Effects of Gonadal Steroids on Ventricular Repolarization and on the Response to E4031

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ABSTRACT

Gonadal steroids are thought to be important determinants of gender-related differences in electrophysiology, such as the longer rate-corrected QT intervals (QTc) than men (Ashman, 1942; Adams, 1936; Merri et al., 1989; Rautaharju et al., 1992). There also are gender-related differences in the incidence of some clinical arrhythmias. We studied the chronic effects of gonadal steroids on cardiac action potentials (APs) using standard electrophysiological techniques. Papillary muscles were removed from the hearts of oophorectomized rabbits that had been treated with placebo, estradiol or dihydrotestosterone (DHT). The electrocardiograms of the three groups did not differ. Papillary muscle APs were studied during drive at cycle lengths of 330 to 5000 msec. The APD30 of the DHT group was significantly shorter than that of the others at cycle lengths of >500 msec. The APD90 of the estradiol group was significantly longer than that of the DHT group at cycle lengths of >1000 msec. The APD90 of the placebo group tended to be intermediate. The effects of the antiarrhythmic drug E4031 (10^{-8}–10^{-6} M) also were examined. E4031-induced prolongation of APD90 and magnitude of early afterdepolarizations was significantly greater in the estradiol-treated than the DHT-treated and placebo groups. In conclusion, in rabbit heart, gonadal steroids are important determinants of base-line electrophysiological properties and the proarrhythmic response to E4031.

Women have faster heart rates and longer rate-corrected QT intervals (QTc) than men (Ashman, 1942; Adams, 1936; Merri et al., 1989; Rautaharju et al., 1992). There also are gender-related differences in the incidence of some clinical arrhythmias. For example, female gender is a risk factor for torsades de pointes associated with cardiovascular drugs that prolong repolarization (Makkar et al., 1993) and for the occurrence of syncope and sudden death in the congenital long QT syndrome (Moss et al., 1985). The mechanisms responsible for gender-related differences in cardiac rhythm and arrhythmias are largely undefined, although Drici et al. (1996a) recently demonstrated that estradiol and DHT down-regulate potassium channel (HK2 and Isk) expression. These results suggest that gonadal steroids determine gender-related differences in electrophysiology at the level of control of ion channels.

Data such as these suggest the need for more intensive investigation of the gender-specific determinants of rhythm and arrhythmias. Therefore, we elected to study the AP characteristics and antiarrhythmic drug responsiveness of oophorectomized young rabbits chronically treated with placebo, estradiol or DHT. We determined estradiol and DHT actions on the electrocardiogram and ventricular action potentials, as well as on the interaction with E4031, an antiarrhythmic drug representative of agents that prolong repolarization via block of the potassium channel, I_{Kr} (Sanguinetti and Jurkiewicz, 1990).

Methods

Female New Zealand White rabbits were oophorectomized at age 40 to 50 days (Turckheim et al., 1983) and randomly divided into three groups: treatment with placebo, estradiol benzonate (10 μg/day) or DHT (10 mg/day) for 3 weeks. We used DHT because it is not metabolized to estradiol by cytochrome P-450 aromatase (Abdelghani et al., 1994). At a dosage of 1 mg/day, DHT produces female androgen levels that are of the same order of magnitude as those of normal males (Nielsen et al., 1982). Estradiol (1 μg/day) is the dosage that stimulates chinning in oophorectomized females (Mariscal et al., 1992). Hormones were dissolved in carthamus oil (0.2 ml) and injected subcutaneously under the nape of the neck. Carthamus oil was used as the placebo. Electrocardiograms were recorded with animals in the conscious state before the start of hormonal replacement and on a weekly basis thereafter. To ensure technically optimal electrocardiograms, standard lead II was recorded with the leads clipped to the rabbits’ ears and forelegs. Measurements of RR, PR, QRS, QT

ABBREVIATIONS: AP, action potential; DHT, dihydrotestosterone; MDP, maximum diastolic potential; \(V_{max}\) maximum rate of rise of phase 0 of the action potential; APD30, action potential duration to 30% repolarization; APD90, action potential duration to 90% repolarization; EAD, early afterdepolarization; CL, cycle length.
and QT intervals were made as previously described (Bazett, 1920), averaging the intervals over a 60-sec period after the rhythm had stabilized.

After 3 weeks of treatment, the rabbits were anesthetized by the intravenous administration of sodium pentobarbital (30 mg/kg). Their hearts were quickly removed and immersed in warm Tyrode’s solution (36°C) equilibrated with 95% O₂/5% CO₂ and containing (in mM) NaCl 131, NaHCO₃ 18, KCl 4, CaCl₂ 1.2, MgCl₂ 0.5, NaH₂PO₄ 1.8 and dextrose 5.5. The Ca²⁺ was reduced in comparison to the Tyrode’s usually used in Purkinje fiber experiments to reduce contractile force. Right ventricular papillary muscles were dissected for electrophysiological study.

In experiments on APs, a stabilization period of 3 to 4 hr was permitted during constant stimulation at CL of 1000 msec. Frequency-dependent changes in the AP were assessed during drive at CL of 5000, 2000, 1000, 500 and 330 msec. MDP, overshoot, V_{max} were displayed on a digital storage oscilloscope and V_{max}, APD₅₀ and APD₉₀ also were determined.

In experiments using E4031, control APs were recorded at CL of 1000 and 2000 msec. Muscles then were driven at CL of 1000 msec and superfused with graded concentrations of E4031 (10⁻⁸–10⁻⁶ M). Equilibration was 30 min at each drug concentration. After superfusion with 10⁻⁶ M E4031, CL was changed again to 2000 msec. In preparations that showed EADs, the number and amplitude of the EAD were determined as previously described (Damiano and Rosen, 1984). In this study, the number of EADs during the last 5 min of superfusion was counted and reported as EADs/min.

**Microelectrode techniques.** Conventional microelectrode techniques were used (Rosen et al., 1973). All preparations were placed in a tissue bath, superfused with Tyrode’s solution equilibrated with 95% O₂/5% CO₂ and warmed to 36°C (pH 7.3 ± 0.05, mean ± S.E.M.). Solutions were pumped through the tissue bath at a flow rate of 12 ml/min, with chamber content changed three times a minute. The bath was connected to ground with a 3M KCl/Ag/AgCl junction. Preparations were impaled with 3M KCl-filled glass capillary microelectrodes with tip resistances of 10 to 20 MΩ. The maximum rate of rise of phase 0 of the AP (V_{max}) was obtained by electronic differentiation with an operational amplifier (Rosen et al., 1973). The electrodes were coupled by an Ag/AgCl junction to an amplifier with high-input impedance and input capacity neutralization (Duo 773; World Precision Instruments, New Haven, CT). Transmembrane APs and V_{max} were displayed on a digital storage oscilloscope and chart recorder. The system was calibrated as previously described (Rosen et al., 1973). For stimulated preparations, standard techniques were used to deliver 1.0-msec square-wave pulses 2 times threshold through bipolar Teflon-coated silver electrodes.

**Statistical analysis.** Statistical analysis was performed using analysis of variance and, where the f value permitted, Bonferroni’s test (Snedecor and Cochrane, 1980). P < .05 was considered significant. Data are reported as mean ± S.E.M.

### Results

**Effects of gonadal steroids on the electrocardiogram.** Electrocardiographic data are summarized in table 1. Although electrocardiograms were recorded weekly, only the baseline (0 week) and 3 week data are shown. Neither estradiol nor DHT affected the electrocardiographic characteristics.

**Effects of gonadal steroids on papillary muscle APs.** All preparations were driven at CL of 330 to 5000 msec. At all CLs, there were no significant differences in MDP, overshoot and V_{max} among placebo, estradiol and DHT groups. Control values for placebo (n = 14), estradiol (n = 15) and DHT (n = 13) groups at CL of 1000 msec were MDP, −86 ± 1, −85 ± 1 and −85 ± 1 mV; overshoot, 24 ± 1, 22 ± 1 and 25 ± 1 mV; and V_{max}, 142 ± 10, 129 ± 12 and 160 ± 13 V/sec, respectively (all P > .05). For all three groups, APD was maximal at CL of 1000 msec and decreased at both longer and shorter CLs (fig. 1). This result is similar to the data of others for rabbit (Kodama et al., 1992) and in marked contrast to data for species such as dog (Elharrar and Surawicz, 1983). Moreover, there were clear differences among groups in APD, as shown in figure 1. First, the values for APD₅₀ of the placebo and estradiol groups did not differ significantly, but both were significantly longer than the DHT group at all CLs of >330 msec. Second, for APD₉₀ the values for the estradiol group were significantly greater than those for the DHT group at CL of 1000 to 5000 msec, and the values for placebo were intermediate. Moreover, APD₉₀ of the estradiol group tended to become longer than that of the placebo group as CL increased, and at CL of 5000 msec, the estradiol group had a significantly longer APD₉₀. Finally, at CL of 330 msec (near that of the heart rate in the conscious rabbit; see table 1), there was no significant difference in APD₉₀ among groups.

We then determined the effects of E4031 on the APs of papillary muscles from animals in each group. E4031 had no effects on MDP, overshoot and V_{max} (data not shown). Figure 2 shows the effects of E4031 (10⁻⁷–10⁻⁶ M) on APD₅₀ (fig. 2A) and APD₉₀ (fig. 2B) at CL of 1000 msec. The prolongation of APD₅₀ and APD₉₀ by E4031 was greater in the estradiol group than the others, with the marked prolongation of APD at E4031 10⁻⁶ M being associated with the occurrence of EAD. Representative experiments are shown in figure 3.

To study EAD in greater detail, we conducted additional experiments with E4031 at CL of 2000 msec. We observed E4031-induced EAD in 47% of 15 papillary muscles in the placebo group, 25% of 8 muscles in the DHT group and 67% of 12 muscles in the estradiol group (P < .05 compared with DHT). These muscles were obtained from a total of 10 rabbits in the placebo group, 6 in the DHT group and 8 in the EST group. Moreover, as shown in table 2, both the numbers of EAD in any sequence and the EAD amplitude were greater in the estradiol group than in the others.

### Discussion

A growing body of evidence suggests there is a fundamental difference in substrate that renders women more suscept-

### Table 1

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<th>RR Baseline</th>
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<th>QRS Baseline</th>
<th>QT Baseline</th>
<th>QTc Baseline</th>
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<tr>
<td></td>
<td>3 Weeks</td>
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<tr>
<td>Placebo (n = 10)</td>
<td>239 ± 8</td>
<td>242 ± 7</td>
<td>63 ± 1</td>
<td>65 ± 2</td>
<td>48 ± 2</td>
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<tr>
<td>ESTR (n = 10)</td>
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<td>237 ± 9</td>
<td>62 ± 2</td>
<td>63 ± 2</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>DHT (n = 10)</td>
<td>229 ± 10</td>
<td>237 ± 7</td>
<td>66 ± 2</td>
<td>67 ± 2</td>
<td>47 ± 2</td>
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tible to cardiac arrhythmias than men. For example, women have faster heart rates than men, and on the electrocardiogram, they have longer QTc intervals (McFarlane et al., 1989). That estrogen and progesterone both exert important and as yet ill-defined effects on substrate is suggested by the following. First, in women without cardiovascular disease, the administration of oral contraceptive agents induces a significantly higher incidence of ventricular ectopy than is seen in controls (Romhilt et al., 1984). Second, in the congenital long QT syndrome, female gender is an important independent risk factor for the occurrence of syncope and sudden death (Moss et al., 1985). There also are important gender-specific differences in mRNA expression of a variety of structural and functional proteins in myocardium (Rosenkranz-Weiss et al., 1994) and hormonal modulation of autonomic function as seen in the effect of 17β-estradiol to reduce the alpha adrenergic responsiveness of vascular tissues (Jilma et al., 1994).

The identification of receptors for gonadal steroids in the heart provides a rationale for investigating direct hormonal modulation of adrenergic receptor responses in this tissue. Androgen receptors have been identified in both atrial and ventricular tissue (McGill et al., 1980), whereas estrogen receptors appear to be largely confined to atrial myocytes (Stumpf et al., 1977). However, the consequences of their functional activation have not been investigated in any detail. Klanfkalaya and Chan (1988) reported that estrogen and progesterone have a synergistic effect to increase muscarinic cholinergic and beta adrenergic receptor density in ovariec-tomized rats. Rokosh et al. (1994) recently reported that postpartum female rats have a lower abundance of the mRNAs encoding the alpha-1B and alpha-1D adrenergic receptors in brain and heart, respectively. However, extensive studies of gonadal steroid regulation of cardiac adrenergic
receptors at the level of agonist responsiveness, as well as gene expression, are not available.

Given the complexities of cardiac-autonomic interactions and the evolving spectrum of cardiac disease, it is clear that any attempt to relate gonadal steroid function to the modulation of normal and abnormal cardiac function represents an attack on a “moving target.” Within this context, arrhythmias and their ongoing therapy are a cause of continued concern. This is true of ventricular arrhythmias in the postinfarction setting and of atrial arrhythmias. Here, again, there are gender-specific differences. For example, atrial fibrillation, a major cause of death in an aging population, is seen in 5% of women and in only 2.3% of men older than 60 (P < .05) (Cobbe, 1994). The response to and the effects of antiarrhythmic drugs differ as well with respect to gender. This may in part reflect gender-specific differences in the cytochrome P-450 system (Giardina, 1992), which is important to the metabolism of many pharmacological (including antiarrhythmic) agents. It also may relate to gender-specific differences in the drug-substrate interaction at the level of the heart. Certainly, drugs that prolong repolarization induce a higher incidence of QT prolongation, torsades de pointes and death in women than in men (Makkar et al., 1993). Moreover, even in settings in which drugs are not administered yet the QT interval is prolonged (as in congenital long QT syndrome), women have a longer QT interval and an increased propensity to torsades de pointes than do men (Lehmann et al., 1997).

In our study, chronic treatment with estradiol and DHT provided a stable environment in which to consider the actions of these hormones alone and their interaction with E4031. DHT shortened the early phase of repolarization (APD90), whereas estradiol tended to prolong APD, especially at long CLs. This effect was mainly due to prolongation of the late portion of phase 3 (APD90). As a result, APD90 of the estradiol group was longer than that of the DHT group at CL of 1000 msec. At CL of 330 msec, AP durations were equivalent in the estradiol, DHT and placebo groups. This may explain the finding that both estradiol and DHT did not change QT interval in the intact rabbits, in which the RR interval is ~240 msec (table 1). That the QT interval during estrogen treatment > DHT > placebo was shown by Drici et al. (1996a). However, these investigations used heart-blocked, Langendorff-perfused rabbit hearts stimulated from the ventricle at a CL of 400 msec. Based on our figure 1, at this CL, differences in QT interval would be expected to occur; hence, the results of the two studies are in fact consistent.

We have not examined the effects of gonadal steroids on the ion channels that are responsible for repolarization of the action potentials. Drici et al. (1996a) showed that HK2 and Isk mRNA were down-regulated in cardiac ventricular tissue from oophorectomized rabbits treated with estradiol or DHT. They also reported that in normal adult rabbits, the IK1 and ICa current densities were lower in females (Drici et al., 1996b). Differences in these repolarizing currents could contribute to the longer AP durations seen in the estradiol-treated rabbits compared with DHT-treated rabbits and provide a basis for the QT prolongation and proarrhythmia seen with some repolarization-prolonging antiarrhythmic drugs in female patients (Waldo et al., 1996; Lehmann et al., 1996).

The potential for chronic effects of estradiol to be deleterious were revealed in the experiments using the Ikr-blocking antiarrhythmic drug E4031. E4031 induced significantly greater prolongation of AP duration and a higher incidence and magnitude of EADs in the estradiol group. These results have direct implications for clinical studies. For example, in the SWORD trial (Waldo et al., 1996), administration of d-sotalol, an Ikr blocker, was associated with a higher incidence of death in women than in men. In addition, recent studies have found female patients to be at a greater risk for QT prolongation (Lehmann et al., 1997; Stramba-Badiale et al., 1997) and sotalol-induced torsades de pointes (Lehmann et al., 1996) than are men. Our own results, with a different Ikr-blocking antiarrhythmic agent, E4031, demonstrate both excess AP prolongation and excess occurrence of EAD in the setting of estradiol treatment. Both these changes are thought to be important to the evolution of torsades de pointes (e.g., Members of the Sicilian Gambit, 1994).

These observations, considered in light of the earlier works of Drici et al. (1996a, 1996b), suggest strongly that further consideration be given both to the mechanisms by which estrogen and DHT may influence channel structure and function and their interaction with antiarrhythmic drugs. They suggest, as well, that new strategies for drug evaluation and therapy be adopted: using E4031 as an example, it would be interesting to learn whether protocols incorporating lower dosages and plasma levels might confer an antiarrhythmic action and proarrhythmic potential equated to those in men or whether the diminution in proarrhythmic potential was accompanied by a comparable reduction in antiarrhythmic activity.

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