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## Do androgens play a beneficial role in the regulation of vascular tone? Nongenomic vascular effects of testosterone metabolites

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**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* 298: H1301–H1307, 2010. First published March 12, 2010; doi:10.1152/ajpheart.00753.2009.—The marked sexual dimorphism that exists in human cardiovascular diseases has led to the dogmatic concept that testosterone (Tes) has deleterious effects and exacerbates the development of cardiovascular disease in males. While some animal studies suggest that Tes does exert deleterious effects by enhancing vascular tone through acute or chronic mechanisms, accumulating evidence suggests that Tes and other androgens exert beneficial effects by inducing rapid vasorelaxation of vascular smooth muscle through nongenomic mechanisms. While this effect frequently has been observed in large arteries at micromolar concentrations, more recent studies have reported vasorelaxation of smaller resistance arteries at nanomolar (physiological) concentrations. The key mechanism underlying Tes-induced vasorelaxation appears to be the modulation of vascular smooth muscle ion channel function, particularly the inactivation of L-type voltage-operated  $\text{Ca}^{2+}$  channels and/or the activation of voltage-operated and  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels. Studies employing Tes analogs and metabolites reveal that androgen-induced vasodilation is a structurally specific nongenomic effect that is fundamentally different than the genomic effects on reproductive targets. For example,  $5\alpha$ -dihydrotestosterone exhibits potent genomic-androgenic effects but only moderate vasorelaxing activity, whereas its isomer  $5\beta$ -dihydrotestosterone is devoid of androgenic effects but is a highly efficacious vasodilator. These findings suggest that the dihydro-metabolites of Tes or other androgen analogs devoid of androgenic or estrogenic effects could have useful therapeutic roles in hypertension, erectile dysfunction, prostatic ischemia, or other vascular dysfunctions.

$5\alpha$ -dihydrotestosterone;  $5\beta$ -dihydrotestosterone; hypertension; vascular relaxation; vasodilation

Numerous clinical and epidemiological studies have established that marked sexual dimorphism exists in a variety of human cardiovascular diseases (CVDs). The well-established clinical observations that hypertension (HT) and coronary artery disease occur more frequently in men than in premenopausal women (26–28, 30, 31, 38, 69) have led to the dogmatic concept that testosterone (Tes) has deleterious effects on the heart and vasculature and exacerbates the development of CVD in males (18, 37, 54). However, clinical and epidemiological studies on the role of Tes in CVD are at best controversial, and recent in-depth reviews and analyses of the role of androgens in CVD reveal that there is little sound evidence from either animal or human studies that androgens shorten men's lives

and recent human studies reveal both acute and chronic beneficial effects of Tes on coronary artery disease (31, 69). While some animal studies suggest that Tes may exert deleterious effects on the vascular wall by enhancing vascular tone through acute (63) or chronic mechanisms (18, 36, 57), accumulating evidence now suggests that Tes and other androgens exert protective effects on both cardiovascular and metabolic functions and may play important roles in the acute regulation of vascular function (44, 66). Indeed, numerous studies have now demonstrated that Tes exerts beneficial effects on cardiovascular function, among other effects, by inducing rapid vasorelaxation of vascular smooth muscle (VSM), as reviewed recently (44, 66). This acute effect of Tes and other androgens has been observed at micromolar concentrations in a variety of large arteries (aorta, coronary and umbilical arteries) as well as small resistance arteries (mesenteric, prostatic, pulmonary, and subcutaneous) from several animal species (rat, mouse, rabbit, pig, and dog) and humans (2, 8, 10, 32, 48, 60, 71). Interestingly, in studies employing small vessel wire myography, it has been reported that micromolar concentrations of Tes induce vasodilation of rat pulmonary arteries (23), human subcutaneous resistance arterioles (32), and porcine small prostatic arteries (43).

It is important to clarify that circulating plasma Tes concentrations in adult men range 11–36 nmol/l, whereas its  $5\alpha$ -reduced metabolite [ $5\alpha$ -dihydrotestosterone ( $5\alpha$ -DHT)] is present in plasma at levels about 10% that of Tes, ranging in most men between 1.0 and 2.9 nmol/l. However, it has been agreed that physiological concentrations of Tes are in the range of 100 pM–100 nM, whereas supraphysiological and pharmacological concentrations exceed 100 nM. In this respect, a significant vasodilation has also been observed at physiological (low nanomolar) concentrations of Tes in rat mesenteric arterioles (64, 68) and human pulmonary resistance arteries (55) in vitro. Furthermore, there is also the evidence that Tes produces coronary or systemic vasodilation in vivo at physiological concentrations (100 pM to 100 nM) in humans (67) and in canine and porcine animal models (3, 39). Moreover, in electrophysiological (patch clamp) experiments measuring ion currents in single VSM cells, Tes acts at nanomolar concentrations (8, 17, 41, 58, 59) and even at circulating (36 nmol/l) concentrations (17, 58, 59) to inhibit  $\text{Ca}^{2+}$  channels.

### Mechanisms of Androgen-Induced Vasodilation

A variety of studies has clearly established that androgen-induced vasorelaxation is a rapid, nongenomic effect on the vascular wall (5, 11, 22, 46). Furthermore, it should be noted that numerous studies have shown that high pharmacological concentrations of Tes (10–100  $\mu\text{M}$ ) induce vasodilation in

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endothelium-denuded vessels, suggesting an endothelium-independent mechanism (8, 10, 12, 24, 47, 48, 60, 63, 73). In contrast, other studies have demonstrated that Tes-induced vasorelaxation from physiological to pharmacological concentrations (100 pM–10  $\mu$ M) is inhibited significantly by the removal of the endothelium or treatment with *N*<sup>G</sup>-nitro-L-arginine methyl ester, both in the rat mesenteric arterial bed (64) and in the human pulmonary artery (55). In this latter study, sensitivity to Tes-induced vasodilation was much higher in endothelium-intact than in endothelium-denuded vessels (1 nM vs. 30  $\mu$ M; Ref. 55). Clearly, these data suggest the presence of an endothelium-dependent mechanism at physiological concentrations of Tes (11–36 nmol/l). This possibility is supported by previous *in vivo* studies that demonstrated that Tes-induced vasodilation of both canine coronary and porcine systemic arteries was nitric oxide (NO) dependent (3, 39). The simplest explanation of this discrepancy is that the vasorelaxation induced by lower physiological concentrations of Tes appears to be, at least in part, endothelium dependent, whereas vasorelaxation induced by higher pharmacological concentrations of Tes (>10  $\mu$ M) in many *in vitro* studies appears to be endothelium independent. An alternate explanation is that Tes-induced vasorelaxation, while NO dependent, may rely on the activation of neuronal NOS in VSM cells. This possibility is supported by studies that employed nonselective NOS inhibitors such as *N*<sup>G</sup>-nitro-L-arginine methyl ester and by more recent studies which clearly established that lower physiological concentrations of Tes activate the formation of NO in VSM cells isolated from porcine coronary and rat mesenteric arterioles (8, 68).

Structure-function studies employing a variety of Tes analogs and metabolites have revealed that Tes-induced vasodilation is a structurally specific nongenomic effect that is fundamentally different than the genomic effects of Tes on reproductive targets (10, 51, 73). Figure 1 summarizes the possible nongenomic mechanisms of androgen action on the vascular wall. A variety of studies has demonstrated that the key mechanism underlying the vasorelaxing action of Tes is associated with the modulation of VSM cell membrane ion channel function, particularly 1) inactivation of L-type voltage-operated  $\text{Ca}^{2+}$  channels (VOCCs) (6, 13, 17, 23, 24, 41–43, 48, 50, 59) or 2) activation of  $\text{K}^{+}$  channels (2, 3, 8, 10, 19, 60, 64, 68, 71, 73), particularly the voltage-operated  $\text{K}^{+}$  channel ( $\text{K}_v$ ) and/or the large-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channel ( $\text{BK}_{\text{Ca}}$ ).

The direct vasorelaxing effect of Tes on VSM cells has also been examined in electrophysiological experiments using the patch-clamp technique, which have confirmed the findings from vascular function studies, i.e., that Tes inactivates VOCCs and/or activates  $\text{K}_v$  and  $\text{BK}_{\text{Ca}}$ . At physiological concentrations (11–36 nmol/l), Tes inhibits VOCC currents in rat A7r5 VSM cells and human embryonic kidney-293 cells (17, 58, 59); these studies also demonstrated that Tes and nifedipine share common molecular requirements for the inhibition of VOCCs (same site of action as dihydropyridines). Although VOCC function may differ in cultured cell lines, the results in primary cultured and freshly dissociated rat aortic myocytes (41) are consistent with the findings of these reports. At physiological to supraphysiological concentrations (10 nM–1  $\mu$ M), Tes exhibits a greater potency than nifedipine to inhibit VOCCs, whereas at pharmacological concentrations (above 1  $\mu$ M), the antagonist action of Tes on VOCCs is reversed to an agonist effect, increasing

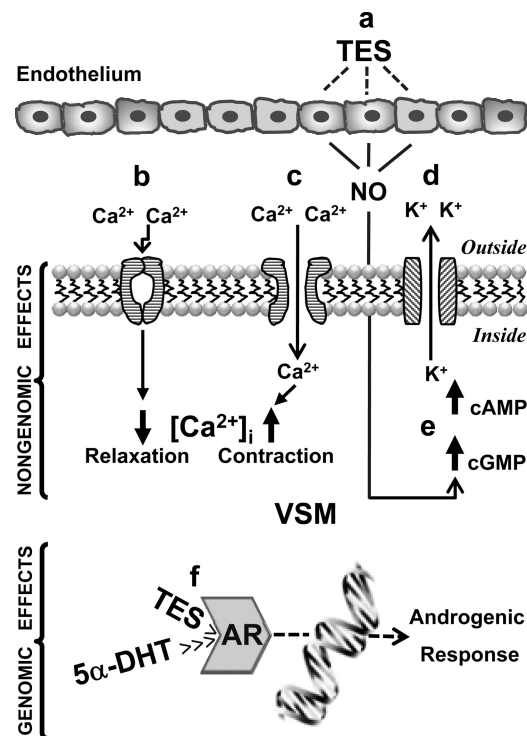


Fig. 1. Genomic and nongenomic mechanisms of action of androgens in the vascular smooth muscle (VSM) cell. *a*: Endothelium-dependent mechanisms, presumably involving endothelium-derived relaxing factors, particularly nitric oxide (NO), would seem to be responsible for the relaxing effect of testosterone (Tes) at physiological (<100 nM) but not at supraphysiological or pharmacological (>100 nM) concentrations. The incremental increase in cGMP reflects NO production, which induces vasorelaxation. Likewise, Tes and its metabolite 5 $\beta$ -dihydrotestosterone (5 $\beta$ -DHT) at pharmacological concentrations (>10  $\mu$ M) induce endothelium-independent relaxation. *b*: Tes shares the same molecular target as the dihydropyridines [the  $\alpha_1\text{C}$ -subunit of L-type  $\text{Ca}^{2+}$  channels; voltage-operated  $\text{Ca}^{2+}$  channel (VOCC)] to elicit a powerful and direct channel blockade at physiological concentrations (36 nM) (17, 58, 59), restricting extracellular  $\text{Ca}^{2+}$  entry and diminishing intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in the VSM cell to induce vasodilation (relaxation). 5 $\beta$ -DHT, a much weaker androgen than Tes, is a pure VOCC blocker at a broad range of concentrations (from nanomolar to micromolar) (41). *c*: Tes but not 5 $\beta$ -DHT, at pharmacological concentrations (above 1  $\mu$ M), activates VOCCs, increasing extracellular  $\text{Ca}^{2+}$  entry and increasing  $[\text{Ca}^{2+}]_i$  to induce vasoconstriction (contraction) (41). *d*: Tes at supraphysiological concentrations (>100 nM) activates voltage-operated  $\text{K}^{+}$  channels or large-conductance  $\text{Ca}^{2+}$ -sensitive  $\text{K}^{+}$  channels, increasing  $\text{K}^{+}$  efflux to induce VSM hyperpolarization and vasorelaxation (8). *e*: Tes but not 5 $\beta$ -DHT at pharmacological concentrations (>30  $\mu$ M) is capable of increasing cGMP and cAMP production (8, 41). *f*: Genomic actions of Tes and 5 $\alpha$ -DHT are mediated by the cytosolic androgen receptor (AR). 5 $\alpha$ -DHT has the highest affinity for the AR and mediates many androgenic effects, whereas 5 $\beta$ -DHT has little affinity and is without biological effects (14).

inward  $\text{Ca}^{2+}$  currents carried by VOCCs (41); this evidence may explain the vasoconstrictor effect of Tes observed by other investigators (18, 36, 57, 63). Furthermore, the authors (41) presented data revealing that the vasodilatory action of the 5 $\beta$ -reduced metabolite of Tes, 5 $\beta$ -DHT, involves the inhibition of VOCCs from nanomolar to micromolar concentrations (100 nM–32  $\mu$ M). In addition, the agonist action of Tes to activate  $\text{K}^{+}$  channels at nanomolar concentrations has also been reported in patch-clamp studies. In porcine coronary myocytes, 200 nM Tes very dramatically activated  $\text{BK}_{\text{Ca}}$  channels, increasing the open probability by more than 10-fold (8). Similarly, in rat mesenteric myocytes, 100 nM Tes markedly



activated  $K_v$  channels (68). In both studies, the selective blockade of  $BK_{Ca}$  or  $K_v$  channels markedly inhibited Tes-induced increases in  $K^+$  channel function.

Several additional mechanisms may also contribute to the Tes-induced relaxation of VSM at pharmacological (micromolar) concentrations in isolated blood vessels. Thus Tes can inhibit T-type  $Ca^{2+}$  currents (17, 59), as well as non-L-type VOCCs such as receptor-operated  $Ca^{2+}$  channels (6, 42, 50) and store-operated  $Ca^{2+}$  channels (24). Moreover, Tes may also cause vasorelaxation by modulating intracellular signal transduction pathways such as increasing the levels of cGMP (8) and cAMP (41), which may indeed evoke vasorelaxation. First, in VSM cells, Tes stimulates NO production (via neuronal NOS), which in turn evokes the formation of cGMP (via guanylyl cyclase) to induce vasorelaxation (8, 68). It has been reported that Tes increases cGMP accumulation and stimulates  $BK_{Ca}$  channel activity at micromolar concentrations (10–50  $\mu$ M) in porcine coronary artery and at nanomolar concentrations (100 nM) in rat mesenteric myocytes to induce vasorelaxation (8, 68). Second, the modulation of intracellular cAMP by Tes may occur via the sex hormone-binding globulin-Tes complex and may be biologically active via its binding to cell-surface sex hormone-binding globulin receptors that evoke an increase in intracellular cAMP. Thus Tes (120  $\mu$ M) stimulates cAMP production in single rat aortic myocytes (41); then, the activation of cAMP induces the phosphorylation of protein kinase A and subsequently protein kinase A-mediated phosphorylation of either phospholamban (a protein that inhibits the sarcoplasmic reticulum ATPase  $Ca^{2+}$  pump), phospholipase C (enzyme responsible for inositol 1,4,5-trisphosphate production), or inositol 1,4,5-trisphosphate receptor, all of which are putative mechanisms of cAMP action.

An unequivocal determination whether androgens inactivate VOCCs or activate  $K^+$  channels is not possible at the present time, since most of the available studies have only explored one but not both of these possible mechanisms. Indeed, androgens may both inactivate inward  $Ca^{2+}$  currents carried by VOCCs (at physiological concentrations, 11–36 nM) and/or activate outward  $K^+$  currents carried by  $K^+$  channels (at physiological concentrations, 1–100 nM) in the VSM cell at different concentrations; however, a definitive answer will require more comprehensive studies that examine the roles of both mechanisms simultaneously. In the meantime, the reader is referred to a review that has examined this controversy in detail (25).

#### Structure-Function Relationship of Androgen-Induced Vasodilation

Since 17 $\beta$ -estradiol causes acute and long-term vasodilation and Tes and estrogens share the same biosynthetic pathway, it has been suggested that Tes-induced vasorelaxation might be an indirect effect mediated by the local conversion of Tes to 17 $\beta$ -estradiol by vascular *P*-450 aromatase (see Fig. 2). However, this possibility has been excluded for several reasons, 1) inhibition of *P*-450 aromatase does not prevent Tes-induced vasorelaxation (8, 63, 64, 73), 2) estrogen receptor antagonism does not alter Tes-induced vasodilation (3, 22, 48), and 3) nonaromatizable metabolites of Tes (e.g., DHT) cause vasorelaxation (8, 10, 47, 48).

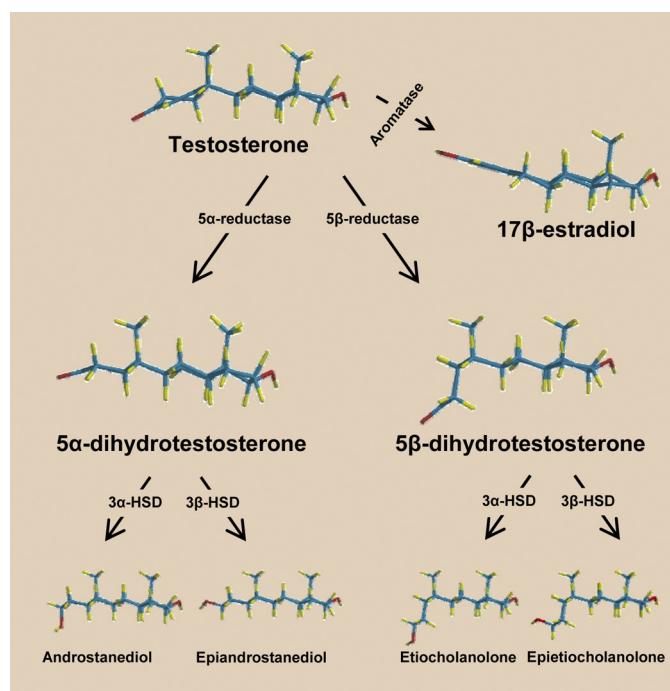


Fig. 2. Metabolic pathways of androgens. Tes can be bioconverted into 17 $\beta$ -estradiol via the enzyme *P*-450-aromatase or into its immediate 5-reduced dihydro-metabolites: 5 $\alpha$ -DHT (via the enzyme 5 $\alpha$ -reductase) and 5 $\beta$ -DHT (via the enzyme 5 $\beta$ -reductase). Subsequently, these dihydro-androgens undergo a 3 $\alpha$ - or 3 $\beta$ -hydroxylation via the enzymes 3 $\alpha$ - or 3 $\beta$ -hydroxysteroid dehydrogenase (HSD) to produce the tetrahydro-androgens (3 $\alpha$ ,5 $\alpha$ -; 3 $\beta$ ,5 $\alpha$ -; 3 $\alpha$ ,5 $\beta$ -; and 3 $\beta$ ,5 $\beta$ -reduced metabolites). Note the 3-dimensional conformation of the androgen molecules:  $\Delta$ 4,3-keto structure (Tes), 5 $\alpha$ /*trans*-conformation (5 $\alpha$ -reduced metabolites), and 5 $\beta$ /*cis*-conformation (5 $\beta$ -reduced metabolites). These molecular conformations reveal that minor changes in the orientation of C<sub>5</sub> in the A-ring can result in major changes in the efficacy and potency of nongenomic vascular effects of the androgen molecule (e.g., 5 $\alpha$ -DHT vs. 5 $\beta$ -DHT; see text for details).

Interestingly, it has been observed that Tes (>10  $\mu$ M) acts as a pulmonary vasodilator with much greater efficacy than 17 $\beta$ -estradiol (12). Moreover, the Tes metabolite 5 $\beta$ -DHT is also more potent than 17 $\beta$ -estradiol in producing relaxation of the rat aorta (50), and both Tes and 5 $\beta$ -DHT are more effective than 17 $\beta$ -estradiol in blocking inward  $Ca^{2+}$  currents in rat aortic myocytes (41). These findings raise the possibility that androgens are more efficacious than estrogens in inducing vascular relaxation.

The metabolism of Tes in various target tissues yields a number of interesting compounds with potential biological relevance (Fig. 2). Apart from its irreversible bioconversion to 17 $\beta$ -estradiol via the enzyme aromatase (CYP19), Tes can also be converted to the 5-reduced dihydro-metabolites (5 $\alpha$  and 5 $\beta$  reductions; Ref. 65), which include 5 $\alpha$ -DHT via the enzyme 5 $\alpha$ -reductase type 1 and 2 (56) and 5 $\beta$ -DHT via the enzyme 5 $\beta$ -reductase, a member of AKR superfamily (9). In this important metabolic pathway, it is emphasized that different tissues appear to have different relative activities of 5 $\alpha$ - or 5 $\beta$ -reductase, resulting in different patterns of Tes metabolism, although it has been hypothesized that the 5 $\alpha$ -reductase pathway has greater importance overall. These dihydro-metabolites subsequently undergo a 3 $\alpha$ - or 3 $\beta$ -hydroxylation (via the enzymes 3 $\alpha$ - or 3 $\beta$ -hydroxysteroid dehydrogenase, respectively) to produce the tetrahydro-metabolites: 3 $\alpha$ ,5 $\alpha$ -; 3 $\beta$ ,5 $\alpha$ -; 3 $\alpha$ ,5 $\beta$ -; and

3 $\beta$ ,5 $\beta$ -reduced metabolites (androstenediol, epiandrostanediol, etiocholanolone, and epietiocholanolone, respectively). Androstenediol (a 3 $\alpha$ ,5 $\alpha$ -androgen) via 17 $\beta$ -hydroxysteroid dehydrogenase is then converted into androsterone, a major excretory metabolite of Tes, and both androstenediol and androsterone are genomically inactive in reproductive targets.

It is important to emphasize that the dihydro- and tetrahydro-androgens are nonaromatizable; thus, they cannot be bioconverted into estrogens (Fig. 2). Since several recent studies have revealed that these nonaromatizable metabolites are fully capable of causing vascular relaxation (8, 10, 47, 48, 50, 73), the established concept that Tes is metabolized to inactive excretory metabolites must then be discarded when considering the effects of androgens on cardiovascular function. As a nonaromatizable dihydro-androgen metabolite of Tes, 5 $\alpha$ -DHT has been frequently used as a tool to verify that the aromatization of Tes to estrogen is not required for this androgen to produce vasorelaxation (3, 8, 10, 64, 73). It has been reported that 5 $\alpha$ -DHT exhibits a lower efficacy and/or potency than Tes to produce vasorelaxation in the rat aorta (10), pig coronary artery (8), and human umbilical artery (48). In marked contrast, the isomer of 5 $\alpha$ -DHT, 5 $\beta$ -DHT, is notably more potent than Tes in the rat aorta and human umbilical artery (41, 47, 48). Whereas the circulating plasma concentration of Tes in adult men ranges 11–36 nmol/l, its 5 $\alpha$ -reduced metabolite (5 $\alpha$ -DHT) is present in the plasma at levels of only about 10% that of Tes (1.0–2.9 nmol/l). It is reasonable to suppose that the plasma levels of the 5 $\beta$ -reduced metabolite (5 $\beta$ -DHT) might be as low as its 5 $\alpha$ -isomer. However, to our knowledge, there is no information available on the plasma concentrations of 5 $\beta$ -DHT; consequently, further research is urgently needed to determine the range of normal plasma concentrations of 5 $\beta$ -DHT. It is also important to recognize that the levels of 5 $\alpha$ - and 5 $\beta$ -DHT in androgen target tissues that express 5 $\alpha$ - and 5 $\beta$ -reductase are likely to be much higher than circulating plasma concentrations, which suggests that these metabolites act mainly as intracrine mediators in the androgen target tissues in which they are formed. For example, in the prostate gland, tissue 5 $\alpha$ -DHT concentrations are 10-fold higher than in plasma. Thus the same may be true in the vascular wall.

With regard to the tetrahydro-androgens, the limited number of studies on their vascular effects has resulted in conflicting findings. Thus, depending on the vascular bed and species, these androgens are reported to be less efficacious and less potent or equipotent than Tes (10, 48, 73), whereas the Tes precursor dehydroepiandrosterone (DHEA) and the Tes excretory metabolite androsterone may be more potent than Tes (48). Although most studies have established that DHEA does produce vasorelaxation, a contradictory vasoconstrictor response has been observed in prepubertal anesthetized pigs (40), which may be due to the different experimental conditions such as in vitro versus in vivo models, the use of adult versus prepubertal animals, the vascular effects produced by anesthetic agents, or the reflex responses activated by a systemic infusion of DHEA.

Nevertheless, the dramatic difference in vasorelaxing potency between Tes and its dihydro-metabolites deserves further consideration, based on their different structural conformations. We have observed that the A-ring of the steroid nucleus is planar in the structure of Tes and in the  $\alpha$ /*trans* configuration at C<sub>5</sub> of reduced metabolites such as 5 $\alpha$ -DHT. In contrast, the

A-ring bends 90° relative to the steroid nucleus when the C<sub>5</sub> hydrogen is  $\beta$ /*cis* oriented, as in the case of 5 $\beta$ -reduced androgens such as 5 $\beta$ -DHT (see structural conformations in Fig. 2). Clearly, the structural change of the 5 $\beta$  configuration is critical for enhanced vasorelaxation efficacy as previously reported (41, 46–48). To avoid confusion, it must be recognized that Tes and its metabolites are clearly distinguishable by their fundamentally different configurations (Fig. 2). Isomerization may play an important role in this respect: molecules with the same chemical composition but with different spatial orientation of their substituents at critical points (e.g., at C<sub>5</sub>) may have totally different binding properties and biological effects. Thus 5 $\alpha$ -DHT is a potent androgen with a strong affinity for the intracellular androgen receptor (AR), whereas its 5 $\beta$ -isomer (5 $\beta$ -DHT), which does not bind to the AR, is totally devoid of androgenic properties but is highly efficacious in producing vasorelaxation. Isomerization can therefore lead either to an inactivation or to a change in the specific biological properties of the original molecule.

Based on the aforementioned data, it is important to emphasize the high vasodilatory efficacy and potency of 5 $\beta$ -DHT, which are notably greater than those of Tes and its 5 $\alpha$ -isomer (5 $\alpha$ -DHT) in VSM as well as uterine smooth muscle (46, 48–50). Since 5 $\beta$ -DHT has little or no affinity for the intracellular AR and is totally devoid of androgenic properties (14), then the acute vasorelaxing effect of 5 $\beta$ -DHT is most likely mediated by an AR-independent, nongenomic mechanism. This line of evidence unequivocally establishes that the marked vasorelaxing effect of 5 $\beta$ -DHT is mediated through a nongenomic mechanism. In contrast, 5 $\alpha$ -DHT possesses a high affinity for the AR and, hence, high androgenic activity (14). This metabolite is a powerful androgen at the genomic level, with higher potency than even Tes, but its nongenomic vasorelaxing efficacy and potency are notably less than those of Tes (8, 10, 48). On this basis, it is tempting to suggest that the two dihydro-metabolites of Tes elicit different biological responses: 5 $\alpha$ -DHT with high genomic-androgenic action and 5 $\beta$ -DHT with high nongenomic-vasorelaxing action. For this reason, the 5 $\beta$ -reduced C<sub>19</sub> steroids and/or functional 5 $\beta$ -DHT analogs, which do not exert estrogenic or androgenic effects, could have useful roles in vascular therapeutics.

### Physiological Relevance

Because of the methodological limitations inherent to in vitro approaches, the ability of androgens to induce vasorelaxation of isolated blood vessels in most studies appears to be a pharmacological effect that occurs at high (micromolar) concentrations; hence, it is critical to consider whether this rapid, nongenomic effect of androgens has physiological relevance. While previous studies identified the potential of androgens to elicit vasorelaxation at pharmacological concentrations, more recent studies on the mechanism(s) of action at near physiological (11–36 nmol/l) concentrations strongly suggest that Tes-induced vasorelaxation is a physiologically relevant phenomenon (55, 64, 68). This suggestion is supported by clinical observations that Tes replacement in hypogonadal men reduces diastolic blood pressure (4, 33, 62, 70) and that serum Tes levels are reduced in both hypertensive men and women (20, 28, 52, 61).

If androgen-induced vasorelaxation reflects the ability of male sex hormones to modulate blood pressure, then physiological Tes replacement therapy in hypogonadal men may be beneficial in the regulation of blood pressure. In this regard, it is reported that serum concentrations of all Tes components decline as adult men age; likewise, plasma Tes levels are consistently lower in men with CVD (reviewed by Liu et al.; Ref. 31). However, little is known about age-dependent changes in Tes metabolites. It has been documented that serum 5 $\alpha$ -DHT levels decline in men at the age of 50–70 years, whereas in women this hormone exhibits a progressive decline between the age ranges of 20–30 and 70–80 years (29); however, it is unclear whether possible reductions in 5 $\beta$ -DHT levels are related to pathophysiological changes with CVD. Notably, the endogenous Tes metabolite 5 $\beta$ -DHT is an efficacious and potent vasorelaxant that acts at nanomolar to micromolar concentrations without estrogenic and androgenic side effects, thus increasing its potential for use in the treatment of HT. In support of this view, 5 $\beta$ -DHT produces vasodepressor responses in pithed rats in vivo (51) and the activity of 5 $\beta$ -reductase, the enzyme that catalyzes the conversion of Tes to 5 $\beta$ -DHT, is significantly lower in essential hypertensive patients compared with their normotensive controls (21). In this context, it is suggested that 5 $\beta$ -reduced androgens (such as 5 $\beta$ -DHT) may play an important role in blood pressure regulation by reducing vascular tone; thus, reduced levels of 5 $\beta$ -reduced steroids may result in increases in vascular tone, contributing to the development of HT. This suggestion is supported by numerous clinical observations that androgen deficiency appears to exacerbate many risk factors and pathologies associated with CVD (66); however, the use of exogenous androgen therapy with Tes potentially may be linked to an increased risk of prostatic carcinoma (15, 45). Pharmacological blockade of the AR is a well-recognized treatment option for prostatic carcinoma, and this approach would still allow for the potential use of 5 $\beta$ -DHT as a safe, nongenomic treatment for patients with CVD.

Androgen-induced vasorelaxation of the human umbilical artery (48) is another potential physiological role for the vascular effects of androgens, which could contribute to the regulation of fetoplacental blood flow, one of the most important rate-limiting factors for normal fetal growth. Indeed, the vasorelaxing capability of Tes and its 5-reduced metabolites, particularly 5 $\beta$ -DHT (48), which are produced in the materno-fetoplacental unit (1), may be physiologically relevant in maintaining a sustained vasodilation in the fetoplacental circulation. This suggestion is consistent with previous findings showing markedly increased plasma levels of DHEA, androstenedione, and Tes throughout pregnancy (16, 35). Therefore, it is reasonable to propose that 1) an insufficiency of androgens during pregnancy, particularly 5 $\beta$ -DHT, could contribute, at least in part, to the development of preeclampsia/eclampsia and 2) exogenously administered 5 $\beta$ -DHT may be therapeutically relevant for the treatment of gestational HT. Not only does Tes induce similar vasorelaxation in isolated vessels from women (2, 48, 55, 71), but the female vasculature is also acutely sensitive to AR-independent vasodilation induced by 5 $\beta$ -DHT (48), which also increases its potential for use as a safe antihypertensive in women.

Finally, it is well known that the serum levels of Tes fall markedly with increasing age in otherwise normal men, and

there is increasing evidence that Tes replacement therapy significantly improves cardiovascular and metabolic functions in hypogonadal aging men (28, 33, 34, 53, 62). Thus androgen replacement therapy with vasoselective androgens such as 5 $\beta$ -DHT, which exert little or no action on the AR receptor, may be an emerging therapeutic option for aging men to prevent vascular dysfunction such as HT, erectile dysfunction, and prostatic ischemia. Although these nongenomic actions may be subject to tachyphylaxis, there are insufficient data at present to unequivocally address this speculation. Indeed, the recent development of selective AR modulators (SARMs) may permit the selective treatment of CVD and the avoidance of androgenic side effects (7, 72) in fulfillment of the proposed clinical applications of androgen therapy.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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