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Androgenic sensitivity of polyamine-oxidizing enzyme activities in female rat tissues

Short Communication

V. Pavlov and **O. Dimitrov**

Department of Human and Animal Physiology, Faculty of Biology, University of Sofia "St. Kliment Ohridski", Sofia, Bulgaria

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Summary. Effects of testosterone (10μg/100g body weight) on polyamine-oxidizing enzyme activities in female rat uterus, liver and kidney were demonstrated. Testosterone-treated rats exhibited 2.07 fold ($p < 0.002$) higher uterine polyamine oxidase (PAO) activity and 1.93 fold ($p < 0.02$) higher diamine oxidase (DAO) activity, as compared to the controls. In the liver, testosterone caused an elevation in PAO (1.39 fold, $p < 0.05$), but not in DAO activity, whereas in kidney the hormone stimulated DAO (1.30 fold, $p < 0.05$), but not PAO activity. The effects observed suggest a possible role for testosterone in the modulation of polyamine levels in the female organs studied and especially in uterus.

Keywords: Amino acids – Testosterone – Polyamine oxidase – Diamine oxidase – Female rat tissues

Introduction

Polyamines putrescine, spermidine and spermine are natural compounds, which play an important role in cell growth and differentiation (Bachrach, 1973; Tabor and Tabor, 1984). Disregarding transport processes, tissue polyamine concentrations are highly regulated by the activities of enzymes involved in their biosynthesis and catabolism. In mammalian tissues, putrescine is synthesized from L-ornithine and this reaction is catalyzed by ornithine decarboxylase (ODC), an initial and key-regulatory enzyme in the polyamine *de novo* biosynthesis (Russell, 1989). An addition of propylamine residues results in the formation of higher polyamines spermidine and spermine, which can be interconverted and degraded back to putrescine by the action of two enzymes: polyamine oxidase (PAO) and spermidine/spermine N^1-acetyltransferase. After acetylation by the later enzyme, N^1-acetylderivatives

of spermine and spermidine are degraded by PAO which splits off 3-acetamidopropanal, generating spermidine and putrescine respectively (Seiler, 1995). It has been shown that the subcellular localization of PAO is associated with peroxisomes (Hölttä, 1977; Pavlov et al., 1991; Van den Munckhof et al., 1995) in rat tissues and the physiological breakdown of polyamines occurs exclusively by peroxisomal PAO (Schipper et al., 1999). The oxidative degradation of putrescine is catalized by the cytoplasmic diamine oxidase (DAO), which is the key enzyme in the terminal catabolism of polyamines (Sessa and Perin, 1994).

In addition to the well-known androgen stimulation of the polyamine *de novo* synthesis (Persson, 1981; Bullock, 1983; Tovar et al., 1995), we have recently demonstrated effects of testosterone on PAO and DAO activities as in male as well as in female mouse kidney (Jotova et al., 1999), suggesting that these enzymes may be of particular significance in the androgen modulation of renal polyamine levels. The fact that testosterone was capable of affecting renal PAO and DAO in female mice provoked our interest in further studying of the androgen influence on the polyamine catabolism in the female organism. Recent investigations (Hess et al., 1997) renewed the interest in the concept that androgens and estrogens are the opposite sides of the same coin, considering that most androgens can be converted in estrogens (Mainwaring et al., 1988). Since we have recently shown that estradiol can stimulate polyamine-oxidizing enzyme activities in rat uterus (Dimitrov et al., 1996), in the light of the above mentioned concept we undertook an investigation of the influence of testosterone on PAO and DAO activities in female rat uterus, liver and kidney.

Material and methods

Chemicals

The chemicals were purchased 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).

Treatment of animals

Immature female Wistar rats, 20 days old, were used. They were given testosterone (10 μg/100 g body weight, dissolved in propylene glycol) by an intraperitoneal injection. The control mice received propylene glycol only. Animals (n = 6 for each group) were decapitated 24 h after injection and the uterus, liver and kidneys were removed immediately.

Enzyme assays

For PAO activity assay the tissues 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 tissues were homogenized (20%, w/v) in 0.01 M sodium phosphate buffer (pH 7.0). The homogenate was heated at 60°C in a waterbath 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 mixture for PAO activity assay 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 in a final volume of 3.0 ml. After incubation at 37°C in a waterbath 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 reaction mixture for DAO activity determination (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 a 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 measured by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Statistical analysis

The statistical significance of differences was determined by the Student's t-test. Differences with $p < 0.05$ were considered significant.

Results

The effects of testosterone on PAO activity in immature rat uterus, liver and kidney are presented in Fig. 1. Testosterone-treated rats exhibited 2.07 fold ($p < 0.002$) higher uterine PAO activity, than non-treated animals. An increase of 1.39 fold ($p < 0.05$) in hepatic PAO activity was estimated in testosterone-treated rats when compared to the controls. No statistically significant effect of testosterone on renal PAO activity was observed.

The hormone effects on DAO activity in the organs studied are shown in Fig. 2. Uterine DAO activity was 1.93 fold higher ($p < 0.02$) in testosterone-treated uterus, than in the controls. The decrease in DAO activity in the liver

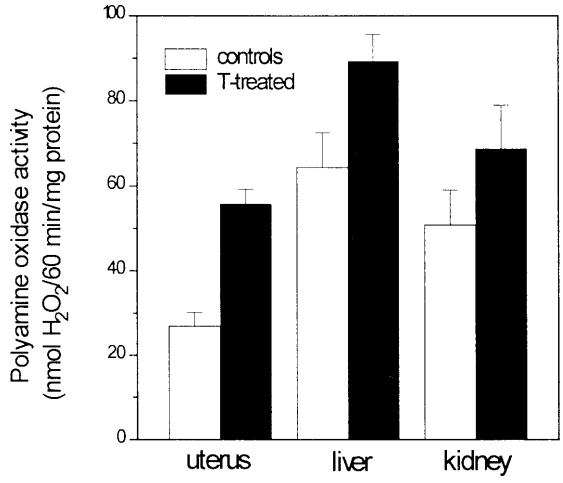

Fig. 1. Effects of testostereone (*T*) on polyamine oxidase activity in female rat tissues. Immature rats were treated with testosterone as described in Material and methods. Uteri from identically animals were pooled into a single homogenate. The liver and kidney from each animal were separately homogenized. Values are means ± SE of 3 experiments

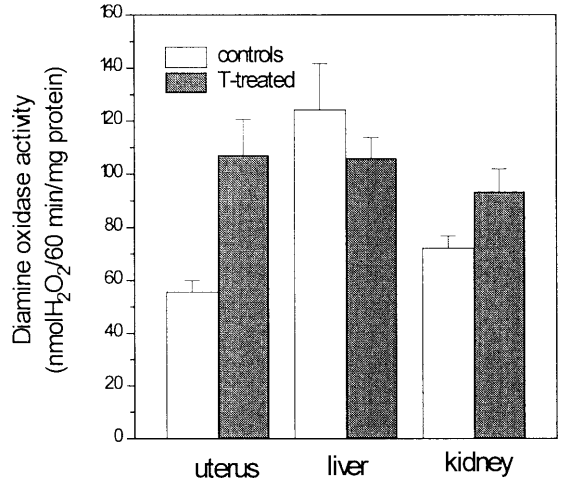

Fig. 2. Effects of testosterone (*T*) on diamine oxidase activity in female rat tissues. Experimental details are described in Fig. 1. Values are means ± SE of 3 experiments

following the administration of testosterone was considered to be not statistically significant. Testosterone treatment caused an increase of 1.30 fold ($p < 0.05$) in renal DAO activity.

Discussion

The data presented show that the male steroid hormone testosterone is able to influence polyamine-oxidizing enzyme activities in female immature

rat tissues. Interestingly, the most significant hormonal influence was observed in such a typical female organ like uterus, where testosterone stimulated both PAO and DAO activities in an equal extent. Organ specificity of the hormonal influence was demonstrated by the fact that in liver testosterone caused statistically significant elevation of the level of PAO, but not of DAO, whereas in kidney the hormone stimulated DAO, but not PAO activity.

The cell can recognize and respond to testosterone by variety of independent mechanisms, some of which have to be defined with regard to metabolite or receptor. Depending on the tissue testosterone may act directly, or to be metabolized (Mainwaring et al., 1988). It is known that androgen receptors are present in uterus (King, 1988). Therefore, it might be suggested that the mechanism of testosterone influence on the uterine polyamine-oxidizing enzymes may involve androgen receptors, which function as ligand-dependent transcription factors. It is well-established that both testosterone and estradiol are present in both sexes. Thus sexual distinctions are not qualitative differences, but rather result from quantitative divergence in hormone concentrations and differential expressions of steroid hormone receptors. We have recently demonstrated that uterine polyamine-oxidizing enzyme activities can be stimulated by estradiol, in an equal experimental model (Dimitrov et al., 1996). Interestingly, the testosterone effect on uterine PAO activity observed in the present study was much stronger than that caused by estradiol (Dimitrov et al., 1996). The magnitude of DAO stimulation by testosterone was respectively equal to that of estradiol. Numerous studies have demonstrated that testosterone can be converted into estrogens in some tissues. For example the major androgen actions on the brain and selected muscles in some species are mediated by estrogens produced from testosterone (Mainwaring et al., 1988). Recent investigations have shown that such a conversion may also occur in rat uterus (Takahashi et al., 1997). Therefore, a suggestion might be done that the androgen influence on uterine polyamine-oxidizing enzyme activities may also involve estrogen receptors. However, additional experiments, using aromatase inhibitors are required to confirm this speculation. The effects of testosterone on the hepatic PAO and renal DAO activities appear to be realized via androgen rather than estrogen receptor mechanism. The fact that estradiol does not affect PAO activity in rat liver (Dimitrov et al., 1996) supports such a suggestion.

The effects observed in the present study suggest that the influence of testosterone on the polyamine-oxidizing enzymes, especially in rat uterus, could be a way of modulating the polyamine pools. This influence might be a regulatory response that acts to reduce intracellular polyamine content according to the metabolic requirements. Further, it would be of interest to investigate the effects of testosterone on the polyamine biosynthetic pathway in the female rat organs studied, in order to describe a more complete picture of androgen influence on polyamine metabolism. Such investigations would also contribute to the better understanding of the physiological functions of androgens in the female organism.

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Authors' address: Dr. Valentin Pavlov, Department of Human and Animal Physiology, Faculty of Biology, University of Sofia "St. Kliment Ohridski", Dr. Tzankov Blvd. 8, 1421, Sofia, Bulgaria

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