Androgen Receptors Mediate Hypertrophy in Cardiac Myocytes

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Background—The role of androgens in producing cardiac hypertrophy by direct action on cardiac myocytes is uncertain. Accordingly, we tested the hypothesis that cardiac myocytes in adult men and women express an androgen receptor gene and that myocytes respond to androgens by a hypertrophic response.

Methods and Results—We used reverse transcription–polymerase chain reaction methods to demonstrate androgen receptor transcripts in multiple tissues and [3H]phenylalanine incorporation and atrial natriuretic peptide secretion as markers of hypertrophy in cultured rat myocytes. Messenger RNA encoding androgen receptors was detected in myocytes of male and female adult rats, neonatal rat myocytes, rat heart, dog heart, and infant and adult human heart. Both testosterone and dihydrotestosterone produced a robust receptor-specific hypertrophic response in myocytes, determined by indices of protein synthesis and atrial natriuretic peptide secretion.

Conclusions—Androgen receptors are present in cardiac myocytes from multiple species, including normal men and women, in a context that permits androgens to modulate the cardiac phenotype and produce hypertrophy by direct, receptor-specific mechanisms. There are clinical implications for therapeutic or illicit use of androgens in humans. (Circulation. 1998;98:256-261.)

Key Words: hormones ■ hypertrophy ■ myocytes

The hearts of men and women are different. Many aspects of cardiac phenotype are distinctive between sexes, and these distinctions have physiological and pathophysiological importance. For instance, even when corrected for body weight, the male heart of many species is hypertrophied relative to the female heart.1 On average, there is a difference in repolarization on the ECG between men and women, with women demonstrating QT prolongation relative to men.2,3 Women also exhibit a greater degree of QT prolongation and propensity to torsade de pointes ventricular tachycardia seen in the setting of genetic, pharmacological, and other factors that slow cardiac repolarization.4–9 The difference in QT duration is not evident before puberty, but it persists after menopause.10–12

Manipulation of the estrogenic and androgenic hormonal milieu in vivo has been shown to affect cardiac weight and ventricular performance.13–15 In athletes using illicit anabolic-androgenic steroids for purposes of enhancing skeletal muscle hypertrophy, adverse cardiac effects, including pathologic hypertrophy, have been reported.16,17 A recent prominent report18 demonstrates that supraphysiological doses of testosterone in normal men produce skeletal muscle hypertrophy and increased strength and suggests that under some circumstances, supraphysiological doses of testosterone might be given to humans for therapeutic purposes. Therefore, clarifying the direct cardiac effects of testosterone is of considerable importance.

Despite the evident importance of sex differences in cardiac phenotype, which are probably dependent on sex steroids, little is known about the molecular processes that underlie these distinctions. Hemodynamic, endocrine, and paracrine factors that mediate cardiac hypertrophy have been extensively examined; cell stretch and the local cardiac renin-angiotensin system are clearly among the important factors.19 To date, there has been no unequivocal evidence that androgens can produce a hypertrophic effect directly on cardiac myocytes independent of other neurohormonal or hemodynamic effects that alter preload and/or afterload, although there is immunohistochemical evidence that androgen receptors are present in some cell types within the myocardium.20,21 Therefore, we undertook the present study to determine whether in adult mammalian heart, androgen receptors are present in a molecular context that permits regulation of cardiac hypertrophy by exogenous androgens. We now report that the androgen receptor gene is expressed specifically in cardiac myocytes and that androgens can mediate a significant hypertrophic response directly in cardiac myocytes.
Methods

Neonatal Myocyte Isolation and Culture

Neonatal rat cardiac ventricular myocytes were isolated by standard techniques from Sprague-Dawley rats with modifications as we have previously described. Neonatal myocytes were plated onto six-well plates (Falcon/Becton Dickenson) in DMEM with 1-glutamine, HEPES, and sodium pyruvate (Gibco BRL/Life Technologies) supplemented with 7% FCS (Gibco BRL/Life Technologies), penicillin/streptomycin (Gibco BRL/Life Technologies), and gentamicin (Sigma Chemical Co). After 24 hours in serum, medium was removed, and cells were rinsed with DMEM and changed to serum-free medium (DMEM supplemented with 0.2% BSA [Sigma], penicillin/streptomycin, and gentamicin) for a minimum of 48 hours. The density of viable neonatal myocytes was ~5 × 10^4 cells/well at the time of harvest. The purity of the culture was confirmed as previously described.

Adult Myocyte Isolation and Culture

Adult rat ventricular myocytes from male Sprague-Dawley rats (200 to 250 g) were isolated as previously described. In brief, both male and female adult Sprague-Dawley rats were anesthetized, and hearts were removed aseptically. Hearts were subjected to retrograde aortic perfusion with low-calcium buffer followed by collagenase treatment, cell isolation, cell purification, and counting as previously described. Cells were plated on laminin-coated (Collaborative Biomedical Products) tissue culture plates (Falcon/Becton Dickenson). Cells were grown in Medium 199 (with Earle’s balanced salts, 25 mMol/L HEPES, and bicarbonate without glutamine; Sigma) supplemented with 0.2% BSA (Sigma), 2 mMol/L L-carnitine (Sigma), 5 mMol/L creatine (Sigma), 5 mMol/L taurine (Sigma), penicillin/streptomycin (Gibco BRL/Life Technologies), and gentamicin (Sigma) and incubated at 37°C until required. The density of viable adult myocytes was ~5 × 10^5 cells/well at the time of harvest. This yielded stable, rod-shaped myocytes easily distinguishable from contaminating cells, which typically compose 1% of the total cells in a preparation. All animal procedures were conducted by protocols approved by the institutional review board.

Infant Human Cardiac Tissue

Tissue from the right ventricular outflow tract was resected as part of the routine complete surgical correction of the tetralogy of Fallot. After pathological examination, it was taken as a discarded pathological specimen, consistent with institutional review board guidelines. The tissue for this study was from a 9-month-old boy and a 9-month-old girl who were otherwise in good health and who had no known genetic anomaly.

Adult Human Cardiac Tissue

Tissue from the left ventricle of hearts from normal adult men and women was obtained and immediately frozen in liquid nitrogen before processing for RNA as described above. The ventricular tissue was from hearts harvested for organ donation but deemed not suitable before transplantation. The tissue was obtained in accordance with approved transplantation protocols and with institutional review board approval.

Isolation of RNA

Total RNA was isolated from intact hearts or from isolated adult and neonatal myocytes by the guanidinium-acid-phenol extraction method described by Farrell. Polyadenylated mRNA was isolated from total RNA by use of oligo(dT) coupled to paramagnetic polystyrene beads (Dynal) and was eluted with 2 mMol/L EDTA (pH 8.0). Samples were quantified by spectrometry, divided into aliquots, and stored in 70% ethanol and 0.3 mMol/L sodium acetate (pH 5.2) at −70°C.

Identification of Androgen Receptor mRNA

For functional androgen receptors to be present in myocytes, it is a requisite that mRNA encoding the protein be present. Accordingly, we used the RT-PCR approach to detect transcripts in heart. mRNA from various sources was reverse-transcribed to cDNA with random primers by means of RNase H− avian myeloblastosis virus reverse transcriptase (Promega); mRNA from male rat heart was used for the RT (−) control. PCR primers were based on the rat epididymal androgen receptor cDNA sequence. The sequence of the upstream primer is 5′-CGAAGCCAGCAGCAGCTGGA-3′, and the sequence of the downstream primer is 5′-GGCGCGGAGATTGTAGTAGT-3′. This corresponds to sequences of bp 1590 to 1611 and 2090 to 2069 of the cDNA described by Tan et al and would be expected to produce a PCR product of 501 bp. The sequence encodes part of the hormone-binding domain of the androgen receptor. Before RT, the RNA was treated with 10 U of DNase I to degrade any residual contaminating genomic DNA. PCR was performed with Taq DNA polymerase for 35 cycles in a Perkin-Elmer 2400 thermal cycler with an annealing temperature of 58°C. Amplification products were subjected to electrophoresis through 2% agarose gels, stained with ethidium bromide, visualized by ultraviolet transillumination, and photographed. Selected PCR products were excised from the gel, purified, and directly sequenced by the Sanger et al dideoxy method with PCR primers as the sequencing primers.

Assessment of Myocyte Hypertrophy

A widely used index of hypertrophy of cardiac myocytes in vitro is relative rate of incorporation of [3H]phenylalanine into myocyte protein. We used this approach to determine whether in vitro exposure to androgens produced myocyte hypertrophy. In brief, neonatal myocytes were exposed to [3H]phenylalanine (0.44 μCi/mL) in the presence or absence of test compound(s) for 48 hours at 37°C. The DMEM contained unlabeled phenylalanine in excess of the amount of [3H]phenylalanine added for incorporation studies. Ice-cold PBS was used to thoroughly wash the myocytes before protein precipitation with 10% trichloroacetic acid for 1 hour at 4°C. Each well was then scraped and the precipitate washed with 95% ethanol before resuspension in 3.0 mL of 0.15N NaOH. [3H]phenylalanine incorporation was determined by scintillation counting. In addition, an aliquot was taken for determination of total DNA with a dsDNA fluorescent quantification reagent (PicoGreen, Molecular Probes Inc). Results were then expressed relative to nanograms of DNA to correct for possible variation in cell number per sample.

Enhanced expression of gene products present in the immature heart is an additional highly characteristic hypertrophic response. Accordingly, at the end of the 48-hour incubation period, myocytes were washed with DMEM before further incubation. We measured secretion of ANP by neonatal myocytes over this time period using a radioimmunoassay as previously described.

Statistics

The response to hormones was analyzed by the Wilcoxon signed rank test for nonparametric data, and where appropriate, the Bonferroni correction was used for multiple comparisons.

Results

Detection of the Androgen Receptor Gene in Cardiac Tissue by RT-PCR

To test the hypothesis that adult mammalian heart of both sexes and neonatal hearts express the androgen receptor gene, we conducted RT-PCR on RNA extracted from myocytes and...
homogenates of adult male and female rat hearts. In addition, we studied RNA from cultured myocytes and pooled neonatal rat heart (the sex of the neonates was not determined). Because mammalian heart contains numerous cell types (vascular smooth muscle, endothelial cells, neurons) in addition to myocytes, it is possible that RT-PCR of heart homogenates may detect the androgen receptor gene from noncardiac muscle cells. Accordingly, we first investigated purified, freshly isolated myocytes from adult male and female rat heart and from cultured neonatal rat heart. RNA isolated from these cells was subjected to RT-PCR. A PCR product corresponding to the androgen receptor was detected in multiple experiments from the myocytes (Figure 1), demonstrating specifically that myocytes from both sexes and from neonatal cells express the androgen receptor gene. The absence of a PCR product when RT was omitted (RT
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; Figure 1) confirms that PCR products did not arise from contamination with genomic DNA. Expected PCR product size is 501 bp. –RT control was consistently negative for RNA from all sources. Experiment was replicated 4 to 7 times with separate cell preparations with similar results.

Figure 1. PCR products from rat cardiac myocytes. Primers for rat epididymis androgen receptor were used to amplify cDNA reverse-transcribed from rat cardiac tissue RNA. Lane 1, DNA size marker. mRNA was from following sources: lane 2, adult female cardiac myocytes; lane 3, adult male cardiac myocytes; lane 4, neonatal myocytes (mixed sexes); lane 5, negative control (adult male myocytes) in which RT step was omitted to exclude possibility of amplification of possible contaminating genomic DNA. Expected PCR product size is 501 bp. –RT control was consistently negative for RNA from all sources. Experiment was replicated 4 to 7 times with separate cell preparations with similar results.

To determine whether other mammalian species of interest also express androgen receptors in ventricle, we isolated mRNA from cultured myocytes and pooled neonatal rat heart (the sex of the neonates was not determined). Because mammalian heart contains numerous cell types (vascular smooth muscle, endothelial cells, neurons) in addition to myocytes, it is possible that RT-PCR of heart homogenates may detect the androgen receptor gene from noncardiac muscle cells. Accordingly, we first investigated purified, freshly isolated myocytes from adult male and female rat heart and from cultured neonatal rat heart. RNA isolated from these cells was subjected to RT-PCR. A PCR product corresponding to the androgen receptor was detected in multiple experiments from the myocytes (Figure 1), demonstrating specifically that myocytes from both sexes and from neonatal cells express the androgen receptor gene. The absence of a PCR product when RT was omitted (RT
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; Figure 1) confirms that PCR products did not arise from contamination with genomic DNA. A PCR product corresponding to the androgen receptor was also identified from homogenates of adult male and female rat ventricle (results from male ventricle shown in Figure 2; n=3 to 5 separate preparations). To confirm that the PCR product was an amplified segment of the androgen receptor cDNA, the PCR product from adult male heart was directly sequenced. The sequence was identical to that for the rat epididymal androgen receptor cDNA (sequence not shown).

To determine whether other mammalian species of interest also express androgen receptors in ventricle, we isolated mRNA from male rat and female dog left ventricle and from infant male and female human right ventricle. Figure 2 demonstrates that RT-PCR detects the androgen receptor mRNA from rat, dog, and human infant right ventricular tissue. To confirm that androgen receptor mRNA is present in the adult human left ventricle, we also performed RT-PCR on mRNA from adult human samples. Figure 3 demonstrates that transcript is present in normal adult male and female left ventricle. Control experiments were performed to exclude spurious amplification of genomic DNA. DNase I treatment of samples before RT and PCR, as well as experiments in which RT was omitted, produced no PCR product.

Figure 2. PCR products from rodent and mammalian ventricular tissue. PCR products from reverse-transcribed mRNA from ventricles from following sources: lane 1, DNA size marker; lane 2, male rat; lane 3, female dog; lane 4, infant male human right ventricle; lane 5, infant female human right ventricle; lane 6, negative control in which RT was omitted. Sequencing of band at ~500 bp confirms that product corresponds to androgen receptor.

Figure 3. PCR products from adult human left ventricle. Montage of PCR products from RNA reverse-transcribed from normal adult human left ventricle and from male rat heart.
The hypertrophic response of the myocardium can be pleomorphic. Therefore, as a marker for stimulation of the fetal gene program that is a virtually universal finding in cardiac hypertrophy, we measured ANP release for 1 hour after Ang II or androgen addition to the cultures. Figure 5 demonstrates that Ang II augments ANP release from myocytes. Dihydrotestosterone increases ANP secretion by neonatal myocytes, but interestingly, testosterone had no effect on this marker of hypertrophy. The androgen receptor antagonist cyproterone abolished the hypertrophic response to testosterone and to dihydrotestosterone (Figure 4B). The receptor antagonist abolished the hypertrophic response to testosterone and to dihydrotestosterone (Figure 4B).

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Discussion
There are two major findings of this study. First, we demonstrate that mammalian cardiac tissues from several species, including humans, unequivocally express the gene encoding an androgen receptor. Androgen receptors are specifically expressed in myocytes, a condition necessary for the cardiac muscle phenotype to be directly regulated by androgenic steroids. However, expression of androgen receptor mRNA is not sufficient to conclusively establish that functional androgen receptor protein is present. Second, we demonstrate for the first time that both testosterone and dihydrotestosterone produce a hypertrophic response by acting directly on cardiac muscle cells, increasing amino acid incorporation into protein. The other well-established marker for cardiac hypertrophy, ANP secretion, was augmented by dihydrotestosterone but not by testosterone. Increased ANP secretion probably reflects increased transcript abundance for ANP, because we have previously demonstrated that in the neonatal myocyte system, increased ANP secretion in response to several different hypertrophic stimuli is invariably associated with an increase in ANP mRNA abundance. The androgen receptor antagonist cyproterone abolished the hypertrophic response, demonstrating that the hypertrophic effects of the androgenic steroids are mediated specifically by hormone binding to the androgen receptor. Thus, in cardiac muscle cells, both necessary and sufficient conditions exist that potentially permit direct modulation of the muscle cell phenotype by androgens in a fashion independent of altered hemodynamics, ventricular loading conditions, or other alterations in neurohormonal milieu.

It is most likely that the hypertrophic response determined by increased amino acid incorporation into protein is the result of testosterone or of dihydrotestosterone binding to the androgen receptors, which are transported to the nucleus, where they modulate gene transcription. Other effectors that produce hypertrophy in this system over a period of 1 hour operate by a genomic mechanism. Nongenomic effects of androgens have been described, including alterations in calcium flux, that possibly could contribute to the hypertrophic response as well. Of note, testosterone and dihydrotestosterone in the concentrations studied had no adverse affect on cell morphology or viability, unlike the findings of Melchert and Welder and Welder et al, who studied much higher concentrations and reported a toxic effect.
The present in vitro findings resolve the ambiguity from previous in vivo studies in which gonadectomy with or without androgen replacement in rats of various genetic backgrounds altered cardiac phenotype.13,15,40–44 With in vivo studies, it is difficult to be certain whether the hypertrophic response is due to direct signaling via myocyte androgen receptors, to altered hemodynamics, or to other alterations in the hormonal milieu that might stimulate hypertrophy by release of growth factors such as Ang II, insulin-like growth factor-1, epidermal growth factor, or norepinephrine.45

A surprising, highly reproducible finding was that the testosterone metabolite dihydrotestosterone was able to increase ANP secretion, but testosterone was without effect. Although it is well established that various organs have differential responsiveness to testosterone and dihydrotestosterone, it is unusual for the markers of hypertrophy, amino acid incorporation into protein, and ANP secretion to be uncoupled. This finding suggests that distinct testosterone and dihydrotestosterone receptor isoforms may be present in myocytes that are identical in the region identified by PCR but have both shared and distinct effects on transcription of specific genes. There is precedent for differential tissue response to dihydrotestosterone but not to testosterone: prostate and perineum.

**Clinical Implications**

There are at least three pertinent areas in which these findings may have clinical implications. First, androgens have a direct growth-promoting effect on the heart, and as such, probably account at least in part for the difference in cardiac mass between men and women, even after other factors are controlled for. We demonstrate that androgen receptors are present in both infant and adult human heart of both sexes, conditions necessary for hypertrophic responses to androgens of children and adults. We did not perform quantitative comparisons of transcript abundance in the tissues studied; relative abundance of transcript for a receptor may or may not correlate with hormone responsiveness of a tissue. The present study and preceding studies make it likely that the important effectors are androgens, not estrogen, because estrogen receptors have been detected in the atria but have been difficult to unequivocally demonstrate in ventricular myocytes.21,46 Moreover, changes in cardiac electrophysiologic properties correspond temporally to changes in the androgen but not estrogen milieu (see below).

Second, for athletes and bodybuilders who use illicit androgen preparations to promote muscle hypertrophy35 and to enhance performance, it is very likely that there are direct effects on the cardiac muscle, including hypertrophy, that may be undesirable and have deleterious long-term clinical effects such as increased ventricular stiffness.17,47 There are many anecdotal reports of adverse myocardial effects of exogenous androgens in humans,26 although this is not a universal finding.36

Third, there are implications for androgen regulation of gene expression in heart beyond cardiac hypertrophy. Our findings help to open an avenue of investigation aimed at elucidating the cellular basis for the increased relative propensity of women to QT prolongation and torsade de points in various settings.4–9 Whereas the rate-corrected QT interval before puberty is indistinguishable between boys and girls, at puberty, repolarization shortens for boys but remains unchanged for girls.12 Similarly, QT shortening and relatively reduced cardiac event rates in boys after puberty is observed in blood relatives from families with congenital long-QT syndrome.27,49 These findings are buttressed by the present observations and those of Morano et al44 that androgens can alter cardiac gene expression, possibly including those encoding ion channels that regulate repolarization. Experimental findings of Drici et al50 are consistent with this hypothesis.

In summary, we have demonstrated that mammalian cardiac myocytes from hearts of both sexes express the androgen receptor gene. Androgens are capable of mediating a hypertrophic response of cultured adult myocytes of a magnitude nearly that of the most efficacious hypertrophic stimuli identified for heart. Androgens must be considered among the neuroeffectors, paracrine factors, and hormones that act directly on the cardiac myocyte and regulate the cardiac hypertrophic response.

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**References**


