Testosterone and Secondary Hypertension
New Pieces to the Puzzle
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The absence of estrogen, in males or after menopause in women, is independently associated with increased cardiovascular risk.1 A number of factors have been implicated as the underlying cause, including effects at the cell-organ level (eg, inflammatory activation, increased oxidative stress, and increased vasoconstriction),1 as well as changes in cardiovascular physiology (eg, sympathetic activation, increased blood pressure, and insulin resistance). All of these parameters are sensitive to modulation by sex steroid hormones, particularly estrogens, androgens, and increased vasoconstriction, as well as changes in cardiovascular physiology.2 Testosterone is the main male sex steroid hormone but is also produced by women.2 Testosterone is converted to 17β-estradiol by aromatase in numerous tissues, including the vasculature and adipose tissue, representing an important local source of estrogen.3 In obesity, aromatase-derived 17β-estradiol formation may represent an important cause of feminization in males and the increased risk of estrogen-sensitive cancers in obese females.3

Androgenic Steroids and Cardiovascular Risk
In many countries, including the United Kingdom, the United States, Canada, and Australia, anabolic androgenic steroids, such as testosterone, are controlled substances, and their nonmedical use is considered drug abuse.4,5 Testosterone has been used since the 1930s for nonmedical, athletic purposes, especially in male and female body builders and swimmers.6 Because testosterone abuse may increase arterial blood pressure,7 leading to left ventricular hypertrophy,8 it should be included among the differential diagnoses of secondary arterial hypertension. Moreover, testosterone abuse has been associated with myocardial infarction because of coronary vasospasm9 or thrombosis.10–12 Among the mechanisms of how exogenous, as opposed to endogenous, testosterone contributes to increased in cardiovascular risk, coagulatory activation, as well as accelerated progression of coronary artery disease, have been described.10 Sex steroids dilate human coronary arteries,13 and diminished vasodilator activity in response to testosterone under hypertensive conditions has been reported,14 yet research into the mechanisms of testosterone’s contribution to or aggravation of hypertension, particularly in the presence of genetic risk, remains scarce.

Novel Links Between Testosterone and Arterial Hypertension
Genomic, as well as rapid, nongenomic effects of sex steroids contribute to cardiovascular homeostasis and likely to cardiovascular protection in premenopausal women and possibly also in men.15 Nongenomic effects of testosterone were described >40 years ago16 and include rapid changes in calcium signaling. Sex steroid-induced vasodilation (reviewed in Reference 17) is mediated via both membrane subpopulations of nuclear steroid receptors, such as the androgen receptor (AR),18 as well as novel G protein–coupled receptors.19,20 AR-mediated rapid effects of testosterone (or its more active metabolite 5α-dihydrotestosterone) have been described; however, other receptors also appear to be involved.18

In the present issue of Hypertension, Chignalia et al21 now present new evidence on genomic and nongenomic mechanisms of testosterone action on vascular smooth muscle cells in arterial hypertension through modulating associated cellular events, thus setting the stage for further aggravation of hypertension. Using animal models of normotension and polygenic hypertension, the investigators found that, in vascular smooth muscle cells from male animals, testosterone regulates cellular processes, such as phosphorylation of the nonreceptor tyrosine kinase, c-src, which mediates vascular contraction and hypertrophy,22 key events contributing to the increased vascular resistance in hypertension.23 Importantly, c-src is upregulated in experimental polygenic hypertension24 and has been implicated in rapid, nongenomic sex steroid signaling.

Chignalia et al21 also observed greater production of reactive oxygen species in response to testosterone in vascular smooth muscle cells from hypertensive as compared with normotensive animals. These effects were not attributed to conversion of testosterone to 17β-estradiol, because the aromatase inhibitor anastrazole had no effect on reactive oxygen species formation. Although testosterone effects on steady-state mRNA levels did not differ much with regard to NADPH oxidase subunits Nox1 and p22phox, the investigators report a striking difference with regard to the expression of Nox4, which was upregulated in response to testosterone only in vascular smooth muscle cells from normotensive but not from hypertensive animals. Although one might consider this
observation counterintuitive, it might not be so. Although vascular NADPH oxidase has been generally implicated in excessive and deleterious reactive oxygen species formation, additional functions of the Nox4 subunit have been identified. Clempus et al25 have shown that, unlike Nox1, Nox4 is responsible for maintaining vascular smooth muscle cells in a differentiated state, that is, countering proliferation and, thus, vascular hypertrophy. In addition, cardiovascular protection can be achieved though Nox4-derived, \( \text{H}_2\text{O}_2 \)-mediated vasodilation through hyperpolarization. Indeed, mice overexpressing Nox4 exhibit increased vasodilator function and lower blood pressure, effects that paradoxically can be abrogated by antioxidant treatment.26 Thus, Nox4 has been recently recognized as a “protective” Nox.27,28 The lacking stimulatory effect of testosterone on Nox4 expression in cells from hypertensive mice reported by Chignalia et al21 thus might indirectly promote vascular smooth muscle cell growth under hypertensive but not normotensive conditions.

**Testosterone and Vascular Smooth Muscle Cell Growth**

Chignalia et al21 also present the novel finding that testosterone stimulates vascular smooth muscle cell migration, although there were no differences between cells obtained from normotensive and hypertensive animals. The finding that testosterone stimulates cell migration as early as 2 hours after application in cells obtained from normotensive or hypertensive animals is surprising perhaps, but the migratory effect might merely represent a physiological response of the cell. What still needs answering is whether testosterone stimulates vascular smooth muscle cell proliferation in vitro as well as in vivo and whether responses are enhanced in cells obtained from hypertensive animals.

An unexpected finding by Chignalia et al21 was the observation that the nongenomic stimulatory effects of testosterone on reactive oxygen species production were insensitive to the AR antagonist flutamide, suggesting that another, yet unidentified, receptor might be mediating this effect. Similar to the G protein–coupled estrogen receptor reported to mediate nongenomic responses to estrogen in 2005,29 the orphan G protein–coupled receptor GPRC6A has been identified recently as mediating nongenomic responses to testosterone and might be a possible candidate to mediate the rapid, AR-independent effects on reactive oxygen species production reported by Chignalia et al.21 The findings reported by Chignalia et al21 are in keeping with recently published work by Nheu et al,29 who found that testosterone stimulates vascular smooth muscle DNA synthesis, a measure of cell proliferation, through a mechanism involving a steroid receptor distinct from AR, whereas the effects of 5α-dihydrotestosterone were inhibited by AR blockade.29 The involvement of a membrane AR such as GPRC6A20 in the regulation of vascular smooth muscle cell growth would also be in keeping with work by Somjen et al30 reporting membrane-mediated growth effects of cell-impermeable BSA-linked testosterone in human vascular cells.

Finally, the question of whether testosterone is proliferative21,29 or antiproliferative30,31 in vascular smooth muscle (or, in turn, prohypertensive) may depend on the steroid dose, sex, age, kidney function,32 or, as shown by Chignalia et al,21 by the genetic environment of the cells. In vivo studies in hypertensive versus normotensive animals could reveal the effects of testosterone on parameters such as arterial hypertension and help assess the effects of endogenous versus exogenous testosterone. Endogenous (as opposed to exogenous) testosterone, after all, may be vasoprotective and renoprotective,33 likely in part through its conversion to 17β-estradiol by aromatase.34,35

**Clinical Implications and Perspectives**

The findings reported by Chignalia et al21 are novel and important because they provide some of the first molecular information on how genetic background might determine the prohypertensive effects of testosterone. In fact, such a genetic vascular predisposition could also underlie the development or aggravation of secondary arterial hypertension associated with testosterone abuse in humans.8 From these findings, one could hypothesize that cells derived from animals (or humans) with a genetic predisposition to arterial hypertension will respond to a greater degree to testosterone, showing greater increases in reactive oxygen species production and insufficient induction of “protective” NADPH oxidase subunit Nox4, resulting in an overall greater oxidative stress burden.

Several questions remain that provide opportunities for further research in this area. Because Chignalia et al21 used only cells from male animals, one obvious question would be to what extent the effects of testosterone would be present in cells from females, which also produce testosterone and 5α-dihydrotestosterone.2 Recently, the role of sex chromosomes as determinants of cellular responses has received increasing attention,36 and often the sex of the cells used is not even determined in studies investigating the effects of sex steroids.37 Such information would be of interest, because females are known to be largely protected from hypertension until menopause.1 Furthermore, this information might be clinically relevant because sex differences in clinical responsiveness to antihypertensive therapy have been described.38 As indicated previously, information from in vivo studies using exogenous testosterone at doses known to cause hypertension in humans is required to assess effects on vascular hypertrophy in vivo.

Finally, identification and characterization of new receptors mediating nongenomic testosterone signaling, for which GPRC6A20 represents a potential candidate, as well as cellular targets such as the “protective” Nox4,28 should be studied further with regard to their role in the effects of testosterone on vascular homeostasis. This might ultimately allow us to understand the mechanisms underlying the testosterone-induced increases in blood pressure in normotensive individuals and those genetically at risk. Finally, the work of Chignalia et al21 reminds us once again that testosterone abuse should be included in the differential diagnoses of secondary hypertension, particularly in young patients, and that fighting testosterone abuse represents an important opportunity in the primary prevention of hypertension.
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Disclosures

None.

References

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