In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits

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Abstract

Objectives: Women have longer rate-corrected QT intervals (QTc) and are at higher risk for developing life-threatening torsades de pointes ventricular arrhythmias than men, especially after taking medications that block cardiac human ether-a-go-go-related gene (HERG)-encoded K+ channels. The purpose of the present study was to determine if the male sex steroid hormone, dihydrotestosterone (DHT), influences QT intervals in orchiectomized (Orch) male rabbits. Methods: ECG and whole-cell patch-clamp analyses were employed to evaluate cardiac repolarization and K+ currents in hearts isolated from orchiectomized (Orch) male New Zealand White rabbits receiving subcutaneous sustained release pellets for either dihydrotestosterone (DHT) or placebo. The efficacy of the treatment paradigm was monitored by measuring plasma DHT concentrations before and after the treatment period (10–14 days). Results: The results show that rate- and drug-induced QT-lengthening is attenuated in hearts from DHT-treated rabbits relative to placebo-treated controls. No significant changes in QRS were observed in response to DHT, thereby indicating that DHT influences QT primarily through an effect on ventricular repolarization. In addition, hearts from DHT-treated rabbits displayed significantly less QT lengthening in response to quinidine challenge compared to placebo controls. Current densities for two important cardiac repolarizing K+ currents, I_{K1} and I_{Kr}, were found to be significantly increased in ventricular myocardium of DHT-treated rabbits. Further, the half-maximal voltage of Kr activation (V_{1/2}) for I_{Kr} was significantly shifted to more negative potentials in myocytes from DHT vs. placebo hearts (1.21±1.12 vs. 1.43±1.4 mV, respectively, n=12, P<0.001). Corresponding changes in rabbit ether-a-go-go-related gene (RERG) mRNA were not found when examined by Northern blot hybridization. Conclusions: These results suggest that the presence of male sex steroid hormones in male rabbits helps to suppress rate- and drug-induced delays in cardiac repolarization. DHT action produces increased current densities for I_{Kr} and I_{K1} and a left-shift in the V_{1/2} for I_{Kr} that could account, at least in part, for the observed QTc differences between males and females. Since little change was seen in ventricular RERG gene expression, DHT action in the heart may influence I_{Kr} via post-transcriptional and/or post-translational mechanisms.

Keywords: ECG; Gender; Gene expression; Hormones; K-channel; Repolarization

This article is referred to in the Editorial by A.F. James and J.C. Hancox (pages 1–4) in this issue.

1. Introduction

Female gender is a risk factor for drug-induced cardiac arrhythmias, with an approximately 2:1 preponderance of women:men developing torsades de pointes (TdP) arrhythm-
mias in response to similar concentrations of a wide variety of drugs [1]. TdP is a potentially fatal form of polymorphic ventricular tachycardia that typically occurs in a setting of increased QT interval duration measured from electrocardiogram (ECG) recordings [2,3]. The generally longer baseline rate-corrected QT (QTc) in women compared to men has long been suspected as a contributory factor for why women are at higher risk than men for developing TdP arrhythmias [4,5].

Evidence from clinical studies suggests that the gender differences in QTc may be primarily due to the influence of male sex steroid hormones since prepubescent boys and girls have QTc values similar to those found in adult women, whereas postpubescent males generally have shorter QTc intervals [6,7]. In addition, a recent study that compared JTC intervals in castrated men to those found in intact men and women showed that the JTC intervals in the castrated male group were significantly longer than those in normal males and were similar to those found in normal females [8]. Moreover, testosterone replacement therapy in the castrated male group produced shortening of JTC similar to that observed in normal males. These results suggest that male sex steroid hormones can influence cardiac repolarization and may, therefore, be responsible for the shorter QTc values observed in men compared to those found in women.

We and others have shown that the rabbit heart is a good model to examine sex differences in cardiac repolarization because rabbits display sex differences in QT intervals and susceptibility to drug-induced TdP arrhythmias [9–13]. In the present study, we compare the effect of DHT versus placebo treatment in orchiectomized (Orch) male rabbits on baseline and drug-induced QT over a wide range of pacing rates using isolated perfused (Langendorff) heart preparations. In addition, current densities of two major repolarizing potassium currents, the rapid component of the delayed rectifier ($I_{K_r}$) and the inward rectifier ($I_{K_{1}}$), are evaluated in ventricular cardiomyocytes isolated from placebo- versus DHT-treated rabbits.

2. Methods

2.1. Animals

New Zealand White rabbits (3–4 months old, weight 3–3.5 kg) were obtained from Covance Labs. (Denver, PA, USA), and orchiectomies were performed at Georgetown University by the veterinary staff. Briefly, rabbits were anesthetized with ketamine–xylazine (50 mg/kg and 10 mg/kg, i.m., respectively). Open castration was performed with hemostasis of tunica cremaster using a 3-clamp technique. Subcutaneous implantation of pellets slowly releasing DHT (200 mg each, Innovative Research of America, Sarasota, FL, USA) at 5 mg/day was performed at the time of orchiectomy as previously described [9]. The animals recovered in a heated environment and were given buprenorphine (0.02–0.05 mg/kg) for perioperative pain relief. Plasma DHT levels were measured from serum samples collected using a commercially available ELISA kit (ALPCO Labs., Windham, NH, USA) immediately prior to surgery and again at the time of sacrifice (10–14 days after surgery/pellet implantation). This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996), and all experiments were conducted in accordance with the guidelines of the Georgetown University Animal Care and Use Committee.

2.2. Langendorff preparation

Langendorff preparations were performed as previously described [10]. Briefly, rabbits were anesthetized with intravenous pentobarbital (35 mg/kg), anticoagulated with heparin injection (500 I.U., i.v.), and sacrificed with each heart being rapidly removed through a median sternotomy and immersed in an ice-cold oxygenated Tyrode’s solution. After the removal of extraneous fat and connective tissue, hearts were perfused by the nonrecirculating Langendorff technique as previously described [10].

2.3. ECG recordings and QT measurements

Four silver–silver chloride electrode wires were positioned in a simulated Einthoven configuration with the reference and ‘foot’ electrodes fixed beneath the heart on the walls of a tissue bath with the approximate diameter of a rabbit thorax. The signals were amplified by an ECG amplifier (Colbourn Instruments, Lehigh Valley, PA, USA), allowing for the simultaneous recording of the three orthogonal signals designated as X, Y, and Z. The ECG signals were filtered selectively at 60 Hz.

2.3.1. Baseline QT measurements

The stability of the electrophysiologic measurements in our Langendorff preparation has been previously reported [10,11]. After about 30 min of continuous pacing at a cycle length of 0.4 s, baseline QT was recorded. To evaluate the cycle length dependence of the baseline QT interval and the effect of a sex steroid hormone, the cycle length was switched to one of the following cycle lengths: 0.8, 1.2 and 2.3 s, and the QT interval was measured when stable (=5% change over a 5-min period).

2.4. Whole-cell patch-clamp recordings

Ventricular myocytes were isolated from rabbit hearts using a modification of the method previously described by Giles et al. [14]. For each treatment group (i.e. placebo and DHT), at least three cells from three different hearts were recorded for analysis of $K^+$ currents. The whole-cell
patch-clamp technique was used to record the membrane currents in single ventricular myocytes as we reported previously [10,15]. Pipettes with tip resistance of 1–4 MΩ were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL, USA) and filled with an intracellular solution containing (mmol/l) KCl 125, NaCl 10, CaCl₂ 1, Mg-ATP 5, EGTA 14, HEPES 10, cAMP 0.1, adjusted with KOH to pH 7.2. A holding potential of −40 mV was used to inactivate fast sodium and T-type calcium currents. The external solution was Tyrode’s solution containing (mmol/l) NaCl 137, KCl 5.4, HEPES 10.0, MgCl₂ 1.0, CaCl₂ 2.0, glucose 10.0, pH 7.4. Cd²⁺ (0.2 mmol/l) was used to block the L-type calcium channel and to shift the I–V relationship of 1ₜₒ and 1ₚₛ to more positive potentials [10].

Cardiac myocytes adhering to glass coverslips were placed in a small chamber mounted on the stage of an inverted microscope and superfused at 1.5 ml/min at room temperature (22–24 °C). Only rod-shaped single cells that were quiescent and exhibiting well-defined, cross-striations were studied. After establishment of the whole-cell configuration and measurement of cell capacitance, series resistance (≈10 MΩ) was compensated by ~70%. Junction potentials under these conditions were approximately −3 mV and not corrected. 1ₚₛ currents were elicited from a holding potential of −40 mV by a series of 1.5-s test pulses from −10 to +50 mV in 10-mV increments. Membrane potential was then returned to the holding potential in order to observe 1ₚₛ, tail currents. The current–voltage (I–V) relationship for 1ₚₛ was constructed by measuring the tail currents.

The inward-rectifier K⁺ currents (1ₚₛ) were elicited from a holding potential of −40 mV by a series of 250-ms test pulses ranging from −90 to −10 mV in 10-mV increments. The amplitude of 1ₚₛ at each voltage was determined by measuring the peak current relative to zero current.

2.5. Northern blot analysis

Total RNA was purified from equivalent regions (apex of ventricle, ~500 mg/sample) from castrated rabbits receiving either placebo or DHT (200 mg) sustained release pellets for a period of 14 days using Trizol™ (Invitrogen, Carlsbad, CA, USA), as previously described [16]. A 25-μg portion of RNA per sample was loaded and separated by agarose gel electrophoresis, transferred to BrightStar-Plus™ (Ambion, Austin, TX, USA) nylon membrane, and hybridized with a radiolabeled 3.5-kb HindIII– BamHI HERG cDNA (GenBank accession no. U04720) fragment isolated from a pSP64 vector (kindly provided by Dr. Mark Curran) [17]. Please note that this HERG probe does not distinguish between the A and B isoforms of HERG since it contains sequences common to both [18,19]. After washing, the membrane was developed and quantitated using a Molecular Dynamics Phosphorimager (Model 445, Rockville, MD, USA). The membrane was then stripped, re-hybridized with a radiolabeled rat β-actin cDNA fragment [16], and reprocessed as above.

2.6. Data analysis and statistics

Data were recorded on a strip chart recorder (Gould Electronics, Cleveland, OH, USA) at a paper speed of 200 mm/s. Four representative beats from each set of XYZ recordings were chosen for QT measurement. The end of the T wave was defined as the latest point of the T wave as it merges with the electrical baseline in any lead [20]. QT intervals were interpreted in a blinded fashion by two investigators. Patch-clamp data were also acquired and analyzed in blinded fashion. All current measurements were normalized for total cell capacitance (i.e. pA/pF) to allow comparison between cells of various sizes. One-way analysis of variance (ANOVA) with Bonferroni posthoc testing for multiple comparisons was used to compare QT and JT measurements at specific pacing cycle rates and current densities at specified voltages. The Student’s t-test was used to compare Northern blot data from placebo and DHT rabbits. Data are reported as mean±S.E.M., with differences between values being considered statistically significant when P<0.05.

3. Results

We previously showed that male rabbit hearts have shorter QT intervals and greater K⁺ current densities (1ₚₛ and 1ₚₛ) than female rabbit hearts [10]. To test the hypothesis that these differences were the result of male sex steroid hormone action(s), we performed a series of experiments on orchiectomized (Orch) male rabbits that were implanted with either placebo or dihydrotestosterone (DHT) sustained release pellets. The efficacy of the treatment paradigm was evaluated by measuring DHT in plasma samples obtained from the rabbits immediately before castration (time of pellet implantation) and again at the time of sacrifice. As shown in Table 1, Orch male rabbits receiving placebo pellets displayed plasma DHT concentrations similar to those found in intact female rabbits. In contrast, those receiving sustained-release DHT pellets had plasma DHT levels that were significantly elevated relative to the placebo-implanted controls (78±9 vs. 19±2 pg/ml, respectively, P<0.001, n=7–8/group). The Orch rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Control (untreated)</td>
<td>205±77 (n=14)</td>
<td>27±4 (n=6)</td>
</tr>
<tr>
<td>Orch male + placebo</td>
<td>19±2 (n=8)</td>
<td>N.a.</td>
</tr>
<tr>
<td>Orch male + DHT</td>
<td>78±9*** (n=7)</td>
<td>N.a.</td>
</tr>
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***, P<0.001 vs. placebo control
N.a., not applicable.
with DHT pellets had, on average, somewhat lower DHT concentrations than those observed prior to surgery (78 ± 9 vs. 205 ± 77 pg/ml, respectively), though these values were not found to represent statistically significant differences (P > 0.05, n = 7–14/group). Thus, the treatment paradigm was effective at both significantly reducing DHT plasma concentrations in placebo-implanted Orch males and reconstituting DHT plasma levels in Orch males implanted with sustained-release DHT pellets.

Initially, we examined QT intervals in isolated perfused rabbit heart preparations (Langendorff) over a range of pacing rates. Sample ECG tracings recorded during these experiments are shown in Fig. 1. The presence of DHT helped to suppress the increases observed in QT interval produced by pacing the hearts at slower rates (increased cycle lengths) (Fig. 2). Normal male and female rabbit heart data [10] are shown for comparative purposes. Note that placebo-treated Orch males behaved like normal females while the DHT-treated Orch males behaved like normal males in this assay. Thus, the presence of DHT can lead to decreases in QT intervals in male rabbit hearts.

Since QT intervals are a measure of the duration of
ventricular depolarization as well as repolarization, we also compared JT intervals to obtain a more specific estimate for ventricular repolarization. We found similar results in these comparisons (Fig. 3). The most pronounced difference ($P<0.05$) occurred at a pacing cycle length of 1.2 s, with hearts from DHT-treated rabbits displaying JT intervals of $199\pm12$ (n=4) while those from placebo-treated rabbits displaying JT intervals of $240\pm8$ (n=4). In addition, no significant differences were observed in QRS (60±1 ms, n=4, vs. 61±3 ms, n=4, for Orch+placebo vs. Orch+DHT, respectively, pacing cycle length=2.3 s, $P>0.05$). Thus, the QT differences observed in response to DHT primarily reflect changes in ventricular repolarization. These results demonstrate that DHT suppresses cycle length-dependent slowing of cardiac repolarization in male rabbit hearts.

To determine if the presence of DHT could also influence drug-induced QT prolongation, we evaluated QT responses to quinidine (1 μM) in the Langendorff preparation. As shown in Fig. 4, quinidine-induced QT-lengthening was attenuated in hearts isolated from DHT-treated Orch rabbits relative to the placebo controls. The biggest difference in degree of quinidine-induced QT prolongation ($\Delta QT$) between placebo and DHT groups occurred at the slowest pacing rate (57±4 vs. 32±2%, respectively, pacing cycle length=2.3s, n=4, $P<0.001$). Thus, DHT appears to enhance the heart’s ability to resist drug-induced QT prolongation.

To investigate potential mechanisms underlying the DHT-induced QT changes in male hearts, we examined repolarizing K$^+$ currents in isolated ventricular cardiomyocytes using the whole-cell patch-clamp recording technique. We previously demonstrated that at least two important K$^+$ currents, $I_{K1}$ and $I_{Kr}$, were present at higher densities in male ventricular cardiomyocytes compared to those observed in females [10], thus we chose to focus on these two currents.

The inward rectifier K$^+$ current, $I_{K1}$, was measured in ventricular cardiomyocytes isolated from Orch male rabbits that had received either placebo or DHT sustained-release pellets. The outward component of $I_{K1}$ was significantly greater in ventricular cardiomyocytes from DHT

![Fig. 3. Comparison of JT intervals in Langendorff-perfused hearts from placebo and DHT-treated Orch animals.](https://academic.oup.com/cardiovascres/article-abstract/57/1/28/375505/312x556)

**Placebo**

**DHT**

<table>
<thead>
<tr>
<th>Pacing Cycle Length (s)</th>
<th>JT (ms)</th>
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</thead>
<tbody>
<tr>
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<td>260</td>
</tr>
<tr>
<td>0.5</td>
<td>250</td>
</tr>
<tr>
<td>1.0</td>
<td>240</td>
</tr>
<tr>
<td>1.5</td>
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</tr>
<tr>
<td>2.0</td>
<td>220</td>
</tr>
<tr>
<td>2.5</td>
<td>210</td>
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</table>

![Fig. 4. Comparison of quinidine-induced QT prolongation in isolated perfused rabbit hearts from placebo and DHT-treated Orch rabbits.](https://academic.oup.com/cardiovascres/article-abstract/57/1/28/375505/312x556)

**Placebo**

**DHT**

<table>
<thead>
<tr>
<th>Pacing Cycle Length (s)</th>
<th>$\Delta QT$ (ms)</th>
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<tbody>
<tr>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>0.5</td>
<td>18</td>
</tr>
<tr>
<td>1.0</td>
<td>16</td>
</tr>
<tr>
<td>1.5</td>
<td>14</td>
</tr>
<tr>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>10</td>
</tr>
</tbody>
</table>

![Fig. 5. Comparison of the inward rectifier K+ current, $I_{K1}$, in ventricular cardiomyocytes isolated from placebo vs. DHT-treated Orch rabbits.](https://academic.oup.com/cardiovascres/article-abstract/57/1/28/375505/312x556)

**DHT**

**Placebo**

<table>
<thead>
<tr>
<th>Test Potential (mV)</th>
<th>Current Density (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-120</td>
<td>2</td>
</tr>
<tr>
<td>-100</td>
<td>2.5</td>
</tr>
<tr>
<td>-80</td>
<td>3</td>
</tr>
<tr>
<td>-60</td>
<td>3.5</td>
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<tr>
<td>-40</td>
<td>4</td>
</tr>
<tr>
<td>-20</td>
<td>4.5</td>
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<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>5.5</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
</tr>
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</table>

Inset: example of an $I_{K1}$ recording is depicted.
rabbit hearts compared with those obtained from placebo hearts (e.g. 2.2±0.1 vs. 1.4±0.1 pA/pF at −60 mV, n = 9–14/group, P<0.05, Fig. 5). No significant differences were observed in the inward portion of \( I_{k1} \) for these two groups. An example of a current tracing for \( I_{k1} \) is shown in the inset. We confirmed that this current was \( I_{k1} \) by using low concentrations of BaCl\(_2\) to block it (data not shown) [14]. Significant increases in \( I_{k1} \) densities were observed between test potentials of −70 and −20 mV in DHT ventricular cardiomyocytes, with the maximum at −60 mV (Fig. 5). These results show that \( I_{k1} \) in ventricular cardiomyocytes from DHT-treated rabbits was up to 50–60% greater than that found in ventricular cardiomyocytes from placebo-treated rabbits.

One of the most striking observations from this study came from our measurements of \( I_{k1} \). An example of \( I_{k1} \) currents recorded from ventricular cardiomyocytes for these experiments is shown in Fig. 6 (inset). Substantially larger tail currents were recorded from the cells isolated from DHT-treated rabbits than those from the placebo group. To illustrate this point, we compared the mean current densities (pA/pF) over a range of activation voltages (mV) for each group and found significant increases in \( I_{k1} \) between activation voltages of +20 and +50 mV in the DHT group relative to the placebo group (Fig. 5). These results show that \( I_{k1} \) densities are up to 40% greater in ventricular cardiomyocytes from DHT-treated versus placebo-treated Orch rabbits. Furthermore, comparison of the half-maximal voltage of activation (\( V_{1/2} \)) for \( I_{k1} \) in myocytes from DHT-treated rabbits versus placebo-treated rabbits reveals that the \( V_{1/2} \) was significantly shifted towards more negative potentials in the DHT group (21.2±1.2 vs. 30.2±1.4 mV, respectively, n = 12, P<0.001). Thus, the presence of DHT facilitates activation of \( I_{k1} \) at lower voltages and increases the density of this current in ventricular myocytes.

To determine if the observed DHT-mediated increases in \( I_{k1} \) were due to changes in RERG gene expression, we examined RERG mRNA levels in rabbit ventricle from placebo and DHT-treated Orch rabbits using Northern blots. RERG mRNA was chosen as the target for this experiment because it encodes the major channel subunit for \( I_{k1} \), [21]. As shown in Fig. 7, no increase in RERG mRNA was detected in DHT-treated hearts relative to placebo-treated controls (P>0.05, n=3/group). Although the probe used to detect RERG mRNA does not distinguish between the A and B isoforms, only one band of approximately 4 kb was detected, indicating that adult rabbit ventricle predominantly expresses only one isoform. This isoform is presumed to be the A isoform because cDNA sequence information (Genbank accession no. U87513) from rabbit ventricular RERG is highly homologous (92.2%) to the A isoform of HERG (Genbank...
accession no. U04720) [22]. The blot was stripped and reprobed for β-actin to ensure the integrity of the RNA samples. Interestingly, β-actin mRNA appeared to be slightly increased in the DHT group relative to placebo controls while RERG mRNA was similar in both groups. Consequently, relative RERG mRNA expression appears slightly decreased in the DHT samples (Fig. 7B). Therefore, the increased $I_{Kr}$ densities observed in ventricular cardiomyocytes from DHT-treated rabbits are likely due to post-transcriptional and/or post-translational modifications of the RERG channel protein.

4. Discussion

The principal finding from this study is that DHT enhances the ability of male rabbit hearts to suppress rate- and drug-induced delays in cardiac repolarization (i.e. DHT-induced shortening of QT–JT intervals). The conventional mechanism of action for DHT and other androgenic steroid hormones is to bind to androgen receptors, which, in turn, leads to changes in the expression of specific genes that are the targets for androgen receptor binding. It is now well-established that androgen receptors are expressed in ventricular tissue from several mammalian species, including humans [23–25] but there is very little information available about the identity of specific gene targets of androgen receptor activity in the heart. There is also evidence that sex steroid hormones such as testosterone and 17-β-estradiol can influence potassium currents through nongenomic mechanisms [26,27], so it remains to be determined which mode of action may be operative in the heart under physiological conditions. Another mitigating factor is the variability of DHT plasma concentration levels, which may not be a particularly good indicator of hormonal influence on cardiac repolarization parameters since their mode of action likely involves alteration in the expression patterns of specific target genes over a period of time.

Although the specific mechanism of DHT action in the heart is not known, its influence leads to increased current densities for at least two key repolarizing $K^+$ currents, $I_{K1}$ and $I_{Kr}$, that had previously been identified as being greater in ventricular cardiomyocytes isolated from male (as compared to female) rabbit hearts [10]. It is clear, however, from this study and earlier work that the increased current density for $I_{Kr}$ cannot be accounted for by a corresponding increase in RERG gene expression either in Orch males (this study) or in Ovx females [9] following DHT administration. Given the fact that the observed DHT-induced changes in $I_{Kr}$ only produced about a 30% increase in current, it may be difficult to detect such relatively small changes in RERG gene expression (if present) in our heart samples. In addition, there may some heterogeneity of RERG gene expression [28] within the rabbit heart that could also influence the RNA results, and it is important to keep in mind that mRNA changes (or lack thereof) do not necessarily reflect alterations in RERG protein expression. These caveats notwithstanding, alternative explanations for how $I_{Kr}$ is increased by DHT could include phosphorylation of existing RERG [29–31], changes in RERG protein trafficking to the membrane [32], and/or alterations in β-subunit association(s) [21,33,34]. This latter possibility is particularly intriguing since association between HERG and a putative β-subunit encoded by the KCNE1 gene (minK) has been shown to increase $I_{Kr}$ density and cause an apparent leftward shift in $V_{1/2}$ [33] similar to our findings with $I_{Kr}$ in myocytes from DHT-treated rabbits. Irrespective of the specific mechanism, however, the greater densities of $I_{Kr}$ and $I_{K1}$ likely account, at least in part, for the enhanced ability of male hearts to buffer rate- and drug-induced prolongation of QT intervals.

Other cardiac ion currents also appear to contribute to the observed gender differences in cardiac repolarization [35], and evaluation of their activities in relation to DHT treatment will need to be performed to determine what role they may or may not play regarding gender differences in susceptibility to drug-induced cardiac arrhythmias. Our study does not, for example, address whether or not the slow component of the delayed rectifier $K^+$ current, $I_{Ks}$, may also play a role. $I_{Ks}$ was generally thought to be the predominant delayed rectifier current in adult rabbit ventricular myocardium [36–38]; however, several reports have shown that $I_{Ks}$ is also present in a limited extent in rabbit ventricular myocytes [12,39,40]. Although it is possible that $I_{Ks}$ could have been present in some of our $I_{Kr}$ recordings since it would have been activated (if present) by the voltage protocol used for our $I_{Kr}$ experiments, two key pieces of evidence strongly suggest that little, if any, $I_{Ks}$ was present. First, we previously showed that the preparation and voltage-clamp methods employed for this study yield exclusively $I_{Kr}$ [10,15]. This was shown by applying the $I_{Kr}$-selective inhibitor, E-4031 (5 μM), which totally abolished the tail currents in ventricular myocytes isolated from male and female rabbit hearts [10].

Despite this, one could argue that the DHT may have augmented dormant or otherwise previously quiescent $I_{Ks}$ in the myocytes that we tested, but, if that were the case, then we should have expected to see a shift in the $V_{1/2}$ for $I_{Kr}$ towards more positive test potentials (right-shifted) since $I_{Ks}$, unlike $I_{Kr}$, continues to increase as the voltage of activation becomes more positive [39]. In fact, we observed exactly the opposite result ($V_{1/2}$ for $I_{Kr}$ was left-shifted in the presence of DHT), thereby providing the second key piece of evidence indicating that $I_{Ks}$ was not likely present in the myocytes that we tested. Consequently, our results in no way imply that $I_{Ks}$ and/or many other cardiac ion currents that were not evaluated in this study may or may not be influenced by DHT. Indeed, the results of a recent study [41] suggest that cardiac $I_{Ks}$ can also be influenced by the sex of the patient.

The ability of DHT to influence the QT and JT intervals...
in Orch male rabbits is consistent with recent results reported by Pham et al. [12], which showed that Orch male rabbits displayed greater cardiac action potential duration in response to the I_{Kr}-selective blocker, dofetilide. In humans, QT intervals tend to become shorter in males near the time of puberty when testosterone and DHT serum levels rise dramatically [6,7]. Perhaps the strongest evidence in this regard comes from a recent study by Bidoggia et al. [8] where they compared JT intervals in normal men and women with those from castrated men and virilized women. Their results showed that castrated men had rate-corrected JT intervals (JTC) similar to those seen in control (normal) women and significantly longer than those found in control (normal) men. Moreover, testosterone administration in the castrated men produced shortening of JTC comparable to that observed in normal men. Our data suggest that similar influences of male sex steroid hormones may be operative in rabbits. These findings indicate that it is primarily the male sex steroid hormones that account for the observed differences in cardiac repolarization between males and females and that the isolated perfused rabbit heart preparation is a useful model for studying this phenomenon in the laboratory.

Acknowledgements

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