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REGULATION OF SEMICARBAZIDE-SENSITIVE AMINE OXIDASE ACTIVITY BY TESTOSTERONE: ROLE OF ANDROGEN RECEPTOR, ORNITHINE DECARBOXYLASE AND NITRIC OXIDE SYNTHASES

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Abstract: Semicarbazide sensitive amine oxidase (SSAO; E.C.1.4.3.6) also known as diamine oxidase (DAO) or vascular adhesion protein-1 (VAP-1) is a key enzyme of the catabolism of polyamines and is partially responsible for the degradation of histamine. Its activity was assayed in male immature rat liver and kidney. It is known that testosterone (T) activates SSAO in murine kidney, an effect that might depend on the intracellular level of polyamines. The aim of this research is to study the participation of androgen receptor, ornithine decarboxylase (ODC) and nitric oxide synthases (NOS) in testosterone regulation of DAO. The rats were treated i.p. 4 hours before measurements with T, T + hydroxyflutamide (HF), an androgen receptor antagonist, T + difluoromethylornithine (DFMO), an inhibitor of ODC or one of two selective NOS inhibitors. It was observed that i) T significantly increased DAO activity in rat kidney; ii) treatment with HF abolished this effect of T; iii) the presence of DFMO had not significant effect on T-dependent influence on DAO activity; iv) the application of NOS inhibitors reduced DAO activity of rat liver and kidney and v) the simultaneously applied T and NOS inhibitor left DAO activity at control level in liver and significantly decreased it in kidney. It is concluded that T may regulate the net (microsomal and soluble) DAO activity in rat kidney and to some extend in rat liver by a mechanism, which includes androgen receptor binding and NO as a downstream signal molecule.

INTRODUCTION

Polyamines are involved in the control of cell cycle and cell growth as regulators of replication, transcription and translation in all cell types (Boyce et al., 2009). Semicarbazide sensitive amine oxidase (SSAO; E.C.1.4.3.6) also known as diamine oxidase (DAO) or vascular adhesion protein-1 (VAP-1) is a key enzyme of catabolism of polyamines and therefore is important reducer

of their intracellular concentration (Boyce et al., 2009; Hernandez et al., 2006). DAO/SSAO common functions are also to generate reactive oxygen species and to facilitate the leucocyte traffic through endothelial vascular layer and thus to support the inflammation by endothelial VAP-1 isoform, to facilitate glucose uptake by an insulin-like effect and others (García-Vicente et al., 2005; Hernandez et al., 2006; Pietrangeli et al., 2009). It is also partially responsible for the degradation of histamine (Boyce et al., 2009).

DAO/SSAO plays an important role in different pathological conditions. It is an important participant in the apoptosis of vascular smooth muscle cell (Hernandez et al., 2006), as well as stimulates the process of fibrosis in kidney (Wong et al., 2013) and liver (Weston et al., 2015). These effects depend mainly on its oxidation products methylglyoxal, formaldehyde, to some extend on the overproduction of hydrogen peroxide H_2O_2 and some other reactive oxygen species by enhanced amounts of VAP-1 and its soluble form - sVAP-1 (Weston et al., 2015; Wong et al., 2013). For that reason enzymes of polyamines' metabolism and mainly DAO seem to be suitable pharmacological targets for therapeutic treatment because their improper activities have been suggested to participate in the early stages of many chronic diseases (Wong et al., 2013).

Nitric oxide (NO), derived from L-arginine by NO synthases (NOS), is an important paracrine regulator of many physiological processes like vascular relaxation and neuronal excitability mainly via its common signal transduction pathway – direct activation of soluble guanylate cyclase (sGC) which downstream generates cGMP as a second messenger (Moncada et al. 1991). Two isoforms are expressed continuously – the endothelial (eNOS) and the neuronal (nNOS), and for that reason they are also known as constitutive isoforms. They operate in tissues under physiological conditions, while the third one – the inducible NOS (iNOS), is important mainly during inflammation (Feng et al., 2012; Lind et al., 2017). Additionally, our previous study reveals that the participation of sGC as a factor for enhanced DAO activity in rat tissues (Mishonova et al., 2017).

The androgens regulate the metabolism of polyamines in mice (Dimitrov et al., 1996; Pavlov & Dimitrov, 2000). T and other androgens have strong immunosuppressive effects (Cutolo & Wilder, 2000) mainly by shifting the cytokine balance towards anti-inflammatory state (Malkin et al., 2004). T supplementation in men with chronic obstructive pulmonary disease increases eNOS and iNOS expression but downregulates iNOS proteins (Chavoshan et al., 2012). T regulates the circulation in some vascular beds as well (Gonzales et al., 2003; Deenadayalu, 2012). These data suggest a T-T receptor-NOS cross-regulation with significant impact on DAO activity.

This study is focused on the role of androgen receptor, ornithine decarboxylase (ODC) and NOS in T-dependent regulation of DAO activity in the liver and kidney of immature male rats. Specific or selective pharmacological tools were used to clarify the participation of these proteins.

MATERIALS AND METHODS

All experimental procedures were conducted in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the Bulgarian Center for Bioethics, the Ethics Committee for Animal Experiments of Sofia University and are compliant with the International Guiding Principles for Biomedical Research Involving Animals, as issued by the Council for International Organizations of Medical Sciences.

In all experiments healthy immature male Wistar rats 30-35 days old and with body weight of 78.4±18.4 g (n = 54) were used. The control animals were injected with 0.2 ml relevant solvent. In the T-tested group the animals were treated i.p. with 20 μg T per rat, dissolved in 0.2 ml propylene glycol. The HF-tested group was injected with 5 mg/kg HF, dissolved in 40 μl DMSO and further mixed with 160 μl saline before application. The T+HF-tested group was injected successively with T and HF. The DFMO-tested group was treated with 2 mg/kg DFMO, dissolved in 0.2 ml saline. The T+DFMO-tested group was injected successively with T and DFMO in same dose. The Nω-Nitro-L-arginine methyl ester (L-NAME)-tested group was treated with 1 mg/kg L-NAME, dissolved in 0.2 ml saline. The T+L-NAME-tested group was injected successively with T and L-NAME. The L-NG-monomethyl arginine (L-NMMA)-tested group was treated with 0.9 mg/kg L-NMMA in 0.2 ml saline. The T+L-NMMA-tested group was injected successively with T and L-NMMA. All animals were treated intraperitoneally 4 h before DAO assay. The liver and kidneys were quickly isolated, weighed, cut into small pieces and homogenized in 10 mM ice-cold sodium phosphate buffer, pH=7.0 in a ratio 1:4 (w:v). The homogenates were heated at 60°C for 10 min and were centrifuged at 20 000 g for 20 min at 40°C. The enzyme activity was measured in supernatant.

Assay of DAO activity

DAO activity was assayed spectrophotometrically in rat liver and kidney as described by Dimitrov et al. (1996) using putrescine as substrate.

Data analysis

The data is expressed as mean±S.E.M. of mainly six independent experiments performed in three parallel samples. The statistical significance was determined using One-way analysis of variance (ANOVA) followed by a Tukey test, if the data were not normally distributed and non-parametric Kruskall-Wallis One Way Analysis of Variance on Ranks test was performed. All statistical analyses were computed using SigmaStat version 3.5.

Chemicals and reagents

Chemicals and reagents were obtained from Sigma-Aldrich, USA. $Na_2HPO_4.2H_2O$ was from Merck, Germany.

RESULTS AND DISCUSSION

Treatment with T increased significantly DAO activity of immature rat kidney (44.01±6.64%, P = 0.002, n=6) but not of the liver (12.72±6.02%, P = 0.065, n=6). The introduction of HF (Fig. 1), an androgen receptor antagonist, decreased DAO activity of rat liver (9.54±4.39%, P = 0.025), while that of the kidney was not changed significantly (-13.7±10.99%, n=4). The application of T and HF together did not change significantly DAO activity neither in liver (-2.00±5.36%, n=8) or in kidney (-2.85±6.81%, P = 0.442, n=8). Thus, the presence of HF abolished the effect of T in kidney DAO activity.

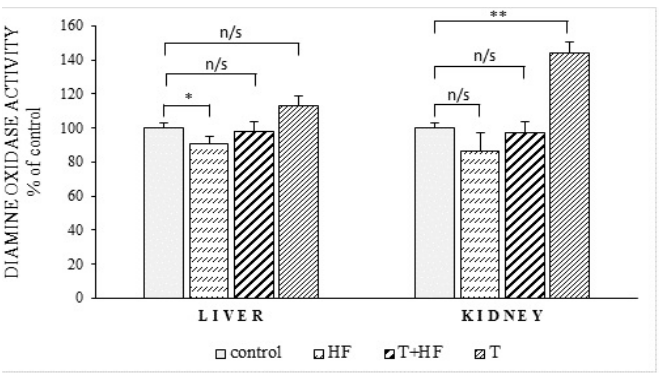

Fig. 1. Effects of hydroxyflutamide (HF) (5 mg per rat), testosterone (T) (20 μg per rat) and combination of T + HF on liver and kidney. Values are mean ± SEM, * P < 0.05, ** P < 0.01.

The introduction of DFMO (Fig. 2), an inhibitor of ODC, was without effect on DAO activity in rat liver (-6.54±14.14%, P= 0.343, n=4) and kidney (3.32±7.42%, P=0.334, n=4). The results of the combination of T and DFMO were similar to those of T alone (5.47±8.24%, P = 0.105, n=8) in liver. In the kidney the application of T+DFMO led to a significant increase of DAO activity (28.24±8.97%, P = 0.009, n=8) that was somewhat lower from T alone (44.01±6.64%, P = 0.002, n=6).

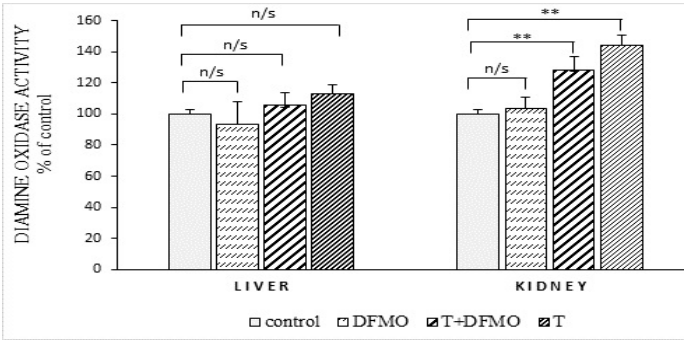

Fig. 2. Effects of DFMO (5 mg per rat), T (20 μg per rat) and the combination of T+DFMO on DAO activity of rat liver and kidney. Values are mean ± SEM, ** P < 0.01.

Another series of experiments studied the role of NO-generating enzymes using NOS inhibitors. The introduction of L-NAME (Fig. 3), a non-selective endothelial and neuronal inhibitor of NOS, induced a significant decrease of DAO activity in both rat liver (28.48±5.61%, P = 0.002, n=6) and kidney (-26.85±3.79%, P = 0.002, n=6). The simultaneous application on another hand expressed tissue differences. T+L-NAME restored the control values of DAO activity in liver (-1.94 ±7.81%, P = 0.671, n=9) and expectedly T+L-NAME showed significant difference vs L-NAME (37.11±10.92%, P = 0.012). In kidney, however, the combination T+L-NAME suppressed DAO activity (-19.60±5.73%, P = 0.010, n=8) as did L-NAME alone. Here L-NAME vs T+L-NAME difference was not observed (9.92±6.68%, P = 0.306).

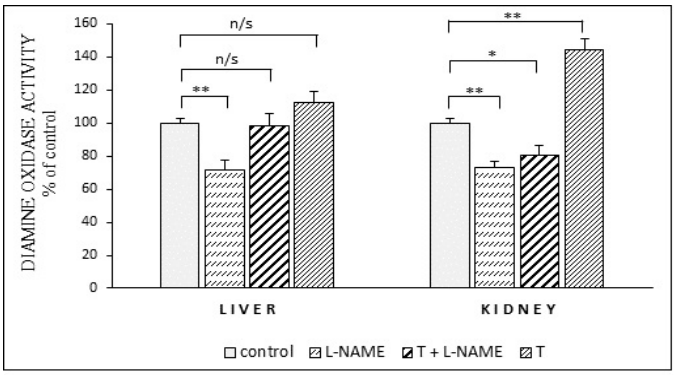

Fig. 3 Effects of L-NAME (1 mg per rat), T (20 μg per rat) and the combination of T+L-NAME on DAO activity of rat liver and kidney. Values are mean ± SEM, * P < 0.05, ** P < 0.01.

A similar pharmacological tool, L-NMMA, a non-selective inhibitor of eNOS, nNOS and iNOS, was also used (see Fig. 4). L-NMMA induced similar effects as L-NAME. In the liver L-NMMA significantly decreased DAO activity compared to its values after the application of T (21.80±3.69%, P = 0.008, n=5). In the same organ the treatment with T+L-NMMA restored DAO activity to controls values (2.68±5.08%, P = 0.69, n=5) and was close to that observed in the presence of T (-8.91±5.04%, P = 0.24).

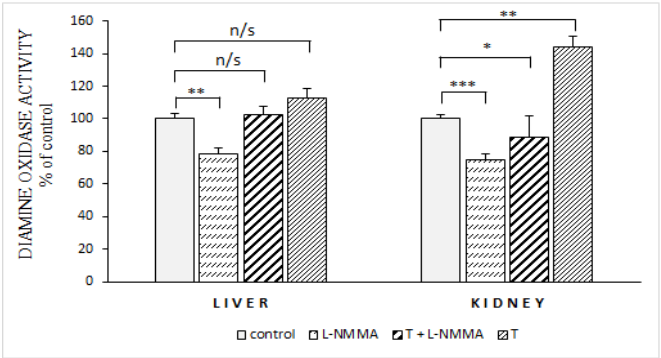

Fig. 4. Effects of L-NMMA (0.9 mg per rat),
T (20 μg per rat) and the combination of T+L-NMMA on DAO activity of rat liver and kidney.
Values are mean ± SEM, * P < 0.05, ** P < 0.01, *** P > 0.001.

In rat kidney (Fig. 4), the treatment with L-NMMA led to significant inhibition of DAO activity (-25.14±3.57%, $P<0.001$, n=5), a difference that was restored in the presence of T. T+L-NMMA vs control is -11.08±12.80% ($P=0.151$, n=5).

Common homeostatic organs, liver and kidney, are androgen targets (Park et al., 2004; Mintziori et al., 2017). Testosterone increases rat kidney DAO activity and restores it after its NOS inhibition to control level in rat liver. This is similar to effects of T in mice (Pavlov & Dimitrov, 2000) and seems to be a general phenomenon for rodents and possibly other mammals. As revealed in this study, this effect depends on T receptor and is not linked to any other possible androgen target. T regulates biosynthesis of polyamines (Jasuja et al., 2014; Lee et al., 2011) and therefore it is reasonable to expect that ODC, once stimulated by T, may enhance the speed of polyamine degradation. This suggestion, however, was not confirmed, as the introduction of specific ODC inhibitor neither induced its own effect on DAO activity in both organs, nor inhibited T-dependent stimulation of DAO enzyme activity.

Selective eNOS and nNOS inhibitor L-NAME and eNOS, nNOS and iNOS inhibitor L-NMMA when applied alone decreased significantly DAO activity. The simultaneous application of NOS inhibitor and T in liver restored DAO activity levels to control values and only slightly enhanced DAO activity in kidney keeping it significantly below the controls and far from those in T alone. The successful inhibition of T effect on DAO by a specific sGC blocker (Mishonova et al., 2017) as well as the data above suggest that T activates DAO mainly by a NOS- and NO-sensitive mechanism, i.e. NOS are unavoidable component of the signal pathway. This suggestion is supported by reports for T-dependent increase of eNOS activity (Li et al., 2016; Ota et al., 2012) which causes acute endothelium-dependent relaxation of some vascular beds (for review Villar et al. 2006). T modulates NO-cGMP signaling in rat Leydig cell (Andric et al., 2010), increases eNOS and iNOS in human (Chavosan et al., 2012) and for that reason it is not a surprise that NOS activities may be responsible for some tissue specific effects of T.

Under our experimental conditions both membrane-bound DAO/SSAO/VAP-1 and plasma sVAP-1 activities were measured. This means that either tissue expression and or higher plasma level due to greater amount of blood can be responsible for the observed higher DAO activity. Further experiments of perfused organs and plasma samples for measurements sVAP-1 are necessary to determine the importance of membrane-bound DAO and sVAP-1.

It is concluded that T, when bound to androgen receptors, increases the activity of soluble and/or microsomal DAO of rat kidney via NOS-sensitive mechanism. Briefly, the mechanism could be described as:
T+androgen receptor→NOS→NO→sGC→cGMP signaling that further activates DAO in the studied tissues DAO. The latter is at least in part mediated by an enhanced local circulation.

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