Testosterone Diminishes the Proarrhythmic Effects of Dofetilide in Normal Female Rabbits

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Background—Recent clinical and experimental data suggest that testosterone may protect males against the deleterious effects of repolarization-prolonging drugs. This study tests the hypothesis that 5α-dihydrotestosterone (DHT) protects normal females against drug-induced excessive prolongation of repolarization.

Methods and Results—We used microelectrode techniques to study isolated preparations of rabbit ventricular endocardium from age-matched normal control female rabbits and female rabbits treated with DHT for 4 weeks. Serum 17β-estradiol levels were identical in the control and DHT-treated animals, whereas DHT levels were high (equaling those in normal males) only in the DHT-treated animals. Basal action potential duration to 90% repolarization (APD90) was significantly shorter in DHT-treated (155±7.4 ms, n=32) than control females (178±6.7 ms, n=29; P<0.05) at cycle length=1000 ms. The increase in APD90 induced by 10⁻⁸ mol/L dofetilide at cycle length=1000 ms was significantly less in DHT-treated females than normal females (ΔAPD90=8±7 and 29±5 ms, respectively, P<0.05). At 10⁻⁶ mol/L dofetilide, the incidence of early afterdepolarizations was 28% in DHT-treated and 55% in normal female rabbits (P<0.05).

Conclusions—Elevating DHT levels diminishes the effects of dofetilide to increase APD and induce early afterdepolarizations in females. Moreover, treatment of females with DHT results in prolongation of APD and an incidence of early afterdepolarization equal to values previously reported by us for dofetilide-treated normal males. That serum levels of 17β-estradiol were the same in DHT-treated and untreated females suggests that estradiol is not involved in the response to dofetilide. Thus, these data suggest that DHT and perhaps other androgenic hormones may protect normal females against the risk of dofetilide-induced arrhythmia. (Circulation. 2002;106:2132-2136.)

Key Words: arrhythmia ■ sex ■ dofetilide ■ hormones

Drugs that prolong the QT interval induce a greater incidence of arrhythmias (ie, torsades de pointes [TdP]) in women than in men.¹⁻³ The greater propensity to drug-induced TdP in females is generally associated with a sex-related difference in ventricular repolarization in the heart such that the rate-corrected QT interval is longer in females than males.⁴⁻⁶ Recent clinical and experimental studies propose that gonadal steroids may modulate the sex-related differences in QT interval and propensity toward drug-induced TdP.⁷⁻¹⁰

Bidoggia et al⁷ showed that women with virilization exhibit a shorter and faster repolarization time than normal women and castrated men, suggesting that testosterone is an important modulator of ventricular repolarization. In addition, Rodriguez et al⁸ demonstrated that women are at greater risk of drug-induced QT prolongation during menstruation and the ovulatory phase of the menstrual cycle, with decreased risk during the luteal phase. Given that progesterone levels are higher during the luteal than the ovulatory and menstruation phases, Rodriguez et al suggest that androgen may determine the risk for drug-induced TdP. Furthermore, in male rabbits, testosterone was determined to be an important protective factor against the effects of an I_{Kr}-blocking drug to prolong repolarization and induce early afterdepolarizations (EADs).¹⁰ In the present study, we treated normal female rabbits with DHT to determine whether DHT can (1) alter the baseline ventricular action potential (AP) at cycle lengths comparable to those of physiological human heart rates and (2) diminish the prolongation of repolarization and decrease the occurrence of EADs induced by the I_{Kr}-blocking drug dofetilide.

Methods
This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US Public Health Service, National Institutes of Health publication No. 85-23, 1996.

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Animals (Harlan, Indianapolis, Ind) were fed water and Rabbit Diet HF 5326 (Labdiet; Purina Mills). Rabbit Diet HF 5326 contains phytoestrogen, which can exert estrogenic effects, but this was a constant in all experiments. Female rabbits received implants of 60-day sustained-release pellets (Innovative Research of America) of vehicle (5 rabbits) or pellets of 5α-dihydroxytestosterone (DHT; the active form of testosterone, not metabolized to estradiol by cytochrome P-450 aromatase; 5 rabbits). Rabbits were treated with DHT for 4 to 5 weeks, at which time they were ready for experimental studies.

Before anesthesia, 2 mL of blood was obtained, and serum was stored at −20°C for analysis. 17β-Estradiol was measured by a solid-phase, chemiluminescent immunoassay (Immulite, Diagnostic Products Co). Assay sensitivity was 20 pg/mL. DHT was measured by radioimmunoassay (Diagnostic Systems Laboratories) coupled with a sample oxidation/extraction procedure to remove testosterone. Assay sensitivity was 4 pg/mL.

Rabbits (3.0 to 3.5 kg at the time of the terminal experiment) were anesthetized with sodium pentobarbital (30 mg/kg IV), and the hearts were excised and immersed in Tyrode’s solution equilibrated at 37°C anesthetized with sodium pentobarbital (30 mg/kg IV), and the hearts were excised and immersed in Tyrode’s solution equilibrated at 37°C with 95% O2/5% CO2. The solution contained (mmol/L): NaCl 131, NaHCO3 18, KCl 4, CaCl2 1.2, MgCl2 0.5, NaH2PO4 1.8, and dextrose 5.5. Right ventricular papillary muscles (3 to 5 mm long and 0.3 to 1 mm in diameter) were dissected and placed in a 4-mL chamber perfused with Tyrode’s solution (37°C, pH 7.4) at a rate of 12 mL/min. Preparations were stimulated via Teflon-coated silver wires with 2-ms, rectangular, twice-threshold current pulses. Stabilization required 1 to 2 hours of stimulation at cycle length (CL) = 1000 ms. We have previously demonstrated the stability of the transmembrane potential throughout the duration of the experiment.13 Conventional microelectrode techniques were used to record transmembrane potentials.

Predrug measurements of transmembrane potentials were made at CL = 1000, 500, and 330 ms, with 3 minutes allowed to achieve steady state at each CL. Thirty minutes was allowed for evolution of drug effects at each concentration before measurements were made.

The IKr blocker dofetilide; a gift from Helopharm (Germany), was dissolved in water to obtain a 10−3 mol/L stock solution before use. Drug effects at each concentration before measurements were made.

Serum 17β-estradiol levels did not differ in the control (55 ± 4 pg/mL) and DHT-treated (66 ± 8 pg/mL) animals, whereas DHT levels were low in control females (17 ± 6 pg/mL) and high in the DHT-treated animals (664 ± 249 pg/mL, P < 0.05). These DHT values were equivalent to those we previously reported in normal males.10

Results

Hormone Levels

Serum 17β-estradiol levels did not differ in the control (55 ± 4 pg/mL) and DHT-treated (66 ± 8 pg/mL) animals, whereas DHT levels were low in control females (17 ± 6 pg/mL) and high in the DHT-treated animals (664 ± 249 pg/mL, P < 0.05). These DHT values were equivalent to those we previously reported in normal males.10

Effects of DHT on Baseline Endocardial APs

At all CLs, there were no significant differences in maximum diastolic potential, AP amplitude, and Vmax of phase 0 between the control and DHT-treated female rabbits. For example, at a CL of 1000 ms, maximum diastolic potential was −79.3 ± 0.7 and −80.3 ± 0.6 mV, AP amplitude was 104.9 ± 1.1 and 107.0 ± 0.8 mV, and Vmax was 177.6 ± 7.3 and 181.6 ± 10.5 V/s in controls and DHT-treated rabbits, respectively. Figure 1 illustrates the CL dependence of AP duration (APD). Control normal females had longer APD to 50% repolarization (APD50) than DHT-treated females (P < 0.05) at a CL of 1000 ms. At rapid pacing rates (CL = 300 ms and 500 ms), APDs were equivalent in normal and DHT-treated rabbits.

Effects of DHT on Response to Dofetilide

Representative AP recordings are shown in Figure 2, and the effects of chronic DHT treatment on dofetilide-induced APD prolongation are presented in Figure 3. Dofetilide had no effect on maximum diastolic potential, AP amplitude, or Vmax (data not shown) but produced concentration-dependent prolongation of APD in both groups (Figure 3). As in our previous study,10 dofetilide exerted its greatest APD-prolonging effects at CLs ≈ 1000 ms. At CLs of 330 and 500 ms, dofetilide (10−7 and 10−6 mol/L) prolonged APD significantly compared with predrug control but did not cause EADs (Figure 3, top and middle panels). At CL = 1000 ms, dofetilide 10−8 mol/L induced significantly greater APD50 prolongation in control females than DHT-treated females.
At higher concentrations, dofetilide induced EADs in both groups, making it difficult to measure APD accurately. EAD incidence was greater in normal (55% at 10^{-6} mol/L dofetilide) than DHT-treated (28% at 10^{-6} mol/L dofetilide, \(P<0.05\)) females. These data are consistent with published data regarding the effects of dofetilide.\(^{16}\) In addition, the data in Figure 3, bottom, suggest that chronic DHT treatment may reduce the reverse use-dependent actions of dofetilide.

**Discussion**

Men have shorter rate-corrected QT intervals than women,\(^{4-6}\) and previous studies have indicated the potential role of sex hormones in modulating this difference.\(^{7-10}\) Recent clinical data\(^{7,9}\) together with experiments in rabbits\(^{8,10}\) suggest that testosterone may provide protection against TdP induced by \(I_K\)-blocking drugs. The present investigation determined that testosterone given to normal female rabbits can shorten baseline APD, lessen the rate-dependence of APD prolongation, and protect against drug-induced excessive prolongation of repolarization.

**DHT Shortens APD and Alters Rate Adaptation**

In this study, we demonstrate that testosterone (DHT) shortens baseline APD\(_{90}\) (CL=1000 ms). Consistent with observations that virilized women have shorter durations of repolarization (QT intervals) than normal women,\(^7\) our results demonstrate that testosterone can indeed modulate ventricular repolarization in females.

Clinical data suggest that sex-related differences in the QT–RR relationship (rate adaptation) may contribute to the longer QTc interval in women. Women exhibit a greater lengthening of the QT interval as heart rate slows, such that the sex-related differences in QT intervals become more pronounced as CL increases.\(^{17}\) Similarly, Liu et al\(^8\) determined that female rabbit hearts demonstrate significantly longer QT intervals at long CL than male hearts. We observed that at short CL (330 and 500 ms), there was no difference in APD, whereas at long CL (1000 ms), DHT-treated females have shorter APD than control females. This may explain the lower rate-dependence of QT prolongation in males compared with females.

**DHT Diminishes Dofetilide-Induced Prolongation of Repolarization and EADs**

Figure 4 summarizes the effects of chronic DHT treatment on 10^{-8} mol/L dofetilide-induced \(\Delta APD_{90}\) (A) and 10^{-6} mol/L dofetilide-induced incidence of EADs (B) in control and orchiectomized males (data from Circulation. 2001;103:2207–2212, Figure 5) and normal females. \(\bullet\) indicates control male; \(\square\), orchiectomized male; \(\triangleleft\), DHT-treated orchiectomized male; \(\odot\), control female; and \(\diamondsuit\), DHT-treated female. \(^*P<0.05\) vs population with high DHT serum levels.
male and female rabbit papillary muscles. At a high DHT level, the effects of dofetilide on AP prolongation (Figure 4A) and incidence of EADs (Figure 4B) diminished. We have previously shown in male rabbits that castration drastically reduces serum DHT levels while accentuating dofetilide-induced APD prolongation and incidence of EADs.\textsuperscript{10} DHT replacement in castrated male rabbits restores the serum DHT concentration and diminishes the effects of dofetilide.\textsuperscript{10} Similarly, in the present study, DHT replacement in normal female rabbits increased serum DHT concentration (equaling those in normal male rabbits) and significantly reduced the AP prolongation and lowered the incidence of EADs induced by dofetilide (Figure 4). Thus, DHT protects against dofetilide-induced excessive AP prolongation and incidence of EADs.

**Mechanism of DHT Action**

Sex hormones have been shown to modify ventricular repolarization and response to \( I_{\text{Kr}} \)-blocking drugs via their modulatory actions on ionic currents important for repolarization.\textsuperscript{18} Recently, Pham et al\textsuperscript{19} demonstrated that estrogen and testosterone can modify the current density, voltage dependence of activation, and transmural dispersion of \( I_{\text{Ca,L}} \) in female castrated rabbits, such that hormones increase epicardial \( I_{\text{Ca,L}} \) density and increase \( I_{\text{Ca,L}} \), transmural dispersion. In castrated male rabbits, however, neither estrogen nor testosterone alters \( I_{\text{Ca,L}} \) properties. Thus, it is unlikely that the protective effects of testosterone noted in this study and our previous work\textsuperscript{10} are a result of modulation of \( I_{\text{Ca,L}} \) by testosterone.

A recent study by Shuba et al\textsuperscript{20} demonstrated that testosterone reduces the maximal blockade of HERG, a human clone of the \( I_{\text{Ks}} \) channel, induced by various neuroleptics (haloperidol, pimozide, and fluspirilene). Testosterone altered the \( I_{\text{Cm}} \) of these neuroleptic drugs. These data\textsuperscript{20} suggest that this may be the mechanism through which testosterone modulates responses to \( I_{\text{Ks}} \)-blocking drugs such as dofetilide. However, Shuba et al\textsuperscript{20} performed their study in a *Xenopus laevis* expression system, where it was determined that testosterone produced a 35% reduction in HERG current. This suggests that testosterone would prolong repolarization, a result inconsistent with the fact that men have shorter QT intervals than women.

Thus far, no available studies have evaluated the modulatory effects of testosterone on native repolarizing potassium current. However, Drici et al\textsuperscript{21} demonstrated that testosterone and estrogen treatment in castrated female rabbits downregulates message levels of certain potassium channels. mRNA levels of HK2 (also known as hKv1.5, a human clone of the ultrarrapidly activating delayed rectifier, \( I_{\text{Kur}} \))\textsuperscript{22} were reduced compared with placebo-treated rabbits. Two different transcripts (3.4 and 0.7 kb) of \( I_{\text{Ks}} \) (or \( I_{\text{minK}} \))\textsuperscript{23} a modulatory subunit of the slowly activating delayed rectifier, \( I_{\text{Ks}} \) are present in placebo-treated female rabbits, but the 0.7-kb transcript was markedly reduced in estradiol- and DHT-treated castrated animals. In addition, estrogen and testosterone did not alter the levels of HERG (\( I_{\text{Ks}} \)) mRNA. However, their mRNA data did not correspond with the changes in QT intervals that they noted.\textsuperscript{21}

Liu et al\textsuperscript{8} evaluated sex-related differences in 3 major repolarizing \( K^+ \) currents (\( I_{\text{Kur}}, I_{\text{Ks}}, \) and \( I_{\text{Kr}} \)) in the heart. They found no difference in \( I_{\text{Kur}} \) density between male and female rabbits and reported a significant difference in \( I_{\text{Ks}} \) at a single voltage, −50 mV (1.46 ± 0.06 pA/pF in females and 1.67 ± 0.08 pA/pF in males), but no difference at any other voltages. With regard to \( I_{\text{Kr}} \) density, Liu et al\textsuperscript{8} determined that female rabbit ventricles have 20% less \( I_{\text{Kr}} \) density than males. Thus, it is possible that testosterone treatment might increase \( I_{\text{Kr}} \) density, thereby contributing to a shorter baseline APD and a diminished dofetilide-induced AP prolongation in males.

An additional important finding is that DHT treatment diminished the reverse use dependence of the effects of dofetilide on APD. A previous study\textsuperscript{24} determined that sensitivity to blockade of \( I_{\text{Kr}} \) by dofetilide was rate independent. Therefore, the reverse use dependence of dofetilide is not directly related to its \( I_{\text{Kr}} \)-blocking effects. The data of Jurkiewicz and Sanguinetti\textsuperscript{24} further suggested that \( I_{\text{Kr}} \), the slowly activating delayed rectifier, is rate dependent (ie, there is more \( I_{\text{Kr}} \) repolarizing current at rapid than at slow pacing rates as a result of \( I_{\text{Kr}} \) accumulation). This may explain why there is a reduced AP-prolonging effect of dofetilide at rapid pacing rates. In light of this study,\textsuperscript{24} our data suggest that testosterone treatment may upregulate \( I_{\text{Kr}} \) in DHT-treated females, thereby diminishing the reverse use-dependent effects of dofetilide. However, the effects of testosterone on these repolarizing potassium currents remain to be elucidated, a limitation that will be addressed in the future.

**Clinical Implications**

Women with virilization have shorter JT intervals than normal women or castrated men.\textsuperscript{7} These results suggest that testosterone influences properties of normal ventricular repolarization. Consistent with this, our data showed that chronic DHT treatment in female rabbits shortens baseline ventricular APD\textsubscript{50} and APD\textsubscript{90} compared with normal female rabbits. In view of this, testosterone indeed modifies the underlying mechanisms that regulate ventricular repolarization. In addition, testosterone modulates the response to \( I_{\text{Ks}} \)-blocking drugs by diminishing the AP-prolonging effects of dofetilide and decreasing the risk for developing EADs. Thus, testosterone protects against the proarrhythmic effects of \( I_{\text{Ks}} \) blockade in both males and females.

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**References**

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