Role of Polyamines and cAMP-dependent Mechanisms on 5α-dihydrotestosterone-elicited Functional Effects in Isolated Right Atria of Rat

Manuel Sánchez, PhD,† Lorena Secades, BS,* Carmen Bordallo, PhD,†‡ Clara Meana, PhD,* José Manuel Rubín, PhD,§ Begoña Cantabrana, PhD,*† and Javier Bordallo, PhD*†*

Abstract: Androgens produce acute vasodilation of systemic, pulmonary, and coronary arteries in several mammal preparations and increase cardiomyocyte contractility. A decrease of the spontaneous beating of sinoatrial cells has also been described. The aim of this study was to characterize the direct effect of 5α-dihydrotestosterone on the spontaneous chronotropism and inotropism in the same preparation as an approach to establish the effect on cardiac output and their mechanism of action. The effects were studied on isolated right atria of Wistar rats placed in an organ bath in Tyrode solution at 37°C and bubbled with carbogen. In male rats, the acute administration of 5α-dihydrotestosterone, a nonaromatizable derivate of testosterone, elicited a positive inotropism, which was associated with a negative chronotropism. As reported in the left atria, polyamines and β-adrenoceptors played a role in 5α-dihydrotestosterone–elicited positive inotropism because the effect was antagonized by α-difluoromethylornithine, an inhibitor of polyamine synthesis, and atenolol, a β_1-adrenoceptor blocker, but not on the negative effect on chronotropism. The androgen increased the sinoatrial node recovery time, suggesting an effect on the mechanisms of spontaneous diastolic depolarization involved in atria pacemaking. These effects of 5α-dihydrotestosterone are not hormonally regulated because they are similarly produced in estrogenized females and gonadectomized male and female rats. These results suggest that the androgen could acutely improve cardiac performance.

5α-dihydrotestosterone, androgens, right atria, negative chronotropism, positive inotropism, heart

(*J Cardiovasc Pharmacol*™ 2009;54:310–318)

Received for publication February 10, 2009; accepted June 16, 2009.

From the *Farmacología, Departamento de Medicina, Universidad de Oviedo; †Instituto Universitario de Oncología del Principado de Asturias; ‡Departamento de Bioquímica y Biología Molecular, Universidad de Oviedo; and §Servicio de Cardiología, Hospital Universitario Central de Asturias, Spain.

M. Sánchez and L. Secades contributed equally to this work.

Supported by a Grant from the Dirección General de Enseñanza Superior y Científica (PB98-1562), Spain. L. Secades and C. Meana were recipients of fellowships from Fundación para la Investigación Científica y Técnica (FICYT) (Principado de Asturias) and Instituto de Salud Carlos III (FISS03-1497), Spain, respectively. The University of Oviedo has contributed to this publication.

The authors report no conflicts of interest.

Reprints: Manuel Sánchez, PhD, Farmacología, Facultad de Medicina, Julián Clavería 6, Oviedo 33006, Spain (e-mail: sanchezf@uniovi.es).

INTRODUCTION

It has been reported that androgens produce changes in the male heart phenotype and on electrophysiological properties, such as shortening QT interval in males after puberty.[1–3] Furthermore, they are associated with cardiac diseases. In this sense, low plasma levels of testosterone have been related to heart failure, leading to its replacement being proposed as a cardiotonic agent for the failing heart.[4] Certainly, clinical benefits have been reported on the clinical parameters of heart failure.[5,6] These effects may be due to androgen activation of intracellular receptors, which have been reported in the atria and ventricles of mammals.[7,8]

The cardiotonic effect of androgens in humans has not been established, neither has the effect of androgen deprivation therapy on cardiac output. However, in humans and experimental animals acute effects of androgens on the cardiovascular system that might enhance cardiac performance have been reported. In this sense, they may improve the clinical parameters of myocardial ischemia by coronary artery dilation[9–12] and decrease afterload, as suggested by the relaxation of the aorta[10,12,13] and systemic circulation, measured as brachial artery relaxation.[14,15] Besides modulating vascular smooth muscles tone, androgens may acutely facilitate the contractility of the myocardium, as reported in isolated left atria of rats[16–18] and cardiomyocytes from ventricles.[19,20] This effect might contribute to increased cardiac output. The nature of the receptor targets that mediate these rapid nongenomic actions of androgens has not been established. However, an interaction of androgens with plasma membrane associated with a pertussis toxin–sensitive G protein has been reported,[18,20] eliciting an intracellular cyclic adenosine monophosphate (cAMP) increase[21] and polyamine synthesis[17,22,23] in the rat atria and an increase in intracellular Ca^{2+} levels dependent on Ca^{2+} released from intracellular stores in ventricular myocytes.[20]

Cardiac output is also dependent on heart rate, and it has been reported that androgens block ion channels of the sinoatrial node involved in pacemaker activity.[24] Consequently, for a better understanding of the overall effect of 5α-dihydrotestosterone on cardiac performance, besides the effect on myocardium contractility, it is also important to establish its effect on chronotropism and its functional consequences. For this, the isolated right atrium of rats was chosen because it has spontaneous activity, which allows the quantification of the effect of 5α-dihydrotestosterone on atria rate and inotropism and to compare whether the effect and mechanisms on

contractility are equivalent to those described on isolated left atria of electrically stimulated rats.

METHODS

Animals and Functional Experiments in Isolated Right Atria of Rats

Three-month-old male Wistar rats, 300–350 g in weight (from the University of Oviedo, Spain, number 3304-13A) were killed by decapitation after placing them in an inhalation chamber filled with CO_2 (Directive 2003/65/CE and Spain RD 1201/2005), following a protocol that was approved by the local Ethical Committee of the University of Oviedo. The right atria were subsequently removed, in some preparations without the sinoatrial node and for specific experiments also the left atria, and placed in an organ bath in 10 mL of Tyrode solution (millimolar composition: NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; and glucose, 5.5) at 37°C and bubbled continuously with a 95% O_2 and 5% CO_2 mixture. The atria were allowed to stabilize for 1 hour under a basal tension of 1 g before experimentation. The isolated left atria were electrically stimulated at 0.5 Hz and the right one, which were not spontaneous beating, from 2 to 3.5 Hz (5 ms and a voltage 20%–30% above the threshold voltage) with a stimulator from C.F. Palmer, Model 8048 (London, United Kingdom). The contractions were recorded on a Letica Uni-graph 50 polygraph through isometric transducers TRI 110.

Specific experiments were carried out in right atria of 3-month-old estrogenized female rats (to avoid the influence of hormonal fluctuations, by 1 mg/kg of estradiol benzoate subcutaneously) 24 hours before the sacrifice[25] and in castrated males and females. These were submitted to bilateral gonadectomy, 30 days before the sacrifice, under anesthesia by intraperitoneal administration of ketamine (75 mg/kg) and diazepam (4 mg/kg).

After the stabilization period, cumulative concentrations of 5α-dihydrotestosterone (1–100 μM), incubated for 6 minutes to record the acute effect, or a single concentration of a submaximal concentration, 60 μM, was added to the organ bath. To avoid long-term effects of the androgen only 1 exposure was performed in each preparation.

The effect of 5α-dihydrotestosterone (30–60 μM) on sinus node recovery time and the effective refractory period were studied.[26] To determine the sinus node recovery time, the atria were stimulated for 30 seconds at an atria rate of 5 Hz (0.5 ms and a voltage 20%–30% above the threshold), then the time elapsed from the last stimulated contraction to the first spontaneously elicited was measured. This was recorded on a chart recorder at a paper speed of 50 millimeters per second. The maximal frequency of stimulation (MFS) was determined, stimulating the atria to progressively increasing frequencies until the contractions did not follow the stimulation. The inverse of this parameter in Hertz was considered as the effective refractory period (ERP), thus ERP = 1/MFS.[26]

For pharmacological characterization of the role of endogenous polyamines and β-adrenoceptors on 5α-dihydrotestosterone–elicited effects on the right atria, an inhibitor of ornithine decarboxylase, α-difluoromethylornithine (1 mM),[27] for 30 minutes or the selective β_1-atenolol (1 μM)[28] and β_2-adrenoceptor, ICI-118,551 (0.3 μM),[29] antagonists for 10 minutes and an inhibitor of adenylyl cyclase, 2′,3′-dideoxyadenosine (30 μM),[30] for 30 minutes were added to the organ bath before the androgen. The effect of β-adrenoceptors desensitization (by long-term exposure, 90 minutes, to high concentrations of isoproterenol (30 μM) and evidenced by the lack of response to subsequent administration of isoproterenol) on 5α-dihydrotestosterone–elicited effects was also studied.

Equally, the functional effect of the polyamines, putrescine and spermine were studied on the right atria, at concentrations that produced effects on the left atria.[23] The effect of 5α-dihydrotestosterone on ornithine decarboxylase activity was studied at different times of incubation in the left atria, from 1 to 6 minutes, and 6 minutes in the left atria, where it was previously characterized,[22] or in the absence (control) of the androgen. Then, the preparations were removed from the organ bath and immediately introduced in liquid nitrogen and preserved at −80°C until used.

To determine the effect of 5α-dihydrotestosterone (60 μM) on intracellular cAMP and to pharmacologically characterize the mechanisms linked in the right atria, the preparations were all incubated for 30 minutes with an inhibitor of phosphodiesterase, IBMX (3-isobutyl-1-methylxanthine) (10 μM),[31] in the absence or the presence of the androgen. Furthermore, the effect of 30-minute incubation with dideoxyadenosine or α-difluoromethylornithine on basal levels and on 5α-dihydrotestosterone modifications on cAMP was also studied. Then, the atria were immediately removed and placed into liquid nitrogen, and kept at −80°C until used.

Ornithine Decarboxylase Assay in Isolated Atria of Rats

Ornithine decarboxylase activity was determined as previously described.[32] Atria were homogenized in a Polytron, 3 times for 10 seconds, in 800 μL of ice-cold buffer containing 10 mM of Tris-HCl, 50 μM pyridoxal 5-phosphate, and 2 mM dithiothreitol, pH 7.2. The homogenate was centrifuged for 15 minutes at ×26,000*g*, at 4°C. Then, 275 μL of the supernatant and 0.250 μCi of L-[I-14C] ornithine (final concentration approximately 22 μM) were incubated for 60 minutes at 37°C in a closed tube equipped with a filter paper wetted in 50 μL 10% KOH to trap released $^{14}CO_2$.

The reaction was terminated by injecting 150 μL of 10% trichloroacetic acid. The tubes were incubated for a further 45 minutes at 37°C to release $^{14}CO_2$ from the incubation buffer and then filter papers were removed and trapped $^{14}CO_2$ was measured by liquid scintillation. To estimate the nonspecific $^{14}CO_2$ released during the incubation, blank tubes were set up by 275 μL of buffer instead of the supernatant, otherwise the tubes were treated similarly. Assays were duplicated in each experiment. The protein content was determined according to the Bradford procedure, and specific ornithine decarboxylase activity was expressed as picomoles of $^{14}CO_2$ evolved per hour and per milligram of protein.

Reverse Transcription–Polymerase Chain Reaction

For these experiments, the right and left atria were placed in the organ bath following the same procedure as described above. Total RNA was isolated from the tissue by the guanidine isothiocyanate method as previously described.[33] RNA from 4 animals was pooled and reverse transcribed twice using random hexamers as primers and SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA) following the instructions of the manufacturer. For each sample, a negative control was prepared without transcriptase.

Target complementary DNAs were amplified by polymerase chain reaction using Taq DNA polymerase (Biotools), and pairs of specific primers for each gene product, 5′-TACTTCCCATCGGACTCTGG-3′ and 5′-CATGAGTTGC CACATTGACC-3′ for ornithine decarboxylase and 5′-CAATACAGGACTCTTTCGAG-3′ and 5′-TTATGGTCG GAACTAACGACG-3′ for 18S ribosomal RNA, used as an internal control for relative reverse transcription–polymerase chain reaction.

Complementary DNA from 2 reactions was amplified in a thermocycler (MyCycler; BioRad) with an initial 4-minute denaturation step at 95°C followed by 35 cycles at 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds in the case of ornithine decarboxylase and 20 cycles for 18S ribosomal RNA. Amplified products were separated by electrophoresis in a 1.2% agarose gel in Tris-Borate-EDTA buffer (Tris, 89 mM; Boric acid, 89 mM and EDTA, 2 mM, pH 8.3). DNA bands were visualized under UV after ethidium bromide staining and photographed using a Vilber Lourmat Photodocumentation system. The size of the specific bands matched the predicted length of the amplicons. Intensity of the bands was quantified using the program PhotoCapt MW 10.1 for Windows (Vilber Lourmat, Marne la Vallée, France).

Determination of Intracellular cAMP Levels in Isolated Right Atria of Rats

For this, the same protocol was followed with the atria in the organ bath. To determine cAMP levels, atria were homogenized with a Polytron in a buffer containing 4 mM EDTA (to prevent enzymatic degradation of cAMP), followed by heating for several minutes in a boiling water bath to facilitate protein coagulation. The extracts were centrifuged at ×18,000*g* for 15 minutes. cAMP in the supernatant was assayed by means of a [^{3}H]AMP radioassay kit following the indications of the manufacturer (GE HealthCare). cAMP levels were expressed as picomoles per milligram of protein.

Drugs

The following drugs were used: 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one), putrescine (tetramethylenediamine), spermine (N, N'-bis[3-aminopropyl]-1,4-butanediamine), atenolol (4-[2′-hydroxy-3′-(isopropylamino)propoxy] phenylacetamide), 2′,3′-dideoxyadenosine, IBMX, and ICI-118,551 hydrochloride ((±)-1-[(2,3-dihydro-7-methyl-1H-inden-4-yl)-3-[(1-methylethyl) amino]-2-butanol hydrochloride) were from Sigma. [3H]AMP radioassay kit from GE HealthCare and L-[I-14C] ornithine from ICN. α-Difluoromethylornithine (DL-α-difluoromethylornithine) donated by Dr. Wooster (Wayne University,). Ketamine (Ketolar) from Pfizer and diazepam (Valium) from Roche.

5α-Dihydrotestosterone, IBMX, and dideoxyadenosine were dissolved in dimethyl sulfoxide; the concentration in the organ bath was ≤0.1%, at which no effect was observed. The rest of the drugs were dissolved in purified water.

Calculations and Statistical Analysis

The effect of 5α-dihydrotestosterone and putrescine on chronotropism, beating per minute, and inotropism, milligram of contraction, was expressed as the percentage of modifications of basal values, before the addition of these drugs to the organ bath. The estimation of the functional modification on isolated rat atria, as a result of the effect of these drugs on the 2 variables, was calculated by multiplying the absolute value of beating per minute by the milligram of contraction. Then, this calculation was normalized considering the basal values as 100%, and the modifications after the addition of the drugs to the organ bath were calculated with respect to this. The data obtained were expressed as the mean ± SEM of at least 5 atria in each case, unless otherwise indicated. The statistical analysis was evaluated by one-way or repeated measures of analysis of variance, depending on the experimental procedure, and Bonferroni test, considering $P < 0.05$ as significant.

The half maximum response to 5α-dihydrotestosterone (EC_{50}) was calculated by fitting the concentration–response curves with the Hill equation (Igor Pro V5.0; WaveMetrics, Inc, OR, USA). The Pearson correlation coefficient (R^2) was analyzed to determine the linear relationship between 2 variables (SPSS 15.0, Chicago, IL, USA).

RESULTS

Effect of 5α-Dihydrotestosterone (1–100 μM) on the Spontaneous Activity of Isolated Right Atria of Rats

In our experimental conditions, the isolated right atria had spontaneous contractions with an average rate of 227.74 ± 11.83 beats per minute. The cumulative administration of 5α-dihydrotestosterone (10–100 μM) to the organ bath, elicited an acute concentration-dependent negative chronotropism and positive inotropism, measured after 6–8 minutes of exposure to the drug (Figs. 1A, 2A, B). Concentrations below 10 μM produced an effect difficult to quantify. This time of incubation was sufficient to reach the steady state of the effect of the androgen, except at 100 μM, the effect of which was maximal on automatism at approximately 10–15 minutes and in some cases led to a complete suppression of automatism. A positive inotropism was produced with an increase in the velocity of contraction and relaxation (Fig. 1B). The effect of 5α-dihydrotestosterone was reversible after the washout of the drug, by replacing with fresh drug-free Krebs solution, except at 100 μM, when a steady state of suppression of chronotropism was produced.

5α-Dihydrotestosterone–elicited modifications on chronotropism and inotropism are well correlated, at any given concentration from 10 to 60 μM, as shown by the Pearson analysis (R^2: 0.785, $P < 0.001$) (Fig. 2A). The effect of the

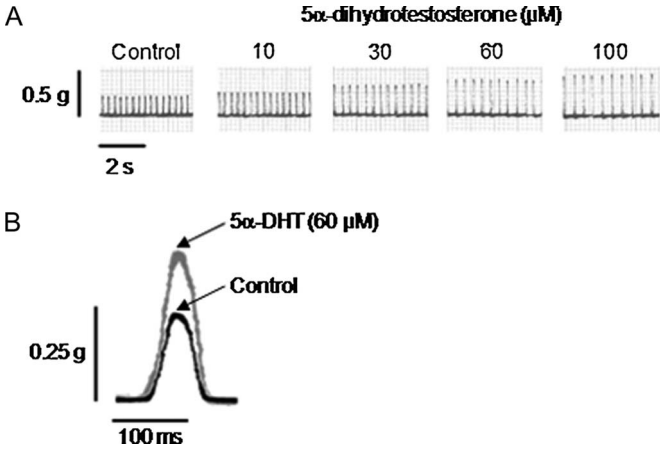

FIGURE 1. A, Recordings of the concentration-dependent effect of 5α-dihydrotestosterone (DHT, 10–100 μM) on spontaneous chronotropism and inotropism of isolated right atrium of rat, and (B) recordings of one trace of control and exposed to 5α-DHT (60 μM).

androgen on inotropism was higher than the theoretically expected if this was due just to the decrease in the frequency of beating. This was studied in isolated right atria lacking sinoatrial nodes, stimulating the preparations at different frequencies from 2 to 3.5 Hz (approximately the spontaneous beats per second of the right atrium) (Fig. 2A). Taking into account the effect on the 2 variables, multiplying the effect of the atria beating rate by the inotropism showed a concentration-dependent increase with an estimated EC_{50} of 26.82 μM (Fig. 2C).

Effect of 5α-Dihydrotestosterone (60 μM) on Sinus Node Recovery Time and Effective Refractory Period in Isolated Right Atria of Rats

The incubation with 5α-dihydrotestosterone, 30 and 60 μM for 6 minutes, increased in a concentration-dependent way, the sinus node recovery time from 326.09 ± 15.95 millisecond, in the absence of the androgen, to 447.71 ± 52.35 millisecond ($P = 0.01$) and 1228.83 ± 218.19 millisecond ($P < 0.001$), respectively, and the ERP from 62.98 ± 2.13 millisecond, in the absence of the androgen, to 64.06 ± 6.69 millisecond and 90.79 ± 15.37 millisecond ($P < 0.01$), respectively.

Effect of α-Difluoromethylornithine (1 mM) on 5α-Dihydrotestosterone (10 to 100 μM)–elicited Effects on Chronotropism and Inotropism in Isolated Right Atria of Rats

The incubation with α-difluoromethylornithine (1 mM), an inhibitor of ornithine decarboxylase, for 30 minutes did not modify the spontaneous activity of isolated right atria. Neither did it modify 5α-dihydrotestosterone (10 to 100 μM)–elicited negative chronotropism, thought it significantly antagonized the effect on inotropism at all the concentrations of the androgen assayed (Fig. 2B).

There exists a positive correlation between the modification in chronotropism and inotropism according to Pearson analysis (R^2: 0.783, $P < 0.001$) (Fig. 2A), and this elicited a significant decrease when the 2 variables were multiplied (Fig. 2C).

Level of messenger RNA Expression of Ornithine Decarboxylase in Isolated Atria of Rat, and Effect of 5α-Dihydrotestosterone on Basal Enzymatic Activity

The measurements of the messenger RNA expression of gene encoding for ornithine decarboxylase and the basal enzymatic activity were higher in the right than the left atria of rats (Fig. 3A). The basal activity of ornithine decarboxylase was higher in the right (27.51 ± 2.44 $pmol \cdot h^{-1} \cdot mg \cdot protein^{-1}$) than the left (19.51 ± 2.62 $pmol \cdot h^{-1} \cdot mg \cdot protein^{-1}$, $P \leq 0.05$) atria of rats, with a ratio of 1:4. The administration of 5α-dihydrotestosterone (60 μM), for 6 minutes to the organ bath, did not increase ornithine decarboxylase activity as it did in the left atria of the same rats, at 100 μM (31.61 ± 2.23, $P < 0.01$), as previously reported.[22] This led us to study it at different times of incubation, 1 and 3 minutes, at which the androgen increased the enzymatic activity (Fig. 3B).

Effect of 5α-Dihydrotestosterone (60 μM) on Intracellular cAMP in Isolated Right Atria of Rats, and Influence of Dideoxyadenosine (30 μM) and α-Difluoromethylornithine (1 mM) on Intracellular cAMP

The acute exposure to 5α-dihydrotestosterone (60 μM) significantly increased the intracellular levels of cAMP, in atria previously incubated with IBMX (10 μM), for 30 minutes. This effect was antagonized by an inhibitor of adenylyl cyclase, dideoxyadenosine (30 μM), or an inhibitor of ornithine decarboxylase, α-difluoromethylornithine (1 mM), added to the organ bath 30 minutes before the androgen. These drugs did not modify basal levels of cAMP (Fig. 4).

Effect of Polyamines on the Spontaneous Chronotropism and Inotropism in Isolated Right Atria of Rats, and the Role of the β-adrenoceptors System

The administration of putrescine (6 mM) to the organ bath produced an acute positive chronotropism and inotropism. The cardiotonic effect, but not the positive chronotropism, was antagonized by dideoxyadenosine (30 μM) and β-adrenoceptors desensitization, by 2 hours exposure to isoproterenol 30 μM (Fig. 5). The administration of spermine (1–10 mM) produced a concentration-dependent decrease of chronotropism and inotropism, and at 10 mM completely suppressed nodal automatism (not shown).

Effect of Atenolol (1 μM) and ICI-118,551 (0.3 μM) on 5α-Dihydrotestosterone (60 μM)–elicited Effects on Chronotropism and Inotropism in Isolated Right Atria of Rats

The incubation for 10 minutes with atenolol (1 μM) significantly decreased the basal inotropism, and ICI-118,551

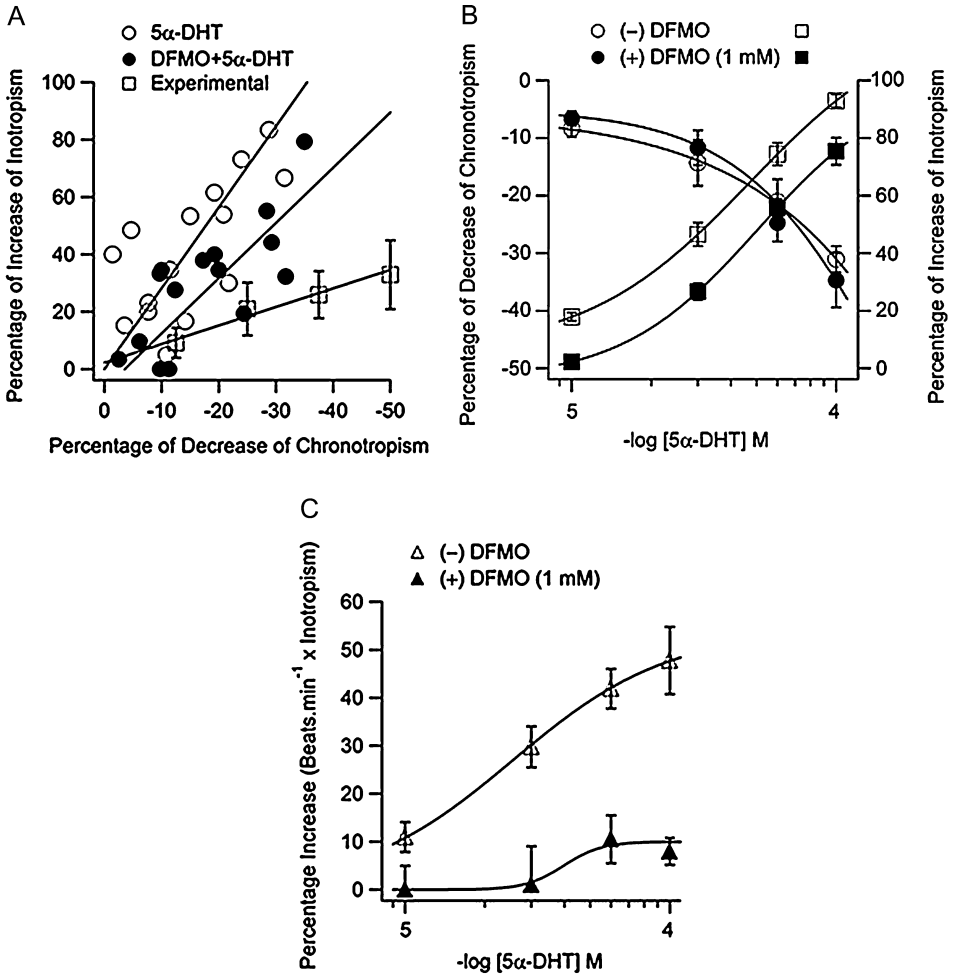

FIGURE 2. A, Experimental correlation between chronotropism and inotropism in electrically stimulated isolated right atria (2–3.5 Hz, 0.5 ms, and a voltage 20%–30% above the threshold), and in spontaneous activity by incubation at any given concentration of 5α-dihydrotestosterone (DHT, 10–100 μM), in the absence and the presence of α-difluoromethylornithine (DFMO, 1 mM). B, Concentration-dependent effect of 5α-DHT (10–100 μM) on the spontaneous chronotropism and inotropism of right atria, circles for chronotropism and square symbol for inotropism, and (C) concentration-dependent effect of the normalized values of beats by inotropism, in the absence and the presence of α-difluoromethylornithine (DFMO, 1 mM). Values represent the mean ± SEM of 7 experiments. The analysis of variance with repeated measures showed a significant difference between curves of $P < 0.001$.

(0.3 μM), decreased the chronotropism. These β-adrenoceptor antagonists did not modify 5α-dihydrotestosterone (60 μM)–elicited negative chronotropism but atenolol antagonized the cardiotonic effect of the androgen (Fig. 6).

Sex and Hormonal Influence on 5α-dihydrotestosterone (60 μM)–elicited Effects on Spontaneous Activity of Isolated Right Atria

There were no significant differences between the spontaneous rate of isolated right atria of control male, estrogenized female, and gonadectomized male and female rats. These were, respectively, in beats per minute ± SEM: 227.74 ± 11.83 for the control males, 226.67 ± 17.02 for castrated males, 235.50 ± 12.82 for estrogenized females, and 236.62 ± 14.49 for castrated females.

Similar to isolated right atria of control male rats, 5α-dihydrotestosterone (60 μM) elicited negative chronotropism and positive inotropism in the right atria of female rats and gonadectomized male and female rats (Fig. 7).

DISCUSSION

The spontaneous beating rate of isolated right atria was similar to other studies.[34] The acute administration of 5α-dihydrotestosterone elicited negative chronotropism and positive inotropism. This interesting finding corroborates previous reports demonstrating the cardiotonic effect of androgens in several isolated preparations of myocardium,[16–20] without an increase in the beating rate.

This study was carried out using 5α-dihydrotestosterone, a nonaromatizable biologically active 5α-reductase derivate of testosterone, to avoid effects of androgens due to their conversion to estrogens.[35] The effective concentrations are higher than the physiological range, but the results found should not necessarily be disregarded on this basis because they might be due to biological reasons regarding the mechanism of action of androgens. For example, the steroids could require a protein for the presentation of the effector target, which might not be present in in vitro studies.[36,37]

5α-Dihydrotestosterone elicited a positive inotropism increasing the velocity of contraction and relaxation, mainly due to a direct effect on contractile myocytes, and not just to a decrease of the atria rate, as expected according to Starling law,[38] which states that the force of contraction depends on the length of cardiomyocytes and this depends on the duration of the diastole. Therefore, the androgen-elicited negative chronotropism may also contribute to the cardiotonic effect, which may contribute to improve cardiac performance. But

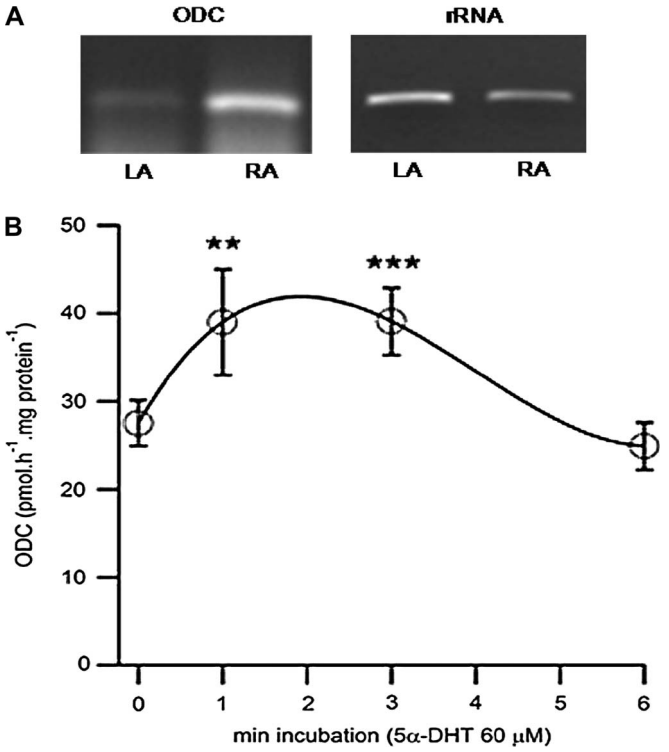

FIGURE 3. A, Representative example of reverse transcription–polymerase chain reaction amplification of ornithine decarboxylase (ODC) and 18S ribosomal RNA genes from left (LA) and right atria (RA). B, Ornithine decarboxylase activity (ODC) (pmol·h^{-1}·mg·protein^{-1}) of isolated right atria in the absence (time = 0) or after varying minutes of incubation with 5α-dihydrotestosterone (DHT, 60 μM). Values represent the mean ± SEM for at least 5 data. $P < 0.01$ and $P < 0.001$ by comparison with the control (in the absence of 5α-DHT), by Bonferroni *t* test.

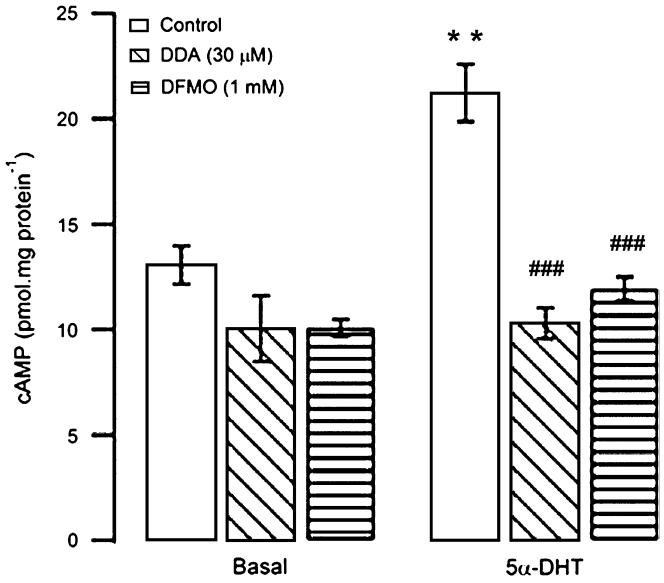

FIGURE 4. Effect of dideoxyadenosine (DDA, 30 μM) or α-difluoromethylornithine (DFMO, 1 mM) on intracellular levels of cAMP in isolated right atria, in the absence (basal) and the presence of 5α-dihydrotestosterone (5α-DHT, 60 μM), pre-incubated with IBMX (10 μM). Values represent the mean ± SEM for at least 5 different experiments. $^{**}P < 0.01$ by comparing with the basal levels, in the absence of the androgen, $\#\#P < 0.01$ by comparing the effect of 5α-dihydrotestosterone (60 μM), by Bonferroni *t* test.

independent of this, even if we subtract the positive inotropism produced experimentally, by decreasing the rate of stimulation, from that of the 5α-dihydrotestosterone–elicited cardiotonic effect, in the right atria, the positive inotropism seems to be higher than that reported in the left atria of rats.[18]

The androgen interference with spontaneous automaticity is associated with an increase in the sinus node recovery time, which suggests a decrease in the slope of phase 4 of the action potential of the nodal cells. This might be due to an interaction with the underlying mechanisms involved in the pacemaker activity,[39] such as T-type Ca^{2+} channel[40–44] and inward rectifier currents[45] and a functional interaction with ionic pumps of plasma membranes.[46] In addition, an increase in the duration of the action potential was observed, which is contrary to the shortening in QT interval reported via genomic mechanisms.[47] These acute effects of the androgen in the right atria are in agreement with an earlier study by Gimeno et al,[16] which reported an increase in the action potential duration and decrease of excitability by acute administration of testosterone.

In addition to the acute effects on ion channels, androgens are also associated with the transcriptional regulation of several ion channels, which might be responsible for the electrophysiological[1,2,3,47] and mechanical[48] differences that exist between sexes. Experimentally, it has been reported that treatment with testosterone for 24–30 hours increased the spontaneous beating frequency of cultured neonatal cardiomyocytes, associated with an increase in the level of expression of T-type Ca^{2+} channels.[40] Their density may, at least in part, account for different heart rates among various mammalian species.[49] This seems not to be the case in the isolated right atria of rats, where neither sexual dimorphism nor sex hormones influence the spontaneous chronotropism or inotropism, such as was reported in the left atria.[50] These were similar in males, estrogenized females, and gonadectomized male and female rats, suggesting that the changes observed in the in vivo studies, by gonadectomy and hormonal replacement, could be related to effects on the mechanisms of modulation of heart rate, such as on cardiac autonomic control.[51–54]

As reported for the left atria of rats,[18,22,23] intracellular polyamines are involved in the positive inotropism elicited by 5α-dihydrotestosterone in the right atria because the effect on inotropism was antagonized by α-difluoromethylornithine, an inhibitor of ornithine decarboxylase,[27] and the androgen elicited an increase in the activity of this enzyme. Although the kinetics of activation was different for the 2 atria, the right atria have higher basal enzymatic activity than the left ones. The measurement of messenger RNA of ornithine decarboxylase was to investigate whether this difference might be due to differences in the levels of expression of this enzyme between both atria. These results are in agreement with previous reports

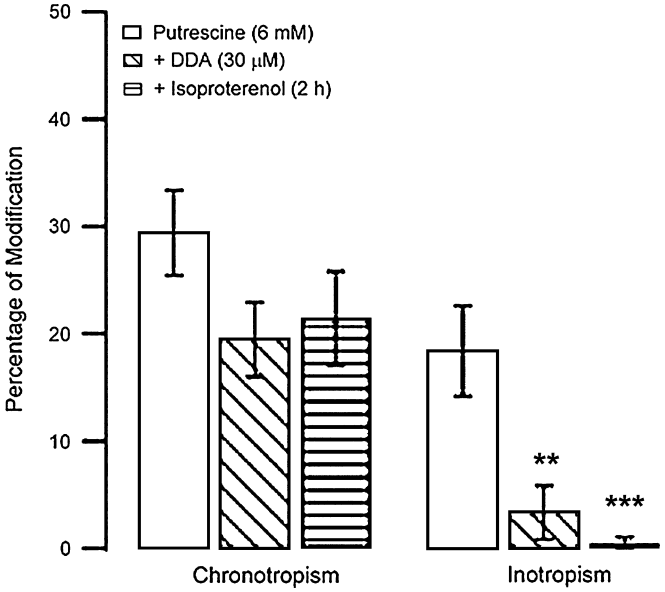

FIGURE 5. Effect of dideoxyadenosine (DDA, 30 μM) or β-adrenoceptor desensitization, by 2 hours incubation with isoproterenol (30 μM) on putrescine (6 mM)–elicited positive chronotropism and inotropism. Values represent the mean ± SEM for at least 5 different experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ by comparing with putrescine, by Bonferroni *t* test.

of differences in the enzymatic activity in different parts of the heart, showing a similar ratio of 1:4 between both atria.[55] In addition, the time course of activation of ornithine decarboxylase activity after 5α-dihydrotestosterone exposure is faster than that obtained in the left atria.[22] These findings may have

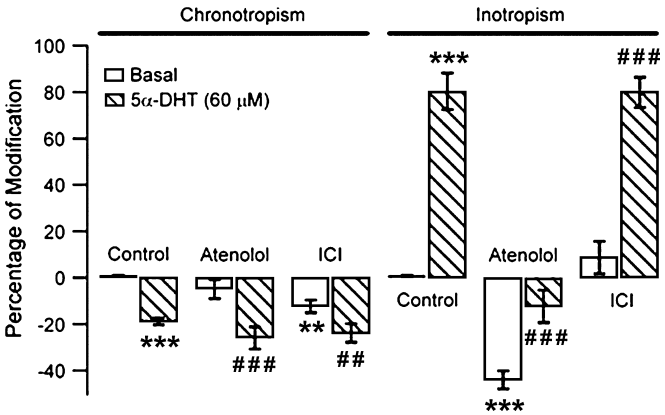

FIGURE 6. Effect of atenolol (1 μM) and ICI-118,551 (ICI, 0.3 μM) on 5α-dihydrotestosterone (5α-DHT, 60 μM)–elicited negative chronotropism and positive inotropism in isolated right atria of rats. Each point represents the mean ± SEM for at least 6 different experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ by comparing with the basal levels (control), $\#\#P < 0.01$ and $\#\#\#P < 0.001$ by comparing the effect of 5α-dihydrotestosterone (60 μM) in the absence or the presence of the β-adrenoceptor antagonist by Bonferroni *t* test.

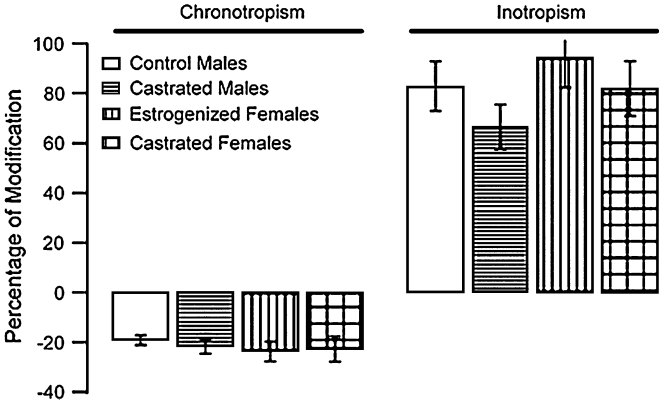

FIGURE 7. 5α-Dihydrotestosterone (DHT, 60 μM)–elicited negative chronotropism and positive inotropism in isolated right atria of control and castrated male rats and estrogenized and ovariectomized females. Values represent the mean ± SEM for at least 5 different experiments.

functional consequences regarding 5α-dihydrotestosterone–elicited cardiotonic effect.

In addition, β_1-adrenoceptors and cAMP-dependent mechanisms are involved in the cardiotonic effect of the androgen in the right atria because this effect was antagonized by atenolol, a selective β_1-blocker of these receptors[28] (but not by the β_2-adrenoceptor antagonist ICI-118,551),[29] and dideoxyadenosine, an inhibitor of adenylyl cyclase.[30]

Intracellular polyamines might modulate β-adrenoceptor–mediated responses[2,23] because α-difluoromethylornithine inhibited 5α-dihydrotestosterone–elicited positive inotropism and the increase of cAMP. This suggests that, as reported in the left atria, [22,23] the androgen could modulate β-adrenoceptor by the increase of intracellular polyamines.

However, neither cAMP-dependent mechanisms nor intracellular polyamines is involved in the negative chronotropism elicited by 5α-dihydrotestosterone. Therefore, different transduction/coupling mechanisms may exist via the androgen to produce the effects in cardiomyocytes and sinoatrial node cells.

The administration of putrescine elicited an acute positive chronotropism and inotropism. The effect of putrescine on the sinoatrial node was independent of β-adrenoceptor activation because it was not affected by dideoxyadenosine or β-adrenoceptor desensitization, but these inhibited the positive inotropism as occurred in the left atria where putrescine behaved as a low affinity agonist of β-adrenoceptors.[56] On the other hand, extracellular spermine produced negative chronotropism and inotropism. The latter has also been described in isolated preparations of myocardium.[56,57] The effect of putrescine on inotropism and the influence of α-difluoromethylornithine on 5α-dihydrotestosterone–elicited cardiotonic effect led us to associate intracellular polyamines as mediators of the cardiotonic effect of the androgen in isolated atria of rats.[23]

CONCLUSIONS

According to these results, 5α-dihydrotestosterone produced bradycardia and may improve cardiac output by

a direct action on the heart. There was dissociation in the coupling mechanisms in the sinoatrial node cells and cardiomyocytes, which were, respectively, independent and dependent of intracellular polyamines, which may modulate β-adrenoceptors and increase cAMP.

This study contributes to a better understanding of the overall effect of androgens on cardiac performance. Although, the functional significance of this experimental study should be confirmed in different mammal species and in humans and in pathological conditions to establish the pharmacological effect of androgens on cardiac performance.

REFERENCES

1. Rautaharju PM, Zhou SH, Wong S, et al. Sex differences in the evolution of the electrocardiographic QT interval with age. *Can J Cardiol*. 1992;8: 690–695.
2. Lehmann MH. QT prolongation in end-stage liver disease: a result of altered sex hormone metabolism? *Hepatology.* 1997;26:244.
3. Locati EH, Zareba W, Moss AJ, et al. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the International LQTS Registry. *Circulation*. 1998;97: 2237–2244.
4. Pugh PJ, Jones RD, West JN, et al. Testosterone treatment for men with chronic heart failure. *Heart*. 2004;90:446–447.
5. Kontoleon PE, Anastasiou-Nana MI, Papapetrou PD, et al. Hormonal profile in patients with congestive heart failure. *Int J Cardiol*. 2003;87: 179–183.
6. Malkin CJ, Jones RD, Jones TH, et al. Effect of testosterone on ex vivo vascular reactivity in man. *Clin Sci (Lond)*. 2006;111:265–274.
7. Lin H, Parmacek MS, Morle G, et al. Expression of recombinant genes in myocardium in vivo after direct injection of DNA. *Circulation*. 1990;82: 2217–2221.
8. Marsh JD, Lehmann MH, Ritchie RH, et al. Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation*. 1998;98:256–261.
9. Jaffe MD. Effect of testosterone cypionate on postexercise ST segment depression. *Br Heart J.* 1977;39:1217–1222.
10. Yue P, Chatterjee K, Beale C, et al. Testosterone relaxes rabbit coronary arteries and aorta. *Circulation*. 1995;91:1154–1160.
11. Webb CM, Adamson DL, de Zeigler D, et al. Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. *Am J Cardiol*. 1999;83:437–439; abstract 439.
12. English KM, Steeds RP, Jones TH, et al. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. *Circulation*. 2000;102:1906–1911.
13. Deenadayalu VP, White RE, Stallone JN, et al. Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am J Physiol Heart Circ Physiol*. 2001;281:H1720–H1727.
14. Ong PJ, Patrizi G, Chong WC, et al. Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. *Am J Cardiol*. 2000;85:269–272.
15. Kang SM, Jang Y, Kim JY, et al. Effect of oral administration of testosterone on brachial arterial vasoreactivity in men with coronary artery disease. *Am J Cardiol*. 2002;89:862–864.
16. Gimeno AL, Gimeno MF, Webb JL. Action of sex steroids on the electrical and mechanical properties on rat atrium. *Am J Physiol*. 1963; 205:198–200.
17. Koenig H, Fan CC, Goldstone AD, et al. Polyamines mediate androgenic stimulation of calcium fluxes and membrane transport in rat heart myocytes. *Circ Res*. 1989;64:415–426.
18. Rubin JM, Hidalgo A, Bordallo C, et al. Positive inotropism induced by androgens in isolated left atrium of rat: evidence for a cAMP-dependent transcriptional mechanism. *Life Sci*. 1999;65:1035–1045.
19. Golden KL, Marsh JD, Jiang Y, et al. Acute actions of testosterone on contractile function of isolated rat ventricular myocytes. *Eur J Endocrinol*. 2005;152:479–483.
20. Vicencio JM, Ibarra C, Estrada M, et al. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology.* 2006;147:1386–1395.
21. Velasco L, Sanchez M, Rubin JM, et al. Intracellular cAMP increases during the positive inotropism induced by androgens in isolated left atrium of rat. *Eur J Pharmacol*. 2002;438:45–52.
22. Bordallo C, Rubin JM, Varona AB, et al. Increases in ornithine decarboxylase activity in the positive inotropism induced by androgens in isolated left atrium of the rat. *Eur J Pharmacol*. 2001; 422:101–107.
23. Velasco L, Secades L, Bordallo C, et al. Role of putrescine on androgen-elicited positive inotropism in the left atrium of rats. *J Cardiovasc Pharmacol*. 2008;52:161–166.
24. Michels G, Hoppe UC. Rapid actions of androgens. *Front Neuroendocrinol*. 2008;29:182–198.
25. Martinez C, Lopez C, Hidalgo A, et al. Gonadectomy eliminates endothelium-dependent diethylstilbestrol-induced relaxant effect in rat aorta. *Pharmacology.* 2003;67:136–142.
26. Tamargo J. Electrophysiological effects of bunaphtine on isolated rat atria. *Eur J Pharmacol*. 1980;62:81–88.
27. Metcalf BW, Bey P, Danzin C, et al. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogs. *J Am Chem Soc*. 1978;100:2551–2553.
28. Coleman AJ, Somerville AR. The selective action of beta-adrenoceptor blocking drugs and the nature of beta1 and beta2 adrenoceptors. *Br J Pharmacol*. 1977;59:83–93.
29. O'Donnell SR, Wanstall JC. Evidence that ICI 118, 551 is a potent, highly Beta 2-selective adrenoceptor antagonist and can be used to characterize Beta-adrenoceptor populations in tissues. *Life Sci*. 1980;27:671–677.
30. Johnson RA, Yeung SM, Stubner D, et al. Cation and structural requirements for P site-mediated inhibition of adenylate cyclase. *Mol Pharmacol*. 1989;35:681–688.
31. Beavo JA, Rogers NL, Crofford OB, et al. Effects of xanthine derivatives on lipolysis and on adenosine 3',5'-monophosphate phosphodiesterase activity. *Mol Pharmacol*. 1970;6:597–603.
32. Lau C, Slotkin TA. Regulation of rat heart ornithine decarboxylase: change in affinity for ornithine evoked by neuronal, hormonal, and ontogenetic stimuli. *Mol Pharmacol*. 1979;16:504–512.
33. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 1987;162:156–159.
34. Shinagawa Y, Satoh H, Noma A. The sustained inward current and inward rectifier K+ current in pacemaker cells dissociated from rat sinoatrial node. *J Physiol*. 2000;523(pt 3):593–605.
35. Veldhuis JD. The hypothalamic pituitary-testicular axis. In: Yen SSC, Jaffe RB, eds. *Reproductive Endocrinology.* 3rd ed. Philadelphia, PA: WB Saunders Co; 1991:409–459.
36. Rosner W, Hryb DJ, Khan MS, et al. Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane. *J Steroid Biochem Mol Biol*. 1999;69:481–485.
37. Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. *Mol Endocrinol*. 2002; 16:2181–2187.
38. Guyton AC, Hall JE. Heart muscle; the heart as a pump and function of the heart valves. In: Guyton AC, Hall JE, eds. *Textbook of Medical Physiology.* 11th ed. Philadelphia, PA: WB Saunders Co; 2005:96–106.
39. Mangoni ME, Nargeot J. Genesis and regulation of the heart automaticity. *Physiol Rev.* 2008;88:919–982.
40. Michels G, Er F, Eicks M, et al. Long-term and immediate effect of testosterone on single T-type calcium channel in neonatal rat cardiomyocytes. *Endocrinology.* 2006;147:5160–5169.
41. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. *J Physiol*. 1988;395:233–253.
42. Zhou Z, Lipsius SL. T-type calcium current in latent pacemaker cells isolated from cat right atrium. *J Mol Cell Cardiol*. 1994;26: 1211–1219.
43. Triggle DJ. The physiological and pharmacological significance of cardiovascular T-type, voltage-gated calcium channels. *Am J Hypertens*. 1998;11:80S–87S.
44. Mangoni ME, Traboulsie A, Leoni AL, et al. Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels. *Circ Res*. 2006;98:1422–1430.
45. Carnes CA, Dech SJ. Effects of dihydrotestosterone on cardiac inward rectifier K(+) current. *Int J Androl*. 2002;25:210–214.

46. Secades L, Cortina R, Velasco L, et al. Interaction of androgens with cardiotonic drugs in isolated left atrium of rat. *Pharmacology*. 2004;70: 118–122.
47. Surawicz B, Parikh SR. Differences between ventricular repolarization in men and women: description, mechanism and implications. *Ann Noninvasive Electrocardiol*. 2003;8:333–340.
48. Golden KL, Marsh JD, Jiang Y, et al. Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. *Am J Physiol Endocrinol Metab*. 2003;285:E449–E453.
49. Ono K, Iijima T. Pathophysiological significance of T-type Ca2+ channels: properties and functional roles of T-type Ca2+ channels in cardiac pacemaking. *J Pharmacol Sci*. 2005;99:197–204.
50. Bordallo J, Secades L, Bordallo C, et al. Influence of gender and sex hormones on 5alpha-dihydrotestosterone elicited effect in isolated left atria of rats: Role of beta-adrenoceptors and ornithine decarboxylase activity. *Eur J Pharmacol*. 2009;604:103–110.
51. Du XJ, Dart AM, Riemersma RA. Sex differences in the parasympathetic nerve control of rat heart. *Clin Exp Pharmacol Physiol*. 1994;21: 485–493.
52. Norton GR, Trifunovic B, Woodiwiss AJ. Attenuated beta-adrenoceptor-mediated cardiac contractile responses following androgenic steroid administration to sedentary rats. *Eur J Appl Physiol*. 2000;81:310–316.
53. El-Mas MM, Afify EA, Mohy El-Din MM, et al. Testosterone facilitates the baroreceptor control of reflex bradycardia: role of cardiac sympathetic and parasympathetic components. *J Cardiovasc Pharmacol*. 2001;38: 754–763.
54. Pereira-Junior PP, Chaves EA, Costa ESRH, et al. Cardiac autonomic dysfunction in rats chronically treated with anabolic steroid. *Eur J Appl Physiol*. 2006;96:487–494.
55. Tipnis UR, Frasier-Scott K, Skiera C. Isoprenaline induced changes in ornithine decarboxylase activity and polyamine content in regions of the rat heart. *Cardiovasc Res*. 1989;23:611–619.
56. Bordallo C, Cantabrana B, Velasco L, et al. Putrescine modulation of acute activation of the beta-adrenergic system in the left atrium of rat. *Eur J Pharmacol*. 2008;598:68–74.
57. Ventura C, Ferroni C, Flamigni F, et al. Polyamine effects on [Ca2+]i homeostasis and contractility in isolated rat ventricular cardiomyocytes. *Am J Physiol*. 1994;267:H587–H592.