Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes

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Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. Am J Physiol Endocrinol Metab 285: E449–E453, 2003. First published April 8, 2003; 10.1152/ajpendo.00054.2003.—Sex-related differences in cardiac function have been well documented. The extent to which sex hormones are responsible for these differences is unclear. The current study was designed to determine whether castration and androgen replacement resulted in changes in functional expression of genes encoding the L-type calcium channel and Na/Ca exchanger in isolated rat ventricular myocytes. Sixteen weeks of castration produced a 50% decline in dihydropyridine receptor expression levels and a 16% (P < 0.05) increase in time to peak shortening. Furthermore, cardiac myocytes isolated from castrated animals also displayed an 18% (P < 0.001) increase in time to relengthening and an 80% decrease in Na/Ca exchanger gene expression when compared with intact controls. Testosterone treatment of castrated animals completely reversed these effects. These results provide the first evidence that androgens regulate functional expression of the L-type calcium channel and the Na/Ca exchanger in isolated ventricular myocytes and thus may play a role in modulating cardiac performance in males and thereby contribute to the observed gender differences in cardiac function.

Cardiac myocyte; castration; testosterone; calcium channel; sodium/calcium exchanger

CLINICAL STUDIES DEMONSTRATE sex disparities in the heart. In addition to the noted sex differences in cardiac dimensions, studies show than women have higher mortality rates postmyocardial infarction and exhibit greater diastolic heart failure when compared with men (10). Other reports demonstrate lower systolic function and greater diastolic compliance in males vs. females (7). Sex variances in cardiac excitation-contraction coupling occur after puberty, suggesting that steroid hormones may play a role in sex differences in cardiac function (14). Studies examining the influence of steroid hormones on cardiac function are limited. Using heart isolation perfusion studies, Scheuer et al. (23) demonstrated that gonadectomy of male and female rats attenuates cardiac performance. Improvement of cardiac performance was achieved with hormone replacement therapy, suggesting that sex hormones regulate cardiac performance. Thus a reduction in circulating sex hormones as a result of therapeutic interventions or as part of the normal aging process may have adverse effects on cardiac performance.

Normal cardiac function is dependent on proper calcium homeostasis. During a cardiac action potential, the influx of calcium through L-type calcium channels activates the release of calcium from the sarcoplasmic reticulum. Relaxation occurs when the influx of calcium ceases and calcium is extruded from the cytoplasm by the Na/Ca exchanger and sequestered in the sarcoplasmic reticulum by the sarco(endo)plasmic reticulum calcium ATPase (1–3). Our laboratory has recently demonstrated that gonadectomy of male rats causes a substantial reduction in the myocardial expression of genes encoding the L-type calcium channel, Na/Ca exchanger, and β1-adrenergic receptor (5). A reversal in the levels of gene expression and cardiac hypertrophy occur after androgen replacement. The goal of the present investigation is to determine whether changes in gene expression of calcium homeostatic proteins in whole heart after gonadectomy and androgen replacement result in functional changes of isolated ventricular myocytes.

MATERIALS AND METHODS

Animal castration. The animal care committee at the Wayne State University School of Medicine approved this study. Age-matched 60-day-old male Sprague-Dawley rats were housed individually, fed and watered ad libitum, and maintained on a 12:12-h light-dark cycle at 23°C. Six rats were anesthetized, and the posterior tip of each scrotum was swabbed with alcohol and betadine solution. A small incision was made in the posterior tip of each scrotum sac. The spermatic cord was tied with 4.0 silk suture, and the testes were removed. The incision was closed with 4.0 silk sutures, and the animal was allowed to recover. Three intact animals were sham operated.

Androgen replacement. Steroid capsules were prepared by cutting Silastic tubing (0.62 ID × 0.125 in. OD; Dow Corning, Midland, MI) in 15-mm lengths, sealing one end with Silastic adhesive and filling the capsule with testosterone propionate (Sigma Chemical, St. Louis, MO). The tubes were then sealed with Silastic adhesive. Immediately before implantation,
capsules were rinsed using 70% ethanol and washed with sterile saline (9). For capsule implantation, a small lateral incision was made on the back of the neck. The skin was bluntly dissected to form a pocket where the Silastic capsule was inserted in three animals immediately after castration. Three castrated control males were implanted with empty Silastic capsules. After implantation (16 wk), the animals were weighed and killed. Blood samples were collected, and hearts were frozen on dry ice for subsequent analysis of mRNA levels. Serum testosterone levels were determined using a commercially available RIA kit (Coat A Count Total testosterone kit; Diagnostic Products). The assay can detect as little as 4 ng/dl. The assay has a broad working range where the coefficient of variation is low and uniform. There were no differences in body weights among groups (data not shown).

Cell isolation procedures. Single ventricular myocytes were enzymatically isolated from three animals in each group (6). Briefly, hearts were removed rapidly and perfused (at 37°C) with Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.25 CaCl2, 1.2 MgCl2, 1.2 KH2PO4, 25 NaHCO3, 10 HEPES, and 11.1 glucose. The KHB was equilibrated with 5% CO2-95% O2.

Hearts were subsequently perfused with a nominally calcium-free KHB buffer for 2–3 min until spontaneous contractions ceased. This was followed by a 20-min perfusion with calcium-free KHB containing 223 U/ml collagenase (Worthington Biochemical, Freehold, NJ) and 0.1 mg/ml hyaluronidase (Sigma Chemical). After perfusion, ventricles were removed and minced, under sterile conditions, and incubated with the above enzymatic solution for 3–5 min. The cells were further digested with 0.02 mg/ml trypsin (Sigma) before being filtered through a nylon mesh (300 µm) and subsequently separated from the enzymatic solution by centrifugation (60 g for 30 s). Myocytes were resuspended in a sterile filtered, calcium-free Tyrode buffer that contained (in mM) 131 NaCl, 4 KCl, 1 MgCl2, 10 HEPES, and 10 glucose, was supplemented with 2% BSA, and had a pH of 7.4 at 37°C. Cells were initially washed with calcium-free Tyrode buffer to remove remnant enzyme, and extracellular calcium was added incrementally back to 1.25 mM. Myocytes with obvious sarcomemmal blebs or spontaneous contractions were not used. After myocyte isolation, cells were divided into two groups. One group of myocytes was used to analyze gene expression of calcium regulatory proteins (see below), whereas only rod-shaped myocytes with clear edges from the remaining group were selected for recording of mechanical properties.

Real-time quantitative PCR. Total RNA was extracted from isolated rat ventricular myocytes with guanidium thiocyanate-phenol-chloroform by the single-step method, as previously described (6). Real-time quantitative RT-PCR was performed on cDNA generated from 300 ng of total RNA using murine Moloney leukemia virus RT (Invitrogen) and random hexamers (6). For the PCR, we used 200 nM of both sense and antisense primers (Genset), 30 ng cDNA and SYBR Green PCR Master Mix (PE Applied Biosystems) in a final volume of 25 µl, and a ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). Sense and anti-sense primers were GATGGAATCTACGCTTATGG and GGCAGCTTTTGTCTCTG for the α1c-subunit of the L-type calcium channel (DHP receptor); GTCGCGATGGCTGATTA and ATTCCTCTACACCTGGTG for the Na/Ca exchanger, and CGCCTACACATCAAGGAA and GTCGAGATTACCGCGCT for 18S. Fluorescent signals were normalized to an internal reference, and the threshold cycle (Ct) was set within the exponential phase of the PCR. The relative gene expression was calculated by comparing cycle times for each target PCR. The target PCR Ct values are normalized by subtracting the 18S Ct value, which gives the ΔCt value. From this value the relative expression level to 18S for each target PCR can be calculated using the following equation: relative gene expression = 2ΔCt.

Cell shortening/relengthening. Mechanical properties of ventricular myocytes were assessed using a SoftEdge video-based edge-detection system (IonOptix, Milton, MA; see Ref. 6). In brief, cells were placed in a Warner chamber mounted on the stage of an inverted microscope (IX-70; Olympus) and superfused (~1 ml/min at 37°C) with a buffer containing (in mM) 131 NaCl, 4 KCl, 1 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES (pH 7.4). The cells were field stimulated with suprathreshold voltage at a frequency of 0.5 Hz and 3-ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to an FHC (Brunswick, NE) stimulator. The myocyte being studied was displayed on the computer monitor using an IonOptix MyoCam camera. SoftEdge software (IonOptix) was used to capture changes in cell length during shortening and relengthening.

Data analysis. For data presented in Figs. 1A and 3B, differences between variables were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA. For data presented in Figs. 1B, 2, and 3C, t-tests were conducted. Data are shown as means ± SE.

RESULTS

After euthanasia, isolated ventricular myocytes from each group of animals were divided into two groups. One group of myocytes was employed for analysis of gene expression, whereas the remaining group was evaluated for possible changes in myocyte contractile properties. At euthanasia, serum testosterone levels were determined using an RIA. As expected, testosterone levels in gonadectomized animals were below detectable limits. Testosterone levels in intact and testosterone-treated gonadectomized animals were 2.1 ± 0.051 ng/ml and 265 ± 0.31 pg/ml, respectively.

Figure 1A shows mRNA levels for the α1c-subunit of the L-type calcium channel (DHP receptor) in ventricular myocytes isolated from intact animals and castrates treated with and without testosterone. Castration produced a 50% decline in DHP receptor expression levels compared with intact controls. Testosterone supplementation to castrates completely restored DHP receptor mRNA levels. To determine whether changes in DHP receptor gene expression resulted in functional changes, we examined cell shortening and relengthening properties. There was no significant difference in myocyte resting length among groups (data not shown). Peak shortening (normalized to cell length) in response to electrical stimulation is shown in Fig. 1B. There was a 14% decrease in peak shortening in myocytes isolated from castrated animals compared with intact controls, which was not statistically significant. Peak shortening of myocytes isolated from castrates supplemented with testosterone was similar to that of intact controls. Although there was no significant difference in peak shortening among groups, castration increased myocyte time to peak shortening by 16%
which was reversed with testosterone administration (Fig. 2).

In addition to the prolonged time to peak shortening, myocytes isolated from castrated animals also displayed an increased time to 90% relengthening compared with intact controls (208 ± 4.10 vs. 170 ± 4.40 ms, P < 0.001; Fig. 3B). Testosterone administration to castrates reversed this effect. The increase in time to relengthening in castrates was associated with a decrease in Na/Ca exchanger gene expression (Fig. 3A).

DISCUSSION

A number of studies imply that sex hormones may contribute to sex differences in cardiac function (13, 17, 20, 25). The importance of androgens as cardioregulatory hormones in males has not been elucidated completely. Published reports document a reduction in contractile performance after gonadectomy of male rats (22). Furthermore, androgens in males may contribute to gender-related differences in excitation-contraction coupling (21). Studies examining the effects of androgens on isolated ventricular myocytes are limited. Marsh et al. (18) were the first to show the presence of functional androgen receptors in isolated cardiac myocytes from several species. Furthermore, by employing both in vitro and in vivo models, it was
shown that testosterone stimulates hypertrophy of cardiac myocytes via androgen receptor-dependent mechanisms (5, 18). In other studies, we demonstrated that testosterone treatment of gonadectomized male rats produces a profound increase in the expression of cardiac calcium regulatory proteins in whole heart (5). The current study was designed to determine whether these changes in gene expression of calcium homeostatic proteins resulted in functional changes of isolated ventricular myocytes.

The α1c-subunit (DHP receptor) is the pore-forming subunit of the L-type calcium channel that determines ion selectivity and voltage sensitivity and provides the binding sites for all of the clinically used calcium channel blockers (15). Consistent with our previously published results, castration reduced DHP receptor mRNA levels, whereas testosterone replacement blocked this effect (5). The decrease in DHP receptor gene expression is associated with a slight decline in cell shortening and a significant increase in the time to peak shortening, which are restored with testosterone treatment. Reduced expression of the L-type calcium channel along with a decrease in myosin ATPase activity may contribute to the observed reduction in left ventricular contractility in gonadectomized male rats reported by Scheuer et al. (23). Work from the laboratory of Liu et al. (15) has shown that the 5′-untranslated region of the gene encoding the α1c-subunit of the L-type calcium channel contains a consensus hormone replacement element. When cardiac myocytes are transfected with a reporter gene construct containing the 5′-flanking region of the calcium channel α1c-gene, there is a marked increase in reporter gene expression after testosterone treatment. Thus a reduction in L-type calcium channel expression after castration may involve a lack of interaction between hormone-bound androgen receptor (AR) and regulatory sequences within the promoter region of the L-type calcium channel. Our results are consistent with those of Koenig et al. (11), who demonstrated that androgen treatment of rat cardiac myocytes augments calcium flux across the sarcolemma. Although we did not measure calcium current or calcium transients, it is probable that the higher intracellular calcium transients reported in cardiac myocytes isolated from males vs. females is the result of the presence of testosterone (4).

Myocardial relaxation occurs when calcium is extruded from the cytoplasm by the Na/Ca exchanger and sequestered in the sarcoplasmic reticulum (3). Here we provide the first evidence that gonadectomy and androgen replacement regulate the expression of the Na/Ca exchanger. Consistent with a decline in Na/Ca exchanger functional expression, isolated myocytes from the same animals displayed an increase in the time to relengthening. Attenuated functional expression of the Na/Ca exchanger in isolated ventricular myocytes may contribute to the observed decrease in the rate of myocardial relaxation in gonadectomized animals reported by Scheuer et al. (23). Consistent with this hypothesis, Hintz et al. (8) showed that cardiac myocytes isolated from gonadectomized animals display a prolonged time to relaxation and a slowed calcium transient decay rate compared with cardiac myocytes isolated from sham-operated controls. The specific transcriptional processes by which testosterone regulates Na/Ca exchanger expression are unclear. The cardiac promoter for the Na/Ca exchanger contains several putative binding sites for a number of transcription factors (19). Gonadectomy and testosterone replacement-induced alterations in Na/Ca exchanger gene expression are likely via complex protein-protein interactions involving AR and a number of cis- and trans-acting factors (16).

Altered circulating testosterone levels occur frequently under physiological and pathophysiological conditions in human males. In addition to the dramatic fall in androgens that occurs in males after gonadectomy as part of treatment for some tumors, a substantial fall in plasma androgen concentration occurs as a part of the normal male aging process. Furthermore, advancing age is associated with the development of myocardial hypertrophy and slower contraction times (12, 24). A reduction in serum testosterone levels may contribute to the observed changes in myocardial structure and function. Interestingly, our studies suggest that only a small amount of circulating testosterone is required to modulate cardiac performance. Clearly, more detailed time-course and dose-response studies are needed to further characterize androgenic action on contractile function. Nevertheless, testosterone replacement therapy in older males has gained considerable attention over the past several years as a means to prevent or reverse aging in the male. However, solid clinical and scientific information regarding the effects of testosterone replacement therapy on modulating cardiac function in older males is lacking. Results from the current study shed some light into the potential role of androgens as cardioregulatory hormones in males. Further examination of the effects of androgen treatment for hypogonadism on cardiac function is needed to design appropriate therapeutic androgenic agents for treating older males. Ideally, these agents will have beneficial effects on cardiac function and other biological systems without having detrimental effects on other organs, such as the prostate.

In summary, we have provided the first evidence that castration and testosterone replacement alter expression of calcium regulatory proteins and contractile properties of isolated rat ventricular myocytes. Because testosterone can be aromatized to estrogen, the possibility that endogenous differences in estrogen may contribute to our observed findings cannot be excluded. Additional studies are needed to determine whether androgens influence calcium sensitivity and/or the expression of other important calcium-regulating proteins, including the calcium release channel and the sarcoplasmic reticulum calcium pump. Furthermore, the extent to which functional alterations and changes in the levels of gene expression in response to castration and androgen replacement are the result of direct signaling via myocyte androgen receptors to modified hemodynamics or to other changes in...
the hormonal milieu warrants further investigation (18). Nevertheless, the ability of testosterone to regulate gene expression and contractile properties of ventricular myocytes may underlie some of the observed sex differences in cardiac function.

DISCLOSURES

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REFERENCES


