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ANDROGEN-PRIMING OF THE HYPERINDUCTIVE ESTROGEN EFFECT ON THE CYTOPLASMIC ACTIVITY OF 3α-HYDROXYSTEROID DEHYDROGENASE IN RAT KIDNEY

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SUMMARY

The behaviour of the estrogen-inducible cytoplasmic NADP-dependent activity of renal 3α-hydroxysteroid dehydrogenase (3α-HSDH) was investigated in male and female rats. Administration of estradiol or diethylstilbestrol to adult gonad-intact male, but not female, rats caused hyperinduction of activity to levels far above the activity found in normal female rats. Administration of estradiol to gonad-intact female, as well as prepubertally or postpubertally gonadectomized rats of either sex, after pretreatment with 5α-dihydrotestosterone also led to hyperinduction. Thus both exogenous and testicular androgens can prime the mechanisms by which estrogen exerts its hyperinductive effect on the renal enzyme activity.

INTRODUCTION

Recent investigations [1–5] have shown that rat kidney cytosol contains an NADP-dependent 3α-hydroxysteroid dehydrogenase (3α-HSDH) catalysing the interconversion of 5α-dihydrotestosterone (DHT) and 5α-androstane-3α,17β-diol (3α-DIOL). The enzyme exhibits a considerably higher affinity for DHT than for the 3α-DIOL [1, 2]. Because NADPH/NADP ratios are greater than 1 in the cytosol of mammalian tissues, De Moor *et al.*[1] concluded that the cytoplasmic activity of 3α-HSDH plays a major role in the transformation of DHT to the 3α-DIOL under *in-vivo* as well as *in-vitro* conditions. In view of the fact that 3α-DIOL competes only weakly with DHT for binding to cytoplasmic androgen receptors isolated from various target organs [6–8], this enzymatic conversion of DHT may be regarded as an inactivation of the highly potent androgen, leading to a metabolite which cannot be translocated and thus, according to the current concept of the mechanism of androgen action [7, 8], is not able to initiate nuclear events.

The NADP-dependent cytoplasmic activity of renal 3α-HSDH is higher in female rats (male:female activity ratio = 1:3) due to the inductive action of estrogens [3–5]. The administration of a single dose of 0.1 mg estradiol valerate to adult male rats with intact gonads and pituitaries leads to an effect on this enzyme activity which has not been observed for any other sexually differentiated renal or hepatic steroid metabolizing enzyme activities, namely a hyperinduction of activity to levels far above those normally found in female rats [5]. The same dose of estradiol valerate did not lead to any significant change in the enzyme activity of adult female rats with intact gonads and hypophysis.

The present investigation was undertaken to elucidate whether this hyperinductive effect of estrogens is the result of estrogen action after obligatory androgen-priming.

EXPERIMENTAL

Chemicals. All steroids and biochemicals were obtained commercially. For further details see [9].

Treatment of animals. Male and female rats of the strain Chbb: THOM were obtained from the Laboratorium für Versuchstierkunde (Firma Dr. K. Thomae, Biberach/Riß). They were orchidectomized (scrotal route) or ovariectomized (abdominal route) prepubertally (day 25 of life) or postpubertally (day 50 of life) under ether narcosis. Estradiol, diethylstilbestrol and 5α-dihydrotestosterone were administered subcutaneously (for further details, see legends of figures and tables). Rats were killed between 7 and 8 a.m. on the day of investigation by stunning and decapitation.

Determination of the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50). NADP-dependent 3α-HSDH activity was determined in the cytosol (100,000 *g* supernatant) of whole homogenates of decapsulated kidneys (1 g tissue + 12 ml 0.25 M sucrose). For details of the fractionation procedure see [3].

Starting with 5α-dihydrotestosterone as substrate, the rate of product, 5α-androstane-3α,17β-diol, formation was determined using a radiometric method (for further details, see [9]). Assays were performed in triplicate; enzyme activity is expressed as pmol.min^{-1}.mg cytosolic protein^{-1}.

Determination of protein concentration. Protein concentration in the cytosols was determined according to Lowry *et al.*[10].

Significance tests. Results are given as means ± SD (number of rats in each group, $n \geqslant 5$). Statistical differences between two mean values were determined using the *t*-test for unpaired results. Unless otherwise stated the use of the word *significant* in the text indicates $P < 0.001$.

RESULTS

Dose-dependent effects of estradiol (E_2) and diethylstilbestrol (DES) in adult gonad-intact male rats (Table 1 and Table 2)

Subcutaneous administration of as little as 0.5 μg E_2/day or 5 μg DES/day to adult gonad-intact male rats over a period of 15 days leads to a significant ($P < 0.001$) induction of the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase (3α-HSDH) to levels far above the normal female activity. Using 50 μg E_2/day or 500 μg DES/day this hyperinduction reaches activity levels which are approximately 20 times higher than those observed in vehicle-treated normal female rats. The reaction of the seminal vesicles to these estrogen regimens is described in [9].

Time course of the effect of a low and a high dose of estradiol (Fig. 1)

Administration of a low dose of 5 μg E_2/day to gonad-intact male rats for 4 days leads to an induction of enzyme activity up to the level of the normal female animal. If the daily administration is continued, hyperinduction can already be observed by day 6, whereafter the rate of enzyme activity increase is practically linear up to the 15th day of treatment. The activity level reached at the end of the estrogen administration remains unchanged for at least 7 days; this is demonstrated by the fact that after seven days estrogen administration it makes no difference whether the activity is assayed 24 h or 8 days later. If a higher daily dose of 50 μg E_2/day is employed, hyperinduction can be recognized by the 4th day of treatment. Between day 4 and 6 a very rapid activity increase occurs, but then no further significant changes are observed with continuing treatment.

Table 1. Dose-dependent effects of systemically administered estradiol on the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase of adult male rats with intact gonads

Treatment	Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1})
Vehicle-treated male rats	126 ± 29
Estradiol-treated male rats	
0.1 (μg/day)	199 ± 23†
0.5	1174 ± 312*†
1.0	1893 ± 614*†
5.0	5240 ± 894*†
10.0	5552 ± 1134*†
50.0	7848 ± 1285*†
Vehicle-treated female rats	389 ± 89*

Estradiol (dissolved in 0.1 ml sesame oil) was given subcutaneously once daily over a period of 15 days. Treatment started on day 75 of life. Rats were killed 24 h following the last injection. The values listed represent means ± SD of at least five animals.

* Indicates $P < 0.001$ versus vehicle-treated male rats.

† Indicates $P < 0.001$ versus vehicle-treated female rats.

Table 2. Dose-dependent effects of systemically administered diethylstilbestrol on the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase of adult male rats with intact gonads

Treatment	Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1})
Vehicle-treated male rats	82 ± 12
Diethylstilbestrol-treated male rats	
1.0 (μg/day)	226 ± 45*
5.0	950 ± 280*†
10.0	1296 ± 216*†
50.0	2610 ± 243*†
100.0	2801 ± 404*†
500.0	5996 ± 350*†
Vehicle-treated female rats	320 ± 80*

Diethylstilbestrol (dissolved in 0.1 ml propylene glycol) was given subcutaneously once daily over a period of 10 days. Treatment started on day 75 of life. Rats were killed on day 90 (i.e. 15 days following the start of treatment). The values listed represent means ± SD of at least five animals.

* Indicates $P < 0.001$ versus vehicle-treated male rats.

† Indicates $P < 0.001$ versus vehicle-treated female rats.

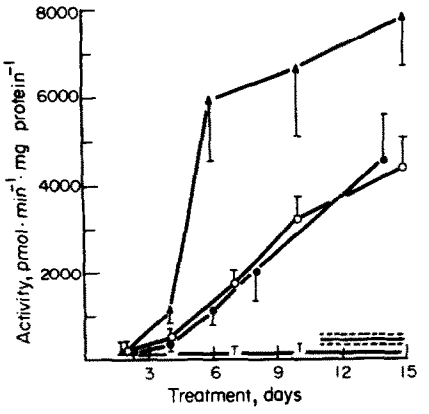

Fig. 1. Time course of the effect of systemically administered estradiol on the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase of adult male rats with intact gonads. Estradiol was dissolved in 0.1 ml sesame oil and given subcutaneously once daily. Treatment started on day 75 of life; (■) vehicle-treated controls killed 24 h following the last injection; (○) estradiol (5 μg)-treated rats killed 24 h following the last injection; (●) estradiol (5 μg)-treated rats killed 15 days following the start of treatment; (▲) estradiol (50 μg)-treated rats killed 24 h following the last injection; (═══) activity level (mean ± SD) of vehicle-treated adult female rats with intact ovaries. The values represent means ± SD of at least five animals.

Influence of androgen pretreatment on the effect of estradiol in the adult female rat (Table 3)

In contrast to the situation in adult male rats, administration of 5 μg E_2/day to gonad-intact adult female animals over a 14 day period does not lead to hyperinduction of the enzyme activity. However, if the females are pretreated with 1 mg 5α-dihydrotestosterone (DHT)/day for 14 days before the 14 day 5 μg E_2/day regimen, then hyperinduction does indeed occur. The fact that simultaneous treatment of gonad-intact males with DHT as well as E_2 prevents the hyperinductive effect of the estrogen, demonstrates the function of androgens in the hyperinduction of the enzyme activity is to prime the organ for estrogen action.

Influence of androgen pretreatment on the effect of estradiol in postpubertally (Table 4) and prepubertally (Table 5) gonadectomized male and female rats

Both postpubertal (day 50 of life) and prepubertal (day 25 of life) gonadectomy lead to a loss of the sex difference in the enzyme activity of adult rats (cf. Table 3). In the case of the postpubertally operated rats this is realized as a decrease in the enzyme acti-

Table 3. NADP-Dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase and the influence of androgen pretreatment on the effect of systemically administered estradiol in adult female rats with intact ovaries

Treatments		Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1})	
Day 1–14	Day 15–28	Male	Female
Vehicle	Vehicle	122 ± 11	380 ± 44
Vehicle	E_2	1998 ± 438*	430 ± 71
Vehicle	E_2 + DHT	180 ± 60†	
DHT	E_2		1981 ± 664*†

Estradiol (E_2, 5 μg/day) and 5α-dihydrotestosterone (DHT, 1 mg/day) were dissolved in 0.1 ml propylene glycol and administered subcutaneously once daily.

E_2 + DHT, indicates simultaneous administration of E_2 and DHT. Treatment of gonad-intact rats started on day 65 of life. Rats were killed 24 h following the last injection. The values listed represent means ± SD of at least five animals.

* Indicates $P < 0.001$ versus respective vehicle-treated group.

† Indicates $P < 0.001$ versus respective group treated with vehicle followed by E_2.

Table 4. NADP-Dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase and the influence of androgen pretreatment on the effect of systemically administered estradiol in adult male and female, postpubertally gonadectomized rats

Treatments		Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1})	
Day 1–14	Day 15–28	Male	Female
Vehicle	Vehicle	104 ± 11	91 ± 7
Vehicle	E_2	1363 ± 443*	290 ± 98*
DHT	E_2	3056 ± 914*†	1498 ± 373*†
DHT	Vehicle	125 ± 28	81 ± 17

Estradiol (E_2, 5 μg/day) and 5α-dihydrotestosterone (DHT, 1 mg/day) were dissolved in 0.1 ml propylene glycol and administered subcutaneously once daily.

Rats were gonadectomized on day 50 of life. Treatment started on day 65. Rats were killed 24 h following the last injection. The values listed represent means ± SD of at least five animals.

* Indicates $P < 0.001$ versus respective vehicle-treated group.

† Indicates $P < 0.001$ versus respective group treated with vehicle followed by E_2.

Table 5. NADP-Dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase and the influence of androgen pretreatment on the effect of systemically administered estradiol in adult male and female, prepubertally gonadectomized rats

Treatments		Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1})	
Day 1–14	Day 15–28	Male	Female
Vehicle	Vehicle	98 ± 8	102 ± 15
Vehicle	E_2	506 ± 124*	342 ± 103*
DHT	E_2	1167 ± 282*†	1047 ± 602*†
DHT	Vehicle	114 ± 11	101 ± 11

Estradiol (E_2, 5 μg/day) and 5α-dihydrotestosterone (DHT, 1 mg/day) were dissolved in 0.1 ml propylene glycol and administered subcutaneously once daily.

Rats were gonadectomized on day 25 of life. Treatment started on day 60.

Rats were killed 24 h following the last injection. The values listed represent means ± SD of at least five animals.

* Indicates $P < 001$ versus respective vehicle-treated group;

† Indicates $P < 001$ versus respective group treated with vehicle followed by E_2.

vity of the female animals to the male level, whereas in the case of the prepubertally gonadectomized rats the enzyme activity simply remains at the undifferentiated immature level (cf. Table 6).

Administration of 1 mg DHT/day for 14 days to prepubertally or postpubertally gonadectomized male or female rats does not cause any significant alteration in the enzyme activity. Administration of estradiol alone at a dose of 5 μg/day for 14 days induces the enzyme activity in male and female prepubertally operated rats as well as postpubertally gonadectomized females up to the level of the adult female gonad-intact rat; postpubertally castrated males react to this estradiol regimen with a hyperinduction of the enzyme activity. Hyperinduction may also be achieved in pre- or postpubertally gonadectomized animals of either sex if the estradiol regimen is *preceded* by treatment with DHT. The effect is most pronounced in postpubertally orchidectomized rats. The fact that postpubertally, but not prepubertally orchidectomized, rats react to estradiol administration with a hyperinduction of activity without prior exogenous DHT treatment, demonstrates that testicular androgens secreted between the onset of puberty and the 50th day of life are sufficient for adequate priming.

Effect of estradiol in immature male and female rats (Table 6)

Administration of 5 μg E_2/day for 10 days to immature rats leads to significant increases only in the male sex. However the level observed in adult female gonad-intact animals was not reached.

DISCUSSION

It has been demonstrated in the present study that the NADP-dependent activity of the cytoplasmic 3α-hydroxysteroid dehydrogenase (3α-HSDH) of rat kidney reacts better to the administration of estrogens (as induction or hyperinduction) in the male than in the female sex under a variety of experimental conditions. The testicular androgens secreted in this sex are sufficient to prime the enzyme activity for an estrogen stimulus at any later stage of development. The duration of testicular androgen influence is obviously the major factor determining the size of estrogen effect. When the duration of testicular androgen influence is limited to the neonatal and immature stages of development, a 10 day period of estradiol administration (from day 19 of life) succeeds in producing a significant increase in activity, but this does not reach the level found in adult gonad-intact females. The enzyme

Table 6. Effect of systemically administered estradiol on the NADP-dependent cytoplasmic activity of renal 3α-hydroxysteroid dehydrogenase of immature male and female rats with intact gonads

Treatment	Renal 3α-hydroxysteroid dehydrogenase activity (pmol.min^{-1}.mg protein^{-1}) Male	Female
Vehicle	94 ± 11	96 ± 21
Estradiol	192 ± 49*	112 ± 8

Estradiol (5 μg/0.1 ml propylene glycol) was given subcutaneously once daily for 10 days. Treatment started on day 19 of life. Rats were killed on day 33 (i.e. 15 days following the start of treatment). The values listed represent means ± SD of at last five animals.

* Indicates $P < 0.001$ versus vehicle-treated group.

activity in immature female rats does not react to estradiol treatment at all at this stage, a direct contrast to the estrogen-responsiveness of the immature rat uterus [11].

The fact that administration of estradiol after the 60th day of life induces the adult female gonad-intact level of activity in prepubertally gonadectomized male and female as well as postpubertally ovariectomized female rats, indicates that the mechanisms underlying this inductive effect of estradiol undergo a maturing process between day 19 and 60 of life.

In the case of postpubertally castrated male rats, however, sufficient testicular androgens have already been secreted by day 50 of life to prime the enzyme activity for an estrogen-stimulated hyperinduction to levels far above the female gonad-intact level. This androgen-priming effect is even more pronounced in rats with intact testes.

The fact that pretreatment of gonad-intact females, as well as both pre- and postpubertally gonadectomized male and female rats, with dihydrotestosterone, always leads to hyperinduction when estradiol is administered, demonstrates that androgen action is a prerequisite for hyperinductive estrogen action. The obligatory sequential order of androgens before estrogens is underlined by the fact that simultaneous administration of dihydrotestosterone to gonad-intact male rats blocks the hyperinduction normally elicited by estradiol (Table 3). In this context it is interesting to note that synergism between androgens and estrogens has also been described in the chick oviduct [12].

Estradiol-induced hyperinduction of NADP-dependent 3α-HSDH activity in the cytosol is accompanied by a decrease in the NADP-dependent activity of 3α-HSDH in the microsomal fraction [9]. The question arises as to whether estrogens merely lead to a redistribution of one enzyme activity between these two cellular compartments. This cannot be the case. Not only are the responses of these two 3α-HSDH activities to other hormonal stimuli quantitatively and qualitatively independent of each other [3–5, 9, 13], but Verhoeven *et al.*[2] have clearly demonstrated that they differ in their kinetic characteristics and some of their physicochemical properties. There can be little doubt that the cytoplasmic and microsomal enzymes are separate entities.

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