardo et al., 1955; Velardo, 1956; Velardo and Sturgis, 1956). Certain 17α-alkyl derivatives and 19-nortestosterone have been reported to be anti-estrogenic (Payne et al., 1956; Edgren and Calhoun, 1957; Sturtevant, 1957; Edgren, 1958; Edgren et al., 1959). Non-steroidal substances such as MER-25 (Lerner et al., 1958) and (di-p-hydroxyphenyl)-butane-pentane : -hexane : -1, 4-pentanediene-3-one (Banay et al., 1955) were also demonstrated to have a certain anti-estrogenic activity.

In the previous papers (Kumagai et al., 1964 and 1967), the authors demonstrated that glycyrrhizin inhibited such biological actions of cortisone as antigranulomatous, pituitary suppressive and glycconeogenetic action. This paper showed evidence that the substance also inhibited the action of estradiol-17β on uterine weight of mice and rats and β-glucuronidase activity in the uterus of mice in a certain dose ratio. Maximal inhibition —50% in uterine weights and greater than that in uterine β-glucuronidase—was observed when the dose ratio of glycyrrhizin : estradiol-17β was 500 : 1 or 1000 : 1.

The inhibitory mechanism remains unknown, but we speculate that glycyrrhizin would inhibitorily act at the site of estrogen action in target tissues.

Glycyrrhizin in higher doses showed less inhibitory effect and even the reverse, i.e., stimulatory effect appeared in rats in a dose ratio of 5000 : 1. Glycyrrhizin was found to inhibit both in vivo and in vitro the degradation of cortisol in the liver (Kumagai et al., 1957) and the augmentation of estrogen action by large amounts of glycyrrhizin might be also explained by the inhibition of estrogen inactivation by glycyrrhizin in the liver, though further experiments must be done to support the hypothesis.

SUMMARY

1. Glycyrrhizin inhibited the action of estradiol-17β on uterine weight and β-glucuronidase activity in ovariectomized mice in a certain dose ratio.
2. Maximal inhibition (50% or more) was observed when the dose ratio of glycyrrhizin : estradiol-17β was 500 : 1 or 1000 : 1.
3. In any higher ratio of glycyrrhizin to estrogen the action was decreased or even reversed in ovariectomized rats.
4. Glycyrrhizin by itself had no effect on either uterine weight or β-glucuronidase activity in ovariectomized mice.
REFERENCES


