Published in final edited form as:

Vascul Pharmacol. 2012 March ; 56(3-4): 176–182. doi:10.1016/j.vph.2012.01.008.

Regional differences in the vasorelaxing effects of testosterone and its 5-reduced metabolites in the canine vasculature

Mercedes Perusquía^{1,*}, Julia Espinoza¹, Luis M. Montaña², and John N. Stallone³

¹Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 04510 México D.F., México

²Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510 México D.F., México

³Michael E. DeBakey Institute-Women's Health Division, Texas A&M University, College Station, TX 77843-4466, USA

Abstract

Although the vasorelaxing effects of testosterone (T) and various androgen metabolites have been observed in a variety of blood vessels and species, previous studies have not systematically compared the vasorelaxing effects of androgen metabolites in different vascular beds within the same species. Therefore, we studied the vasorelaxing effects of T and its 5-reduced metabolites (5 α - and 5 β -DHT) on KCl-induced contractions of the canine left coronary artery, femoral artery and saphenous vein, using standard isometric recordings. KCl contractions were inhibited by each androgen in a concentration-dependent manner from 1.8 to 310 μ M. Vascular sensitivity and efficacy were expressed as inhibitory concentration 50 (IC₅₀) and maximal relaxation (R_{max}), respectively. The coronary artery was significantly more sensitive to androgen-induced vasorelaxation than the saphenous vein or femoral artery. These vasorelaxing responses were unaffected by an antiandrogen (Flutamide) or the sulfhydryl reagent, *N*-ethylmaleimide, suggesting a nongenomic mechanism independent of signaling mediated by the androgen receptor G proteins. Concentration-response curves were unchanged in endothelium-denuded preparations; thus, the endothelium appears to have no role in androgen-induced vasorelaxation. 5 β -DHT was the most potent androgen in both coronary and femoral artery, but all three androgens were equipotent in the saphenous vein. It is concluded that: 1) significant regional differences exist in vasorelaxing effects of androgen metabolites in the canine vasculature; 2) structural differences in these androgens determine their vasorelaxing efficacy; and 3) regional differences in androgen-induced vasorelaxation may account for some of the conflicting findings reported on the vasorelaxing effects of the androgens.

Keywords

androgens; testosterone; DHT; vasoactive androgens; nongenomic effects

© 2012 Elsevier Inc. All rights reserved.

*Address for reprint requests and other correspondence: M. Perusquía, Dept. of Cell Biology and Physiology, Institute for Biomedical Research, P.O. Box: 70228, National Autonomous University of Mexico, Mexico D.F. 04510 (perusqui@unam.mx).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Androgens are endogenous sex steroid hormones with physiological actions on a variety of tissues including bone, cardiovascular, reproductive, and skeletal muscle. Notably, it has been documented that several androgen metabolites appear to be important mediators of cardiovascular function in both sexes. As reviewed recently (Perusquía and Stallone 2010), numerous studies have now demonstrated that testosterone (T) elicits beneficial effects on cardiovascular function by inducing rapid (3–5 min onset), nongenomically mediated vasorelaxation *in vitro* at a wide range of concentrations (from nM to μ M) in numerous vascular beds (coronary, umbilical arteries, aorta, and small resistance arteries such as: mesenteric, prostatic, pulmonary, and subcutaneous) from a variety of species (rat, mouse, rabbit, pig, dog), including humans. Likewise, androgen-induced vasodilation *in vivo* has been well-established in coronary and systemic circulations in dogs, pigs, and humans (Webb et al., 1999). That the vasodilatory action of testosterone is nongenomic is supported by a wide variety of evidence which shows that testosterone-induced vasorelaxation persists: a) when testosterone is covalently bound to albumin and cannot cross the cell membrane; b) in the presence of inhibitors of DNA transcription (actinomycin D) or mRNA translation (cycloheximide); c) in the presence of antagonists of androgen receptors (AR) such as Flutamide; and d) in AR-deficient Testicular-feminized rats (Perusquía and Stallone 2010). The acute vasodilatory action of the androgens is particularly noteworthy because T and its 5 β -reduced metabolite (5 β -dihydrotestosterone; 5 β -DHT) exhibit greater potency than female sex steroids (estrogens and progestins) to induce vasorelaxation (English et al., 2001; Montañó et al., 2008; Perusquía and Villalon 1999; Perusquía et al., 1996).

Despite the widespread vasodilatory effects of androgens observed in numerous vascular beds and species, there is significant variability in the efficacy of these steroids among the vascular beds and species studied. The apparent variability in the vasodilatory efficacy of some androgen metabolites may thus result from differences among the species and blood vessels studies as well as the agonists used to precontract the vessels for *in vitro* measures of vasorelaxation and the chemical structure of the androgens being studied. This plethora of differences makes accurate and valid comparison of the acute vascular actions of the androgens quite difficult.

Likewise, the role of the endothelium in androgen-induced vasorelaxation has been a matter of debate and remains controversial, with evidence that it is an endothelium-dependent (Chou et al., 1996; Costarella et al., 1996; Geary et al., 2000; Rowell et al., 2009; Tepareenan et al., 2002) as well as -independent mechanism (Deenadayalu et al., 2001; Ding and Stallone 2001; English et al., 2001; Jones et al., 2003; Perusquía et al., 1996; Perusquía et al., 2007; Seyrek et al., 2007; Teoh et al., 2000; Yue et al., 1995). Similarly, very few studies have compared the vasodilatory actions of androgen analogues and metabolites, which are known to exhibit substantial differences in the efficacy and site of action to induce vasorelaxation (Deenadayalu et al., 2001; Ding and Stallone 2001; Montañó et al., 2008; Perusquía and Villalon 1999; Perusquía et al., 1996; Perusquía et al., 2007; Yue et al., 1995).

Remarkably, at present, there are no published studies which have compared the vasorelaxing effects androgens on different vascular beds from the same species, using the same agonist to precontract the vessels for *in vitro* measures of vasorelaxation. Therefore, the aim of the present study was to compare the vasorelaxing effects of T and several key metabolites (5-reduced metabolites), under identical experimental conditions in the canine coronary and femoral arteries and saphenous vein precontracted by KCl. The potential role of AR, G-proteins and the vascular endothelium in androgen-induced vasorelaxation of these canine blood vessels was also examined.

2. Methods

Male mongrel dogs, weighing 15–30 kg, obtained from the Section of Surgical Teaching at the Faculty of Medicine Veterinary and Zootechnics, National Autonomous University of Mexico (UNAM), were killed by rapid exsanguination from the common carotid artery under deep anesthesia with sodium pentobarbital (30 mg/kg, i.v.). All animal protocols were approved by the Institutional Care and Use Committee of the Institute for Biomedical Research, UNAM, and experiments were conducted in accordance with the published *Guiding Principles in the Care and Use of Animals* approved by the American Physiological Society.

2.1. Vascular tissue preparation and contractile force measurements in vitro

Following sacrifice, the heart was rapidly removed, and the left anterior descending coronary artery, the femoral artery (both conduit vessels), and the saphenous vein (a capacitance vessel) were isolated. Arteries and veins were immediately placed in ice cold low- Ca^{2+} Ringer's Solution of the following composition (mM): NaCl (110), KCl (5.0), CaCl_2 (0.16), MgCl_2 (2), NaHCO_3 (10), KH_2PO_4 (0.5) NaH_2PO_4 (0.5), HEPES (12), glucose (10) and EDTA (0.49), resulting in a pH of 7.0. The blood vessels were stored at 4 °C for 24–48 h after isolation and then, the blood vessels were transferred to Krebs-Henseleit bicarbonate solution (KHS) with the following composition (mM): NaHCO_3 (24.9), NaCl (119.5), KCl (4.7), KH_2PO_4 (1.2), MgSO_4 (1.2), CaCl_2 (2.5) and glucose (12.0); this solution was gassed continuously with 95% O_2 in 5% CO_2 to maintain pH 7.4 and constant temperature (37 °C). The blood vessels were cleaned of the surrounding fat and adventitia and cut into rings 3.0 mm in length. Care was taken not to injure the endothelium during the preparation (preparations with endothelium); however, to assess the role of the endothelium, some vascular rings were denuded of their endothelium before mounting by gently rubbing the luminal surface with a frayed nylon string (preparations without endothelium). The presence or absence of endothelium was evaluated functionally in all experiments (as described below).

Vascular rings with or without endothelium from the different vessels were mounted in 10-mL tissue chambers containing KHS at 37 °C, continuously gassed with O_2/CO_2 95:5. The resting force was adjusted to 1 g (10 mN). Contractions were recorded with an isometric force transducer (FTO3C, Grass Instruments, Quincy, MA, USA) connected to a computerized data acquisition and analysis system (MP 150; Biopac Systems, CA, USA). Under these conditions, the vascular rings were equilibrated for 2 h before conducting the experiments.

Following equilibration, the endothelial integrity was assessed in each preparation. The vessels were precontracted with different agonists to ensure the generation of sustained tone (coronary artery, 1 μM prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$); femoral artery and saphenous vein, 1 μM phenylephrine (Phe). Endothelial integrity was confirmed when 10 μM acetylcholine (ACh) or 10 μM carbachol (CCh) produced relaxation of at least 40% in the precontracted vessels. Neither ACh nor CCh (0.5, 1 or 10 μM) produced relaxation of saphenous vein rings precontracted with Phe, $\text{PGF}_{2\alpha}$, KCl, noradrenaline, or serotonin. Therefore, in these vessels, deletion of the endothelium was confirmed histologically by light microscopy (hematoxylin-eosin) after the experiments. As a positive control in this vessel, the effects of the NO donor, sodium nitroprusside (SNP; 0.001 to 10 μM in a cumulative manner) were tested on Phe-induced contraction at 1 μM in de-endothelized preparations.

After functional evaluation of the endothelium, the preparations, both with and without endothelium, were washed with KHS and allowed to return to the basal tension. Tissues were allowed to equilibrate for 60 min and then a contraction was evoked with high

potassium solution (KCl 60 mM; KHS with an equimolar substitution of 60 mM KCl and 64.7 mM NaCl). This contraction was repeated three times to verify the reproducibility of the tissue response. After the KCl contractions, the tissues were washed with KHS to re-equilibrate for 60 min. The KCl-induced contraction was repeated for 90 min (control response) and the tissues were washed. Before any treatment the KCl contraction was evoked again and after a stable contractile tension was attained, cumulative concentrations (1.8, 3.1, 5.6, 10, 18, 31, 56, 100 and 310 μ M) of T, 5 α - or 5 β -dihydrotestosterone (-DHT), dissolved in absolute ethanol (0.1% vol/vol), were applied during the sustained phase of tension. The response to androgens at each concentration was evaluated after 30 min. Finally, the rings were washed and re-equilibrated for 60 min before inducing the last contraction to KCl, recorded during 90 min (second control to verify tissue viability). Afterward, the concentration-response curves to each androgen were plotted, and the inhibitory concentration 50 (IC_{50} ; value for androgen concentration to inhibit 50% of KCl-induced contraction) was calculated to compare the sensitivity of androgen-induced vasorelaxation. Likewise, the highest concentration to induce the maximal relaxation (R_{max}) on KCl-induced contraction was obtained for each androgen to determine their efficacy. In a separate group of experiments, blood vessels were exposed to the equivalent ethanol solvent (0.1% vol/vol) on KCl-induced contraction. Only one treatment was made in each experiment, which means that the concentration-response curves to the different androgens were determined independently in samples from different animals; thus, each n refers to one dog.

Some experiments were performed to study the role that cytosolic (genomic) AR and G-proteins may play in androgen-induced vasorelaxation. The three blood vessels were pretreated with 10 μ M Flutamide (an androgen receptor antagonist) or 30 μ M *N*-ethylmaleimide (NEM; a sulfhydryl alkylating agent, which uncouples G-proteins) 10 min after the KCl stimulus. Thirty min later, the R_{max} for each androgen was evaluated for 30 min. These responses were compared with the vasorelaxation induced by each androgen without any pretreatment (i.e., vehicle-control).

2.2. Data presentation and statistical analysis

Each experiment was performed on vascular rings prepared from individual dogs. All tension measurements in the text and figures were expressed as a percentage of inhibition to 60 mM KCl contraction, each point represents the mean $\pm$ SEM ($n = 6$ experiments, where $n = 1$ represents one dog). Thus, the vasorelaxing effects of androgens on KCl-induced contraction and their relative potency (IC_{50}) and efficacy (R_{max}) were evaluated as described in previous studies (Montaño et al., 2008; Perusquía and Villalon 1999; Perusquía et al., 1996; Perusquía, 2003; Perusquía et al., 2007). Nonpaired Student *t*-test was used to compare the responses between two groups. To compare IC_{50} and R_{max} between groups, a two-way ANOVA followed by Dunnett's test were used. Significance was accepted at $P < 0.05$.

2.3. Drugs and chemicals

With exception of sodium pentobarbital (Anestosal; Pfizer México), the remaining compounds were purchased from Sigma (St. Louis, MO) and included: Testosterone (T; 17 β -hydroxy-4-andosten-3-one), 5 α -DHT (17 β -hydroxy-5 α -androstan-3-one), 5 β -DHT (17 β -hydroxy-5 β -androstan-3-one), prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$; ((*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(*E*,3*S*)-3-hydroxyoct-1-enyl]cyclopentyl]hept-5-enoic acid), phenylephrine hydrochloride (Phe) carbachol chloride (CCh), acetylcholine chloride (ACh), sodium nitroprusside (SNP; Sodium pentacyanonitrosylferrate(II)), Flutamide (2-methyl-*N*-[4nitro-(3-trifluoromethyl)phenyl]propanamide) and *N*-ethylmaleimide (NEM).

The steroids and $\text{PGF}_{2\alpha}$ were separately prepared as stock solution in absolute ethanol and then diluted in absolute ethanol to the concentration needed for each experiment; final ethanol concentration in the tissue baths never exceeded 0.1% v/v of vehicle, and the remaining drugs were dissolved in distilled water. Phe and SNP were kept in the dark until use in order to avoid light-induced degradation.

3. Results

3.1. Effects of androgens on vascular tone

The three androgens tested were found to relax canine saphenous vein, as well as coronary and femoral arteries, precontracted by KCl, in a concentration-dependent manner. In the coronary and femoral arteries, no significant differences were found in the relaxation responses to androgens with or without endothelium (Fig. 1). The vasorelaxing effect induced by every androgen at each concentration tested in the three blood vessels was significantly different ($p < 0.05$) vs. the vehicle control. Although all androgens tested produced a concentration-related vasorelaxation in each of the three vascular beds, marked differences in sensitivity were observed: i) the detectable vasorelaxing response induced by each androgen starting at different concentrations in each blood vessel and; ii) unlike concentration required to achieve the $R_{\max}$. Clearly, the analysis of these data indicates that the vasorelaxing potency (IC_{50}) and efficacy ($R_{\max}$) of each androgen in the three blood vessel types was different (Table 1).

3.2. Blood vessels sensitivity to androgen-induced vasorelaxation

Vascular sensitivity was determined when the IC_{50} of each androgen was compared in every blood vessel. The coronary artery was significantly more sensitive to androgen-induced vasorelaxation than that observed in the saphenous vein and the femoral artery ($P < 0.0001$ and $P < 0.00001$, respectively). The order of vascular sensitivity was: coronary artery > saphenous vein > femoral artery.

3.3. Vasorelaxing potency and efficacy of each androgen

The potency was expressed as the IC_{50} of each androgen in the same blood vessel. Thus, the selective androgens produced vasorelaxation with different potencies (Table 1). The order of potency was: 1) coronary artery, $5\beta\text{-DHT} \gg 5\alpha\text{-DHT} > \text{T}$; 2) femoral artery, $5\beta\text{-DHT} \gg \text{T} \gg 5\alpha\text{-DHT}$; and 3) saphenous vein, $\text{T} = 5\beta\text{-DHT} = 5\alpha\text{-DHT}$.

Table 1 also shows the vasorelaxing efficacy of each androgen, expressed as $R_{\max}$. The order of efficacy was: 1) coronary artery, $5\beta\text{-DHT} > \text{T} = 5\alpha\text{-DHT}$; 2) femoral artery, $5\beta\text{-DHT} = \text{T} > 5\alpha\text{-DHT}$; 3) saphenous vein, $5\beta\text{-DHT} = \text{T} > 5\alpha\text{-DHT}$.

3.4. Vasorelaxing effect of androgens in the presence and absence of endothelium

In this study, we observed that androgens caused concentration-dependent inhibition of KCl contractions in the coronary and femoral arteries with and without endothelium. However, the endothelium had an atypical function in the saphenous vein since neither ACh nor CCh, which are NO-dependent vasodilators, relaxed the preparations with or without endothelium; on the contrary, they had the opposite effect by inducing an increment on the tone of KCl-induced contraction. To further understand the unusual behavior of these NO-dependent vasorelaxants, we examined the responses elicited by a NO donor as a positive control. Thus, we observed that SNP-induced concentration-dependent relaxation (from 0.001 to 10 μM) in the endothelium-denuded saphenous vein (Fig. 2).

3.5. Androgen-induced vasorelaxation in the presence of an antiandrogen and an agent which uncouples G proteins from several receptors

Pretreatment with 10 μ M Flutamide (antagonist of androgen receptors) or 30 μ M NEM (uncouples G proteins) did not block the vasorelaxing effect induced by androgens at the concentrations eliciting $R_{\max}$ in the blood vessels studied (Table 2).

4. Discussion

In canine blood vessels we observed an important relaxing effect induced by T and its 5-reduced metabolites which was concentration-dependent; however, the sensitivity to the vasorelaxation induced by androgens differed markedly with the vascular bed studied and with molecular structure of the androgens tested. The results of this study indicate that all the androgens tested are capable of inducing relaxation of the canine vasculature, but that there are regional differences in the vasorelaxing activity, potency and efficacy of androgens under the same experimental conditions. Furthermore, we observe that an androgen receptor antagonist (Flutamide) did not block androgen-induced vasorelaxation, which implies that the classic intracellular AR is not involved in the vasorelaxing effect elicited by these three androgens in the three vascular districts studied.

We observed androgen-induced vasorelaxation at supra-physiological (micromolar) concentrations, which is consistent with a number of observations in the literature which used isolated large vessels and isometric myography. Experiments in small vessels using wire myograph have reported that T has a significant vasorelaxing effect physiological male concentrations (nanomolar range). These studies have to be interpreted carefully since the vasorelaxing response to T is clearly detected only in pharmacological concentrations (micromolar range) in pulmonary arteries from rats constricted with $\text{PGF}_{2\alpha}$ (Jones et al., 2002), human subcutaneous resistance arteries precontracted by KCl (Malkin et al., 2006), pig prostatic small arteries constricted with noradrenaline (Navarro-Dorado et al., 2008), and human pulmonary arteries and veins constricted by KCl (Rowell et al., 2009). Moreover, this last study also reported that the men blood vessels may exhibit vasorelaxation to T at lower concentrations compared to vessels from women. However, collaterally, it was reported that perfusion of T causes acute vasorelaxation at physiologically relevant concentrations in the male rat mesenteric arterial bed constricted by methoxamine (Tepareenan et al., 2002). In addition, it has been documented that infusion of T produces coronary or systemic vasorelaxation *in vivo* at physiological concentrations in humans (Webb et al., 1999) and in canine (Chou et al., 1996) and porcine (Molinari et al., 2002) animal models. These studies reveal that the range of concentration to observe vasorelaxation depends on the mode of T administration. There is also some evidence that T and its dihydro-metabolites inhibit voltage-gated calcium channels at physiological male concentrations in single vascular myocytes using the patch-clamp technique (Hall et al., 2006; Montañó et al., 2008; Scragg et al., 2004; Scragg et al., 2007). Therefore, it is well recognized that discrepancies exist between: i) the concentrations of T required to produce effects *in vitro* and *in vivo*; ii) its mode of T administration; iii) the different size of blood vessels examined; iv) the vasoconstrictor agent used to precontract the vessels; and v) the chemical type of androgen tested.

4.1. Different sensitivity of blood vessels to androgen-induced vasorelaxation

The literature is unclear on the sensitivity of blood vessels to the androgens. In the present study we were able to detect differences in sensitivity of the blood vessels in the same species to different androgens using the same constrictor agonist to precontract the vessels (KCl). The coronary artery was more sensitive to androgen-induced vasorelaxation than the other blood vessels studied; the femoral artery was less sensitive than the saphenous vein to

androgens. The high sensitivity of coronary artery is similar to previous reports in which both human and animal experiments have demonstrated that T elicits marked coronary vasodilatation both *in vivo* and *in vitro* (Chou et al., 1996; Crews and Khalil 1999; Deenadayalu et al., 2001; English et al., 2002; Webb et al., 1999; Won et al., 2003). Our results indicate that there are regional differences in the reactivity of vascular beds from the same species which may have implications for androgen-induced vasodilation and its effects on blood pressure. Therefore, the differential sensitivity of the blood vessels examined to androgen-induced vasorelaxation deserves further consideration.

4.2. The potential role of endothelium in the vasorelaxing effect of androgens

The endothelium appears to play a major role in the regulation of vascular tone by releasing relaxing and contracting factors (Furchgott and Vanhoutte 1989). Thus, the vasorelaxing effects of androgens may be associated with the release of NO from the endothelium. In this study we observed that removal of the endothelium did not alter the vasorelaxing responses to the androgens. This is in accordance with previous results (reviewed by Perusquía and Stallone 2010), where androgens were used to induce relaxation in endothelium-denuded vascular preparations. The results of the present study clearly demonstrate that androgen-induced vasorelaxation in the canine blood vessels studied is endothelium-independent and does not involve NO. The role of the endothelium in androgen-induced vasorelaxation has been a matter of debate and remains controversial. Experimental evidence suggests that T-induced vasorelaxation is an endothelium-dependent mechanism (Chou et al., 1996; Costarella et al., 1996; Geary et al., 2000; Rowell et al., 2009; Tep-areenan et al., 2002) whereas the majority of studies suggest that this vasorelaxing mechanism is endothelium-independent (Deenadayalu et al., 2001; Ding and Stallone 2001; English et al., 2001; Jones et al., 2003; Perusquía et al., 1996; Perusquía et al., 2007; Seyrek et al., 2007; Teoh et al., 2000; Yue et al., 1995). Again, this uncertainty might be due, at least in part, to the relatively small number of experimental studies that have compared the vasorelaxing effects of androgens in the same blood vessels from several species.

In the present study, the coronary and femoral arteries demonstrated an endothelium-dependent vasorelaxation to ACh or CCh, whereas the saphenous vein did not and a vasoconstriction was evoked. ACh is known indirectly to cause vasorelaxation via interaction with muscarinic receptors on endothelial cell plasma membrane with subsequent release of NO. However, when the endothelium is damaged or is dysfunctional, ACh may couple with muscarinic receptors on the vascular smooth muscle, resulting in vasoconstriction as in the case of the saphenous vein in the present study. Moreover, in this vessel, we were able to verify that the NO donor (SNP) induces vasorelaxation which suggests that the endothelium is dysfunctional or that it does not express cholinergic receptors. Further, there is a recent report that the vascular endothelium in the human saphenous vein is not easily categorized as typical in its responses to traditional NO-dependent vasodilators (Webb et al., 2010). Similarly the dog saphenous vein seems to have an unusual class of alpha adrenoceptors (reviewed in Low et al., 1999). Therefore, the relaxing effect of androgens on the dog saphenous vein is important because this blood vessel shows close similarities to the human saphenous vein, which acts as a conduit in human coronary artery bypass graft surgery. The present findings provide evidence that it is also important to consider the heterogeneity in endothelial properties between species, organs, vessel classes and even within individual vessels and, moreover, within the different vascular segments of each organ.

4.3. Chemical structure-vasorelaxing activity relationship of androgens

The present findings reveal that the vasorelaxing potency and efficacy of each androgen differed between the two arteries. 5 β -DHT displayed a high potency to relax the contraction

induced by KCl in coronary and femoral arteries, but with the same efficacy as its precursor T in femoral artery and saphenous vein. With respect to the 5α -reduced metabolite of T (5α -DHT), its potency and efficacy were always lower than T or its 5β isomer. With regard to the markedly high potency and efficacy of 5β -DHT in coronary and femoral arteries, our own research group has previously reported this powerful vasorelaxing action of 5β -DHT in the rat thoracic aorta, (Perusquía et al., 1996), human umbilical artery (Perusquía et al., 2007) and nonvascular smooth muscle (Perusquía, 2001; Perusquía et al., 2005). In fact, the differences in potency and efficacy of various androgens in the coronary and femoral arteries suggest a chemical structure-function activity relationship as reported previously (Ding and Stallone 2001; Perusquía, 2003; Perusquía and Stallone 2010). In these studies, it was determined that the molecular changes in androgen structure are crucial, in part because when the C₅ hydrogen is β -oriented (β /*cis* configuration in the 5β -DHT) efficacy and/or potency to induce vasorelaxation are much better than when it is α -oriented (α /*trans* configuration in the 5α -DHT) or in the Δ 4,3-keto structure of T. It is also tempting to suggest that this difference could be a consequence of the 5β -configuration, which may be highly specific for activation of nongenomic effects. Indeed, this view is supported by the established fact that 5α -DHT is a highly potent androgen, with high affinity for the cytosolic AR, but has only moderate potency and efficacy as a vasodilator. In contrast, 5β -DHT has virtually no affinity for the AR and is devoid of androgenic actions, yet is a highly potent and efficacious vasodilator, an action which clearly is not mediated by the classical cytosolic AR. Notably, all three androgens studied were equipotent in the saphenous vein. This may be due in part to differences in receptor density or coupling efficiency for signal transduction pathways in arteries versus veins. We also conclude that responses of the saphenous vein to androgens are atypical compared to virtually all arteries studied.

4.4. Androgen-induced vasorelaxation may depend on the preconstrictor agonist used

The above lines of evidence clearly reveal regional differences in androgen-induced vasorelaxation on the background of KCl-elicited precontraction. Nevertheless, another possible difference in the effect of androgens might involve the vasoconstrictor agent used or the amount of precontraction. We have documented that the vasorelaxation in response to androgens is more pronounced in blood vessels depolarized by high KCl solution than in agonist-stimulated blood vessels (Perusquía and Villalon 1999; Perusquía et al., 2007). In this respect, it has been widely recognized that membrane depolarization by high KCl is known to stimulate Ca^{2+} entry from extracellular space mainly through voltage operated Ca^{2+} gates to induce sustained smooth muscle contraction. In contrast, the contractile response generated by different agonists such as $\text{PGF}_{2\alpha}$, Phe, serotonin, or noradrenaline may each be dependent upon different mechanisms of contraction (i.e., intracellular Ca^{2+} release vs. extracellular Ca^{2+} entry). In general, agonist-induced vasoconstriction is known to be activated by phospholipase C, an enzyme that causes the breakdown of phosphatidylinositol-4,5-bisphosphate to inositol 1,4,5-triphosphate (IP_3) and 1,2-diaclyglycerol (Berridge and Irvine 1984). IP_3 stimulates Ca^{2+} release from intracellular stores and causes a transient increase in $[\text{Ca}^{2+}]_i$ (Suematsu et al., 1984), whereas, 1,2-diaclyglycerol activates the enzyme protein kinase C (Nishizuka, 1992). In particular, $\text{PGF}_{2\alpha}$ is proposed to trigger extracellular calcium entry via receptor- and voltage-operated Ca^{2+} channels (Priest et al., 1998). Phe has been shown to stimulate both Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx (Khalil and van Breemen, 1988). The vasoconstrictor effect of 5-HT elicits an enhanced influx of extracellular Ca^{2+} via voltage- or receptor-operated Ca^{2+} channels (Medeiros and Calixto 1991); whereas the contraction generated by noradrenaline may also promote extracellular Ca^{2+} entry through the receptor-operated Ca^{2+} channels, L-type voltage-operated Ca^{2+} channels and non-L-type Ca^{2+} channels (Cauvin et al., 1985). The sensitivity of the relaxing response to T varies with these contractile agonists, which generate different signaling pathways; however, T is capable of

producing vascular relaxation with all of them. It is important to highlight that these contractile agents produce stimulation of Ca^{2+} entry from extracellular space predominantly through voltage-operated Ca^{2+} channels. Admittedly, T may, in fact, inhibit additional contractile mechanisms, such as Ca^{2+} entry through receptor-operated Ca^{2+} channels and/or modify the internal signaling pathways (Montaño et al., 2008). In addition, our present findings show that NEM, which uncouples G proteins from membrane receptors, did not block androgen-induced vasorelaxation and this excludes the possibility that androgens interact with signaling mediated by G protein-coupled receptors. On these bases, comparisons of the responses to androgens in different studies with blood vessels contracted by a diverse number of vasoconstrictor agonists has been inappropriate. However, the different degrees of vasorelaxation induced by androgens in the presence of different constrictor agonists could give some clue to the vasorelaxing mechanisms of action of androgens. In this regard, the postulated mechanism of androgen-induced vasorelaxation suggest that androgens either activate K^{+} channels to increase efflux and/or inhibit Ca^{2+} channels causing hyperpolarization and subsequent vasorelaxation (reviewed by Perusquía and Stallone 2010). It is important to highlight that the involvement of these ion channels in androgen-induced vasorelaxation may also depend upon the pathways activated by the contractile agent used. Moreover, given the different responses by T and its metabolites it would appear inappropriate to conclude that a general mechanism of vasorelaxing action of all androgens could be inferred from the findings with T alone.

5. Conclusion

Regarding the inconsistent available information, the present findings unequivocally indicate that the vasorelaxing response to androgens depends on the vascular bed studied and the different molecular structure of each androgen tested. Likewise, another determining factor such as the precontractile agonist used and the variance in the species should be taken into account.

Acknowledgments

We are grateful to the Veterinarians Ciriaco Tista Olmos and Rosa Marina Alvarez Mascareño and the staff of Section of Surgical Teaching, Faculty of Veterinary Medicine and Zootechnics, UNAM. Supported by a grant from *Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica/Dirección General de Asuntos del Personal Académico* (PAPIIT/DGAPA), Project No. IN205511-3 to M. Perusquía and by a grant from the U.S. National Institutes of Health (Heart, Lung, Blood Institute), Project HL-080402 to J.N. Stallone.

Abbreviations

ACh	acetylcholine
AR	androgen receptors
CCh	carbachol
5α-DHT	5 α -dihydrotestosterone
5β-DHT	5 β -dihydrotestosterone
NEM	<i>N</i> -ethylmaleimide
IC₅₀	inhibitory concentration 50
IP₃	inositol 1,4,5-triphosphate
NO	nitric oxide
Phe	phenylephrine

PGF_{2α}	prostaglandin F _{2α}
SNP	sodium nitroprusside
R_{max}	maximal relaxation
T	testosterone

References

- Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. 1984; 312:315–321. [PubMed: 6095092]
- Cauvin C, Lukeman S, Cameron J, Hwang O, van BC. Differences in norepinephrine activation and diltiazem inhibition of calcium channels in isolated rabbit aorta and mesenteric resistance vessels. *Circ Res*. 1985; 56:822–828. [PubMed: 2408777]
- Chou TM, Sudhir K, Hutchison SJ, Ko E, Amidon TM, Collins P, Chatterjee K. Testosterone induces dilation of canine coronary conductance and resistance arteries *in vivo*. *Circulation*. 1996; 94:2614–2619. [PubMed: 8921808]
- Costarella CE, Stallone JN, Rutecki GW, Whittier FC. Testosterone causes direct relaxation of rat thoracic aorta. *J Pharmacol Exp Ther*. 1996; 277:34–39. [PubMed: 8613939]
- Crews JK, Khalil RA. Antagonistic effects of 17β-estradiol, progesterone, and testosterone on Ca²⁺ entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol*. 1999; 19:1034–1040. [PubMed: 10195933]
- Deenadayalu VP, White RE, Stallone JN, Gao X, Garcia AJ. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am J Physiol Heart Circ Physiol*. 2001; 281:H1720–H1727. [PubMed: 11557563]
- Ding AQ, Stallone JN. Testosterone-induced relaxation of rat aorta is androgen structure specific and involves K⁺ channel activation. *J Appl Physiol*. 2001; 91:2742–2750. [PubMed: 11717242]
- English KM, Jones RD, Jones TH, Morice AH, Channer KS. Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Horm Metab Res*. 2001; 33:645–652. [PubMed: 11733866]
- English KM, Jones RD, Jones TH, Morice AH, Channer KS. Testosterone acts as a coronary vasodilator by a calcium antagonistic action. *J Endocrinol Invest*. 2002; 25:455–458. [PubMed: 12035943]
- Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J*. 1989; 3:2007–2018. [PubMed: 2545495]
- Geary GG, Krause DN, Duckles SP. Gonadal hormones affect diameter of male rat cerebral arteries through endothelium-dependent mechanisms. *Am J Physiol Heart Circ Physiol*. 2000; 279:H610–H618. [PubMed: 10924060]
- Hall J, Jones RD, Jones TH, Channer KS, Peers C. Selective inhibition of L-type Ca²⁺ channels in A7r5 cells by physiological levels of testosterone. *Endocrinology*. 2006; 147:2675–2680. [PubMed: 16527846]
- Jones RD, English KM, Pugh PJ, Morice AH, Jones TH, Channer KS. Pulmonary vasodilatory action of testosterone: evidence of a calcium antagonistic action. *J Cardiovasc Pharmacol*. 2002; 39:814–823. [PubMed: 12021575]
- Jones RD, Pugh PJ, Jones TH, Channer KS. The vasodilatory action of testosterone: a potassium-channel opening or a calcium antagonistic action? *Br J Pharmacol*. 2003; 138:733–744. [PubMed: 12642373]
- Khalil RA, van Breemen C. Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation? *J Pharmacol Exp Ther*. 1988; 244:537–542. [PubMed: 3346836]
- Low AM, Lu-Chao H, Loke JC, Kwan CY, Daniel EE. Insights into the unusual alpha adrenoceptor subtype in dog saphenous vein using phenoxybenzamine. *J Pharmacol Exp Ther*. 1999; 288:148–156. [PubMed: 9862765]

- Malkin CJ, Jones RD, Jones TH, Channer KS. Effect of testosterone on ex vivo vascular reactivity in man. *Clin Sci (Lond)*. 2006; 111:265–274. [PubMed: 16722820]
- Medeiros YS, Calixto JB. Influence of calcium entry blockers and calmodulin inhibitors on 5-hydroxytryptamine-, potassium- and calcium-induced contractions in human umbilical artery in-vitro. *J Pharm Pharmacol*. 1991; 43:411–416. [PubMed: 1681054]
- Molinari C, Battaglia A, Grossini E, Mary DA, Vassanelli C, Vacca G. The effect of testosterone on regional blood flow in prepubertal anaesthetized pigs. *J Physiol*. 2002; 543:365–372. [PubMed: 12181306]
- Montaño LM, Calixto E, Figueroa A, Flores-Soto E, Carbajal V, Perusquía M. Relaxation of androgens on rat thoracic aorta: testosterone concentration dependent agonist/antagonist L-type Ca^{2+} channel activity, and 5β -dihydrotestosterone restricted to L-type Ca^{2+} channel blockade. *Endocrinology*. 2008; 149:2517–2526. [PubMed: 18276759]
- Navarro-Dorado J, Orensanz LM, Recio P, Bustamante S, Benedito S, Martinez AC, Garcia-Sacristan A, Prieto D, Hernandez M. Mechanisms involved in testosterone-induced vasodilatation in pig prostatic small arteries. *Life Sci*. 2008; 83:569–573. [PubMed: 18801377]
- Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science*. 1992; 258:607–614. [PubMed: 1411571]
- Perusquía M. Nongenomic action of steroids in myometrial contractility. *Endocrine*. 2001; 15:63–72. [PubMed: 11572328]
- Perusquía M. Androgen-induced vasorelaxation: a potential vascular protective effect. *Exp Clin Endocrinol Diabetes*. 2003; 111:55–59. [PubMed: 12746753]
- Perusquía M, Hernández R, Morales MA, Campos MG, Villalón CM. Role of endothelium in the vasodilating effect of progestins and androgens on the rat thoracic aorta. *Gen Pharmacol*. 1996; 27:181–185. [PubMed: 8742519]
- Perusquía M, Navarrete E, González L, Villalón CM. The modulatory role of androgens and progestins in the induction of vasorelaxation in human umbilical artery. *Life Sci*. 2007; 81:993–1002. [PubMed: 17804019]
- Perusquía M, Navarrete E, Jasso-Kamel J, Montano LM. Androgens induce relaxation of contractile activity in pregnant human myometrium at term: a nongenomic action on L-type calcium channels. *Biol Reprod*. 2005; 73:214–221. [PubMed: 15758151]
- Perusquía M, Stallone JN. Do androgens play a beneficial role in the regulation of vascular tone? Nongenomic vascular effects of testosterone metabolites. *Am J Physiol Heart Circ Physiol*. 2010; 298:H1301–H1307. [PubMed: 20228257]
- Perusquía M, Villalón CM. Possible role of Ca^{2+} channels in the vasodilating effect of 5β -dihydrotestosterone in rat aorta. *Eur J Pharmacol*. 1999; 371:169–178. [PubMed: 10357254]
- Priest RM, Robertson TP, Leach RM, Ward JP. Membrane potential-dependent and -independent vasodilation in small pulmonary arteries from chronically hypoxic rats. *J Pharmacol Exp Ther*. 1998; 285:975–982. [PubMed: 9618397]
- Rowell KO, Hall J, Pugh PJ, Jones TH, Channer KS, Jones RD. Testosterone acts as an efficacious vasodilator in isolated human pulmonary arteries and veins: evidence for a biphasic effect at physiological and supra-physiological concentrations. *J Endocrinol Invest*. 2009; 32:718–723. [PubMed: 19535892]
- Scragg JL, Dallas ML, Peers C. Molecular requirements for L-type Ca^{2+} channel blockade by testosterone. *Cell Calcium*. 2007; 42:11–15. [PubMed: 17173968]
- Scragg JL, Jones RD, Channer KS, Jones TH, Peers C. Testosterone is a potent inhibitor of L-type Ca^{2+} channels. *Biochem Biophys Res Commun*. 2004; 318:503–506. [PubMed: 15120629]
- Seyrek M, Yildiz O, Ulusoy HB, Yildirim V. Testosterone relaxes isolated human radial artery by potassium channel opening action. *J Pharmacol Sci*. 2007; 103:309–316. [PubMed: 17380036]
- Suematsu E, Hirata M, Hashimoto T, Kuriyama H. Inositol 1,4,5-trisphosphate releases Ca^{2+} from intracellular store sites in skinned single cells of porcine coronary artery. *Biochem Biophys Res Commun*. 1984; 120:481–485. [PubMed: 6610416]
- Teoh H, Quan A, Leung SW, Man RY. Differential effects of 17β -estradiol and testosterone on the contractile responses of porcine coronary arteries. *Br J Pharmacol*. 2000; 129:1301–1308. [PubMed: 10742284]

- Tep-areenan P, Kendall DA, Randall MD. Testosterone-induced vasorelaxation in the rat mesenteric arterial bed is mediated predominantly via potassium channels. *Br J Pharmacol*. 2002; 135:735–740. [PubMed: 11834621]
- Webb CM, McNeill JG, Hayward CS, de ZD, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation*. 1999; 100:1690–1696. [PubMed: 10525487]
- Webb CM, Moat NE, Chong CF, Collins P. Vascular reactivity and flow characteristics of radial artery and long saphenous vein coronary bypass grafts: a 5-year follow-up. *Circulation*. 2010; 122:861–867. [PubMed: 20713903]
- Won E, Won J, Kwon S, Lee Y, Nam T, Ahn D. Testosterone causes simultaneous decrease of $[Ca^{2+}]_i$ and tension in rabbit coronary arteries: by opening voltage dependent potassium channels. *Yonsei Med J*. 2003; 44:1027–1033. [PubMed: 14703612]
- Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation*. 1995; 91:1154–1160. [PubMed: 7850954]

Fig. 1.

Concentration-response curves to testosterone (T), 5 α - or 5 β -dihydrotestosterone (-DHT) on the contraction induced by KCl (60 mM) in left coronary artery (A), femoral artery (B) and saphenous vein (C) with (+E) and without (-E) endothelium. Responses of E+ and E- for each vessel type did not differ significantly ($P > 0.05$). Each symbol in the graph represents the mean $\pm$ SEM of six independent experiments. Each concentration tested was significantly different ($P < 0.05$) as compared with that of vehicle (absolute ethanol 0.1% vol/vol).

Fig. 2. Vasorelaxing effect of sodium nitroprusside (SNP) on the contraction induced by phenylephrine (Phe; $1 \mu\text{M}$) in endothelium-denuded saphenous vein of dog. Each point represents the mean $\pm$ SEM; $n = 6$.

Table 1

Maximal relaxation ($R_{\max}$) and half maximal relaxation (IC_{50}) values of canine blood vessels in response to androgens on KCl-induced contraction.

Androgen	Preparation	$R_{\max}$ (% of relaxation)	IC_{50} (μM)	Potency	r
T					
Coronary artery	+E	95.39 $\pm$ 1.30	26.19 $\pm$ 1.31	1.00	0.97
	-E	92.68 $\pm$ 2.37	26.35 $\pm$ 1.66	1.00	0.97
Femoral artery	+E	78.73 $\pm$ 2.73 [#]	119.77 $\pm$ 9.28 [#]	1.00	0.94
	-E	81.72 $\pm$ 1.74 [#]	114.72 $\pm$ 5.32 [#]	1.00	0.95
Saphenous vein	+E	91.58 $\pm$ 2.81 [#]	65.11 $\pm$ 1.10 ^{#§}	1.00	0.94
	-E	87.92 $\pm$ 1.47 [#]	66.49 $\pm$ 3.22 ^{#§}	1.00	0.95
5α-DHT					
Coronary artery	+E	93.09 $\pm$ 1.58	16.05 $\pm$ 0.74 [*]	1.63	0.98
	-E	91.25 $\pm$ 2.17	15.30 $\pm$ 0.94 [*]	1.72	0.97
Femoral artery	+E	49.51 $\pm$ 1.42 ^{**#}	483.18 $\pm$ 19.68 ^{**#T}	0.24 ^T	0.92
	-E	52.33 $\pm$ 1.17 ^{**#}	405.07 $\pm$ 39.65 ^{**#T}	0.28 ^T	0.94
Saphenous vein	+E	63.60 $\pm$ 2.20 ^{**§}	66.92 $\pm$ 5.98 ^{#§}	0.81	0.91
	-E	66.11 $\pm$ 1.12 ^{**§}	68.76 $\pm$ 5.59 ^{#§}	0.96	0.93
5β-DHT					
Coronary artery	+E	98.25 $\pm$ 1.11 [*]	9.10 $\pm$ 0.58 [*]	2.87	0.96
	-E	98.45 $\pm$ 1.23 [*]	9.30 $\pm$ 0.59 [*]	2.83	0.97
Femoral artery	+E	80.08 $\pm$ 2.42 [#]	58.80 $\pm$ 3.13 ^{**#}	2.05	0.95
	-E	82.19 $\pm$ 2.41 [#]	57.72 $\pm$ 2.66 ^{**#}	2.00	0.95
Saphenous vein	+E	86.10 $\pm$ 2.15 [#]	70.30 $\pm$ 1.56 ^{#§}	0.94	0.92
	-E	88.69 $\pm$ 0.89 [#]	66.00 $\pm$ 4.36 ^{#§}	1.00	0.96

The values are mean (n=6) $\pm$ SEM in blood vessel with (+E) and without (-E) endothelium. IC_{50} was calculated by straight-line regression from every noncumulative concentration-response curve. Pearson's correlation coefficient (r) represents the fitness of the straight line. The potency was calculated from IC_{50} by the formula: $IC_{50} T/IC_{50}$ metabolite assuming a value of 1.00 to T;

* $P < 0.001$,

**

$P < 0.0001$ as compared with T;

$P < 0.01$,

§ $P < 0.001$ as compared with coronary and femoral arteries, respectively.

T Theoretical value, obtained by extrapolation of the straight.

Table 2

Maximal relaxation (% of relaxation on KCl-induced contraction) elicited by androgens in the absence (control) and in the presence of different pretreatments in canine blood vessels.

Androgen (concentration)	Control	Pretreatment	
		Flutamide (10 μM)	NEM (30 μM)
Coronary artery			
T (100 μM)	95.39 ± 1.30	99.00 ± 1.37	95.97 ± 4.74
5α-DHT (56 μM)	93.09 ± 1.58	97.31 ± 1.95	95.45 ± 4.76
5β-DHT (56 μM)	98.25 ± 1.11	100.7 ± 0.36	94.97 ± 2.50
Femoral artery			
T (310 μM)	78.73 ± 2.73	79.32 ± 5.24	77.40 ± 2.55
5α-DHT (310 μM)	49.51 ± 1.42	46.62 ± 1.33	43.29 ± 4.77
5β-DHT (180 μM)	80.08 ± 2.42	88.91 ± 2.91	88.89 ± 3.58
Saphenous vein			
T (310 μM)	87.92 ± 1.47	89.14 ± 4.67	89.22 ± 0.95
5α-DHT (180 μM)	66.11 ± 2.12	64.24 ± 2.76	71.77 ± 3.58
5β-DHT (310 μM)	88.69 ± 0.89	88.64 ± 9.17	81.06 ± 1.07

T (testosterone), 5 α -DHT (5 α -dihydrotestosterone) and 5 β -DHT (5 β -dihydrotestosterone). Flutamide (antagonist of androgen receptors), NEM (*N*-ethylmaleimide; a sulfhydryl alkylating agent, which uncouples G proteins). The values are mean (n $\leq$ 4) $\pm$ SEM.