Testosterone Inhibition of Estradiol-Induced Stimulation of Adrenal 11β- and 18-Hydroxylation (34504)

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The regulatory roles of estrogen in corticosteroidogenesis have been studied in numerous research efforts and found to be both stimulatory (1, 2) and inhibitory (3–5). Androgens are known to effect adrenal (6, 7) and liver hydroxylation of steroids (8, 9). Sex differences in adrenal size (10), steroid half-life (11, 12), and plasma steroid content (11) are well known. The reasons for these differences involve varied abilities to produce, maintain, and metabolize corticosteroids, but the basic mechanisms underlying the differences elude definitive exposition. The present paper presents studies on one aspect of steroidogenesis, namely 11β- and 18-hydroxylation of DOC by adrenal homogenates after *in vivo* administration of testosterone and 17β-estradiol and suggests an antagonism between the actions of these two hormones on hydroxylation by adrenal mitochondria.

Materials and Methods. Alternating left and right control adrenals were removed from Sprague-Dawley or Holtzman rats and pooled. Immediately thereafter each animal received an iv injection of 1 μg 17β-estradiol in 2 μl of propylene glycol and 0.25 ml normal saline. Thirty minutes later contralateral glands were removed, pooled, and homogenized in 0.25 M sucrose to a 0.5% adrenal suspension. Control or estradiol-treated adrenal homogenates were added to incubation flasks to give a final concentration of 1 mg adrenal per ml of incubation medium which was composed of 8 mM phosphate buffer (pH 7.3–7.4), 3.85 mM KCl, 0.9 mM $MgCl_2$, 3.85 mM NaCl, and 20 mM succinate. All preparatory steps were performed at 0–4°. Incubation flasks were preincubated 5 min with shaking at 37° immediately after addition of homogenate. Deoxycorticosterone (DOC) (2 μM, 0.005 μCi/flask) was then added and incubation proceeded for 8 min, after which metabolic activity was terminated by addition of 3 vol of cyclohexane. Samples were shaken and the cyclohexane extract removed, dried, and counted in a Packard Tricarb liquid scintillation spectrometer. The aqueous phase was then again similarly treated with 3 vol of chloroform. Duplicate extractions were performed previously in which the incubation products were separated by paper chromotography in a benzene: formamide system. Comparison of the two methods showed the cyclohexane extract to contain DOC and the chloroform fraction to contain corticosterone (B) and 18-OH DOC. Recoveries were 93.2 ± 1.9% for the DOC and 92.1 ± 2.5% for the B and 18-OH DOC (13).

Percentage conversion to B and 18-OH DOC was used to calculate nanomoles of DOC converted per mg protein per minute. Protein determination was by the method of Lowry *et al.* (14).

Orchiectomies were performed via midline scrotal incision. Depot testosterone[1] was administered immediately after surgery, which was 7 to 17 days prior to removal of the adrenals.

Results. Adrenal homogenates from intact female rats treated with 1 μg 17β-estradiol iv 30 min prior to sacrifice hydroxylated more DOC than control adrenals from the same animals, the average values for 10 experiments being respectively 0.62 and 0.75

[1] Depo-Testosterone Cypionate, The Upjohn Company, Kalamazoo, Michigan.

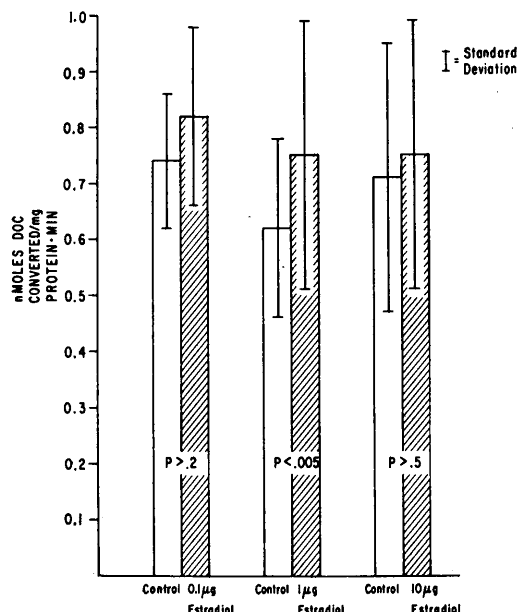

FIG. 1. Amount of 18-OH DOC and corticosterone produced by incubating DOC with adrenal homogenates from female rats. Control values represent results from 8-min incubations with pools of adrenal homogenates taken prior to iv injection of 0.1, 1.0, and 10.0 μg of estradiol. The estradiol values were obtained by incubating DOC for 8 min with glands removed 30 min after hormone injection.

nmoles of DOC converted per mg protein per min ($p<.0005$) (Fig. 1). The amount of estradiol appears to be critical since neither 0.1 μg nor 10 μg caused a significant increase in hydroxylation, with values of 0.74 and 0.82 nmoles DOC converted per mg protein per min for the lower dose ($p>.2$) and values of 0.71 and 0.75 for the higher dose ($p > .5$) (Fig. 1).

When the amount of estradiol which stimulated steroidogenesis in the female (1 μg) was administered to male rats, the stimulation of hydroxylation was not seen (Table I). Control adrenals converted 0.88 nmoles of DOC per mg protein per min while estradiol-treated glands converted 0.92, a difference which is not statistically significant ($p = 0.5$). However, if the male rats were castrated 7–17 days prior to the experiment, the stimulatory effect was apparent (Table I). In this situation, control adrenals converted 0.75 nmoles DOC per mg protein per min whereas estradiol-treated glands converted 0.90 nmoles. This difference is significant below the .025 level. Moreover, if the castrated males received replacement therapy in the form of 10 mg of depot testosterone[1] at the time of orchiectomy, the stimulatory effect disappeared (Table I). The conversions were then 0.87 for controls and 0.81 for estradiol-treated rats. In addition to this, female rats treated with 10 mg of long-acting testosterone also lost the ability to respond to acute dosages of estradiol (Table I). These animals converted 0.77 (control) and 0.83 (estradiol-treated) nmoles DOC per mg protein per min ($p > .2$).

Discussion. The ability of intravenously administered estradiol to stimulate 11β- and 18-hydroxylation by adrenal glands *in vitro* has been reported previously (13). The relationship between estradiol and steroidogenesis has been known for many years and demonstrated in a great variety of situations. Sprunt and Nelson (15), in a system very similar to ours, showed increased conversion of DOC to B by adrenals from rats treated chronically with ethinyl estradiol. Fonzo *et al.* (16) demonstrated that 10^{-5} *M* estradiol added *in vitro* stimulated hydroxylation of

TABLE I. Hydroxylation of DOC by Male and Female Rat Adrenal Homogenates.

Sex	Condition	Treatment	Average nmoles DOC converted per mg protein/min				p
			Control ± SD		Estradiol ± SD		
Female	Intact		0.62	.16	0.75	.24	<.005
Male	Intact		0.88	.20	0.92	.21	.5
Male	Castrated		0.75	.068	0.90	.079	<.025
Male	Castrated	10 mg testosterone	0.87	.22	0.81	.14	>.2
Female	Intact	10 mg testosterone	0.77	.13	0.83	.084	>.2

DOC by adrenal glands in a succinate-supported system. These results have been extended to show that estradiol can act acutely and at a much lower level *in vivo* than *in vitro*.

The narrow dose range of estradiol possessing this stimulatory effect is not surprising. The failure of a larger dose of estradiol to increase hydroxylation appears to agree with Kitay who reported (11) that adrenal venous B production was 2.5 times greater in the female than in the male at rest, but that administration of estradiol caused a diminution of production by the female. Moreover, B production by adrenal slices from female rats was also diminished after estradiol administration *in vivo* while that from males was enhanced (1). He concluded that low levels of estradiol were stimulatory to steroidogenesis, whereas high levels were inhibitory.

The loss of the estradiol effect in male rats could be explained in two ways. Either the adrenal requires the constant presence of moderate levels of circulating estradiol to maintain it in a condition in which it is responsive to the estrogen, or testosterone exerts an antagonistic action. The fact that orchiectomy restores the responsiveness to acute estradiol administration favors the latter explanation, as does the finding of a depression of the effect after testosterone administration in intact females and castrated males. Rembiesa (6) observed that 17α-methyltestosterone suppressed corticosterone formation *in vitro* by rat adrenal glands and that two synthetic testosterone derivatives decreased the conversion of progesterone to aldosterone, 18-OH DOC, 18-OH B, and B by more than half (7).

The exact nature of the antagonistic effect of androgenic hormones on the hydroxylating mechanism is in doubt. Kato and Takanaka (17), investigating liver microsomal hydroxylating capacity, found increased inactivation of hexobarbital in male vs female rats. Paralleling this was an increased content of cytochrome P-450. Skelton *et al.* (18) found decreased levels of cytochrome P-450 in adrenal mitochondria obtained from rats treated with large amounts of androgen. The mitochondria are the sites of 11β- and 18-hydroxylation of steroids and, in fact, the decreased P-450 occurred concomitant with diminished mitochondrial 11β- and 18-hydroxylation. Testosterone could be postulated to prevent the estradiol induced increase in steroid hydroxylation by diminishing P-450 formation. If P-450 represents a rate limiting step, this would produce an impediment to any stimulatory effect of estradiol occurring at P-450 or some earlier point in the hydroxylation pathway.

There are effects of estradiol, however, which cannot be readily explained on the basis of testosterone antagonism. Sprunt *et al.* (19) and Mestman and Nelson (20) have demonstrated an antagonism of estradiol to the methopyrapone-induced block of 11β-hydroxylation. Yates *et al.* (12) found ring A reductase in the livers of female rats to be more active than in males. Kitay has shown that female rats have higher levels of plasma B than male rats, both at rest and in response to stress, and that oophorectomy lowers this level (10). In our experiments, estradiol stimulated hydroxylation of DOC in castrated male rats as well as in intact females. Estradiol may combine with cytochrome P-450 as is suggested by our findings of spectral changes in rat adrenal mitochondria (trough 388, peak 423) on addition of estradiol. The evidence therefore suggests a direct effect of estradiol on steroid hydroxylation which is antagonized by testosterone.

Summary. Adrenal homogenates from intact and castrated male and female rats treated with 1 μg 17β-estradiol iv were tested for ability to convert exogenous DOC to B and 18-OH DOC. Estradiol, which causes a significant increase in hydroxylating capacity in intact female rats, produced no such increase in intact males. Orchiectomized males, however, responded to estradiol with increased ability to hydroxylate. The estradiol response was lost after administration of testosterone to both orchiectomized males and intact females.

The authors thank Naila Asali, Robert Clubb, Robert Hutchins, and Thea Kay Young for their excellent technical assistance. This work supported by USPHS grants #AM11605, and AM05604 and by American Cancer Soc. Grant #P-506.

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Received Oct. 13, 1969. P.S.E.B.M., 1970, Vol. 133.