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17α-OXIDOREDUCTASE ACTIVITY IN KIDNEYS OF MALE AND FEMALE MICE OF VARIOUS AGES

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Summary—Male and female mice at 0–120 days of age were used. Homogenates of kidneys were incubated with [^{14}C]4-androstene-3,17-dione, and 17α-oxidoreductase activity per g tissue was examined. The activities of 17α-oxidoreductase in the kidneys of both sexes increased markedly with age during sexual development by up to 150-fold and reached the maximum values (2700 and 1500 nmol/g/h in male and female kidneys, respectively) at 60 days of age. In the adult male mouse kidney, the activity in isolated cortex fractions was 14 times as high as the activity in isolated medulla fractions; in the medulla fractions renal tubules from the cortex accounted for 3–15% of the total tissue. Furthermore, histochemical examination showed the activity present only in the cortex, at which much higher levels in the tubules than in the glomerulus.

Activity at 35–120 days of age was significantly higher in male kidneys than in female kidneys. The difference appears to be induced by testicular androgens during sexual development, since neonatal castration in males resulted in decreases of activity to levels similar to those in female kidneys. However, castration at 60 days of age showed no significant effect on the activity.

The present results show that the activity per g tissue of 17α-oxidoreductase in the mouse kidney increases markedly with age, and that the activity is largely confined to the renal tubules of the cortex.

INTRODUCTION

17α-Hydroxy-4-androsten-3-one (epitestosterone) is considered to be a biologically inactive steroid. It was first reported to be formed in rabbit liver slices in 1947 [1] and has since been demonstrated to be a relatively minor product in a number of biological systems, including human blood and urine [2–4]. In kidneys, the formation of epitestosterone from 4-androstene-3,17-dione (androstenedione) was marked in the rabbit kidney [5] and the adult female hamster kidney [6], but below detection levels in human and rat kidneys [5]. In the adult male mouse kidney, the major metabolite of androstenedione and testosterone was found by Arimasa and Kochakian [7] to be epitestosterone. However, changes with age in activity of 17α-oxidoreductase (the relevant enzyme), and hormonal regulation and intrarenal localization of this enzyme, have not been previously described in mouse kidney.

EXPERIMENTAL

Animals

Male and female (WB × C57 BL/6)F_1 hybrid mice of various ages were raised in our laboratory from parental strains described previously [8]. Some male mice were castrated within 24 h after birth (neonatal castration). Some male mice were castrated at 60 days of age (adult castration) and killed at 120 days of age. Both intact and neonatally castrated mice were weaned at 25 days of age.

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Chemicals

[^{14}C]Androstenedione (52 mCi/mmol) obtained from New England Nuclear Corporation, Boston, MA, U.S.A was purified by paper chromatography in a hexane–formamide system [9] just before use. The purified radioactive substrate contained very small amounts (<0.1%) of contaminating radioactive steroids. Non-radioactive steroids were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. Other reagents were of analytical grade.

Estimation of activities of 17α- and 17β-oxidoreductases

Kidneys from 0, 20, 35, 60 and 120-day old male and female mice were homogenized, and the homogenates (1–4 mg tissue) were incubated with purified [^{14}C]androstenedione (7.7 nmol; 0.4 μCi/tube) and NADPH in air at 37°C for 15 min in 0.8 ml incubation mixture, as previously described [10]. The incubation mixture consisted of 0.15 M potassium phosphate buffer (pH 7.4), 0.13 M sucrose, 0.03 M nicotinamide, 1 mM $MgCl_2$, 0.5 mM EDTA and 1.5 mM NADPH. NADPH was used as cofactor, since NADPH was shown to be more effective than NADH for the estimation of 17α-oxidoreductase activity in the adult mouse kidney [6, 7].

The incubation mixture was extracted three times with ether–chloroform (4:1, v/v). To the extract, 50 μg quantities of androstenedione, epitestosterone and testosterone were added. Paper chromatography was performed using the hexane–benzene (1:1, v/v)–formamide system [9] to separate radioactive products in the extract. Products were separated into

androstenedione, epitestosterone and testosterone fractions. Since 17-hydroxy-5α-C_{19}-steroids were produced in lesser quantities (especially 17α-hydroxy-5α-products under the present incubation conditions), the formation of these 17-hydroxy-5α-C_{19}-steroids was not examined. Radioactivity in the androstenedione, epitestosterone and testosterone fractions was measured using aliquots of eluates. Finally, all the separated [^{14}C]steroids in other aliquots of the epitestosterone and testosterone fractions were recrystallized to constant specific activity with 15 mg of epitestosterone and testosterone, respectively, in order to determine accurate levels.

The activities of 17α- and 17β-oxidoreductases were expressed as epitestosterone and testosterone formed from androstenedione, respectively. The data in Table 1 demonstrate that the assay of 17α-oxidoreductase met quantitative criteria when at least 80% of substrate remained and the incubation time did not exceed 15 min. The methods used for the estimation of 17β-oxidoreductase activity were similar to those reported previously [11]. The assay also met quantitative criteria under the same incubation conditions. Under the assay conditions used, the rate of production of epitestosterone or testosterone was proportional to the amount of tissue and incubation time, when a sufficient amount (1.5 mM) of NADPH was added. Enzyme activities were expressed as nmol of epitestosterone or testosterone formed per g wet tissue per h. Because the concentration of substrate (7.7 nmol/l–4 mg tissue) was supraphysiological, the assumption was made that endogenous levels of the steroid intermediates can be disregarded when estimating enzyme activities (for example, endogenous levels of testosterone estimated by radioimmunoassay in kidneys of various ages were less than 0.002 nmol/10 mg tissue).

Isolation of cortex and medulla from adult mouse kidney

For the isolation of cortex and medulla from kidneys of 60-day old male mice, the central part of frozen kidney was cut into slices of about 2 mm in width. The cortex and medulla fractions were isolated carefully with the naked eye. Isolated cortex and medulla were used for both estimation of enzyme activities and histological examination.

Histology

The kidneys of various ages and the isolated cortex and medulla from 60-day old male kidneys were fixed in 10% formalin, embedded in paraffin, serially sectioned and stained with hematoxylin and eosin.

Histochemical demonstration of 17α-oxidoreductase

The distribution of 17α-oxidoreductase was studied in the kidneys of 60-day old male mice, using epitestosterone and both NADP and NAD. Both NADP and NAD were used as cofactors, since previous studies [6, 7] on the formation of epitestosterone from androstenedione in the adult mouse kidney reported that NADPH was a more effective cofactor for a small amount of substrate, while NADH was more effective for a very large amount of substrate. The methods used for the histochemical demonstration of 17α-oxidoreductase were the same as previously reported for steroid dehydrogenases [12], except for the substrate used. The substrates were dehydroepiandrosterone and 3β-hydroxy-5-pregnen-20-one for 3β-hydroxysteroid dehydrogenase, and testosterone and oestradiol-17β for 17β-hydroxysteroid dehydrogenase, in the previous study [12]; in the present study we used epitestosterone for 17α-hydroxysteroid dehydrogenase.

RESULTS

Weight of mouse kidneys during postnatal maturation

Mean weights of both kidneys of mice during postnatal maturation are shown in Fig. 1. The weights of kidneys increased with age and reached the maximum values at 60 days of age in normal male and female mice, and in neonatally castrated male mice. The most conspicuous increase in weight was found from day 0 to 20 days of age. The weights of kidneys in females and neonatally castrated males were significantly lower than those in male mice at 35 and 60 days of age.

Activity of 17α-oxidoreductase in kidneys of male and female mice at various ages

The specific activity of 17α-oxidoreductase (nmol/g/h) in the kidneys of male and female mice increased markedly with age. The specific activity at day 60 after birth was up to 150 times the specific activity at day 0. The most conspicuous increase was found from 20 to 35 days of age, at which time the specific activity of 17α-oxidoreductase appeared to reach a near plateau (Fig. 2). Since the weight of kidney increased considerably during postnatal maturation (Fig. 1), the total activity of

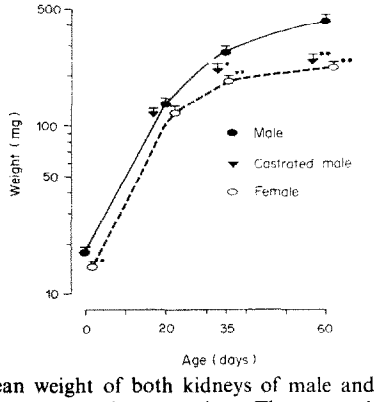

Fig. 1. Mean weight of both kidneys of male and female mice during postnatal maturation. The castration was carried out at day 0 after birth. The kidneys were removed from normal males, normal females and the neonatally castrated males at 0–60 days of age. Each point is the mean ± SD of 4 mice. Differences from male mice (P): * < 0.05, ** < 0.01 (t-test).

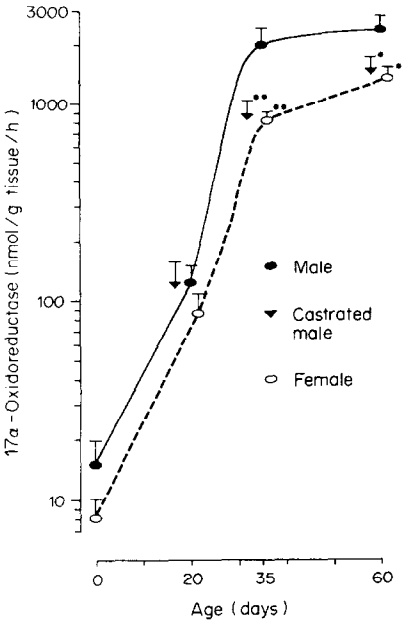

Fig. 2. Activities of 17α-oxidoreductase in kidneys of males, females and neonatally castrated males at different ages. Homogenates of kidneys (1–4 mg) were incubated with [^{14}C]androstenedione (7.7 nmol; 0.4 μCi/tube) and NADPH at 37°C for 15 min in 0.8 ml incubation mixture. For the estimation of enzyme activity, all radioactive products found in epitestosterone fractions were recrystallized to constant specific activity with 15 mg non-radioactive epitestosterone. Each point is the mean ±SD of 4 separate estimations. Differences from male mice (P): * < 0.05, ** < 0.01 (t-test).

17α-oxidoreductase per kidney increased with age remarkably. The total activity at day 60 was up to 3000 times the total activity at day 0.

The specific activity of 17α-oxidoreducatase in male kidneys was significantly higher than in female kidneys at 35 and 60 days of age. At birth and at 20 days of age the differences between sexes were not statistically significant (Fig. 2).

Effect of castration on 17α-oxidoreductase activity in kidneys of male and female mice

Mouse kidney is an androgen responsive tissue [3]. Since the 17α-oxidoreductase activity in male mouse kidney was significantly higher than in the female (Figs 2 and 3), the effect of castration on the enzyme activity was examined. Castration at 60 days of age resulted in a significant decrease in the weight of kidneys in the males at 120 days of age, but not in the females. However, the specific activity of 17α-oxidoreducatase was not significantly changed by castration (Fig. 3).

Effect of the neonatal castration on the 17α-oxidoreductase activity in the male mouse kidney was then examined. Neonatal castration resulted in significant decreases in specific enzyme activity at 35 and 60 days of age, with levels in neonatally castrated males similar to those in the females (Fig. 2).

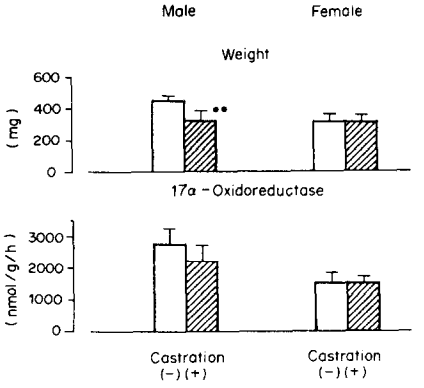

Fig. 3. Effects of castration at day 60 after birth on the weight and 17α-oxidoreductase activity of mouse kidneys. The castrated mice and normal mice were killed at 120 days of age. Incubation conditions are shown in Fig. 2. Each point is the mean ±SD of 4 separate estimations. Difference from non-castration (P): ** < 0.01 (t-test).

Localization of 17α-oxidoreductase activity in kidney of 60-day old male mouse

In the kidneys of adult male mice, 17α-oxidoreductase activity was largely confined to the cortex (Fig. 4). Although the isolated medulla fractions contained a low activity (7% that of cortex on a weight basis), this activity may well reflect contamination by the cortex. On histological examination renal cortical tubules accounted for 3–15% of the total tissue in the isolated medulla fractions. Furthermore, histochemical examination of the intrarenal localization of the 17α-oxidoreductase activity showed the activity in the cortex but not in the medulla in the kidneys of 60-day old male mice (Fig. 5). In the cortex, 17α-oxidoreductase activity was much higher in the renal tubules than in the glomerulus. NAD was the preferred cofactor for the histochemical demonstration of 17α-hydroxysteroid dehydrogenase.

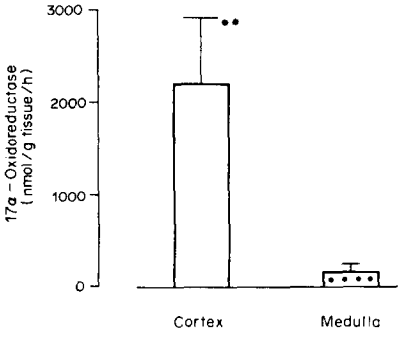

Fig. 4. Activities of 17α-oxidoreductase in the isolated cortex and medulla fractions from kidneys of 60-day old male mice. Incubation conditions are shown in Fig. 2. In the medulla fractions, renal tubules from the cortex accounted for 3–15% of the total tissue. Each point is the mean ±SD of 6 separate estimations. Difference from medulla (P): ** < 0.01 (t-test).

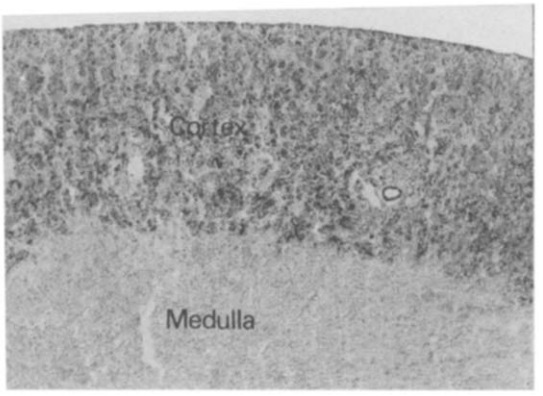

Fig. 5. Histochemical demonstration of 17α-hydroxysteroid dehydrogenase with epitestosterone and NAD in the kidney of 60-day old male mouse. The activity is present only in the cortex (×35).

Table 1. Formation of epitestosterone from androstenedione by varying amounts of mouse kidney and NADPH at different incubation times

Amount of tissue (mg wet weight)	Incubation time (min)	NADPH (mM)	(pmol)	(pmol/mg tissue/h)
0.5	15	3	423	3380
1	15	3	839	3360
2	15	3	1530	3060
4	15	3	2530	2530
8	15	3	3050	1530
2	7.5	3	778	3110
2	15	3	1460	2920
2	30	3	2690	2690
2	60	3	3540	1770
2	15	0.5	1610	3220
2	15	0.0	77	154
0	15	3	0	0

Homogenates of kidneys from 60-day old male mice were incubated with [^{14}C]androstenedione (7.7 nmol; 0.4 μCi/tube) at 37°C in 0.8 ml incubation mixture. Values were obtained after recrystallization to constant specific activity.

Activity of 17α-oxidoreductase in spleen and liver of 60-day old male mouse

The activities of 17α-oxidoreductase in the spleen and the liver were almost undetectable (0.02 and 1.6 nmol/g/h, respectively), though the activity in the kidney was very high (about 3000 nmol/g/h).

Activity of 17β-oxidoreductase in kidneys of male and female mice at different ages

Although the specific activity of 17α-oxidoreductase in the kidneys of both sexes increased markedly with age during sexual development, by up to 150-fold (Fig. 2), the specific activity of 17β-oxidoreductase in mouse kidneys increased with age only slightly (less than 2-fold). Therefore, the specific 17β-oxidoreductase activities in the kidneys were much lower than the specific 17α-oxidoreductase activities at 35 and 60 days of age. The activity of 17β-oxidoreductase in both female and neonatally castrated male kidneys was slightly lower than in male kidneys (Table 2).

DISCUSSION

In the adult male mouse kidney, epitestosterone was reported in 1973 to be the major metabolite of androstenedione [7]. The present study demonstrates for the first time the intrarenal localization of the 17α-oxidoreductase activity, which is required for the formation of epitestosterone from androstenedione.

The 17α-oxidoreductase activity in the adult male mouse kidney was shown to be largely confined to the cortex (Figs 4 and 5), in which the activity was much higher in the tubules than in the glomerulus. Furthermore, the present study demonstrates for the first time that the 17α-oxidoreductase activity per g kidney tissue increases markedly with age during sexual development of male mice by up to 150-fold, and reaches the very high level at maturity (Fig. 2) previously reported by Arimasa and Kochakian in 1973 [7]. A similar marked increase with age in 17α-oxidoreductase activity was also found in the female mouse kidney (Fig. 2). A physiological role of the enzyme in the mouse kidney, and factor(s) regulating its marked increase with age, remain to be determined.

In contrast with the mouse kidney, the 17α-oxidoreductase activity in the hamster kidney was found to be higher in females than in males [6]. It has been reported [3] that the mouse kidney is induced to grow by androgen, but that the hamster kidney shows no detectable dependence on androgen for growth. In mice, the specific activity of 17α-oxidoreductase was significantly higher in the male kidney than in the female kidney and the neonatally castrated male kidney (Fig. 2). The difference in the specific activity between the male and female mouse kidneys thus seems to be induced by androgens secreted from neonatal, immature and

Table 2. Activities of 17β-oxidoreductase in kidneys of male and female mice of various ages

Age (days)	Normal males	Normal females Mean + SD (nmol/g tissue/h); $n = 4$	Neonatally castrated males	Castrated[a] males	Castrated[a] females
0	64 ± 19	65 ± 19			
20	112 ± 36	56 ± 14[b]	55 ± 10		
35	117 ± 28	67 ± 13[b]	70 ± 14[b]		
60	125 ± 46	71 ± 14	65 ± 8		
120	114 ± 48	110 ± 60		133 ± 26	120 ± 30

Homogenates of kidneys (1–4 mg) were incubated with [^{14}C]androstenedione (7.7 nmol; 0.4 μCi/tube) and NADPH at 37°C for 15 min in 0.8 ml incubation mixture.

[a]Castration was carried out at 60 days of age.

[b]$P < 0.05$ vs normal males (t-test).

pubertal testes. Although Arimasa and Kochakian [7] reported that adult castration of male mice produced only a suggestive slight increase in the 17α-oxidoreductase activity, adult castration had no significant effect on the specific activity of 17α-oxidoreductase in the present study (Fig. 3). The activity of 17β-oxidoreductase in the male guinea-pig kidney was decreased by castration and restored by androgen administration [13]. These previous findings in the guinea-pig are not inconsistent with the present findings on the 17β-oxidoreductase activities in the male and female mouse kidneys (Table 2).

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