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EFFECT OF STEROID HORMONE TREATMENT ON ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN THE SYRIAN HAMSTER KIDNEY

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Abstract—Aryl hydrocarbon hydroxylase (AHH) activity was determined in castrate and intact male Syrian hamster kidney and liver microsomes following *in vivo* treatment with either diethylstilbestrol (DES) or 17β-estradiol as well as other steroid hormones. After 1 month of estrogen treatment, there was a 5-fold decline in AHH activity in castrated hamster kidneys compared with untreated castrate levels. The amount of AHH activity in the kidney was depressed more than 75% of untreated castrate levels even after the estrogen had been withdrawn for 6 days. Consistent with a nearly 2.5-fold higher renal AHH activity observed in intact male hamsters compared to castrates was the finding of a 1.7-fold elevation in the activity of this enzyme after treatment of castrated animals with androgen[5α-dihydrotestrosterone (5α-DHT)] for 1 month. Moreover, following withdrawal of estrogen from intact hamsters, the increase in AHH activity in the kidney essentially paralleled the rise in serum testosterone levels. In castrated animals, the depression of AHH activity by estrogen was partially reversed by concomitant 5α-DHT treatment. However, no appreciable changes were seen in liver AHH activity with androgen treatment in the presence or absence of estrogen. Similarly, the level of AHH activity, which was nearly 7- and 14-fold higher than intact and castrate kidney levels, respectively, was not altered by estrogen treatment. Neither progesterone nor cortisone had any effect on the levels of AHH activity in either the kidney or liver. Therefore, AHH activity in the male hamster kidney, but not the liver, is responsive to both estrogens and androgenic hormone.

A major pathway for the oxidative metabolism of polycyclic aromatic hydrocarbons to possible reactive carcinogenic intermediates in mammalian tissues involves the microsomal multisubstrate monooxygenase aryl hydrocarbon (benzo[a]pyrene) hydroxylase. Chemical similarities between these carcinogenic agents and estrogenic hormones have been proposed [1]. This correlation has suggested to us that the multisubstrate monooxygenase system in the kidney or liver of hamsters might be affected by estrogens, hormones capable of inducing nearly a 100% incidence of renal carcinomas in this species [2]. Previous studies in our laboratory have demonstrated the presence of a high affinity estrogen receptor in cytosols of untreated hamster kidneys [3] which has been shown by others to translocate into the nuclear compartment [4]. In addition, this receptor is elevated in the kidney following continuous estrogen administration [3] and, as a consequence of this enhanced interaction of estrogen with its receptor, appears to induce appreciable quantities of progesterone receptor [5, 6]. These results demonstrate that the hamster kidney is hormonally responsive to estrogens. However, our recent observation [7] that estrogen-induced renal tumorigenesis can be inhibited by α-naphthoflavone, an inhibitor of multisubstrate monooxygenase activity, suggests that nonhormonal mechanisms may also be involved in the transformation of the hamster kidney.

In the present study, we investigated changes in aryl hydrocarbon hydroxylase (AHH) activity in the male hamster kidney and liver following treatment with various estrogenic hormones and the modulation of this enzyme in the kidney by androgen.

MATERIALS AND METHODS

Chemicals. 17β-Estradiol, estrone, and estriol were provided by Calbiochem (San Diego, CA). Other steroids, diethylstilbesterol (DES), benzo[a]pyrene and co-factors (NADP, NADPH, and nicotinamide) were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals and organic solvents were of the highest grade and obtained from the Fisher Scientific Co. (Chicago, IL).

Animals and treatment. Adult intact and castrate male Syrian golden hamsters (LAK:LVG), weighing 90–100 g (50–60 days old) each, were obtained from the Charles River Lakeview Hamster Colony, Wilmington, MA. Hamsters were castrated at least 2 weeks prior to treatment or killing. Pellets (20 ± 1.7 mg) of pure hormone were prepared without-binder and implanted subcutaneously as described previously [8]. The effects of both natural and synthetic estrogens and other steroid hormones on the activity of AHH were studied in groups of castrated hamsters receiving: (1) DES, (2) 17β-estradiol, (3) estrone, (4) 17α-estradiol, (5) hexestrol, (6) α-

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dienestrol, (7) 5α-dihydrotestosterone (5α-DHT), (8) 5α-dihydrotestosterone + DES, (9) progesterone, and (10) cortisone acetate. Pellets were implanted for 36–42 days, and then the hormone was withdrawn for 65 hr before killing for enzyme activity determination. In another series, groups of castrated hamsters received the following estrogenic hormones for 40 days before study: (1) DES, (2) 17β-estradiol, (3) 17α-estradiol, (4) estrone, and (5) estriol. The pellets of these animals were withdrawn at various intervals before killing for enzyme activity assessment. The mean daily absorption for DES, 17β-estradiol, estrone, estriol, 17α-estradiol, hexestrol, α-dienestrol, 5α-dihydrotestosterone, progesterone, and cortesone acetate was 170 ± 20, 140 ± 30, 130 ± 10, 120 ± 10, 160 ± 10, 190 ± 10, 160 ± 10, 110 ± 10, 190 ± 10 and 180 ± 10 μg (S.E.) daily respectively.

Microsome preparation. Male hamsters were killed by decapitation under light ether anesthesia. The livers and kidneys were removed immediately and rinsed. Subsequently, the tissues were minced, blotted on filter paper, and then weighed and homogenized in chilled glass–Teflon tissue grinders with 3 vol. of isotonic KCl (15 mM). While livers from individual animals were assayed for AHH activity separately, the kidneys were pooled from two to three hamsters and treated as one sample. Homogenates were centrifuged at 9,000 g for 15 min and the resultant supernatant fractions were then centrifuged in a Spinco L2 ultracentrifuge for 40 min at 165,000 g. The microsomal fraction, free of glycogen, was washed once and then resuspended in isotonic KCl. The washed microsomes were then diluted to 0.5–0.7 mg protein/ml.

Enzyme assay. The AHH activity was assayed essentially by the method described by Wattenburg *et al.* [9]. Briefly, the incubation mixture consisted of NADH (0.7 mM), NADPH (1.2 mM), nicotinamide (60 mM), and phosphate buffer (50 mM) in a final volume of 1.0 ml. The reaction, initiated by the addition of 2.0 ml of microsomes (0.5–0.7 mg protein/ml) containing benzo[a]pyrene (20 mM), was carried out aerobically for 20 min at 37° and then terminated by the rapid addition of cold acetone. The metabolites formed were extracted with hexane at 37° for 10 min. The samples were stored in the dark at 4° overnight. An aliquot of the solvent was removed (0.5–1.0 ml) and the BP-hydroxy derivatives were extracted with 1 N NaOH. The fluorescence of the aqueous extract was determined in a Farrand photoelectric fluorometer model A with a primary filter which transmits light maximally at 400 nm and a secondary interference filter with a peak wavelength of 522 nm and a half-band width of 14 nm. Quinine sulfate solution (0.3 μg/ml of 0.1 N H_2SO_4) was used as a fluorescent standard. All determinations were made in triplicate with appropriate blanks consisting of either boiled microsomes or in the absence of benzo[a]pyrene. A unit of enzyme activity is equivalent to the fluorescence of 1 ng of 3-hydroxy-BP formed per min per mg protein.

Protein and testosterone determinations. Protein concentration of the microsomal fraction was assessed by the method of Lowry *et al* [10] using bovine serum albumin as a standard. Serum testosterone levels were measured by RIA using rabbit anti-testosterone-30-carboxymethyl oxime-human serum albumin (anti-T-3HSA) and [1,2,6,7-^{3}H]testosterone (New England Nuclear Corp., Boston, MA) as described by McMillin *et al.* [11].

Statistical analyses. Values are expressed as the mean ± S.E.M. The data were analyzed by paired comparison using Student's t-test.

Table 1. Effects of various steroid hormone treatments on the activity of aryl hydrocarbon hydroxylase in the Syrian hamster kidney and liver*

Treatment†	N	Kidney	Liver
Intact	7	3.5 ± 0.44	18.0 ± 1.28
Castrate	18	1.4 ± 0.13‡	18.5 ± 0.62
+ DES	11	0.3 ± 0.02§	16.2 ± 1.14
+ 5α-DHT	5	2.4 ± 0.14	21.2 ± 1.66
+ 5α-DHT + DES	4	0.7 ± 0.15‖	17.3 ± 0.57
+ Progesterone	3	1.7 ± 0.04	21.1 ± 0.87
+ Cortisone acetate	3	1.1 ± 0.02	18.0 ± 0.75

* Enzyme activity is expressed in units per mg of protein, mean ± S.E.M. N represents the number of individual determinations of animals used in each group.

† Castrate animals were implanted with appropriate hormone pellets (20 mg) for 36 days and then the hormone was withdrawn for 60 hr before killing for enzyme activity determinations.

‡ $P < 0.001$ vs intact.

§ $P < 0.001$ vs castrate.

‖ $P < 0.001$ vs 5α-DHT-treated group.

RESULTS

The effects of prolonged administration of different steroid hormones on kidney and liver aryl hydrocarbon hydroxylase (AHH) activities in castrate and intact male hamsters are summarized in Table 1. The level of AHH activity in kidney microsomes of intact hamsters was nearly 2.5-fold greater than in castrated animals. The administration of DES for 1 month to castrated hamsters depressed the activity of AHH 4- to 5-fold from untreated castrate levels, whereas the administration of 5α-DHT to similar groups of hamsters about the same period increased the level of enzyme activity in the kidney to essentially intact untreated levels. When castrated hamsters were treated simultaneously with DES and 5α-DHT also for about 1 month, the level of renal AHH activity was approximately one-half the amount found in untreated castrated hamsters, indicating a partial antagonism of the estrogenic response. Neither progesterone nor cortisone treatment for a similar period of time had any appreciable effect on AHH activity in the kidney. The levels of the AHH activities in the livers of intact and castrated hamsters were essentially the same and nearly 13-fold greater than in the kidney. None of the above-mentioned hormonal treatments effected any change in the level of AHH activity in the livers. The abilities of other synthetic as well as natural estrogens to diminish the activity of AHH were examined in castrated hamsters (Table 2). Priming animals with these estrogens resulted in a decline in AHH activity in the kidney similar to that shown following DES treatment. In

Table 2. Effect of various estrogens on the activity of aryl hydrocarbon hydroxylase in the Syrian hamster kidney and liver*

Treatment†	N	Kidney	Liver
Castrate	4	1.5 ± 0.08	18.5 ± 1.05
+ DES	6	0.3 ± 0.07‡	18.8 ± 0.76
+ 17β-Estradiol	5	0.3 ± 0.01‡	16.5 ± 2.55
+ Estrone	4	0.3 ± 0.01‡	17.4 ± 2.26
+ Estriol	5	0.3 ± 0.03‡	22.0 ± 0.58
+ 17α-Estradiol	7	0.4 ± 0.02‡	17.0 ± 0.59
+ Hexestrol	3	0.7 ± 0.09§	20.4 ± 0.20
+ α-Dienestrol	3	0.4 ± 0.03‡	19.0 ± 1.75

* Enzyme activity is expressed in units per mg of protein, mean ± S.E.M. N represents the number of individual determinations of animals used in each group.

† Castrate animals were implanted with estrogen pellets (20 mg) for 42 days, and then the hormone was withdrawn for 65 hr before killing for enzyme activity determinations.

‡ $P < 0.001$ vs castrate control.

§ $P < 0.01$ vs castrate control.

contrast, the liver AHH activity levels remained unaltered from castrate levels with all hormonal treatments. Various estrogens were withdrawn from groups of 40-day hormone-treated castrated hamsters at a period of maximum inhibition of renal AHH activity, and the amount of enzyme activity was determined at daily intervals for 6 days (Fig. 1). The suppression of hamster renal AHH activity by these estrogens continued despite withdrawal of the hormones throughout the 6-day period. The time

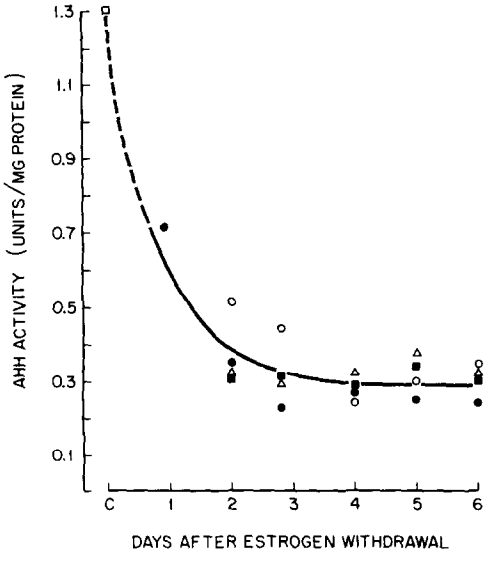

Fig. 1. Kidney AHH levels in hamsters treated with estrogens. Castrate male hamsters were treated with 17β-estradiol (●), 17α-estradiol (○), estrone (■), and estriol (△) for 40 days, and the hormone pellets were withdrawn at 1, 2, 3, 4, 5 and 6 days before killing for AHH activity determination. C (□) indicates the level of enzyme activity in kidneys of castrated untreated hamsters. Data represent the mean of at least three separate determinations. A unit of enzyme activity is equivalent to the fluorescence of 1 ng of 3-hydroxy-BP formed per min per mg protein.

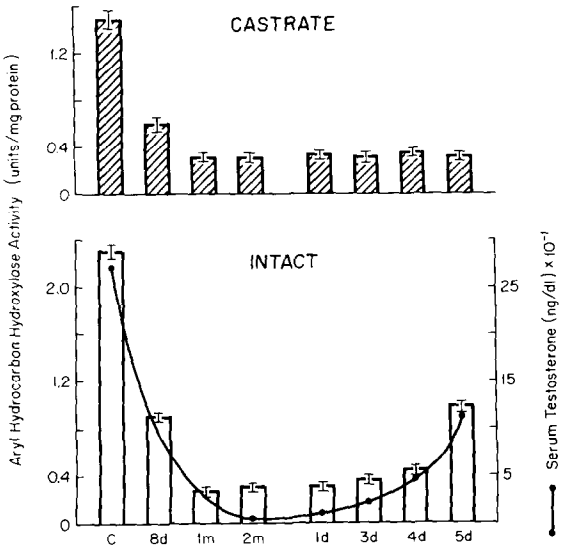

Fig. 2. Effect of DES on kidney AHH activity in castrated vs intact hamsters. Castrate and intact male hamsters were treated with DES for 8 days (8d), 1 (1m) and 2 months (2m), and the pellets were withdrawn 1 (1d), 3 (3d), 4 (4d) and 5 days (5d) before killing for enzyme activity determination. Testosterone levels were monitored in the serum of the intact hamsters. C represents the levels of kidney AHH activity in control untreated hamsters. Data represent the mean ± S.E.M. of at least three separate determinations. A unit of enzyme activity is equivalent to the fluorescence of 1 ng of 3-hydroxy-BP formed per min per mg protein.

course for estrogen suppression of renal AHH activity was determined by comparing the response of DES treatment and withdrawal in both castrated and intact hamsters (Fig. 2). After 8 days of estrogen treatment, kidney AHH activity was decreased more than 60% of castrated levels. A subsequent decline (75% of castrate levels) in enzyme activity occured at 1 month with continued hormone administration as indicated previously, with no further decrease following 2 months of estrogen treatment. Hormonal withdrawal did not affect the reduced levels of AHH activity in the castrate kidney. Although the amount of AHH activity in the kidney of intact animals exhibited a similar decrease following DES treatment for 8 days and a more marked decline after 1 to 2 months of treatment, a gradual elevation of enzyme activity was observed upon hormone withdrawal which was most evident on day 5. Similar results were obtained in intact animals treated under comparable conditions as described using 17β-estradiol (not shown). The inhibition of renal AHH activity by estrogens in the intact hamster paralleled the decline in serum testosterone levels which most probably occured as a consequence of the suppression of testicular function by the exogenous estrogen treatment (Fig. 2). In contrast to the behavior of AHH activity in the castrate kidney following hormonal withdrawal, there was a significant rise in renal AHH activity in intact hamsters which paralleled the increase in serum testosterone levels as testicular function began to return to normal following continued hormonal withdrawal. These findings

are consistent with the enhanced levels of AHH activity in castrated hamsters receiving androgen treatment. No significant changes were observed in liver AHH activity from intact or castrated hamsters following essentially the same hormonal treatment.

DISCUSSION

Depending on the species, organ, and mode of exposure, different responses have been obtained for the effect of steroid hormones on AHH activity. A recent report demonstrated a 1- to 2-fold increase in AHH activity in rat liver and intestine following chronic *in vivo* administration of 17β-estradiol but a decrease in the activity of this enzyme in the lung [12]. Similarly, estrogen-treated ovariectomized mice also exhibited enhanced liver AHH activity, whereas testosterone administration resulted in a decline in the level of this enzyme in the same tissue [13]. On the other hand, Kramer *et al.* [14] demonstrated that testosterone treatment enhanced benzo[a]pyrene hydroxylation in rat liver, while 17β-estradiol lowered AHH activity when administered to castrated animals. Moreover, these investigators indicated that these hormonal effects are dependent on pituitary involvement. In contrast, Nebert *et al.* [15] reported no significant changes in liver and skin AHH activities of castrated male mice following 1 week of 17β-estradiol treatment. In other studies, the administration of either 17β-estradiol or testosterone to various mouse tissues in culture, including kidney and liver, resulted in a marked inhibition of AHH activity [15, 16]. Similar *in vitro* additions of sex steroids to various rat tissues also effected a depression of AHH activity [17]. However, increases in AHH activity were observed in fetal rat liver primary cultures when exposed to DES in the medium [18]. It is pertinent to note that none of the above rodent species which have received the most attention regarding the influence of sex steroids on AHH activity are known to be susceptible to the carcinogenic effects of estrogens either in the kidney or liver. Our finding of markedly reduced levels of renal AHH activity in hamsters as a consequence of estrogen treatment and its promotion following androgen administration in castrated hamsters appears to be a tissue-specific response since the level of the liver enzyme is not affected by these hormonal regimens. Therfore, this observation may have particular relevance to the carcinogenic activity of estrogens in the hamster kidney. Of the natural and synthetic estrogens examined which caused a fall in AHH activity in the present study, all are capable of inducing renal carcinomas in hamsters [19]. Moreover, consistent with this concept is that androgens prevent renal tumorigenesis when administered concomitantly with estrogen [2]. The ability to induce or depress AHH activity may have either a beneficial or harmful effect on any given carcinogenic response in a system. Therefore, it is conceivable that the specific suppression of AHH activity in the hamster kidney by estrogens may either inhibit the conversion of estrogenic intermediates to less carcinogenic metabolites or perhaps facilitate the metabolism of estrogens to more reactive forms.

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