Testosterone and Secondary Hypertension: New Pieces to the Puzzle

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Testosterone, Estrogen, and Cardiovascular Disease

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 (e.g. inflammatory activation, increased oxidative stress, increased vasoconstriction),¹ as well as changes in cardiovascular physiology (e.g. sympathetic activation, increased blood pressure, insulin resistance). All these parameters are sensitive to modulation by sex steroid hormones,¹ particularly 17β-estradiol, testosterone and its more active metabolite, 5α-dihydrotestosterone, 5α-DHT, which is produced from testosterone by 5α-reductase.¹,²

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 females.³

Androgenic Steroids and Cardiovascular Risk

In many countries, including the UK, the United States, Canada, and Australia, anabolic androgenic steroids such as testosterone are controlled substances and their non-medical use is considered drug abuse.⁴,⁵ Testosterone has been used since the 1930s for non-medical, athletic purposes, especially in male and female body builders and swimmers.⁶ As 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 due to coronary vasospasm⁹ or thrombosis.⁸,¹⁰,¹¹,¹² 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. Sex steroids dilate human coronary arteries, and diminished vasodilator activity in response to testosterone under hypertensive conditions has been reported, 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, non-genomic effects of sex steroids contribute to cardiovascular homeostasis and likely to cardiovascular protection in premenopausal women and possibly also in men. Non-genomic effects of testosterone were described more than 40 years ago, include rapid changes in calcium signaling and testosterone-induced vasodilation (reviewed in mediated via both membrane subpopulations of nuclear steroid receptors such as androgen receptor (AR) as well as novel G protein-coupled receptors. AR-mediated rapid effects of testosterone (or its more active metabolite 5α-DHT) have been described, however other receptors appear also involved.

In the current issue of Hypertension, Chignalia et al. now present new evidence on genomic and non-genomic mechanisms of testosterone action on vascular smooth muscle cells in arterial hypertension through modulating associated cellular events, and 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 non-receptor tyrosine kinase, c-src, which mediates vascular contraction and hypertrophy, key events contributing to the increased vascular resistance in hypertension. Importantly, c-src is upregulated in experimental polygenic hypertension and has been implicated in rapid, non-genomic sex steroid signaling.

Chignalia et al. also observed greater production of reactive oxygen species in response to testosterone in vascular smooth muscle cells from hypertensive as compared to normotensive animals. These effects were not due to conversion of testosterone to 17β-estradiol, since the aromatase inhibitor anastrazole had no effect on reactive oxygen species formation. While 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. While vascular NADPH oxidase has been generally implicated in excessive and deleterious ROS formation, additional functions of the Nox4 subunit have been identified. Griendling and associates have shown that unlike Nox1, Nox4 is responsible for maintaining vascular smooth muscle cells in a differentiated state, i.e. counteracting proliferation and thus vascular hypertrophy. In addition, cardiovascular protection can be achieved though Nox4-derived, H2O2 -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. Thus, Nox4 has been recently recognized as a “protective” Nox. The lacking stimulatory effect of testosterone on Nox4 expression in cells from hypertensive mice reported by Chignalia et al. thus might indirectly promote vascular smooth muscle cell growth under hypertensive but not normotensive conditions.

**Testosterone and Vascular Smooth Muscle Cell Growth**

Chignalia et al. also present the novel finding that testosterone stimulated 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 cell 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 al. was the observation that the non-genomic stimulatory effects of testosterone on reactive oxygen species production were insensitive to the androgen receptor antagonist flutamide, suggesting another – yet unidentified - receptor might be mediating this effect. Similar to the G protein-coupled estrogen receptor reported to mediate non-genomic responses to estrogen in 2005, the orphan G protein-coupled receptor GPRC6A has been recently identified as mediating non-genomic 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. The findings reported by Chignalia et al. are in keeping with recently published work by Nheu et al. who found that testosterone stimulated 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-DHT were inhibited by AR blockade. The involvement of a membrane androgen receptor such as GPRC6A in the regulation of vascular smooth muscle cell growth would also be in keeping with work by Somjen et al. reporting membrane-mediated growth effects of cell-impermeable bovine serum albumin (BSA)-linked testosterone in human vascular cells.

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

**Clinical Implications and Perspectives**

The findings reported by Chignalia et al. are novel and important since 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. 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. Since Chignalia et al. 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-DHT. Recently, the role of sex chromosomes as determinants of cellular responses has received increasing attention, and often the sex of the cells used is not even determined in studies investigating the effects of sex steroids. Such information would be of interest since females are known to be largely protected from hypertension until menopause. Furthermore, this information might be clinically relevant since sex differences

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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 non-genomic testosterone signaling – for which GPRC6A \(^{20}\) represents a potential candidate – as well as cellular targets such as the “protective” Nox4 \(^{28}\) should be studied further with regard to their role for 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 Chignalia et al. 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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**Abbreviations**

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<th>AR</th>
<th>androgen receptor</th>
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<td>5α-DHT</td>
<td>5α-dihydrotestosterone</td>
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