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Antihypertensive effects of androgens in conscious, spontaneously hypertensive rats

Mercedes Perusquía[a,*], Nieves Herrera[a], Mercedes Ferrer[b,c], John N. Stallone[d,e]

[a] Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Biología Celular y Fisiología, México City 04510, Mexico
[b] Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
[c] Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Madrid, Spain
[d] Women's Health Division, Michael E. DeBakey Institute, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4466, USA
[e] Department of Veterinary Physiology & Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4466, USA

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ABSTRACT

Androgens are vasoactive steroids that induce acute vasodilation in a number of isolated vascular beds from different species, but the effects of these hormones on systemic blood pressure (BP) have been studied little. Although it has been reported that androgens exert systemic hypotensive effects through peripheral vasodilation in normotensive rats, there have not been any reports of systemic hypotensive effects of androgens in animals with hypertension. This study was designed to evaluate the acute effects of testosterone (TES) and its 5-reduced metabolites on systemic BP in hypertensive rats and to test the hypothesis that hypotestosteronemia may be involved in the pathogenesis of hypertension. Chronic, indwelling catheters were implanted in carotid artery and jugular vein of 18–21-week-old male spontaneously hypertensive rats (SHR) and normotensive-control Wistar-Kyoto (WKY) rats, for BP recording and drug administration, respectively. Bolus injections of TES, 5α- or 5β-dihydrotestosterone (5α- and 5β-DHT), were administrated cumulatively to conscious rats at doses of 0.1–100 μmol kg^{-1} min^{-1}. 5β-DHT was also administrated during the pressor effect of Bay K 8644, an L-type voltage-operated Ca^{2+} channel (L-VOCC) agonist. In separate experiments, BP of orchidectomized normotensive male WKY and Wistar rats, with or without androgen-replacement therapy, was evaluated weekly for 10 weeks by tail-cuff plethysmography. TES and its metabolites reduced BP in a dose-dependent manner, while heart rate was reduced with some androgens at the highest doses. The hypotensive effects of androgens were markedly greater in SHR, with a rank order potency of: 5β-DHT > TES > 5α-DHT. 5β-DHT, the most potent antihypertensive androgen, abolished the pressor response to Bay K 8644 in SHR. TES deprivation by orchidectomy increased BP in normotensive WKY and Wistar rats, but this hypertension was prevented by TES replacement therapy. BP responses to androgens are androgen structure-dependent. These data indicate that: 1) androgens play a significant role in the control of BP and may contribute to the pathogenesis of hypertension; 2) blockade of L-VOCC is involved in the antihypertensive effects of androgens, which are non-genomically mediated; and 3) hypotestosteronemia may be a risk factor for hypertension.

1. Introduction

It is well recognized that essential or primary hypertension is one of the most important public health challenges worldwide. This disease is considered to be a multifactorial disorder with many genetic, environmental, and demographic factors contributing to the severity of the increases in blood pressure (BP). Likewise, it has been well documented that the gonadal steroid hormones may also play an important role in the regulation of BP. Although there is a well-established dogmatic view that the androgens are

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; TES, testosterone; 5α-DHT, 5α-dihydrotestosterone; 5β-DHT, 5β-dihydrotestosterone; BP, blood pressure; L-VOCC, L-type voltage-operated Ca^{2+} channels; Tfm, testicular-feminized male; AR, androgen receptor; SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial blood pressure; HR, heart rate; BPM, beats per minute; ED_{50}, effective dose 50; E_{max}, maximal effect; ORX, orchidectomized; ADT, androgen deprivation therapy.

* Corresponding author at: Dept. of Cell Biology and Physiology, Institute for Biomedical Research, P.O. Box: 70228, National Autonomous University of Mexico, Mexico City 04510, Mexico.
E-mail address: perusqui@unam.mx (M. Perusquía).

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pro-hypertensive and exacerbate cardiovascular diseases in men, clinical studies have yet to report higher levels of testosterone (TES) or other androgens in hypertensive men. Of particular interest are the recent studies that have identified beneficial effects that male sex steroids (androgens) may exert on the control of BP and on the development of cardiovascular diseases. Indeed, associations between lower plasma levels of TES and hypertension in men and/or women have been reported in numerous studies [1–6], which suggest beneficial effects of androgens on cardiovascular health. These clinical observations, reinforced with subsequent experimental findings in isolated blood vessels, have led to increasing acceptance of the idea that the vascular system is a target for direct effects of the androgens. It is now well established that androgens produce acute, non-genomically mediated, vaso-relaxation of isolated blood vessels from both males and females of several species, including humans (reviewed by [7–10]). In addition, it has now been clearly established that the vasorelaxing effects of TES, its analogs, and its metabolites, are structurally specific effects, with efficacies and potencies fundamentally different from those for the genomic effects on reproductive targets [8,11–13]. Interestingly, the immediate 5α-reduced metabolite of TES (5α-dihydrotestosterone, 5α-DHT) is genomically active and exerts potent androgenic actions on reproductive targets, but only moderate vasorelaxing effects on isolated blood vessels, while its stereoisomer, 5β-DHT, is genomically inactive but exerts major vasorelaxing effects, with the highest efficacy and potency of all androgens tested for vasorelaxation of isolated vessels from both animals and humans [8,10,14–16] and for reduction of systemic BP in normotensive rats [11].

The simplest interpretation of the acute vasorelaxing effects of TES and its metabolites *in vitro* is that they could reduce systemic arterial BP through direct vasodilation of the systemic vasculature; however, data to support this idea are limited to a few studies in normotensive animal models. In the first such study, intravenous (*iv*) bolus administration of 5-reduced metabolites of TES blocked the vasopressor responses to noradrenaline or Bay K 8644 in anesthetized vagosympathectomized, pithed rats; and 5β-DHT was more potent than 5α-DHT or the 3α,5β-reduced metabolite to induce rapid vasodepressor responses [12]. Similarly, in anesthetized pigs, intra-arterial infusion of TES produced increases in coronary, mesenteric, renal and iliac blood flow [17]. More recently, it was demonstrated that in conscious, ganglionic-blocked male Sprague-Dawley and testicular-feminized male (Tfm) rats, which exhibit a defective intracellular androgen receptor (AR), 5β-DHT produced greater reductions in systemic BP than TES, which were attributed to direct vasodilatory effects of these androgens through activation of neuronal nitric oxide synthase [11]. While these studies strengthen the idea that androgens can decrease BP through direct vasodilation of the systemic vasculature, their potential antihypertensive properties have not been investigated in experimental animal models of hypertension.

Therefore, the present study was designed to determine the direct effects of TES and its 5-reduced metabolites (5α- and 5β-DHT) on systemic BP of conscious, male Spontaneously Hypertensive Rats (SHR), one of the most thoroughly studied animal models of human essential hypertension. The potential antihypertensive effects of the androgens were also studied in normotensive-controls animals (the Wistar-Kyoto rat, WKY). Since androgens appear to block L-type voltage-operated Ca^{2+} channel (L-VOCC) function in single vascular myocytes [18–21], the role of L-VOCC in the potential systemic hypotensive effects of androgens was also examined in male SHR. Finally, the possible association between long-term androgen deficiency and hypertension, inferred from previous human clinical studies, was explored in conscious, male normotensive WKY and Wistar rats, either castrated or castrated with androgen-replacement therapy.

2. Methods

2.1. Animals

The study was conducted in the Department of Cell Biology and Physiology, Institute for Biomedical Research, National Autonomous University of Mexico (UNAM). Animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 86-23, revised 2014). All protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the Institute for Biomedical Research, UNAM.

2.2. Animal surgical preparation

2.2.1. Chronic indwelling vascular catheters

Male (SHR or WKY) rats, 18–21 weeks old were obtained from the Animal Center of the Institute of Cell Physiology, UNAM. The rats were anesthetized with a combination of Ketamine (80 mg/kg) and Xylazine (10 mg/kg) given intraperitoneally, and chronic indwelling catheters were placed in the left carotid artery and right jugular vein, using polyurethane tubing (internal diameter 0.36 mm, external diameter 0.84 mm, "Microrenathane", Braintree Scientific, Inc., USA). The catheters were passed subcutaneously to the dorsal surface of the neck and exteriorized, and the rats were then fitted with polyester cloth vests with Velcro closures that surrounded the chest and neck to protect the catheters.

2.2.2. Castration of male WKY and Wistar rats

In a separate experiment, male WKY rats (18–22 week old) underwent a bilateral orchidectomy surgery. Briefly, the rats were anesthetized with 80/10 mg/kg of Ketamine/Xylazine *i.p.* Each testis was excised through a small incision at the posterior end of the scrotum, the spermatic cord was then ligated with silk suture and transected distal to the ligature to remove the testis. The transected cord was allowed to retract into the inguinal canal and the scrotum was then closed with silk suture. Rats were randomly divided into two groups: orchidectomized WKY rats (oWKY), and oWKY + TES replacement rats. Immediately following surgery, the latter group was administrated androgen replacement therapy using TES propionate (8.75 mg/Kg; dissolved in extra virgin olive oil), given once per week by the *s.c.* injection during the next 10 weeks. In order to detect possible strain differences in the effects of castration on BP, a third group of 18–21-week-old male Wistar rats was castrated (oW) and changes in BP followed weekly during the 10 weeks following orchidectomy surgery.

2.3. Evaluation of antihypertensive effect of androgens in SHRs and WKY

After the SHR and WKY rats had completely recovered from anesthesia and catheter implant surgery (24–48 h), they were placed in clear plexiglass rodent restrainers, which allowed some free movement, and allowed to acclimate to experimental conditions for 90 min. The carotid arterial catheter was connected to a pressure transducer (Grass P23 XL) adapted to an MP150 Research System (Biopac Systems Inc., CA), and the output combined with AcqKnowledge software for data acquisition and analysis. When hemodynamic variables had been stable for a period of at least 90 min, baseline values of BP, *i.e.*, systolic BP (SBP), diastolic BP (DBP) and mean arterial BP (MAP) in mmHg and heart rate (HR) in beats per minute (beats min^{-1}, BPM) were monitored continuously during the experiment and calculated in the