# EFFECT OF ACTH ON ADRENAL ESTROGENS

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**Abstract**—Tissue levels of rat adrenal estrogens during the estrous cycle were determined by radioimmunoassay, and effect of ACTH on the adrenal estrogen levels was examined. The levels of estrone and estradiol in proestrus were 2.75±0.61 pg/mg tissue and 1.0± 0.19 pg/mg tissue (mean±SE), respectively. The higher level of estrone but not of estradiol was inclined to appear in proestrus rather than in other phases. Injection of 250 IU/kg of $\alpha^{1-24}$-ACTH significantly increased the estrone level at 15 and 30 min, and estradiol at 15 min. Administration of reserpine, which was used as the ACTH releaser, produced an increase in the estrone level but did not change the estradiol level. While dexamethasone injection decreased the estrone level significantly at 8 hours after the treatment, the level of estradiol remained unchanged. Hypophysectomy produced a considerable decrease in both steroid levels, particularly estrone. These results indicate that the major estrogen in the rat adrenal gland is estrone and that the level is regulated by hypophyseal ACTH.

It has been reported that the plasma or urinary estrogen level remained unchanged in ovariectomized and postmenopausal women, and also that the level increased in subjects with adrenocortical tumor or hyperplasia. In addition, it has been reported that estrogens were produced in adrenal glands besides the ovary, on the basis of the increase of plasma or urinary estrogens with ACTH treatment and decreases seen with dexamethasone treatment (1–5). However, a distinct function of the rat adrenal estrogens has not been clarified. Our study was carried out to determine the level of estrogens in rat adrenal gland during estrous cycle. The interaction between ACTH and adrenal estrogens was also examined using reserpine as the ACTH releaser and both dexamethasone injection and hypophysectomy as ACTH depressor, in order to provide more information on the rat species.

## MATERIALS AND METHODS

Adult, female, Wistar rats weighing 200 g, were housed in a room with controlled temperature (24±1°C) and light (from 6:30 a.m. to 6:30 p.m.). Food and water were given freely. Rats with 3–4 successive regular 4-day estrous cycle were used, and were decapitated at 11:00 a.m., in all the experiments. Rats were used for 48 hr after hypophysectomy by the external auditory canal method, under ether anesthesia. There were 10 rats per group in all the experiments.

After $\alpha^{1-24}$-ACTH (Daiichi Pharmaceutical Co. Ltd.) had been given s.c., in a dose of 250 IU/kg, dissolved in physiological saline, the rats were sacrificed at 15, 30, and 60 min with minimum disturbance. Dexamethasone phosphate (Sankyo Co. Ltd.) was also given s.c. in a dose of 100 μg/kg, and the rats were sacrificed at 2, 4, 8, and 24 hr after adminis-

tration. Control rats were given a comparable volume of the vehicle. Reserpine (Nippon Chemiphar Co. Ltd.) dissolved in 0.2% carboxymethylcellulose (CMC) was administered p.o. in a volume of 1 ml, and the rats were sacrificed at 1, 3, 6, and 18 hr after this administration. The control rats were treated with 0.2% CMC only.

Four adrenal glands, removed and cleaned from fat, were weighed and immediately homogenized in 4 ml of ice-cold 30% ethanol solution. The homogenate was centrifuged at 2000 rpm for 10 min and 3 ml of the supernatant was extracted twice with 4 ml of dichloromethane. The extract was evaporated to dryness and the residue was chromatographed over Sephadex LH-20 ($12\times0.5$) using benzene: methanol (85:15) as an elution solvent for separation of estrone and estradiol (6). Estrone and estradiol were measured by radioimmunoassay using anti-estrone or anti-estradiol serum (Teikoku Zoki Pharmaceutical Co. Ltd.). The extraction and chromatographic recovery of estrone [6, 7-$^3$H] and estradiol [6, 7-$^3$H] (New England Nuclear Corp.) was $85\pm4.4$% and $81\pm4.0$% ($N=10$), respectively. These methodological losses were corrected. All samples from each experiment were measured within a single assay and the values are shown in mean$\pm$SE.

## RESULTS

The result of estrogen determination in estrous cycle is given in Table 1. The estrone level was approx. 3 times higher than that of estradiol. A low level of estriol was detected ($0.21\pm0.057$ pg/mg tissue) throughout the estrous cycle ($N=10$, data not shown). Estrone but not estradiol tended to exhibit a regular high level in proestrus rather than in other phases. Fig. 1 shows the effect of ACTH on adrenal estrogen levels. ACTH injection induced a significant increase in both steroid levels. Estrone level was increased 318% and 351% of the control at 15 and 30 min, respectively. The estradiol level was also increased 246% of the control at 15 min, but rapidly reached the control level by 30 min. The effect of reserpine, which was used as an ACTH releaser, on adrenal estrogen level is demonstrated in Fig. 2. Estrone level increased at 3 and 6 hr after the injection while estradiol level remained unchanged. The effect of dexamethasone injection on adrenal estrogen level is shown in Fig. 3. The estrone level decreased gradually and reached a maximum supression of 23% of the control at 8 hr, and recovered to 2- and 4-hr levels at 24 hr. In contrast, estradiol was inhibited little in rats treated with dexamethasone. Fig. 4 also shows the depression of adrenal estrogen levels by hypophysectomy. Levels of adrenal estrogens were so low after hypophysectomy that adrenals collected from 10 rats were used as 1 sample and

TABLE 1. Level of adrenal estrogens during estrus cycle

| Phase of cycle | Estrone (pg/mg tissue) | Estradiol |
|---|---|---|
| Prosetrus | $2.75\pm0.61$ | $1.0\pm0.19$ |
| Estrus | $2.32\pm0.70$ | $0.8\pm0.08$ |
| Metoestrus | $2.61\pm0.51$ | $0.8\pm0.12$ |
| Diestrus | $2.02\pm0.75$ | $0.8\pm0.15$ |

The values are the mean$\pm$SE of 10 animals.

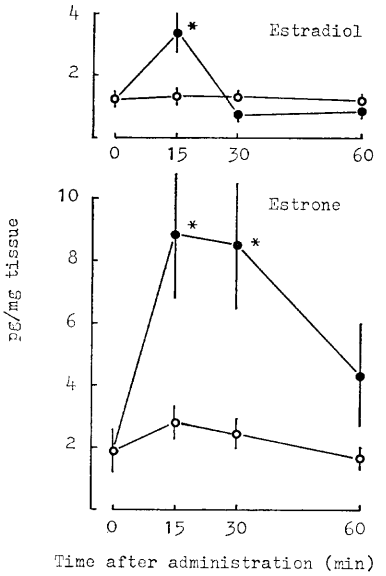


FIG. 1. Effect of ACTH on adrenal estrogen levels. ACTH (250 IU/kg) was given s.c. ●——● ACTH, ○——○ Control. Each point represents the mean of 10 animals. Vertical bar indicates SE of the mean. Significantly different from the control at *P<0.05.

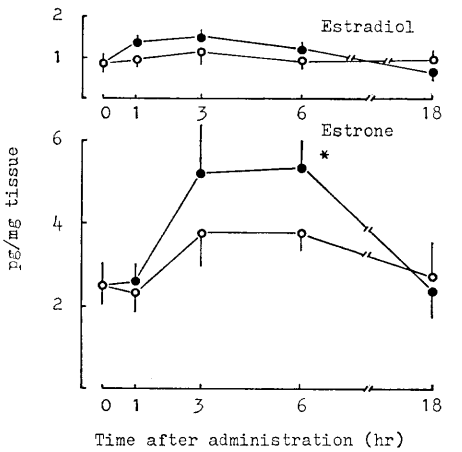


FIG. 2. Effect of reserpine on adrenal estrogen levels. Reserpine (50 mg/kg) was administered p.o. ●——● Reserpine, ○——○ 0.2% CMC. Each point represents the mean of 10 animals. Vertical bar indicates SE of the mean. Significantly different from the control at *P<0.05.

the mean is given in Fig. 4. Estrone and estradiol levels decreased considerably 48 hr hypophysectomy. The former was inhibited from 2.43±0.64 pg/mg tissue of the control level to 0.11 pg/mg tissue and the latter from 1.48±0.15 pg/mg tissue in the control to 0.20 pg/mg tissue.

## DISCUSSION

The present study demonstrated that the major functional estrogen in rat adrenal gland was estrone. This result is in agreement with other reports, except for species differences in rats, humans (7), and Rhesus monkey (8). Levels of estrone were higher during proestrus than in other phases. Shaikh and Shaikh (9) reported that estradiol rather than estrone in rat adrenal venous blood was at a high level during proestrus. This result suggested that cyclic changes may be influenced by reproductive hormones excreted from the ovary, similar to the reports on the function of adrenal corticosterone or progesterone (10–13). Recently, an estradiol receptor was found in adrenal glands from rats and mice (14, 15).

$\alpha^{1-24}$-ACTH injection induced a marked increase in the levels of both estrone and estradiol (Fig. 1). The increase and duration of the high level were much larger than that seen with estradiol. Simultaneous determination of corticosterone after 250 IU/kg of ACTH showed a maximum level of corticosterone, also at 15 min (data not shown). Since ACTH stimulates corticosteroid production in the biosynthetic reaction from cholesterol to pregnenolone (16),

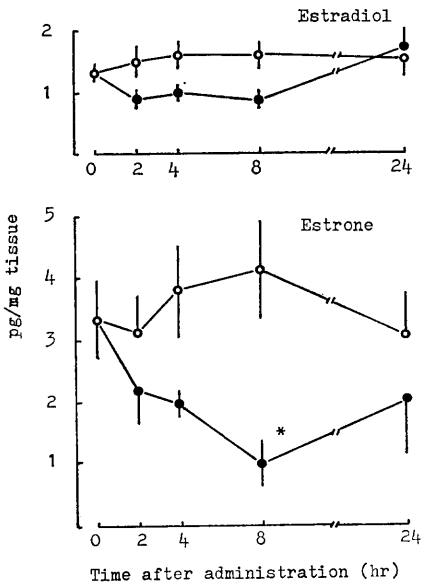


FIG. 3. Effect of dexamethasone on adrenal estrogen levels. Dexamethasone (100 μg/kg) was given s.c. ●——● Dexamethasone, ○——○ Control. Each point represents the mean of 10 animals. Vertical bar indicates SE of the mean. Significantly different from the control at *P<0.05.

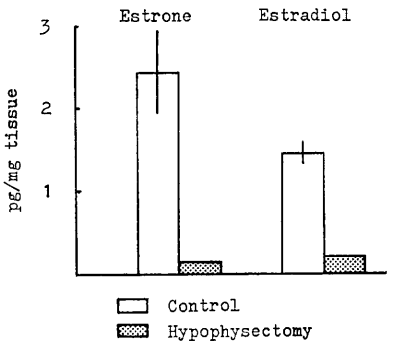


FIG. 4. Effect of hypophysectomy on adrenal estrogen levels. Hypophysectomy was carried out 48 hr before the experiment.

it seems reasonable that ACTH would stimulate adrenal estrogens at the same site as in the case of corticosterone. Moreover, ACTH increases adrenal androstenedione rather than testosterone, and estrone may be converted from androstenedione in normal adrenal gland of rats. In this respect, Frieden et al. (17), using rat tissue slices, reported that aromatization of androstenedione occurred in the adrenal gland.

As shown in Fig. 4, reserpine increased adrenal estrone at 3 and 6 hr. It was reported that reserpine can stimulate a powerful and prolonged secretion of ACTH (18–20). Montanari and Stockham (19) reported that a single dose of 2.5 mg/kg of reserpine raised the plasma and adrenal corticosterone and that these effects did not occur in the hypophysectomized rat. Thus, it can be assumed that reserpine has no direct action on the adrenal gland but does act as a stimulator for ACTH. Reserpine also stimulates prolactin (21, 22), and it was reported that human prolactin rather than ACTH stimulated estrogen production, especially estradiol, by feminizing adrenal neoplastic cells (23). This finding indicates, however, that such action of prolactin may exist under pathological conditions.

Adrenal suppression by dexamethasone treatment (24) and experimental states of hypophysectomy (25) further confirmed that adrenal estrogens were regulated by hypophyseal ACTH. Concerning depression by dexamethasone injection, it was reported that urinary estrone and estradiol levels were inhibited 55% and 2%, respectively, in postmenopausal women (2). Thus, the physiological role of adrenal estrogens remains to be defined.

In conclusion, the present results indicate that the main adrenal estrogen is estrone, and that the level was regulated by hypophyseal ACTH, similar to the case with corticosterone.

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