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ANDROGENIC CONTROL OF THE MICROSOMAL 3α-HYDROXYSTEROID OXIDOREDUCTASES IN RAT KIDNEY

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SUMMARY

The influence of gonadal hormones on the microsomal NADPH-linked and the microsomal NADH-linked 3α-hydroxysteroid oxidoreductase activity has been investigated in rat kidney. It has been concluded that the high levels of activity observed in male animals can be accounted for by testicular hormones. Castration reduces both enzyme activities and androgen replacement restores them. Several 5α-reduced androgen derivatives are able to induce both enzymes. 5α-Dihydrotestosterone is the most effective one. Cyproterone acetate inhibits the induction. The activity of both enzymes has been studied as a function of age between day 1 and 360. The NADPH-linked enzyme closely reflects the activity of the testis. The NADH-linked oxidoreductase is subject to complex developmental changes.

INTRODUCTION

Recently we identified three 3α-hydroxysteroid oxidoreductases able to interconvert 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) and 5α-androstane-3α,17β-diol (3α-androstanediol) in rat kidney: a microsomal NADPH-dependent enzyme, a microsomal NADH-dependent enzyme and a soluble NADPH-linked enzyme [1]. All three enzymes display clear cut sex differences. The microsomal NADPH-3α-hydroxysteroid oxidoreductase is 35 times more active in male rats, its NADH-linked counterpart is 2–3 times more active in male animals. The soluble NADPH-linked enzyme on the contrary is more active in female kidney cytosol. In the present paper the influence of sex hormones on the two oxidoreductases associated with the microsomal fraction is further explored. Particular attention has been paid to the usefulness of these enzymes as parameters of androgen activity in rat kidney.

EXPERIMENTAL

Animals. Rats of two different Wistar strains were used in these studies. The animals differed in the activity of the microsomal NADH-linked enzyme [1]. They are conveniently referred to as Wistar I (conventional Wistar R/A with high NADH-linked activity) and Wistar II (specific pathogen free Wistar Af-Han-Pfd with low NADH-linked activity). Castration (by the abdominal route), ovariectomy and adrenalectomy were performed essentially as described by Zarrow [2].

Chemicals and methods. 17β-hydroxy-5α-androstan-3-one propionate 5α-androstane-3α,17β-diol dipropionate and 5α-androstane-3β,17β-diol dipropionate were obtained from Steraloids. Cyproterone acetate was a gift from Schering A.G. [1,2-^{3}H]-5α-dihydrotestosterone (48 Ci/mmol) was obtained from New England Nuclear (Langen, Germany). Routine procedures for the preparation of microsomes and for the radiochemical determination of microsomal NADPH- and NADH-linked 3α-hydroxysteroid oxidoreductase activity using [^{3}H]-5α-dihydrotestosterone as substrate have been reported in detail [1]. Unless stated otherwise these assays were used without modification.

RESULTS

Influence of castration in adult life on microsomal 3α-hydroxysteroid oxidoreductases in rat kidney. The influence of castration of adult male rats on microsomal 3α-hydroxysteroid oxidoreductases has been summarized in Table 1. The activity of the NADPH- and NADH-linked enzyme decreases rapidly during the first days after gonadectomy. The NADH-linked oxidoreductase already attains definitely female levels six days after castration. The NADPH-dependent activity disappears more slowly. Even after 3 months of androgen deprivation the values observed in male animals tend to be higher than those encountered in female rats. The effect of adrenalectomy, although not statistically significant suggests that adrenal hormones might account for the residual sex difference.

Influence of prepubertal gonadectomy and androgen replacement on rat kidney 3α-hydroxysteroid oxidoreductases. In order to avoid the influence of adult sex hormones on 3α-hydroxysteroid oxidoreductases in rat kidney, male and female animals were gonadectomized immediately after weaning (day 21). On day 70 some of them were killed. Oxidoreductases were

Table 1. Influence of castration in adult life on microsomal 3α-hydroxysteroid oxidoreductases

Treatment	3α-Hydroxysteroid oxidoreductase activity NADPH	NADH
	μmol g protein^{-1} h^{-1}	μmol g protein^{-1} h^{-1}
Control males	56.68 ± 2.67	140.57 ± 7.66
Control females	2.13 ± 0.61	65.58 ± 0.88
Male rats gonadectomized:		
for 3 days	36.90 ± 2.72	71.64 ± 1.77
for 6 days	22.06 ± 3.62	69.10 ± 4.69
for 13 days	9.24 ± 2.70	56.24 ± 7.14
for 24 days	6.96 ± 2.63	58.12 ± 2.25
for 3 months	5.43 ± 1.19	49.29 ± 3.68
Male rats GX for 3 months + AdX for 7 days	2.46 ± 0.43	53.64 ± 2.95

Male and female Wistar II rats were assayed for microsomal 3α-hydroxysteroid oxidoreductase activities at the age of 80 days. Some of the male animals were gonadectomized (GX) at the same age and analyzed after various periods of androgen deprivation. Some of the animals underwent adrenalectomy (AdX) 7 days before being killed. All values represent the mean ± S.E. of 4 individual determinations

assayed in the microsomal fraction and in the cytosol. From that day onwards the rest of the male animals received androgen substitution. Groups of 4 animals were sacrificed after various periods of treatment. It can readily be observed that prepubertal gonadectomy markedly reduces microsomal dehydrogenase activity in male animals. In the ovariectomized female rats the activity is as low as in untreated female animals (Fig. 1). These findings confirm that the high levels of NADPH- and NADH-linked 3α-hydroxysteroid oxidoreductase activity in adult male animals can be accounted for by testicular hormones. It is worthwhile noting, however, that after prepubertal gonadectomy the microsomal NADPH-linked activity is also significantly ($P < 0.01$) higher in male animals than in female ones. Substitution with testosterone propionate (TP) yields a marked increase in the two microsomal oxidoreductases whereas in cyto-

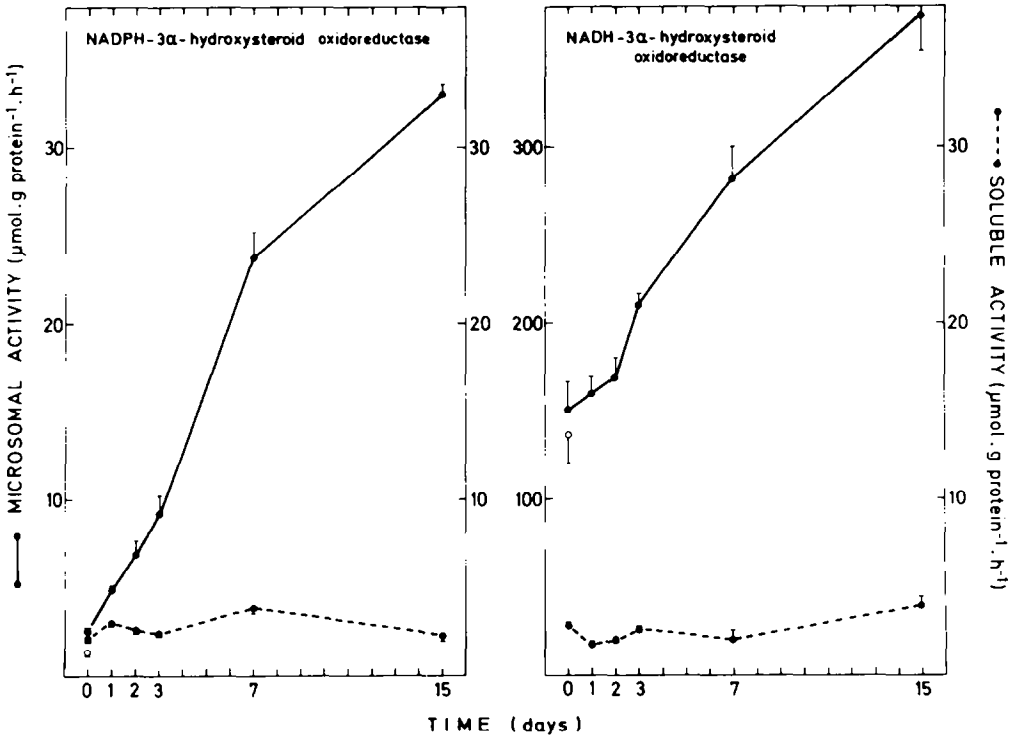

Fig. 1. Microsomal and soluble 3α-hydroxysteroid oxidoreductase activity in rat kidney after prepubertal gonadectomy and androgen substitution. Wistar I rats of both sexes were gonadectomized on day 21. On day 70 NADPH- and NADH-linked 3α-hydroxysteroid oxidoreductase activity was measured in microsomes and cytosol from male (●) and female (○) animals. Some of the male rats received androgen substitution (750 μg of TP/day, subcutaneously injected in 0.2 ml of olive oil) and was killed after various periods of treatment. Each point represents the mean ± S.E. of 4 individual animals. The left hand scale of each panel and the solid lines represent microsomal activity. The corresponding soluble activity is given by the broken lines and the right hand scale.

Table 2. Comparison of the influence of prepubertal gonadectomy and androgen replacement on NADPH-3α-hydroxysteroid oxidoreductase activity in kidney microsomes from male and female rats

Treatment	Number	Male rats	Female rats	Sex difference	Treated male vs control	Treated female vs control
		μmol g protein^{-1} h^{-1}				
Sham operated day 21	4	60.7 ± 1.4	1.5 ± 0.1	$P < 0.001$		
GX day 21 (control)	8	6.8 ± 0.5	2.9 ± 0.2	$P < 0.001$	-	
GX day 21 14 days oil	4	7.0 ± 1.2	2.4 ± 0.1	$P < 0.01$	NS	NS
GX day 21 24 h TP 2.1 mg m^2	4	7.8 ± 1.1	2.5 ± 0.3	$P < 0.005$	NS	NS
GX day 21 48 h TP 2.1 mg·m^2	4	8.8 ± 0.2	3.4 ± 0.4	$P < 0.001$	$P < 0.025$	NS
GX day 21 7 days 2.1 mg·m^2	4	13.9 ± 2.5	3.3 ± 0.3	$P < 0.01$	$P < 0.0025$	NS
GX day 21 14 days 2.1 mg m^2	4	16.1 ± 1.0	4.9 ± 1.2	$P < 0.001$	$P < 0.005$	$P < 0.025$
GX day 21 24 h TP 21 mg m^2	4	9.1 ± 0.9	2.6 ± 0.1	$P < 0.001$	$P < 0.025$	NS
GX day 21 48 h TP 21 mg m^2	4	13.9 ± 2.7	4.8 ± 0.5	$P < 0.01$	$P < 0.0025$	$P < 0.0025$
GX day 21 7 days 21 mg·m^2	4	27.0 ± 5.0	18.8 ± 6.1	NS	$P < 0.0005$	$P < 0.0025$
GX day 21 14 days 21 mg m^2	4	32.6 ± 0.8	25.6 ± 3.5	NS	$P < 0.0005$	$P < 0.0005$

Male and female Wistar I rats were gonadectomized (GX) or sham operated on day 21. From day 70 on some of the gonadectomized animals received either a low or a high dose of testosterone propionate (TP). The administered doses have been corrected for body surface. The values indicated represent the mean ± S.E. of the indicated number of determinations. Male and female animals, and treated and control animals were compared using Student's *t*-test (NS = $P > 0.05$).

sol both NADPH- and NADH-linked activity remain essentially unchanged. The microsomal NADPH-linked activity is raised significantly ($P < 0.005$) as early as 24 h after the initiation of androgen treatment. The microsomal NADH-linked activity responds more slowly. Significant ($P < 0.01$) stimulation is observed from 72 h after the initiation of treatment. Even after 15 days of androgen administration adult levels of 3α-hydroxysteroid oxidoreductase activity are not attained for any of the microsomal enzymes.

Sex differences in microsomal 3α-hydroxysteroid oxidoreductase activity persisting after prepubertal gonadectomy. As already mentioned in the preceding paragraph and as repeatedly confirmed during subsequent experiments (Fig. 1, Tables 2 and 3) the sex difference in the activity of the particulate NADPH-3α-hydroxysteroid oxidoreductase was not completely abolished by gonadectomy on day 21. Further exploration of this observation was severely hampered, however, by the very low levels of enzyme activity in gonadectomized rats. In order to minimalize experimental variation, the subsequent series of experiments were performed using male and female animals derived from the same litters. Both the preparation of microsomes and the assay of oxidoreductase activity was performed completely in parallel.

In a first experiment it was investigated whether the residual sex difference noted after prepubertal gonadectomy could also be observed at the higher and easily detectable levels of NADPH-3α-hydroxysteroid oxidoreductase activity observed after androgen replacement. Male and female animals gonadectomized on day 21 were injected with a high or a low dose of TP for 1–14 days. It is observed that significant sex differences persist at any time following the *low dose* treatment. At the high dose of TP female animals tend to approach male levels of

Table 3. Influence of adrenalectomy on sex differences in NADPH-3α-hydroxysteroid oxidoreductase activity persisting after prepubertal gonadectomy

Treatment	NADPH-3α-hydroxysteroid oxidoreductase activity
	μmol g protein^{-1} h^{-1}
Male rats	
GX (50 days)	2.73 ± 0.26
GX (50 days) + AdX (7 days)	3.40 ± 0.69
GX (50 days) + AdX (14 days)	1.33 ± 0.32
Female rats	
GX (50 days)	1.53 ± 0.23
GX (50 days) + AdX (7 days)	1.21 ± 0.22
GX (50 days) + AdX (14 days)	2.18 ± 0.37
Student's *t*-test	
Sex difference	
GX (50 days)	$P < 0.01$
GX (50 days) + AdX (7 days)	$P < 0.0125$
GX (50 days) + AdX (14 days)	NS
Influence of adrenalectomy	
Male rats AdX (7 days)	NS
Male rats AdX (14 days)	$P < 0.01$
Female rats AdX (7 days)	NS
Female rats AdX (14 days)	NS

Male and female Wistar II rats were gonadectomized (GX) on day 21 and adrenalectomized (AdX) on day 56 or 63. The animals were killed and assayed for microsomal oxidoreductase activity between day 70 and 72. The values listed represent the mean ± S.E. of 6 animals.

Table 4. Influence of 5α-reduced active androgen metabolites on rat kidney 3α-hydroxysteroid oxidoreductases

Treatment		3α-hydroxysteroid oxidoreductase activity		Body and organ weight		
Inducer	Time	NADPH	NADH	Body	Kidney	Prostate
		μmol g protein^{-1} h^{-1}		g	g	mg
TP	7 d	26.5 ± 2.8	434.3 ± 32.9	216 ± 2	1.54 ± 0.05	71 ± 4
DHT-P	7 d	38.4 ± 3.3	633.5 ± 32.7	201 ± 5	1.46 ± 0.03	69 ± 4
3α-DIOL-P	7 d	16.2 ± 0.8	323.7 ± 17.8	198 ± 5	1.40 ± 0.07	23 ± 1
3β-DIOL-P	7 d	7.4 ± 0.6	236.7 ± 6.9	212 ± 7	1.39 ± 0.05	14 ± 2
Oil	7 d	3.8 ± 0.7	187.2 ± 2.8	187 ± 3	1.34 ± 0.03	6 ± 1
TP	14 d	37.3 ± 1.2	573.0 ± 15.9	218 ± 8	1.66 ± 0.07	136 ± 2
DHT-P	14 d	57.5 ± 4.6	899.1 ± 72.0	198 ± 5	1.52 ± 0.05	132 ± 9
3α-DIOL-P	14 d	43.0 ± 3.1	573.8 ± 37.0	214 ± 9	1.50 ± 0.08	59 ± 6
3β-DIOL-P	14 d	13.2 ± 2.5	303.6 ± 21.7	213 ± 8	1.39 ± 0.06	11 ± 1
Oil	14 d	6.1 ± 0.5	225.4 ± 7.5	206 ± 7	1.46 ± 0.03	5 ± 1

Male Wistar I rats were castrated on day 21. From day 70 on the animals were treated for 7 or 14 days either with olive oil 0.2 ml, or with 1 mg/day of one of the following steroids: testosterone propionate (TP), dihydrotestosterone propionate (DHT-P), 3α-androstanediol dipropionate (3α-DIOL-P) or 3β-androstanediol dipropionate (3β-DIOL-P). Values represent the mean ± S.E. of 4 individual animals.

activity from the 7th day of treatment on. Nevertheless, at the high as well as at the low dose of TP a significant stimulation of NADPH-linked microsomal activity is observed earlier in male than in female animals.

In a subsequent experiment attempts were made to explore further the origin of this residual sex difference. Since the adrenal gland is another source of androgen and since there are well-known sex differences in the elaboration of glucocorticoids [3] and androgens [4] by this organ we investigated the influence of adrenalectomy on NADPH-linked oxidoreductase activity of prepubertally gonadectomized rats (Table 3). Male and female animals were gonadectomized on day 21 and assayed on day 70–72. Some of the animals were adrenalectomized 7 or 14 days before sacrifice. Fourteen days after adrenalectomy male rats showed considerably lower NADPH-3α-hydroxysteroid oxidoreductase activity than non-adrenalectomized or 7 days adrenalectomized animals. No sex differences could be shown in the groups of gonadectomized/14 days adrenalectomized animals.

Influence of 5α-reduced active androgen metabolites on rat kidney 3α-hydroxysteroid oxidoreductases. Prepubertally castrated male rats were injected daily with 1 mg of TP, 5α-dihydrotestosterone proprionate and 3α- or 3β-androstanediol dipropionate. Control animals were treated with 0.2 ml of the vehicle (olive oil) only. After 7 or 14 days of treatment, the animals were sacrificed and assayed for microsomal NADPH- and NADH-linked oxidoreductase activity (Table 4). Dihydrotestosterone propionate was by far the most effective inducer and proved to be the only compound able to raise the NADPH-linked activity to adult male levels. After 14 days of treatment with the latter androgen NADH-linked oxidoreductase activity was twice as high as in normal male controls. 3β-Androstanediol dipropionate was a very weak inducer while the 3α-epimer was as potent as TP after 14 days of treatment. After 7 days of treatment 3α-androstanediol was less effective than testosterone. This might reflect the slower resorption and/or hydrolysis of the dipropionate. It is worthwhile noticing that under the experimental conditions outlined above the ratio of the activity of testosterone and the activity of 3α-androstanediol is considerably higher in prostate than in kidney. This may reflect differences in the

Table 5. Influence of non-androgenic potential substrates and antiandrogens on renal microsomal 3α-hydroxysteroid oxidoreductases

Treatment	3α-hydroxysteroid oxidoreductase activity NADPH	NADH
	μmol g protein^{-1} h^{-1}	μmol g protein^{-1} h^{-1}
Experiment I		
Control	1.13 ± 0.09	47.24 ± 1.50
5α-pregnane-3,20-dione	1.87 ± 0.20	48.92 ± 1.93
11β,17,21-trihydroxy-5α-pregnane-3,20-dione	1.59 ± 0.20	46.61 ± 3.90
Experiment II		
Control	0.97 ± 0.10	40.52 ± 1.85
5α-dihydrotestosterone	19.92 ± 2.85	181.11 ± 7.35
Cyproterone acetate	0.76 ± 0.10	47.74 ± 6.00
5α-dihydrotestosterone + cyproterone acetate	5.69 ± 0.96	88.84 ± 7.17

Female Wistar II rats received 7 daily SC injections of 2 mg of 5α-pregnane-3,20-dione, 11β, 17, 21-trihydroxy-5α-pregnane-3,20-dione or 5α-dihydrotestosterone and/or 10 mg of cyproterone acetate. The former steroids were injected as an aquaeous suspension, the antiandrogen was dissolved in olive oil/benzyl benzoate (90/10). Control animals were injected with one (Experiment I) or both of the vehicles (Experiment II). When two substances or vehicles were injected simultaneously they were administered at different injections sites. Values represent the mean ± S.E. of 4 or 5 determinations.

ability of both organs to use testosterone or 3α-androstanediol as a precursor for 5α-dihydrotestosterone.

Prepubertal orchiectomy causes marked hyperplasia of the adrenal gland and testosterone treatment lowers adrenal weight to control levels [3]. This effect can be accounted for by the inhibitory action of androgens on pituitary ACTH secretion [5]. Our data (Table 4) show a pronounced effect of all 5α-reduced androgen derivatives tested on this parameter of androgen activity. The effectiveness of the 3β-diol contrasts sharply with its low androgenic activity and merits further investigation. In fact, the latter compound has also been shown to be a very potent suppressor of LH secretion [6].

Substrate induction or androgen effect. The inducers of the renal microsomal 3α-hydroxysteroid oxidoreductases described in the preceding paragraph are all either substrates for these enzymes or at least potential precursors for such substrates. Accordingly it was attempted to answer the following question. Is the stimulation of these oxidoreductases an example of substrate induction comparable to the well known inductions in bacterial systems, or is it really an androgen effect that finds its expression, more or less fortuitously in the stimulation of an androgen metabolizing enzyme? The first alternative was tested by studying the influence of 5α-pregnan-3,20-dione and 11β,17α,21-trihydroxy-5α-pregnan-3,20-dione on the induction of both enzymes. Both substances are potential substrates for 3α-hydroxysteroid oxidoreductases. Moreover the first one effectively inhibits NADH-linked 3α-reduction of 5α-dihydrotestosterone whereas the second one inhibits NADPH-linked hydrogenation. The second alternative was tested by studying the influence of an antiandrogen, cyproterone acetate, on the induction of these oxidoreductases. This antiandrogen has been demonstrated to interfere with the androgen receptors involved in the stimulation of classical androgen target organs [7, 8]. The results obtained suggest that the stimulation of both enzymes is a specific androgen effect (Table 5). Neither of the 5α-pregnane derivatives influences the oxidoreduc-

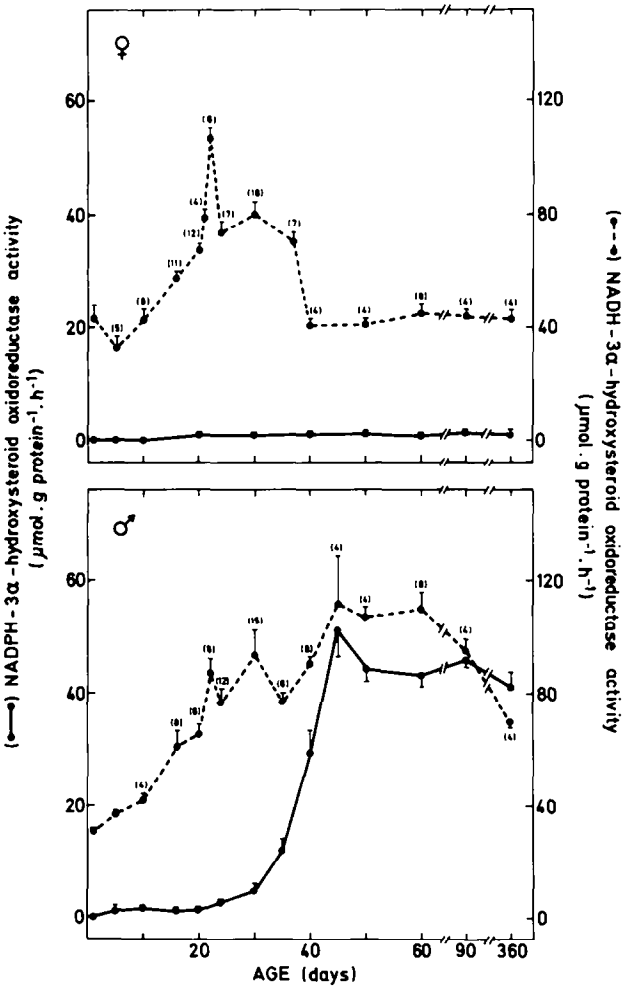

Fig. 2. NADPH- and NADH-linked 3α-hydroxysteroid oxidoreductase activity as a function of age in Wistar II rats. Values for NADPH-3α-hydroxysteroid oxidoreductase activity are the mean ± S.E. of 4 or 8 individual determinations. The number of determinations of NADH-linked activity in each age group has been indicated in parentheses.

tases and cyproterone acetate inhibits the androgen effect.

Microsomal 3α-hydroxysteroid oxidoreductase activity as a function of age. The microsomal NADPH- and NADH-linked 3α-hydroxysteroid oxidoreductases behave quite differently as a function of age (Fig. 2). In female animals the NADPH-dependent activity remains barely detectable throughout life. In male animals this enzyme cannot be found at birth. From day 25 on its activity increases sharply and parallels testicular androgen secretion [9]. The NADH-linked activity, on the contrary, is clearly detectable at birth in male as well as in female animals. Its activity increases steadily during prepubertal life. Around day 20 a first surge in NADH-linked oxidoreductase activity may be observed in male animals. A second surge parallels pubertal testicular activity. In female animals a remarkable upsurge of 3α-hydroxysteroid oxidoreductase activity was observed between day 20 and 22. The levels of activity occurring at this age may even be much higher than indicated in Fig. 2 at least in some litters. In fact, two female litters which displayed activities as high as 213 ± 25 ($n = 4$) and 199 ± 5 ($n = 4$) μmol g protein^{-1} h^{-1} have not been included in this figure. The wide variations observed in the NADH-dependent activity at this age may reflect either a very short and sudden surge in the activity of this enzyme or the interference of uncontrolled factors such as stress with the measurements in the period after weaning. After the age of 37 days a sudden fall in the activity of the NADH-linked dehydrogenase can be noted in female animals. From 40 days on the enzyme reaches adult levels of activity in female rats. In male rats its activity declines slowly after the age of three months.

DISCUSSION

The microsomal 3α-hydroxysteroid oxidoreductases as parameters of androgen activity in the rat kidney. The data presented unequivocally demonstrate that the high levels of NADPH- and NADH-dependent 3α-hydroxysteroid oxidoreductase activity observed in microsomal preparations from adult male rats are physiologically maintained by testicular androgens. As far as the NADPH-linked enzyme is concerned this is in accordance with the results obtained by Ghraf *et al.*[10]. Although several androgen dependent sex differences have been described in mouse and rat kidney, only very few of the enzymes involved have been studied in detail. This is particularly true for the rat. In this species most of the known sex differences are small and/or also under the influence of other non-androgenic modulating factors. In this context we investigated whether the above mentioned oxidoreductases can be used as parameters of androgen activity in the rat kidney.

All available data indicate that the NADPH-linked oxidoreductase may serve as a sensitive index of androgen action in the kidney. This enzyme is 35 times more active in male animals and several findings suggest that androgens are the immediate as well as the main or only stimuli for this dehydrogenase. In female animals it is barely detectable throughout life. In male rats its activity is reduced to very low levels after prepubertal as well as after postpubertal gonadectomy. NADPH-3α-hydroxysteroid oxidoreductase activity can easily be induced by androgens in gonadectomized animals of either sex and in untreated female animals. Other potential substrates are ineffective inducers. The hypophyseal hormones have at most a small potentiating effect on the inducibility of the enzyme [10, 11]. Finally there are some indications that stimulation of this enzyme proceeds along the same lines as stimulation of classical androgen target organs such as the prostate. 5α-Dihydrotestosterone is the most effective inducer and cyproterone acetate a potent competitor for the prostatic 5α-dihydrotestosterone receptor [7, 8] interferes with the enzyme induction. The latter finding disagrees with the results of Ghraf *et al.*[10]. The lower dose of cyproterone acetate used by these investigators might explain this discrepancy.

The NAD-linked oxidoreductase is a less valuable parameter of androgen action in the kidney. The sex differences in the activity of this enzyme are less pronounced. Moreover, both the complex alterations during prepubertal development and the paradoxical stimulation sometimes observed after administration of cyproterone acetate (not shown) suggest that the activity of this enzyme is controlled by non-androgenic factors also.

Although the influence of androgens on the renal microsomal oxidoreductases is quite clear, the ultimate biological significance of the androgenic control of the activity of these enzymes remains obscure.

The "enzymic differentiation" of the microsomal 3α-hydroxysteroid oxidoreductases in rat kidney. As already mentioned, the activity of the *NADPH-3α-hydroxysteroid oxidoreductase* closely parallels the pubertal secretion of androgens by the testis [9]. Only the small but reproducible sex difference in the activity of this enzyme persisting after prepubertal (day 21) as well as after postpubertal gonadectomy deserves some attention. In fact, this sex difference resembles similar differences observed for several steroid metabolizing enzymes in the liver of animals gonadectomized either before or after puberty [12]. As far as the liver is concerned it has been recorded that those residual differences do not depend on circulating extragonadal androgens, but that they are a delayed effect of androgens secreted by the testis in the neonatal period. In an elegant series of experiments Denef [13] demonstrated that their expression is mediated by hypophyseal factors the secretion of which is irreversibly programmed by the neonatal testis. Although definitive conclusions concerning the origin of this sex difference in the kidney will need the elaboration of more sensitive detection methods, the results obtained after adrenalectomy suggest that

the adrenal gland may account at least in part for the observed differences.

The differentiation of the *NADH-3α-hydroxysteroid oxidoreductase* is more complex. The enzyme is detectable at birth and its activity increases before the onset of puberty in animals of both sexes. In terms of "enzymic differentiation" the prepubertal rise in NADH-linked oxidoreductase activity belongs to the "late suckling cluster" a series of enzyme adaptations accompanying the nutritional and hormonal changes occurring about the time of weaning [14]. Several other kidney enzymes display marked increases in activity in this period, e.g.: glutaminase, carbonic anhydrase and alkaline phosphatase [15] arginase [16] and ornithine aminotransferase [17]. These changes are paralleled by an important rise in the activity of the thyroid [18] and in the secretion of corticosterone [19] as well as several other unknown secretion products of the adrenal gland [20]. The influence of the adrenal and the thyroid on steroid metabolizing enzymes in the liver has been documented extensively [21]. The actual contribution of these glands to the increased renal NADH-3α-hydroxysteroid oxidoreductase activity observed about day 20 and their role in the very sudden and pronounced upsurge in activity on day 20–22 in female rats and the decline in rats of the same sex after day 37 remains to be investigated. It cannot be excluded that androgens also play a role in the alterations in NADH-3α-hydroxysteroid oxidoreductase activity preceding puberty. An early testosterone surge has been described in male rats about day 19 [9]. while in female rats very high circulating levels of 3α- and 3β-androstanediol have been noted between day 22 and 24 [22]. Supposing this early rise in activity is indeed mediated by androgens it remains to be explained, however, why no parallel increase in NADPH-dependent activity is apparent about day 20. In fact, when prepubertally gonadectomized rats are stimulated with TP in adult life, NADPH-linked oxidoreductase activity responds earlier than NADH-linked activity.

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