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Developmental aspects of polyamine-oxidizing enzyme activities in the mouse kidney. Effects of testosterone

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Summary. In the present study developmental patterns of renal polyamine-oxidizing enzymes polyamine oxidase (PAO) and diamine oxidase (DAO) in male and female ICR mice were demonstrated. The effects of testosterone (10μg/100g body weight) on renal PAO and DAO activities were also studied. The differences between sexes in both PAO and DAO activities were most clearly expressed in the immature kidney. At the age of 20 days PAO and DAO activities were 1.52 fold ($p < 0.01$) and 1.75 ($p < 0.02$) respectively higher in male mouse kidney than in female. Maturational processes reflected in significant increases in polyamine- oxidizing enzyme activities mainly in female mouse kidney, comparable with the gain in the kidney wet weight. Our data show that testosterone is able to influence renal PAO and DAO activities in addition to the well-known stimulation of polyamine biosynthesis. The hormonal effects were sex and age dependent. The influence of testosterone on renal PAO activity was mainly age dependent. The slight stimulation of renal PAO activity observed in 20- and 50-day old mice, 24h after testosterone administration, change with a decrease in the enzyme activity at the age of 70 days. The effects of testosterone on renal DAO activity were mainly sex dependent. Testosterone caused stimulation of DAO activity with a very close magnitude (nearly twice) in female mouse kidney, independently of the age of mice. In contrast, in male mice the hormone treatment resulted in a statistically significant increase in renal DAO activity at the age of 70 days (1.3 fold, $p < 0.05$) only. It could be suggested that our data indicate the different contribution of renal PAO and DAO in androgen regulation of polyamine levels, depending on sex and the stage of the postnatal development.

Keywords: Amino acids – Polyamine catabolism – Polyamine oxidase – Diamine oxidase – Testosterone – Mouse kidney

Introduction

The polyamines putrescine, spermidine and spermine are ubiquitous cell constituents that are intimately involved in cell growth and development (Bachrach, 1973; Tabor and Tabor, 1984). The parent polyamine, the diamine putrescine is synthesized from L-ornithine by ornithine decarboxylase (ODC, EC 4.1.1.17) which is the key regulatory enzyme in polyamine biosynthesis (Bachrach, 1973; Russell, 1989). The addition of propylamine residues results in the formation of spermidine and spermine. Oxidation represents a crutial reaction by which polyamines enter catabolic routes for functional inactivation and elimination. Polyamines and their N^1-acetyl derivatives are degraded by the action of two enzymes: polyamine oxidase (PAO, EC 1.5.3.3) and diamine oxidase (EC 1.4.3.6). PAO is an integral part of polyamine interconversion cycle in the course of which spermine and spermidine are first acetylated at N^1-position by spermidine/spermine N^1-acetyl transferase (EC 2.3.1.57) and subsequently cleaved by PAO to form spermidine and putrescine respectively (Seiler, 1995). Moreover, recent findings have proved that not only N^1-acetylated polyamines but spermine itself is also oxidative degraded by PAO in vivo (Sarhan et al., 1991). The putrescine formed in the polyamine interconversion can be reutilized for synthesis of higher polyamines spermidine and spermine. DAO is responsible for the oxidative degradation of putrescine to products, that are not reutilizable in polyamine pathway. The enzyme is considered to be the rate-limiting enzyme in the terminal oxidation of polyamines in vivo (Sessa and Perin, 1994).

Numerous studies have shown that ODC activity and polyamine levels are significantly elevated in mouse kidney following androgen treatment (Persson, 1981; Goldstone et al., 1982; Bullock, 1983). The increased ODC activity and polyamine accumulation in mouse kidney have been proposed to be at least partially responsible for kidney hypertrophy caused by testosterone (Goldstone et al., 1982), although this matter is controversial (Berger and Porter, 1986). Recently, Sanches-Capelo et al. (1994) have demonstrated a significant sexual dimorphism in the pattern of renal ODC ontogenity after the 3rd week of the postnatal development of mice. An ontogenic pattern of renal ODC response to testosterone administration in male mice has also been demonstrated (Pass et al., 1981).

Whereas the testosterone influence on ODC activity has been studied extensively, information on androgen effects on polyamine-oxidizing enzymes is only indirect (Persson, 1981). No information is also available on the ontogenity of renal PAO and DAO. We have recently demonstrated androgen influence on polyamine-oxidizing enzyme in rat tissues (unpublished observations) and the question of whether testosterone is able to affect polyamine-oxidizing enzyme activities in mouse kidney has attracted our interest.

In the present study we have investigated the levels of renal PAO and DAO activities during the postnatal development of male and female mice. The influence of testosterone on polyamine-oxidizing enzymes in the developing kidneys was also studied in order to elucidate the potential importance of

polyamine oxidative degradation as another way of modulating polyamine levels in mouse kidney.

Material and methods

Chemicals

Chemicals used in this study were obtained from the following sources: testosterone, N^1-acetylspermine trihydrochloride, putrescine dihydrochloride, semicarbazide hydrochloride, HEPES sodium salt, MOPS sodium salt, peroxidase, bovine serum albumin, sucrose and EDTA from SIGMA Chemicals Co. (St. Louis, MO, USA), 4-aminoantipyrine and propylene glycol-1,2 from Fluka Chemie AG (Buch, Switzerland), phenol from Ferak Laborat. GMBN (Berlin, Germany).

Animals and treatment

Pregnant ICR mice were housed individually in breeding cages with food and water ad libitum. Newborn mice were bred at 12-hour light/dark cycles and fed after weaning standard pellet diet and water ad libitum. Animals (n = 6 for each group) were decapitated on different days after birth and kidneys were removed immediately.

Mice of both sexes at different age were given testosterone (10 μg/100 g body weight, dissolved in propylene glycol) by intraperitoneal injection. The control mice received propylene glycol only. Animals (n = 6 for each group) were decapitated 24 h after injection and kidneys were removed immediately.

Enzyme assays

For PAO activity assay the kidneys were homogenized (5% w/v in ice-cold 0.25 M sucrose containing 10 mM HEPES (pH 7.2) and 1 mM EDTA. The homogenate was centrifuged at 3,500 × g for 10 min, and the supernatant was collected and centrifuged at 20,000 × g for 20 min. The resulting pellet was suspended in 0.25 M sucrose containing 2 mM MOPS (pH 7.2), 5 mM EDTA, and thus was used as a source for PAO activity assay.

For DAO activity assay, the kidneys were homogenized (20%, w/v) in 0.01 M sodium phosphate buffer (pH 7.0). The homogenate was heated at 60°C in a water-bath for 10 min and centrifuged at 20,000 × g for 20 min. The supernatant was used as an enzyme source for DAO assay.

PAO and DAO activities were determined by the method of Hayashi et al. (1989) as reported previously (Dimitrov et al., 1996). Hydrogen peroxide, formed in the amine oxidase reaction, was measured photometrically by coupling 4-aminoantipyrine with phenol in the presence of peroxidase.

The standard reaction mixture for PAO activity assay (3.0 ml final volume), contained 50 mM glycine-NaOH buffer (pH 9.5), 0.82 mM 4-aminoantipyrine, 10.6 mM phenol, 12 IU of peroxidase, 2.5 mM N^1-acetylspermine and 300 µl enzyme preparation. After incubation at 37°C in a water bath with shaking for 60 min, the reaction was stopped by chilling the tubes on ice. The absorbance was measured at 500 nm in a cuvette of 10 mm light path, against a blank containing all components except the substrate.

The standard reaction mixture for DAO activity assay (3.0 ml final volume), just before the photometric measurement, contained 0.1 M sodium phosphate buffer (pH 7.4), 0.82 mM 4-aminoantipyrine, 10.6 mM phenol, 12 IU peroxidase, 2.5 mM putrescine, 1.0 mM semicarbazide and 300 μl enzyme preparation. The enzyme assay was carried out as follows: the blanks, (containing buffer, enzyme source and peroxidase) were preincubated with semicarbazide (a specific DAO inhibitor) at 37°C in the waterbath for 20 min. Samples containing the same components except semicarbazide were also preincubated under the same conditions. After adding 4-aminoantipyrine, phenol and

putrescine to the blanks and samples, all tubes were incubated at 37°C in the waterbath with shaking for 60 min. The reaction was stopped by chilling the tubes on ice and semicarbazide was added to the samples. The absorbance of samples was measured at 500 nm in a cuvette of 10 mm light path, against a blank.

Determination of protein content

Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Statistical analysis

The statistical significance of differences between two groups of data was estimated by the Student's t-test. The values were considered significant when $p < 0.05$.

Results

The levels of renal PAO activity at different stages of the postnatal development of male and female mice are shown in Fig. 1. At the age of 20 days PAO activity was 1.52-fold ($p < 0.01$) higher in male mouse kidney than in female. A certain, but not statistically significant decrease in the level of renal PAO activity with the age of male mice was observed. In contrast to male, PAO activity in female 50- and 70-day old mice was 1.7 fold ($p < 0.01$) and 1.64 fold ($p < 0.002$) respectively higher than the level in 20-day old animals. The values of renal PAO activity determined in 50- and 70-day old female mice even exceeded the corresponding values in males. The difference in the levels of PAO activity between sexes was statistically significant at 70 days ($p < 0.05$).

DAO activity in immature kidney was 1.75 fold ($p < 0.02$) higher in male than in female mice (Fig. 2). The enzyme activity in both male and female

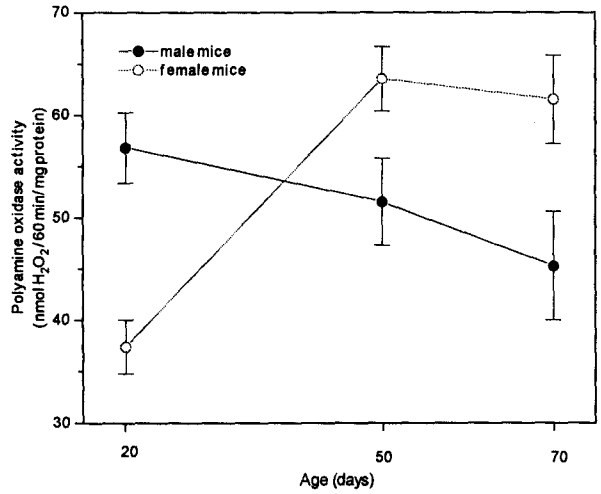

Fig. 1. Polyamine oxidase activity during mouse kidney development. Values are means ± SE of 3 experiments. For details see Material and methods

mice showed tendency to an increase with age. In male mice only a negligible (not statistically significant) elevation in DAO activity at 50 days was observed when compared to the level at 20 days. However, in female mice the enzyme activity was significantly elevated (1.91 fold, $p < 0.001$) when compared to that in immature mice. The highest levels of renal DAO activities were determined in 70-day-old male and female animals.

The kidney wet weight (determined simultaneously with the enzyme assays) increased with the age of animals and was always higher in male than in female mice (data not shown).

The renal PAO and DAO responses to testosterone administration were investigated in 20-, 50- and 70-day-old animals of both sexes. The influence of testosterone on renal PAO activity during the postnatal development of mice is shown in Fig. 3 (for males) and in Fig. 4 (for females). The tendency of hormonal stimulation of PAO activity, observed in 20- and 50-day-old male and female mice, changed with a decrease in the enzyme activity following testosterone treatment of 70-day-old animals. The increase in PAO activity, caused by testosterone was statistically significant in 20-day-old male (1.26 fold, $p < 0.02$) and in 50-day-old male (1.43 fold, $p < 0.002$) and female (1.32 fold, $p < 0.05$) mice. The decrease in renal PAO activity as a result of hormone administration in 70-day-old animals was 1.25 fold ($p < 0.05$) (for male) and 1.45 fold ($p < 0.002$) (for female) respectively.

The effects of testosterone on renal DAO activity during the postnatal development of male mice are summarized in Fig. 5. The hormone treatment resulted in a statistically significant increase in renal DAO activity in 70-day-old animals (1.3 fold, $p < 0.05$) only. In female mice testosterone provoked a marked increase in DAO activity (Fig. 6). The hormonal stimulation was expressed as follows: 1.96 fold ($p < 0.02$) in 20-day-old; 1.82 fold ($p < 0.02$) in 50-day-old and 1.86 fold ($p < 0.002$) in 70-day-old female mice. No statistically

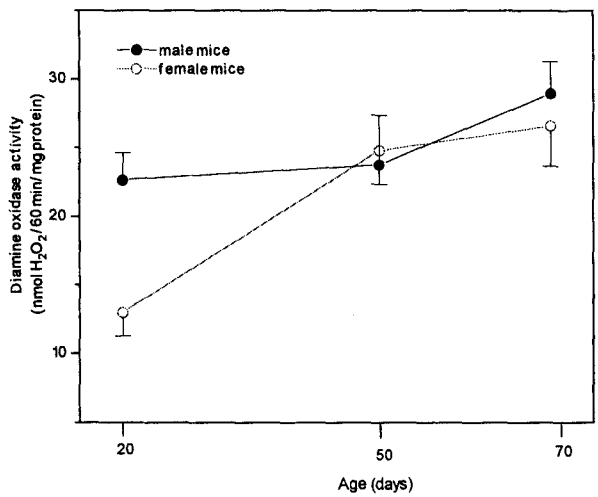

Fig. 2. Diamine oxidase activity during mouse kidney development. Values are means ± SE of 3 experiments. For details see Materials and methods

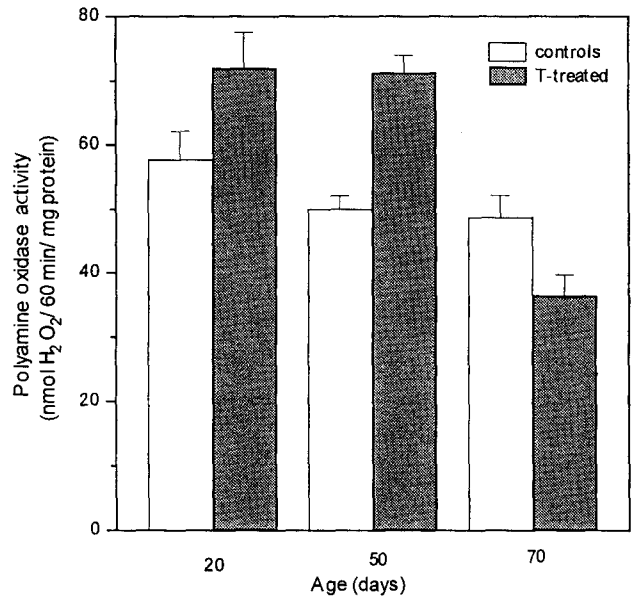

Fig. 3. Effect of testosterone on renal polyamine oxidase activity in male mice. Animals were intraperitoneally injected with testosterone (10μg/100g body weight, dissolved in propylene glycol). The control animals received propylene glycol only). Results are means ± SE of 3 experiments. For details see Material and methods

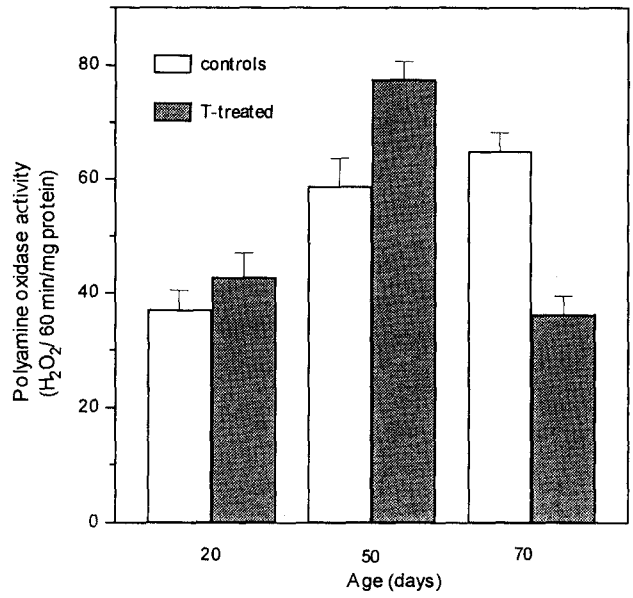

Fig. 4. Effects of testosterone on renal polyamine oxidase in female mice. Animals were intraperitoneally injected with testosterone (10μg/100g body weight, dissolved in propylene glycol). The control animals received propylene glycol only). Results are means ± SE of 3 experiments. For details see Material and methods

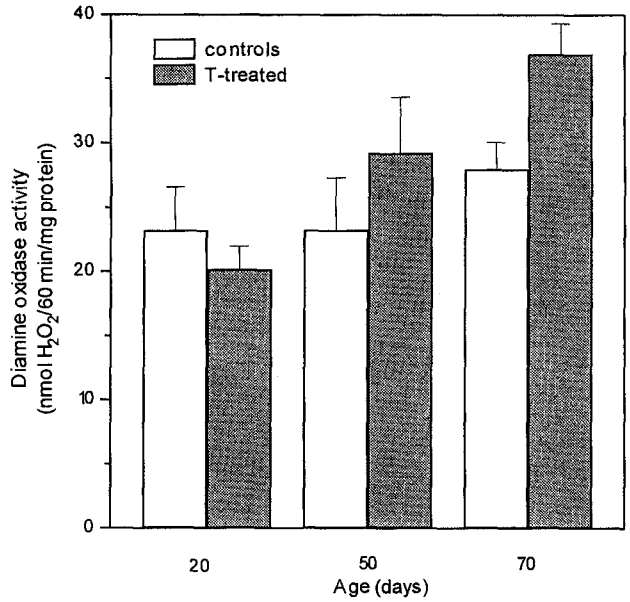

Fig. 5. Effect of testosterone on renal diamine oxidase in male mice. Animals were intraperitoneally injected with testosterone (10 μg/100 g body weight, dissolved in propylene glycol). The control animals received propylene glycol only). Results are means ± SE of 3 experiments. For details see Material and methods

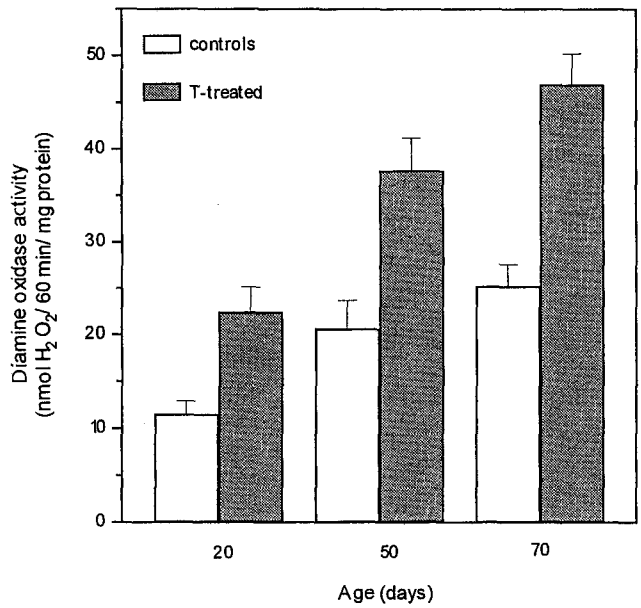

Fig. 6. Effect of testosterone on renal diamine oxidase in female mice. Animals were intraperitoneally injected with testosterone (10 μg/100 g body weight, dissolved in propylene glycol). The control animals received propylene glycol only). Results are means ± SE of 3 experiments. For details see Material and methods

significant changes in kidney wet weight were observed after testosterone treatment of male and female mice at different age (data not shown).

Discussion

In the present study developmental patterns of renal polyamine-oxidizing enzymes PAO and DAO in male and female ICR mice were demonstrated. Among the enzymes involved in polyamine metabolism in mouse kidney only the developmental ontogenity of the key regulatory enzyme in polyamine biosynthesis ODC has been investigated at present (Sanches-Capelo et al., 1994). Recently, significant differences between sexes in renal ODC ontogenity during the postnatal development of CD1 mice have been also shown (Sanches-Capelo et al., 1994). It have been demonstrated an identical relatively low magnitude in the levels of ODC activity in male and female immature kidney. In female kidney ODC activity has remained a relatively low, while in male a marked increase of renal ODC activity as a function of age has been demonstrated. Our results show that a sexual dimorphism obtained for the ontogenity of renal ODC is also valid for the developmental ontogenity of renal polyamine-oxidizing enzyme activities. However, in contrast to ODC the differences between sexes in both PAO and DAO activities were most clearly expressed namely in the immature kidney, while in adult mice no marked differences were observed. The only exception was the PAO activity, which was higher ($p < 0.05$) in female kidney at the age of 70 days. It could also be noted that the most significant changes in both renal PAO and DAO activities as a function of age were observed after maturation of female mice. Maturational processes reflected in significant increases in polyamine-oxidizing enzyme activities in female mouse kidney, comparable with the gain in the kidney wet weight. It is well-known that tissue polyamine concentrations are regulated by biosynthetic, catabolic enzymes and transport. The present data may indicate an important role of catabolic enzymes PAO and DAO in the complex regulation of polyamine pools during the development at least of female kidney. Such a suggestion is in accordance with the notion that polyamine concentrations in female mouse kidney change significantly during the postnatal period, while the level of renal ODC remains a relatively constant (Sanches-Capelo et al., 1994). It is difficult to estimate the significance of the changes in polyamine-oxidizing enzyme activities for the physiology of the mouse kidney. Nevertheless, it could be suggested that the increase in PAO activity may be of particular importance for preventing the accumulation of potentially toxic concentrations of polyamines according to the metabolic needs of the developing kidney.

Numerous studies have shown that testosterone, which is a major determinant of extragenital sexual dimorphism is able to affect the renal ODC activity in both male and female mice. An ontogenic pattern of the renal ODC response to testosterone treatment of male Nya: NILAR mice has been also demonstrated (Pass et al., 1981). The lack of information in literature on the androgenic sensitivity of polyamine-oxidizing enzymes, prompts us to under-

take that investigation of the testosterone influence on renal PAO and DAO activities and its sexual and developmental aspects.

The results obtained show that testosterone is able to influence renal PAO and DAO activities in addition to the well-known stimulation of polyamine biosynthesis. The hormonal effects were sex and age dependent. The influence of testosterone on renal PAO activity was mainly age dependent. The slight stimulation of renal PAO activity in 20- and 50-day old mice, 24h after testosterone administration, changed with a decrease in the enzyme activity at the age of 70 days. These observations may indicate the different contribution of renal PAO in androgen regulation of polyamine levels, depending on the stage of the postnatal development. Interestingly, such a decrease in PAO activity at 24h after prolactin treatment was recently demonstrated by Ferioli and Pinotti (1997) in rat liver.

Our data represent a direct evidence for the androgenic sensitivity of renal DAO activity, which has been suggested by Persson (1981) using a DAO inhibitor aminoguanidine in testosterone-treated male NMRI mice. The effects of T on renal DAO activity were dependent on the sex, female being more affected than male mice. T caused stimulation of DAO activity with a very close magnitude in female mouse kidney, independently of the age of mice. It could be noted that Pass et al. (1981) have reported a similar developmental pattern for renal ODC response to testosterone treatment in Nya: NILAR male mice; however the hormonal stimulation has increased progressively with the age.

The androgen effects reported were manifested by a relatively low hormonal dose, close to the physiological concentrations of testosterone and to the doses used in our investigations concerning effects of sex steroids on polyamine catabolism in rat tissues. Investigations of the effects of several testosterone concentrations are now in progress in our laboratory in order to describe a more complete picture of the androgen influence on mouse kidney polyamine-oxidizing enzymes.

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