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Testosterone relaxes abdominal aorta in male Sprague–Dawley rats by opening potassium (K^+) channel and blockade of calcium (Ca^{2+}) channel

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Abstract

Aim: To investigate the direct effect of testosterone and its precursor/derivative dehydroepiandrosterone (DHEA) on isolated rat abdominal aortic rings. **Materials and methods:** 3 mm abdominal aortic rings that were obtained from 3 months old male Sprague–Dawley rats were suspended in organ baths containing Hepes buffered PSS bubbled with 100% oxygen. Relaxation response to testosterone and DHEA was studied in noradrenalin pre-contracted rings. The role of aromatase and androgen receptor was assessed by inhibition using aminogluthetemide and blockade using flutamide respectively. Relaxation responses of the rings to testosterone in the presence of L-NAME, indomethacin, barium chloride, apamin, charybdotoxin, iberiotoxin, and nifedipine were also determined. **Results:** Both aromatase inhibition and androgen receptor blockade did not block the relaxing effect of testosterone on rings from rat abdominal aorta. Also there was no significant difference between testosterone relaxation response in the presence or absence of L-NAME and indomethacin. However 3 μM, $BaCl_2$ almost completely abolished the aortic ring relaxation response to testosterone while 1 μM, nifedipine potentiated the vasorelaxing effect of testosterone. **Conclusion:** Testosterone relaxes abdominal aorta directly via a non-genomic pathway which is independent of endothelial derived vasoactive substances, but involves activation of inward rectifying potassium channel (K_{IR}) and blockade of L-type calcium channel.

Keywords: Testosterone; Non-genomic; Dehydroepiandrosterone; Vascular relaxation; Potassium channel; Calcium channel

1. Introduction

Many studies have reported that there is pronounced sexual dimorphism in many cardiovascular diseases, most notably hypertension and coronary artery diseases [1], as it has been reported that there is a greater incidence of hypertension and coronary artery disease in men and post-menopausal women when compared with pre-menopausal women [2]. Higher male susceptibility to cardiovascular disease may be due to genetic, hormonal or lifestyle factors or a combination of mechanisms. Out of these factors, hormonal effects are the most tractable for practical therapeutic purposes, considering the various reproductive steroids that are available [1]. Furthermore it has been reported that testosterone injection has a relieving effect on patient with angina pectoris [3,4] is beneficial to men with coronary artery disease [5] or congestive heart failure [6,7]. This suggests that testosterone may have an effect on vascular function. Although the classical pathway of androgen action involves steroid binding to the androgen receptor (AR) [8,9], there are now considerable evidence for rapid, non-genomic effect of steroids including androgens [10]. Non-genomic effects of androgen usually involve the rapid induction of conventional second-messenger signal transduction pathways, such as increases in cytosolic calcium and activation of protein kinase A (PKA), protein kinase C (PKC), and mitogen activated protein kinase (MAPK), leading to diverse cellular effects which includes smooth muscle relaxation [9]. No membrane AR has been characterized, but preliminary evidence of a low affinity microsomal

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membrane binding site for alkylated androgens [11] and an endothelial cell plasma membrane dehydropiandrostenodione (DHEA) binding site [12] still require functional proof of specific receptor status. Also a plasma membrane SHBG membranes and initiating intracellular cAMP signaling has been described in humans [13]. The sex hormone binding globulin (SHBG) receptor remains to be fully characterized, and it is not clear whether it has any physiological role in species like rodents that lack circulating SHBG. Sex steroids (oestrogen, progesterone and testosterone) receptors have been identified in blood vessels of human and other mammals and have been localized in the plasmalemma, cytosol and nuclear compartment of various cells including the endothelium and the vascular smooth muscle [2,14]. Most of the non-genomic effects of androgen on the vascular tone have been described in rabbit coronary arteries and aorta, where testosterone relaxes these vessels [15,16], as well as in rat thoracic aorta [17]. Recently, Kouloumenta et al. [18] reported an *in vitro* relaxing effect of testosterone on rabbit airway smooth muscle while the vasorelaxing effect of testosterone on human radial artery [19] and pulmonary artery [20] have also been reported. Differences exist in the responses of vascular cells from different parts and different species to vasoactive substances, for example, rabbit aorta is more responsive to norepinepherine than its branches, a fact that has been ascribed to variations in response to extracellular calcium ions along the aorta [21,22], while rat thoracic aorta and mesenteric artery exhibit differential responses to norepinepherine and serotonin [23]. Therefore this study was designed to investigate the direct effect of testosterone and its precursor/derivative dehydroepiandrosterone (DHEA) on isolated abdominal aortic rings from male Sprague–Dawley rats in the presence or absence of flutamide, aminogluthetemide, L-nitro arginine metyl ester (L-NAME), indomethacin, barium chloride ($BaCl_2$) tertiapin-Q, iberiotoxin, charybdotoxin, apamin and nifedipine.

2. Materials and methods

Experimental protocol was approved by the Animal Research and Ethics Committee of the Biomedical Wing Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala, India.

2.1. Tissue preparation

Ten inbred adult male Sprague–Dawley rats obtained from Department of Laboratory Animal Sciences (DLAS), BioMedical and Technology (BMT) wing Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) Kerala, India, weighing 250–300 g were killed by cervical dislocation. Thereafter the thoracic cage was opened and the aorta was cut at the visible ends and quickly placed in a Petri dish containing cold (4 °C) Hepes-buffered PSS solution. The aorta was carefully freed of connective tissue and the abdominal portion below the diaphragm was cut into 3 mm rings segment. The ring was then mounted between two fine stainless steel rods, with the small S-shaped attached to a thread. The upper part of the rod was attached to the clamp of the micropositioner, while the thread was attached to the isometric force transducer (top force transducer MLT 050/D from ADInstruments, Australia). The rings were superfused in 20 ml organ bath (Panlab LETICA series 01), with Hepes buffer solution at 37 °C and gassed with 100% oxygen. The pH of the Hepes buffer was between 7.35 and 7.40, and all baths used simultaneously had a parallel connection to the source of Hepes buffer. The composition of the solution in mmol/L was NaCl 133, KCl 3.6, $CaCl_2$ 1.8, $MgCl_2{\cdot}6H_2O$ 1.2, glucose 16, Hepes 30, and KH_2PO_4 1.18. After mounting the ring, a passive tension of 2 g was applied to each ring and then allowed to equilibrate for 90 min, during which each ring was subjected to a sub-maximal dose (0.1 μM) of noradrenalin at 30 min interval. Isometric tension was then measured using the top force transducer which was connected to a Powerlab 2/25 recorder (ADInstruments, Australia).

2.2. Experimental procedure

At the end of the 90 min stabilization period, cumulative concentration–response curves to testosterone propionate and dehydroepiandrosterone (DHEA) (0.1–100 μM) were obtained in aortic rings that were pre-contracted with 0.1 μM noradrenalin. The role of aromatase (CPY19), an enzyme that converts testosterone to estradiol in the peripheral tissues, as well as androgen receptor was assessed by inhibition using aminogluthetemide (5 μM) and blockade using flutamide (10 μM) respectively. The involvement of endothelial vasoactive substances on the effect of testosterone was assessed by incubating some aortic rings with L-NAME (100 μM) an endothelial nitric oxide synthase (eNOS) inhibitor, and indomethacin (10 μM) a cyclooxygenase-2 (Cox-2) inhibitor. Similarly, concentration–response curves to testosterone propionate were obtained after incubation of aortic rings in some potassium channel blockers; barium chloride (3 μM), a non-selective inward rectifying K^+ channel (K_{IR}) blocker, tertiapin-Q (100 nM), a selective inward rectifier K^+ channel (Kir 1.1, Kir 3.x subfamilies of K_{IR}) blocker, apamin (1 μM), charybdotoxin (1 μM), and iberiotoxin (25 nM) which are, selective small, intermediate and large conductance calcium-activated K^+ channel blockers respectively. On the other hand, the role of voltage dependent calcium channel (VDCC) on the effect of testosterone was assessed by incubating some aortic rings in nifedipine (1 μM), a conventional L-type calcium channel blocker. Finally, in order to evaluate if calcium channel blockade augments the relaxing effect of testosterone on the aortic rings, we compared its calcium channel blocking potential with that of nifedipine, a conventional L-type calcium channel blocker. Cumulative concentration–response curves to nifedipine (0.1–100 μM), after incubation of the aortic ring in 1 μM testosterone for 30 min, were then obtained.