Effects of Testosterone and Estradiol *in Vivo* on Hepatic Cortisol Metabolism *in Vitro*[1] (36956)

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(Introduced by Thomas F. Dougherty)
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Testosterone and estradiol are known to exert essentially opposite effects on the hepatic metabolism of drugs and steroid hormones. Testosterone stimulates the activity of steroid side chain reductases and inhibits the activity of A-ring reductases, while estradiol has the reverse effect (1, 2). Androgens have also been shown to stimulate the activity of the mixed function oxidase system that is responsible for the oxidative metabolism of drugs and steroids (3, 4), while estradiol inhibits the androgen-induced activity (3) and prolongs the half-life of circulating corticosteroids (5). After treatment of castrated rats with depot testosterone, Kato *et al.* (3) and Jacobson *et al.* (6) demonstrated increased hydroxylation of testosterone and progesterone, while Jellinck *et al.* (7) observed increased hydroxylation of estradiol. The corticosteroids may also be hormone sensitive substrates for microsomal enzymes since increased 6β-hydroxycortisol[4] excretion has been observed in the neonate (8), in pregnant women (9), and in patients with a variety of diseases (10–12). The present investigation has attempted to determine the effects of the sex hormones on the metabolism of cortisol to more highly polar derivatives.

Materials and Methods. Livers were obtained from 200–250 gram male and female Holtzman rats. Castrations were performed two weeks prior to sacrifice and subcutaneous injections of 1.0 mg of depot estradiol or 5.0 mg of depot testosterone in vegetable oil were given one week prior to sacrifice. Control animals received an equal volume of vegetable oil. Animals were sacrificed by decapitation and their livers removed, rinsed, and placed in ice cold 0.25 *M* sucrose. All procedures prior to incubation were carried out at 0–5°.

Incubation flasks with 10.0 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, contained 10^{-2} *M* glucose-6-phosphate (Sigma), 10^{-3} *M* TPN (Sigma) and 3.45 μM cortisol-4-^{14}C (0.1 μCi). Two hundred mg pieces from each liver were minced and incubated for 5 hr with gentle shaking in a 37° water bath, under an atmosphere of 95% O_2 and 5% CO_2. Incubations were terminated by the addition of five drops of glacial acetic acid. Steroid products were extracted twice with two volumes of ethyl acetate, dried under N_2 and chromatographed in the Y system of Frantz *et al.* (13). Radioactive metabolites were counted in an Actigraph III (Nuclear-Chicago) strip counter with a digital integrator and per cent transformations calculated. Recovery of total 4-^{14}C steroid, labeled 6β-hydroxycortisol, and labeled β-cortol[4] was determined by extracting known amounts of authentic steroids from a system deficient in substrate and counting them in a Packard Tricarb liquid scintillation counter.

The identity of steroid metabolites was

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[3] Work done in partial fulfillment of requirements for the Ph. D. degree in the Department of Anatomy, University of Utah, during tenure as a Title IV National Defense Education Act Predoctoral Fellow.

[4] Trivial names used: 6β-hydroxycortisol = 4-pregnen-6β, 11β, 17α, 21-tetrol-3, 20-dione; β-cortol = 5β-pregnan-3α, 11β, 17α, 20β, 21-pentol.

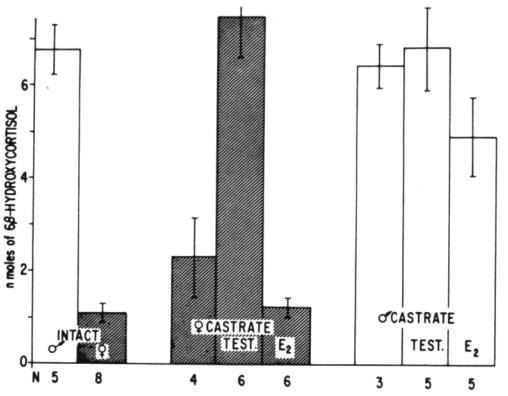

FIG. 1. The effect of testosterone and estradiol on the formation of 6β-hydroxycortisol by liver minces from intact rats and rats castrated two weeks prior to sacrifice. Treatment consisted of a single depot injection of either 1.0 mg estradiol or 5.0 mg testosterone one week prior to sacrifice. Controls received an equal volume of vegetable oil. Bars represent mean nmoles of 6β-hydroxycortisol formed $\pm$ 1 SE.

established by comparing mobilities with authentic compounds in 4 paper and 2 thin-layer chromatographic systems and with authentic acetates in 2 paper chromatographic systems.

Hepatic reticuloendothelial (R.E.) and parenchymal cells from female castrates were separated by a modification (14) of the procedure of Rous and Beard (15). Equal volumes of packed cells were used in each incubation. Microsomal P-450 was measured by the method of Omura and Sato (16), and protein was determined by the method of Lowry *et al.* (17).

Results. Recoveries of 94% for 6β-hydroxycortisol and 95% for β-cortol were obtained from male or female liver minces. Total recovery of radioactive steroid was 86% for males and 85% for females. Thus sex differences found in cortisol metabolism were not due to differential extraction of steroid products. Formation of 6β-hydrocortisol and β-cortol was linear up to three hours.

There was a highly significant sex difference in the production of 6β-hydroxycortisol (Fig. 1), with 6.8 nmoles per flask produced by male livers and only 1.1 nmoles by livers from females ($p < 0.005$). In female castrates testosterone exerted a stimulatory effect, increasing 6β-hydroxylation from 2.3 nmoles to 7.5 nmoles ($p < 0.005$). In males, however, castration (6.4 nmoles, $p > 0.4$) and testosterone (6.8 nmoles, $p > 0.5$) had no effect. Up to 120 days following orchidectomy no decrease in 6β-hydroxylase activity was observed compared to intact controls of the same age. Estradiol did not have a significant effect on the 6β-hydroxylase activity of castrated males ($p > 0.1$) or females ($p > 0.2$).

Liver minces from intact males produced 10.1 nmoles of β-cortol per flask (Fig. 2) compared to 1.7 for females ($p < 0.005$). Testosterone administration to female castrates stimulated β-cortol formation (7.6 nmoles vs 4.2 nmoles, $p < 0.025$). Estradiol on the other hand inhibited its formation in castrated males and females. Livers from ovariectomized females produced 4.2 nmoles of β-cortol compared to 1.7 nmoles for intacts ($p < 0.025$). Treatment of female castrates with estradiol resulted in a significant decrease (1.15 nmoles, $p < 0.025$) in β-cortol formation. Treatment of male castrates (9.0 nmoles) with estradiol also resulted in a decrease in β-cortol formation to 2.4 nmoles ($p < 0.005$). Although a two-week period following orchidectomy was insufficient to alter the ability of liver minces to produce β-cortol, 120 days following castration its formation was significantly de-

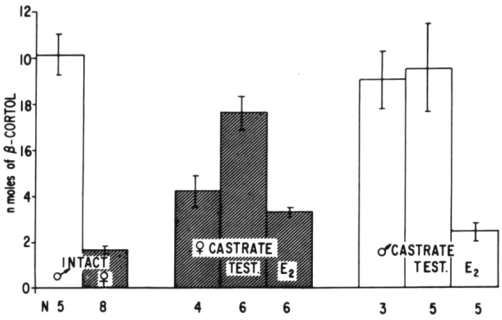

FIG. 2. The effect of testosterone and estradiol on formation of β-cortol by liver minces from intact rats and rats castrated two weeks prior to sacrifice. Treatment consisted of a single depot injection of either 1.0 mg estradiol or 5.0 mg testosterone one week prior to sacrifice. Controls received an equal volume of vegetable oil. Bars represent mean nmoles of β-cortol formed $\pm$ 1 SE.

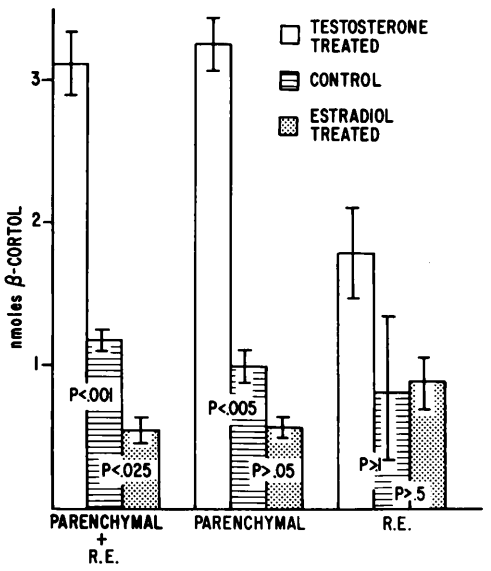

FIG. 3. Effect of testosterone and estradiol on the formation of 6β-hydroxycortisol hy hepatic parenchymal and reticuloendothelial cells. Each bar represents the mean nmoles 6β-hydroxycortisol formed ± 1 SE. The mean includes 3 separate incubations of liver cells from female rats which were castrated two weeks prior to sacrifice and received 5 mg of depot testosterone, 1 mg of depot estradiol or vegetable oil sc one week prior to sacrifice.

creased from 9.7 to 4.6 nmoles per flask ($p < 0.005$).

No difference in the production of 6β-hydroxycortisol or β-cortol by liver minces was seen when incubations were carried out in the presence of 10^{-8} *M* or 10^{-5} *M* estradiol or 10^{-8} *M* testosterone *in vitro*. 10^{-5} *M* testosterone prevented any steroid metabolism in this system.

The hepatic parenchymal cell was the site of the metabolic activities leading to 6β-hydroxylation and β-cortol formation. Parenchymal cells alone metabolized more cortisol than preparations of mixed parenchymal and R.E. cells containing equal volumes of packed cells. Although the R.E. cell fraction also converted small amounts of cortisol, the preparations all contained small numbers of parenchymal cells. Testosterone stimulated 6β-hydroxycortisol production in the parenchymal cell (8.28 nmoles per flask vs 0.67, $p < 0.001$) but had no such effect on the R.E. cell (1.39 nmoles vs 0.49 nmoles, $p >$ 0.2) (Fig. 3). The effects on β-cortol production were essentially the same as in Fig. 3, with all levels being proportionately lower.

Microsomes from male rats contained 2.79 nmoles cytochrome P-450/mg protein while those from females contained 1.58 nmoles/mg protein ($p < 0.005$). Neither 2 weeks oophorectomy ($p > 0.5$) nor 1 week of testosterone treatment ($p > 0.5$) influenced the cytochrome P-450 level.

Discussion. The present investigation has demonstrated that the effect of the sex hormones on the hydroxylation of cortisol is similar to that reported for the hydroxylation of testosterone, progesterone (3, 4) and estradiol (7). Testosterone stimulated the production of 6β-hydroxycortisol in castrated female rats, but had no effect in male castrates. Presumably, once established, the 6β-hydroxylase system requires very little testosterone for maintenance or is metabolized very slowly since it does not show diminished activity 120 days after orchidectomy.

A more definitive effect of the gonadal hormones was seen in the formation of the totally reduced metabolite, β-cortol, which is stimulated by endogenous testosterone in intact males and by administered testosterone in female castrates, and which is depressed by endogenous estradiol in intact females and by exogenous estradiol in castrates of both sexes. These results suggest that testosterone is capable of stimulating a rate-limiting enzyme in the reduction of cortisol. The lack of effect of estradiol or testosterone *in vitro* suggests either a long induction time for the enzymes for steroid hydroxylation and reduction or that factors lacking in the *in vitro* system which are present in the animal are required for increased activity.

Previous investigations have revealed significant differences in the capacity to reduce the A-ring and conjugate with glucuronic acid between the hepatic parenchymal cell and the hepatic reticuloendothelial cell (14, 18). The present investigation presents further evidence of differences in that only the hepatic parenchymal cell was capable of converting cortisol to β-cortol and 6β-hydroxycortisol. The experimental evidence that differences in microsomal content of cytochrome P-450 can not account for the several-fold

increases in 6β-hydroxylase activity support similar findings by Kato *et al.* (3), Schenkman *et al.* (19), and Jacobson and Kuntzman (6) that factors other than the level of cytochrome P-450 determine the activity of the mixed function oxidase system.

Summary. Estradiol inhibited the ability of the liver parenchymal cell to transform cortisol to reduced metabolites whereas testosterone increased both reductive and oxidative metabolism of cortisol. The stimulatory effect on 6β-hydroxylation was not due to an increase in cytochrome P-450 content.

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