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Hormonal regulation of 3α-hydroxysteroid/dihydrodiol dehydrogenase in rat liver cytosol

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Abstract—In rat liver, dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase activities are catalyzed by the same protein. This study shows that estrogen and growth hormone can induce the enzyme in hypophysectomized rats. This implies that estrogen can exert an effect on hepatic steroid and carcinogen metabolism in the absence of the anterior pituitary.

Dihydrodiol dehydrogenase (DD*; EC 1.3.1.20) prevents the formation of *anti*-diol epoxides of polycyclic aromatic hydrocarbons (ultimate carcinogens) by converting their *trans*-dihydrodiol precursors to reactive *ortho*-quinones [1–3] which can be detoxified as glutathionyl and mercapturic acid conjugates [4, 5]. In rat liver, DD is identical to 3α-hydroxysteroid dehydrogenase (3α-HSD; EC 1.1.1.50) [6]. Understanding the mechanisms which induce 3α-HSD/DD activity may have important implications for the regulation of chemical carcinogenesis and steroid hormone metabolism. Previous studies have shown that this enzyme is not regulated by classical inducers of polycyclic aromatic hydrocarbon metabolism such as 3-methylcholanthrene [7, 8]. The present communication shows that estrogens and growth hormone have a direct effect on the induction of rat hepatic 3α-HSD/DD.

Materials and Methods

Androsterone, testosterone and progesterone were products of Steraloids (Wilton, NH). Benzenedihydrodiol (*trans*-1,2-dihydroxy-3,5-cyclohexadiene) was synthesized as described [9]. β-NAD^+ and $NADP^+$ (monosodium salts) were obtained from Pharmacia PL Biochemicals (Piscataway, NJ). Enzyme grade ammonium sulfate and sucrose were products of Schwarz/Mann, Inc. (Spring Valley, NJ). 17β-Estradiol-3-sulfate and growth hormone (bovine somatotrophin) were purchased from Sigma (St. Louis, MO).

All animals were obtained from Charles River (Wilmington, MA). Female rats were ovariectomized on day 28 and treated 2 weeks later. Immature male and female rats were hypophysectomized on day 21, and animals that demonstrated no weight gain over 10 days were then treated. Animals received growth hormone (1.5 units bovine somatotrophin s.c./day for 6 days) or a single dose of one of the following: 17β-estradiol-3-sulfate, testosterone or progesterone (1.0 mg of steroid in saline + 2% EtOH, s.c.). Animals were decapitated 48 hr after the last treatment. Control animals received vehicle alone which had no effect on enzyme activity.

Livers (1 g) from single animals were homogenized in 50 mM Tris–HCl pH 8.6, containing 250 mM sucrose, 1 mM EDTA and 1 mM 2-mercaptoethanol. Cytosol was prepared by differential centrifugation and a 40–75% ammonium sulfate fraction was prepared which precipitated all the soluble 3α-HSD/DD activity [6].

3α-HSD (androsterone oxidation) and DD (benzenedihydrodiol oxidation) activities were measured spectrophotometrically with the 40–75% ammonium sulfate fraction as described previously [9]. Reactions were initiated by the addition of the enzyme preparation. Activities were expressed as nanomoles substrate oxidized per minute per milligram protein. Protein determinations were made by the method of Lowry *et al.* [10].

Results and Discussion

We have shown that livers from adult female rats contain twice as much 3α-HSD/DD activity as adult males and that this difference is reflected by a comparable increase in enzyme protein [9]. Enzyme activity in adult female rats can be reduced to the level observed in males by ovariectomy [9], confirming previous reports that soluble 3α-HSD activity is elevated in female rat liver cytosol [11]. Furthermore, treatment of ovariectomized female rats with 17β-estradiol-3-sulfate restores enzyme activity to the level observed in normal adult female rats [9]. Administration of 17β-estradiol-3-sulfate to intact males also produced a significant increase in enzyme activity, suggesting that feminization of the enzyme phenotype has a dependence on estrogen (see Fig. 1). Similar findings have been reported for the regulation of soluble 3α-HSD in female rat kidney [12].

* Abbreviations: DD, dihydrodiol dehydrogenase, *trans*-1,2-dihydrobenzene-1,2-diol:dehydrogenase (EC 1.3.1.20); 3α-HSD, 3α-hydroxysteroid dehydrogenase, 3α-hydroxysteroid:$NAD(P)^+$ oxidoreductase (EC 1.1.1.50); benzenedihydrodiol, *trans*-1,2-dihydroxy-3,5-cyclohexadiene; androsterone, 5α-androstan-3α-ol-17-one; 17β-estradiol-3-sulfate, 1,3,5(10)-estratriene-3,17β-diol-3-sodium sulfate; GH, growth hormone; IGF-1, insulin-like growth factor I; and HYP, hypophysectomized.

Since immature female rats (day-21) have low estrogen levels and will respond to the administration of this hormone by undergoing a dramatic increase in uterine growth, the level of 3α-HSD/DD activity was measured in immature animals with the expectation that the enzyme would be uninduced. However, immature male rats were found to contain 2- to 3-fold higher levels of enzyme activity than that observed in adult males (Fig. 1). In immature females, enzyme activity was at the elevated level observed in adult females. An ontogenetic study indicated that enzyme activity rises to a peak in male animals by day 21 before it falls to reach the levels observed in adult males (Fig. 2). Collectively these data imply that in both male and female animals, 3α-HSD/DD activity is up-regulated by growth and development. Further, maintenance of the female enzyme phenotype has a dependence on estrogens.

To further examine the regulation of 3α-HSD/DD, experiments were performed using hypophysectomized immature male and female rats. These animals displayed between one-third and one-eighth of the activity present in their respective intact controls (Fig. 1). Enzyme activity was increased approximately 2-fold by the administration of growth hormone to hypophysectomized male rats or by the administration of growth hormone or 17β-estradiol-3-sulfate to female rats (Fig. 3). The specificity of the steroid hormone response was confirmed by showing that the administration of testosterone or progesterone to hypophysectomized female animals failed to increase enzyme activity. In fact, progesterone significantly decreased enzyme activity in hypophysectomized female rats.

It is our current hypothesis that estrogens and growth

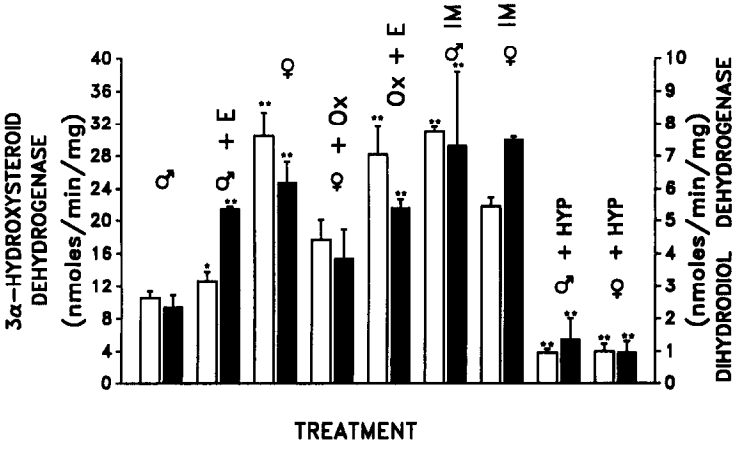

Fig. 1. Effects of sex, estrogen, age and hypophysectomy on rat hepatic 3α-HSD/DD activity. 3α-HSD (□) and DD (■) activities were measured in the 40–75% ammonium sulfate fraction of rat liver cytosol as described in Materials and Methods. Abbreviations: E, 17β-estradiol-3-sulfate; Ox, ovariectomized rats; IM, immature rats; and HYP, hypophysectomized rats. Each determination is the mean ± SD (N = 4). Statistical significance was confirmed using Student's *t*-test and was either greater than $P < 0.05$ (*) or greater than $P < 0.01$ (**). The comparisons used to reject the null hypothesis were intact adult males versus males + E or females; Ox females versus Ox females + E; adult males versus immature males; immature males and females versus hypophysectomized males and females.

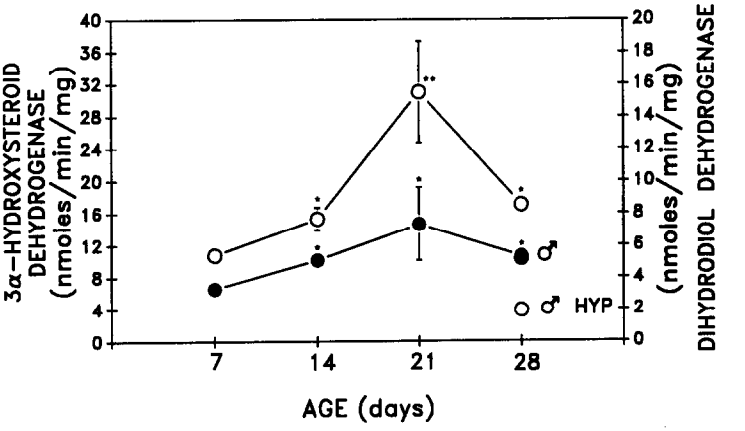

Fig. 2. Ontogenetic regulation of 3α-HSD/DD activity. 3α-HSD (○) and DD (●) activities were measured in the 40–75% ammonium sulfate fraction of male rat liver cytosol prepared from rats 7–28 days of age. The enzyme activities observed at 28 days of age were maintained throughout the life of the adult rats (data not shown). Each determination is the mean ± SD (N = 4). Statistical significance was confirmed using Student's *t*-test and was either greater than $P < 0.05$ (*) or greater than $P < 0.01$ (**). Comparisons used to reject the null hypothesis were intact adult males versus the activity observed at the age indicated. The level of androsterone activity observed in intact adult males (♂) and hypophysectomized males (♂HYP) is also shown.

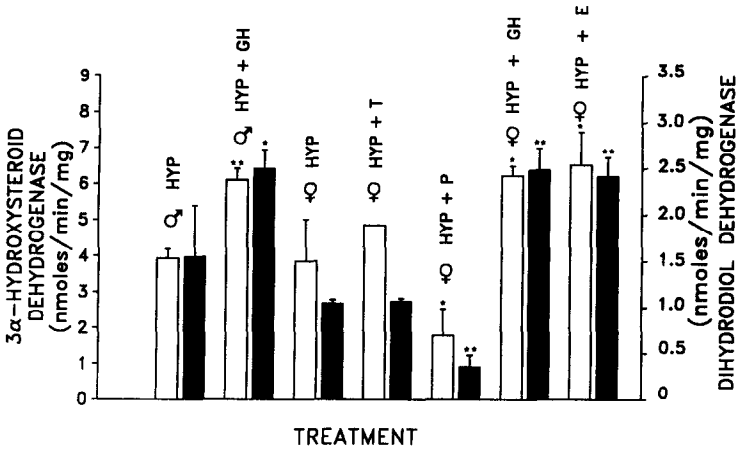

Fig. 3. Hormonal regulation of 3α-HSD/DD activity in hypophysectomized rats. 3α-HSD (□) and DD (■) activities were measured in the 40–75% ammonium sulfate fraction of rat liver cytosol as described in Materials and Methods. Abbreviations: HYP, hypophysectomy; GH, growth hormone; E, 17β-estradiol-3-sulfate; T, testosterone; and P, progesterone. Each determination is the mean ± SD (N = 4). Statistical significance was confirmed using Student's *t*-test and was either greater than $P < 0.05$ (*) or greater than $P < 0.01$ (**). The comparisons used to reject the null hypothesis were hypophysectomized males versus hypophysectomized males + GH; and hypophysectomized females versus hypophysectomized females that had received hormonal treatment.

hormone increase 3α-HSD/DD activity. Since these hormones increase enzyme activity in hypophysectomized females (i.e. in the absence of the anterior pituitary and functional gonads), these hormones must have a direct effect on hepatic 3α-HSD/DD activity. It is of interest to contrast our studies with those conducted by Gustafsson and his colleagues [13–15] which describe the hormonal mechanisms that contribute to the feminization of rat hepatic steroid hormone metabolism (i.e. elevated 15β-hydroxylase and 5α-reductase activities and decreased 16α-hydroxylase and 5β-reductase activities). This is of relevance since Lax *et al.* [11] have proposed that the increase in 3α-HSD activity in female rat liver cytosol is part of this feminization phenotype. Gustafsson and his colleagues have shown that estrogen will cause a continuous release of growth hormone from the anterior pituitary which in turn is responsible for the changes in hepatic enzyme activity [13–15]. Recently, Bullock *et al.* [16] have shown that the sex-differences in hepatic steroid hormone-metabolizing enzymes (hydroxylases and reductases) are imprinted in dwarf rats which are deficient in synthesizing growth hormone. These findings suggest that factors other than gonadal regulation of growth hormone secretion contribute to the feminization of rat hepatic steroid hormone metabolism. The regulation of 3α-HSD/DD activity described here would support this conclusion.

Our studies show that estrogens and growth hormone regulate hepatic 3α-HSD/DD activity by exerting a direct effect on the liver. Our data could fit one of three hypotheses. First, estrogen could exert its effect via the hepatic estrogen receptor, and growth hormone could exert its effect independently via insulin-like growth factor I (IGF-1). Second, IGF-1 could be the common mediator of the effects of estrogen and growth hormone on 3α-HSD/DD activity. This hypothesis would predict that estrogens may regulate IGF-1 expression. It has been found that growth hormone and estrogen will increase IGF-1 mRNA levels in estrogen target tissues (rat uterus) while in rat liver growth hormone will increase IGF-1 mRNA but estrogen is without effect [17]. However, these earlier results were obtained by treating rats with doses of 17β-estradiol which we find insufficient to increase hepatic 3α-HSD/DD activity. Our third hypothesis is that growth hormone primes rat liver to respond to estrogen. This may explain why either 17β-estradiol-3-sulfate or growth hormone alone are unable to restore 3α-HSD/DD activity in hypophysectomized animals to the level observed in intact controls. Norstedt *et al.* [18] have shown that GH and prolactin can increase hepatic estrogen receptor biosynthesis in hypophysectomized rats. The ability of progesterone to reduce hepatic 3α-HSD/DD activity in hypophysectomized female rats also suggests that estrogen and progesterone may exert opposing effects on 3α-HSD/DD activity via their receptors. The interplay of multiple factors may be required for maximal induction and repression of 3α-HSD/DD activity. This situation may be similar to that described by Aronica and Katzenellenbogen [19] in which estrogens, IGF-1 and cAMP work independently to regulate progesterone receptor biosynthesis in rat uterine cells. With the availability of a rat 3α-HSD/DD cDNA probe [20] our three hypotheses can be tested further. Each of these hypotheses would represent novel mechanisms for the regulation of an enzyme that plays an important role in polycyclic aromatic hydrocarbon and steroid hormone metabolism.

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A link between extracellular reactive oxygen and endotoxin-induced release of tumour necrosis factor α *in vivo*

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Abstract—Pretreatment with the reactive oxygen species scavengers superoxide dismutase (SOD) and catalase or with the xanthine oxidase inhibitor allopurinol protected mice against hepatitis induced by the combined administration of lipopolysaccharide (endotoxin) and D-galactosamine. In the sera of protected animals no tumor necrosis factor (TNFα) was detectable in contrast to abundant amounts in the sera of injured control animals. A similar protection by the suppression of systemic TNFα was observed following the pretreatment of mice with polystyrene-coupled SOD prior to endotoxic challenge. Both pretreatments were ineffective when hepatitis was evoked by administration of the mediator TNFα instead of endotoxin. These findings indicate that the formation of extracellular reactive oxygen species is a condition needed to induce the release of TNFα and thus to mediate endotoxin-induced toxicity.