Prolonged Administration of Glycyrrhetinic Acid in Rats

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Prolonged administration of glycyrrhetinic acid was studied in immature female rats, castrated male donor parabionts, and mature cyclic rats. Glycyrrhetinic acid treatment for 7 days interfered with the uterotrophic response of immature rats to estradiol benzoate but had no effect on the pituitary-ACTH content. It did not alter gonadotropin release or estradiol-induced gonadotropin inhibition in male castrated donors. One month of treatment did not alter the estrus cycles. It is apparent that the antiestrogenic effect of glycyrrhetinic acid is limited to exogenous estrogen and its effect on the female accessory reproductive tract.

THIS LABORATORY previously reported that β-glycyrrhetinic acid restricted the response of the uterus (1, 2) and the vagina (2) to exogenous steroidal estrogens. It was not capable of interfering with the uterotrophic response to endogenous sex steroids in the pregnant mare serum stimulated animal (2) or female recipient parabionts (1). Short term administration (3 days) which depressed the uterine growth response of immature intact (1, 2), adrenalectomized (1), and ovariectomized (2) rats had no effect in hypophysectomized rats (2). The pituitary-adrenal axis was not affected by short term administration (3). Prolonged administration (7 days) interfered with the pituitary-adrenal axis (3) and also increased the uterine response of female recipient parabionts (2).

Further investigations on the effect of long term administration were undertaken in an attempt to clarify mode of action. These studies consisted of the effect of (*a*) 7 days of treatment on the uterotrophic response as well as pituitary-ACTH of immature female rats, (*b*) 8 days of treatment on gonadotropin release in male castrated donor parabionts with and without estrogen, and (*c*) 1 month of treatment on the estrus cycles of mature cyclic rats.

MATERIALS AND METHODS

Rats were maintained under constant lighting (14 hours artificial light) at 25° and fed Purina laboratory chow and water *ad libitum*. Hypophysectomized rats were given 5% glucose in the drinking water. All experimental animals were of the Wistar strain. Hypophysectomized Sprague-Dawley male rats (120–140 Gm.) used in the ACTH assay were received 24 hours after surgery and used 48 hours postoperatively. Both β-glycyrrhetinic acid[1] and estradiol benzoate[2] were prepared in an ethanol–sesame oil vehicle (1) and administered subcutaneously.

Immature Female Rats.—Immature female rats (35–50 Gm.) were divided into four groups—controls, glycyrrhetinic acid only, estradiol benzoate only, and glycyrrhetinic acid plus estradiol benzoate. The 7 days of glycyrrhetinic acid treatment was the previously determined pituitary-adrenal blocking dose (3). Glycyrrhetinic acid (2 mg.) or vehicle (0.2 ml.) was administered daily for 6 days with a double dose on the seventh day. Estradiol benzoate was administered to two groups as 0.1 mcg. daily during the last 3 days of treatment. Animals were sacrificed on the eighth day (72 hours after the first estradiol dose), and the uteri were weighed. The pituitaries of two groups (controls and glycyrrhetinic acid only) were removed for ACTH assay. Each pituitary was placed in 0.5 ml. of 0.1 *N* HCl and finely macerated with a motor driven Potter tissue grinder. The final concentration of pituitary suspension was made by adding 4.5 ml. of physiological saline to each 0.5 ml. of pituitary suspension, thus producing a suspension of 0.1 pituitary per 0.5 ml. of 0.01 *N* HCl in saline. The dose of 0.1 pituitary (0.5 ml.) per 100 Gm. of body weight was administered subcutaneously. Animals were sacrificed after 3 hours and the adrenal ascorbic acid determined (4). Each sample was administered to two test animals. The adrenal ascorbic acid was compared with that of untreated hypophysectomized rats to determine the degree of depletion.

Gonadotropin Release in Male Castrated Donor Parabionts With and Without Estrogen.—Parabiotic rats (23–25 days old) consisted of a castrated male donor and an immature female recipient. Rats were anesthetized with pentothal (50 mg./Kg. i.p.). After castrating the male partner, a skin incision was made laterally from the ear to the tail region (right side of the male and left side of the female). The pair was joined anteriorly by a mattress suture through the left scapula of the female and the right scapula of the male and posteriorly by a mattress suture through the gluteus maximus muscle. The two ventral skin flaps were closed with skin clips; a small lateral incision was made in the peritoneum of each animal. The four cut surfaces were sutured together with fine silk thread and the dorsal skin flaps were then closed with skin clips. Each parabiont was given 20,000 units of penicillin intramuscularly immediately postoperatively and again on the third and fifth days. The parabiotic rats were divided into six groups. Male parabionts were treated for 8 days with either vehicle (0.2 ml.) glycyrrhetinic acid (2 mg.), estradiol benzoate (0.25 mcg. or 0.5 mcg.), or glycyrrhetinic acid (2 mg.), plus either dose of estradiol benzoate. Animals were sacrificed on the ninth postoperative day and the ovaries were weighed.

Mature Cyclic Rats.—Estrus cycles were determined by wet vaginal smears taken daily at 9:00 a.m. in mature cyclic rats. Animals were only used after three consecutive normal cycles had been observed. Glycyrrhetinic acid (4 mg.) or vehicle (0.4 ml.) was administered three times a week for 1 month. There were 16 animals in each group.

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[1] β-Glycyrrhetinic acid, supplied by S. B. Penick and Co., New York, N. Y.

[2] Estradiol benzoate, supplied by Organon, Inc., Nutley, N. J.

TABLE I.—EFFECT OF PROLONGED ADMINISTRATION OF GLYCYRRHETINIC ACID ON THE UTEROTROPHIC RESPONSE OF IMMATURE RATS

Group	G.A.,[a] mg.	E.B.,[b] mcg.	N	Uterine Wt., mg. ± S.E.	p
1	0	0	7	40.69 ± 3.85	...
2	16	0	8	42.34 ± 3.27	...
3	0	0.3	16	110.45 ± 4.54	...
4	16	0.3	15	72.20 ± 6.59	<0.001

[a] Glycyrrhetinic acid (2 mg.) or vehicle (0.2 ml.) s.c. daily for 6 days with a double dose on the seventh day. [b] Estradiol benzoate 0.1 mcg. s.c. daily during the last 3 days of treatment. Sacrificed on eighth day.

TABLE II.—ASSAY OF ACTH FROM PITUITARIES OF UNTREATED AND GLYCYRRHETINIC ACID TREATED IMMATURE FEMALE RATS

Pituitary,[a] 0.1/100 Gm. B.W.	N[d]	Adrenal Ascorbic Acid, mcg./100 mg.
None	8	412.11 ± 17.94
Controls[b]	7	325.11 ± 21.27
G.A. treated[c]	8	333.90 ± 27.93

[a] Pituitary suspension of 0.1 pituitary/0.5 ml. of 0.01 *N* HCl in saline made by grinding one pituitary in 0.5 ml. of 0.1 *N* HCl and diluting to 5 ml. with 4.5 ml. of physiological saline. Administered s.c. to hypophysectomized Sprague-Dawley rats 48 hours postoperatively. Adrenal ascorbic acid determined 3 hours after injection. [b] Pituitaries from animals receiving vehicle (0.2 ml. daily for 6 days with double dose on seventh day). [c] Pituitaries from animals receiving glycyrrhetinic acid (2 mg. daily for 6 days with double dose on seventh day). [d] N represents the number of pituitaries tested. Each pituitary was administered to two test animals.

TABLE III.—OVARIAN RESPONSES OF FEMALE RECIPIENT PARABIONTS

Group	G.A.,[a] mg.	E.B.,[a] mcg.	N	Ovarian Wt., mg. ± SE.
1	0	0	6	42.67 ± 2.86
2	2	0	6	37.03 ± 8.47
3	0	0.5	9	12.64 ± 0.80
4	2	0.5	7	14.11 ± 0.63
5	0	0.25	6	14.00 ± 1.40
6	2	0.25	5	11.72 ± 2.88

[a] Daily dose administered s.c. to male castrated donor starting on the day of surgery. Controls received an equivalent volume of vehicle. Sacrificed on ninth postoperative day.

RESULTS AND DISCUSSION

Immature Female Rats.—The uterine growth response to estradiol benzoate was significantly reduced by prolonged administration of glycyrrhetinic acid (Table I). This previously determined pituitary-adrenal blocking dose (3) did not reverse the antiestrogenic effect and had no effect on the unstimulated uterus (Table I).

Seven days of treatment with glycyrrhetinic acid had no effect on the pituitary-ACTH content (Table II). Pituitaries from the treated animals produced the same degree of adrenal ascorbic acid depletion as pituitaries from control animals (Table II). An increase in the resting adrenal ascorbic acid reported at this dosage schedule (3) might have been due to a decreased secretory rate or decreased adrenal responsiveness to the ACTH secretory rate. It certainly cannot be attributed to decreased ACTH reserve or synthesis.

Gonadotropin Release in Male Castrated Donor Parabionts With and Without Estrogen.—Ovarian weights of female recipients receiving gonadotropin from untreated male donors were equal to those receiving gonadotropins from glycyrrhetinic acid treated donors (Table III). Estradiol benzoate administered to male donors depressed the ovarian response of female recipients even when glycyrrhetinic acid had been simultaneously administered (Table III). Glycyrrhetinic acid therefore did not interfere with gonadotropin release nor the estrogenic inhibition of gonadotropin release in male donors. It can only exert an antiestrogenic effect on the response of the female accessory reproductive tract and not the pituitary target organ. This confirms the previous results (1) of lack of interference with estrogenic adrenal stimulation and estrogenic testicular atrophy.

Mature Cyclic Rats.—One month of glycyrrhetinic acid treatment did not alter the estrus cycles of mature rats. Normally established cycles continued throughout the entire period of treatment. Alteration of the estrus cycle could not have been anticipated on the basis of gonadotropin inhibition. These results indicate that glycyrrhetinic acid does not interfere with the response of the vaginal mucosa to endogenous sex steroids. The lack of antagonism of endogeneous sex steroids previously reported (2) and reproduced here infers that the mechanism of action as an estrogen antagonist lies in interference with absorption or metabolism of exogenous estrogen. This apparentiy can only influence the target organs of the accessory reproductive tract. For some reason still unexplained, there is no alteration in the response of the pituitary target organ to exogenous estrogen.

SUMMARY AND CONCLUSIONS

Prolonged administration of glycyrrhetinic acid to immature female rats did not reverse the antiestrogenic effect, did not alter the pituitary-ACTH content, had no effect on the unstimulated uterus, and antagonized the uterine response to exogenous estrogen.

Glycyrrhetinic acid administered to male castrated donor parabionts for 8 days did not interfere with gonadotropin release nor estradiol inhibition of gonadotropin release.

One month of glycyrrhetinic acid treatment did not alter the estrus cycles of mature cyclic rats.

Glycyrrhetinic acid therefore restricts the response of the uterus to exogenous estrogen regardless of duration of treatment. Its antagonism is limited to the response of the female accessory reproductive tract to exogenous estrogen and does not extend to other target organs such as the pituitary. It cannot alter estrus cycles either through interference with gonadotropin release or alteration in the response of the vaginal mucosa to exogenous sex steroids.

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