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Review

Sex, hormones, and repolarization

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Abstract

There is increased awareness of the impact of gender and gonadal steroids on human cardiac rhythm and arrhythmias; e.g., drugs that prolong repolarization induce torsades de pointes (TdP) more frequently in women than men; female gender is an independent risk factor for syncope and sudden death in the congenital long QT syndrome; and the higher propensity toward arrhythmia in normal females is associated with fundamental differences in repolarization such that rate-corrected QT intervals are longer in females than males. Mechanisms underlying these differences are incompletely defined but are believed to involve gonadal steroids. This review discusses recent advances and prospects for further elucidation of the influence of gender and gonadal steroids on ventricular repolarization and arrhythmias.

Keywords: Arrhythmia (mechanisms); Gender; Hormones; Long QT syndrome; Ventricular arrhythmias

1. Introduction

There is an ever-increasing appreciation of the extent to which cardiac function is influenced by gender and gonadal steroids. One of the most dramatic and potentially lethal differences is that seen in repolarization of the heart. Clinical and experimental studies have begun to identify the extent of and the mechanisms for these differences, as shall be reviewed.

2. Repolarization in the human heart: influence of gender and age (see Table 1)

Women have faster resting heart rates and longer rate-corrected QT intervals (QTc) than men [1–5]. Bazett was the first to describe the gender difference in QTc interval, noting that of women to be 24 ms (6%) greater than that of men. Subsequent studies confirmed this finding, reporting mean differences of 2–6% [4,5]. Additional gender differences in T-wave shape and rate dependence of QT intervals have been identified on ECG. The interval from the S-wave offset to the peak of the T-wave is significantly longer in women than men [4], and the maximum instantaneous slopes of the ascending and descending limbs of the T-wave are less steep in women than men at heart rates of 60–80 bpm [6]. These data suggest that gender influences both early and late repolarization.

Abbreviations: APD, action potential duration; DHT, 5α-dihydrotestosterone; E-4031, specific blocker of the rapidly activating delayed potassium rectifying current; EAD, early afterdepolarization; ECG, electrocardiogram; ER, estrogen receptor; ERKO, estrogen receptor knock-out; FSH, follicle stimulating hormone; GnRH, gonadotrophin-releasing hormone; HK2 (Kv1.5), human clone of the ultra-rapidly activating delayed potassium rectifying channel; HERG, human clone of the rapidly activating delayed potassium rectifying channel; HRE, hormone response element; $I_{Ca,L}$, L-type calcium current; I_{K1}, inward rectifying potassium current; I_{Kr}, rapidly activating delayed potassium current; I_{Ks}, slowly activating delayed potassium current; I_{Kur}, ultra-rapidly activating delayed potassium rectifying current; IsK (minK), modulatory subunit of the slowly activating delayed rectifying current; I_{to}, transient outward potassium current; KvLQT1, human clone of the slowly activating delayed potassium channel; LH, luteinizing hormone; QTc, rate-corrected QT interval; SHR, spontaneously hypertensive rat; SWORD, survival with oral *d*-sotalol; TdP, torsades de pointes

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Table 1
Gender-related differences in ventricular repolarization in humans

Measurement of repolarization	Condition or treatment	Summary	Refs.
QTc duration	***Normal***	QTc duration is longer in women than men	[1–5]
	Rate dependence	Steeper QT/RR ratio in women than men	[7,8]
	Age		
	Neonates (1 to ~12 years)	QTc duration is the same	[5,10–12]
	~13–50 years	QTc duration is longer in women than men	[5,13–15]
	50 years<	QTc duration is the same	[5]
JT duration	***Gonadal steroids:***		
	Virilization	JT duration is shorter in virilized women than normal women	[15]
	Orchiectomy	JT duration is shorter in normal men than orchiectomized men	[15]
ST duration	Normal	ST duration is longer in women than men	[4]
T-wave	***Ascending and descending slope***	Less steep in women than men	[6]
Risk of developing TdP	***Drugs that prolong repolarization (i.e., quinidine, sotalol, etc.):***		
	Normal	Lower risk for developing TdP in men than women	[16,19–21]
	Menstrual cycle	Lower risk for developing TdP in women during luteal phase than menstruation and ovulation phases	[22]

Other investigations suggest gender-related differences in the QT-RR relationship (rate-adaptation) may contribute to the longer QTc duration in women. Women manifest a greater lengthening of the QT interval as heart rate slows such that the gender-based differences in QT intervals became more pronounced as cycle length increases [7]. Stramba-Badiale et al. [8] confirmed this finding demonstrating that the QT/RR ratio was steeper in women than men. At a 1000-ms cycle length QT intervals were longer in women and at a 600-ms cycle length QT intervals were equal in both genders.

These differences in QTc intervals appear to have a developmental component. Whereas male and female neonates and children before age 10 [9,10] show no difference in QTc [11] from puberty through adulthood, women have faster heart rates than men and have correspondingly longer QTc. Rautaharju et al. [5] suggested that the gender-related difference in repolarization after puberty reflects abbreviation of QTc intervals in males rather than prolongation in females. The QTc duration in men gradually increases from puberty until age 50 when it is again similar to that of women. A similar age-associated influence was seen in some families with autosomal dominant congenital long QT syndrome [12,13]. For chromosome 7q (HERG)- and 11p (KvLQT1)-linked families, QTc intervals were shorter in men than women and children [13]. In addition, there was an age-dependent reduction in the percentage of male but not female long QT syndrome patients manifesting QT intervals >440 ms [14]. These data suggest the change in level and activity of sex hormones at puberty may contribute to the appearance of the gender-related differences in QTc.

3. Sex hormones and gender differences in QT interval and arrhythmias: studies in human subjects

The shortening of the QT interval during puberty in males implies that androgen (specifically testosterone) rather than estrogen may contribute to gender differences in QTc. This hypothesis is supported by the observation of longer JT intervals in orchiectomized than non-orchiectomized men [15]. This same study reported that women with virilization have shorter JT intervals than castrated men and normal women.

With respect to estrogen and progesterone, the situation is more complex. Lehmann et al. [16] reported a similar propensity for drug-induced torsades de pointes (TdP) in pre- and post-menopausal women. Another retrospective study found no significant effects of hormone replacement therapy on QTc intervals in postmenopausal women [17]. These data argue against a contribution of estrogen to gender-based differences in ventricular repolarization. However, women taking oral contraceptives reportedly have a higher incidence of ventricular ectopy than untreated controls [18], suggesting that estrogen or progesterone may be arrhythmogenic. In this study, a correlation

between hormone levels and incidence of ventricular ectopy was not tested.

The major complication attributed to QTc prolongation is life-threatening TdP, usually consequent to administration of repolarization-prolonging drugs. For example, the antiarrhythmic drug, *d*-sotalol, was associated with a greater incidence of death in both genders, with a higher incidence in women [19,20]. Female gender is also a risk factor for syncope and sudden death in the autosomal dominant congenital long QT syndrome [21], and TdP is more prevalent in women than men during complete heart block [22]. Although it is generally accepted that the greater risk for TdP of all causes in women is associated with longer baseline rate-corrected QT intervals [1–5], Makkar et al. [23] found a greater propensity for cardiovascular drug-induced TdP in women even when baseline QT intervals did not differ across gender.

Women are most at risk of drug-induced QT prolongation during menstruation and the ovulatory phase of the menstrual cycle, with risk decreasing during the luteal phase [24]. Although estrogen levels are lower during menstruation and ovulation, the ratio of progesterone to estrogen is increased during these phases. This has led to the suggestion that the ratio per se rather than absolute hormone levels determines the extent of risk [22].

4. Estrogen and androgen receptors in the heart

Estrogen and androgen receptors are found in myocardium. Tritiated estradiol and 5α-dihydrotestosterone (DHT) were localized to the nuclei of atrial and ventricular myocardial cells of baboons [25,26]. Receptor binding was isoform-specific; only tritiated DHT localized to the cell nuclei of baboon atria and ventricles [27]. Stumpf et al. [28] showed that tritiated estradiol concentrated in the cell nuclei of the atria but not the ventricles of rat hearts. Recent work [29] using immunoblots and immunofluorescence demonstrated estrogen receptor protein expression in both male and female rat neonatal cardiomyocytes and fibroblasts. Grohe et al. [29] described nuclear translocation of estrogen receptors after exposure to 17β-estradiol, and estradiol induced a significant increase in reporter activity in cells transfected with an estrogen-responsive reporter plasmid. These findings demonstrate that cardiomyocytes express gonadal steroid receptors and that the receptors are functional and can modulate gene expression in these cells.

Several studies have demonstrated modulatory effects of estrogen on mRNA level of a slow K^+ channel in rat uterus, rabbit oviduct and dog coronary artery smooth muscle [30–32]. In addition, there is an increase in [^{3}H]nitrendipine binding sites in hearts from chronic estradiol-treated spontaneously hypertensive rats (SHR) compared to untreated oophorectomized SHR [33]. These data suggest estradiol can modulate expression of cardiac calcium currents. Yet, surprisingly, data are scarce regarding the in vitro cardiac electrophysiological effects of androgen, progesterone and estrogen.

5. Impact of gender on ventricular repolarization in animals

Recent studies, largely in the rabbit, have examined the effects of 17β-estradiol and 5α-dihydrotestosterone on repolarization. Data reviewed in the following sections with regard to the rabbit heart are summarized in Tables 2–5. Tables 2 and 3, respectively, summarize gender and gonadal steroid-related differences in repolarization parameters, and Tables 4 and 5, respectively, summarize gender and gonadal steroid-related differences in ventricular ionic currents.

5.1. Gender and cardiac electrophysiology

Liu et al. [34] demonstrated differences in the QT duration in electrocardiograms recorded from isolated perfused male and female rabbit hearts. At a cycle length of 400 ms (approximating the rabbit's normal sinus rate), QT intervals were similar between male and female rabbits. However at a cycle length of 2.3 s, the mean QT interval was significantly longer in hearts from females than males. Liu et al. [34] also noted a steeper cycle length dependence on the QT interval in female compared to male rabbits, as is seen in humans [7,8]. Furthermore, in the rabbit, female gender is associated with a higher incidence of TdP, and potassium channel blockade and QT prolongation appear to be contributory factors [35].

There are gender-related differences in the right ventricular action potentials of rabbit heart, such that early repolarization (APD_{30}) is longer in papillary muscles of females than males [36]. These disparities in APD_{30} may contribute to the gender-related differences in the slope of the ascending limb of the T-wave reported in humans. Yang et al. [6] noted men have significantly greater maximal absolute slopes (dV/dt) of the ascending and descending limbs of the T-wave compared to women. Given that the T-wave reflects the aggregate electrical activity during repolarization of ventricular cells [37], a difference in the ascending and descending slopes of the T-wave [6] would suggest differences in phases 2 and 3 of repolarization. These voltage–time differences in the T-wave may reflect a spatial change (e.g., transmural dispersion or regional dispersion) in ventricular repolarization as predicted by Shimizu et al. [38]. For example, in the long QT syndrome low amplitude, broad, and/or bifurcated T-waves are indicative of diminished repolarizing forces and increased dispersion of repolarization [39]. A longer APD_{30} and greater APD_{30} dispersion in female compared to male rabbits could result in diminished repolarization forces in female rabbit ventricles. This may correspond

Table 2
Gender-related differences in ventricular repolarization in the rabbit

Preparation	Measurement of repolarization	Condition or treatment	Summary	Refs.
Male/female rabbits Isolated Langendorff hearts	QT duration	CL=400 ms	QT duration is equivalent between females and males	[34]
		CL=2300 ms	QT duration is longer in females than males	
	ΔQT duration	*d*-Sotalol; Quinidine	QT increase is greater in females than males	[40]
Male/female rabbits Isolated right ventricle	APD_{30}	CL=330, 500, 1000 ms	APD_{30} is greater in females than males	[36]
	APD_{90}	CL=330, 500, 1000 ms	APD_{90} is equivalent between females and males	
	ΔAPD_{90} CL=1000 ms	Chromanol 293B (10^{-7}–10^{-5} M)	No change in APD_{90} in females and males	[36]
		Dofetilide (10^{-8} M)	APD_{90} increase is greater in females than males	
	Incidence of EAD (CL=1000 ms)	Dofetilide (10^{-6} M)	Greater incidence of EAD in females than males	[36]
	Transmural dispersion of APD_{90}	Control	Epicardial APD_{90} is equivalent to endocardial APD_{90} in both females and males	[36]
		Dofetilide (10^{-6} M)	APD_{90} transmural dispersion is greater in females than males	

with a less steep ascending slope of the T-wave in female than in male human subjects.

A male–female difference in APD_{30} [36] implies gender-related differences in the ionic currents responsible for early repolarization (approximately −20 to +10 mV). We are aware of only one report of repolarizing K currents related to gender (as opposed to hormones). Liu et al. [34] demonstrated a smaller I_{K1} density at −50 mV and a smaller I_{Kr} density in female than male rabbit ventricle. These results may explain the longer APD_{30} in females. Moreover, a 2–5-ms (~3%) longer APD_{90} is seen in normal and oophorectomized females than occurs in normal and orchiectomized male rabbits. This difference in APD_{90} is consistent with the 2–6% differences in QTc generally reported between men and women [1,2,4].

6. Drugs and gender

In intact male and female rabbit hearts, the I_{Kr}-blocking drugs, quinidine and *d*-sotalol, prolonged QT intervals more in females than males [40]. In isolated rabbit ventricles, the I_{Kr}-blocking antiarrhythmic drug, dofetilide, induced greater prolongation of APD_{90}, a higher incidence of early afterdepolarizations (EAD) (Fig. 1), and greater dispersion of repolarization in female than male rabbits [36]. These factors may underlie the greater risk for induction of torsades de pointes [41,42] in female patients [42].

The transmural dispersion resulting from I_{Kr} blockade [36] suggests epi-endocardial differences in I_{Kr} or other ionic currents contributing to repolarization. Although some studies demonstrate a transmural gradient in repolarization [43–45] and outward K^+ currents [46–50] in various species, none of this information has been stratified for gender. With regard to inward currents, the L-type Ca^{2+} current ($I_{Ca,L}$) contributes to the induction of EAD [51]. In experiments exploring gender-based differences in $I_{Ca,L}$ [52], a transmural gradient for $I_{Ca,L}$ occurred in female but not male hearts. Such a transmural gradient could contribute to dispersion of repolarization, and to transmural differences observed in the occurrence of EAD [36].

7. The effects of gonadectomy and hormone replacement on repolarization

Gonadectomy has a dramatic effect on the response of rabbit papillary muscles to the I_{Kr}-blocking drug, dofetilide [36] (Table 3). In male rabbits, orchiectomy resulted in decreased DHT levels and an increase in dofetilide-induced incidence of EAD (Fig. 2). This is consistent with the hypothesis of the protective role of testosterone suggested by Drici et al. [53] and supported by data from our laboratory [54]. However, these earlier studies did not measure DHT levels and hence could not test whether DHT is protective against the effects of I_{Kr} blockade. Subsequent measurement of DHT levels [36] demonstrates the importance of this hormone in limiting the effect of I_{Kr} blockade on repolarization in males.

In female rabbits, oophorectomy reduced the risk for dofetilide-induced APD prolongation and EAD [36] (Fig. 1). The consistently low serum estradiol levels argue

Table 3
Effects of gonadal steroids on ventricular repolarization in the rabbit

Preparation	Measurement of repolarization	Condition or treatment	Summary	Refs.
Gonadectomized male/female rabbits: isolated right ventricle	APD_{30}	CL=330, 500, 1000 ms	OVX-females>ORCH-males	[36]
	APD_{90}	CL=330, 500, 1000 ms	OVX-females=ORCH-males	
	ΔAPD_{90}	Dofetilide (10^{-8} M); CL=1000 ms	APD_{90} increased; ORCH-males>OVX-females	
	Incidence of EAD	Dofetilide (10^{-6} M); CL=1000 ms	ORCH-males>OVX-females	
OVX-female rabbits treated with EST, DHT, or PLA: isolated Langendorff hearts	QT durations	CL=400 ms	EST=DHT>PLA	[53]
	ΔQT durations	Quinidine; CL=400 ms	EST=PLA>DHT	
	$MAPD_{90}$	Control; LV vs. RV (all groups)	LV=RV	[36]
		Control; CL=330 ms	EST>DHT=PLA	
	$\Delta MAPD_{90}$	Azimilide (2 and 5 μM); CL=330 ms	$MAPD_{90}$ increased; EST>DHT=PLA	
OVX-female rabbits treated with either EST, DHT, or PLA: isolated right ventricle	APD_{30}	CL=330 ms	EST=DHT=PLA	[53]
		CL=500–5000 ms	PLA=EST>DHT	
	APD_{90}	CL=330 ms	EST=DHT=PLA	
		CL=500–2000 ms	EST>DHT	
		CL=5000	EST>DHT=PLA	
	APD_{90}	E4031 (10^{-6} M); CL 1000	EST>DHT=PLA	
	Incidence of EAD	E4031 (10^{-6} M); CL 2000	EST>DHT=PLA	
ORCH-male rabbits treated with either EST, DHT, or PLA: isolated right ventricle	APD_{90}	Control; CL 1000 ms	EST=DHT=PLA	[36]
	ΔAPD_{90}	Dofetilide (10^{-8} M); CL=1000 ms	ΔAPD_{90} increased; PLA=EST>DHT	
	Incidence of EAD	Dofetilide (10^{-6} M); CL=1000 ms	PLA=EST>DHT	

Table 4
Gender-related differences in ionic currents in the rabbit ventricle

Preparation	Ionic current	Property	Summary	Refs.
Male/female rabbits Disaggregated LV myocytes	$I_{Ca,L}$	Current density	Transmural gradient in females; Absent in males	[52]
		Whole cell conductance	Transmural gradient in females; Absent in males	
		Steady-state activation	Equivalent in both females and males; no transmural differences	
		Steady-state inactivation	Equivalent in both females and males; no transmural differences	
		Tau of peak current decay	Equivalent in both females and males; no transmural differences	
		Tau of recovery from inactivation	Equivalent in both females and males; no transmural differences	
	I_{Kr}	Current density	Males ~20%>than females	[34]
	I_{K1}	Current density	Greater in males than females at −50 mV; at all other voltage points males and females have equal I_{K1} density	[34]

Table 5
Effects of gonadal steroids on ventricular ionic currents in the rabbit

Preparation	Ionic current	Property	Summary	Refs.
OVX-female rabbits treated with either EST, DHT, or PLA: disaggregated LV myocytes	$I_{Ca,L}$	Current density: Transmural gradient	EST=DHT>PLA	[52]
		Whole cell conductance: Transmural gradient	EST>DHT=PLA	
		Steady-state activation:	Epi>Endo (EST and DHT)	
		Negative shift	PLA=EST=DHT (Endo)	
		Steady-state inactivation	PLA=EST=DHT	
		Tau of peak current decay	PLA=EST=DHT	
		Tau of recovery from inactivation	PLA=EST=DHT	
ORCH-male rabbits treated with either EST, DHT, or PLA: disaggregated LV myocytes	$I_{Ca,L}$	Current density: Transmural gradient	PLA=EST=DHT	[52]
		Whole cell conductance: Transmural gradient		
		Steady-state activation		
		Steady-state inactivation		
		Tau of peak current decay		
		Tau of recovery from inactivation		
OVX-female rabbits treated with EST, DHT or PLA: RNA from ventricle	HERG (I_{Kr})	mRNA levels	PLA=EST=DHT	[53]
	IsK (minK)− regulatory subunit of I_{Ks}	mRNA levels	PLA>EST=DHT	
	HK2 (Kv1.5; I_{Kur})	mRNA levels	PLA=EST=DHT	

against a unique estrogenic basis for the greater risk of females to the proarrhythmic effects of I_{Kr} blockade. Given that oophorectomy blunts the actions of dofetilide, it is probable that a non-estrogenic ovarian and/or pituitary–hypothalamic factor promotes the proarrhythmic response. This factor (or mechanism) may be influenced by progesterone, which is reduced by gonadectomy, or gonadotrophins such as luteinizing hormone (LH) and follicle stimulating hormone (FSH), whose levels could be altered after gonadectomy. These suggestions are supported by the observation that oophorectomy results in increased endogenous gonadotrophin-releasing hormone (GnRH), LH and FSH levels in rabbit [55].

In oophorectomized female rabbits chronically treated with either 17β-estradiol or 5α-dihydrotestosterone (DHT), gonadal steroids can modulate repolarization [53,54]. Isolated hearts from oophorectomized female rabbits treated chronically with estradiol and DHT had significantly longer QT intervals than hearts from placebo-treated rabbits [53]. In a related study, differences were found in APD of papillary muscle isolated from oophorectomized female rabbits chronically treated with either placebo, estradiol or DHT [54]. A difference in cycle length dependence of APD was noted such that at CL= 1000 ms and longer, the estradiol-treated group exhibited longer APD_{90} than the DHT-treated group, while values for placebo-treated animals were intermediate. At cycle lengths less than 500 ms these differences were negligible.

Gonadal steroids also modulate the response to drugs that block the delayed rectifier K^+ current (I_{Kr}). Drici et al. [53] found that quinidine's effects on QT intervals of estradiol-, DHT- and placebo-treated oophorectomized female rabbits differed among the three groups. In all, quinidine prolonged QT intervals compared to baseline. However, quinidine prolonged QT intervals in estradiol- and placebo-treated animals more than DHT-treated animals. Conversely, Hara et al. [54] showed that E-4031, a specific I_{Kr} blocker, caused more extensive prolongation in estradiol-treated than placebo- and DHT-treated groups (Fig. 3). In addition, E-4031 induced a higher incidence of EAD in the estradiol group than the others. Both studies demonstrate that estradiol-treated rabbits undergo a consistently greater drug-induced prolongation of repolarization than DHT-treated animals. However, the response to drug in placebo-treated rabbits is at odds in the two studies. This may be explained by the different pharmacological actions of quinidine and E-4031. E-4031 specifically blocks I_{Kr}, whereas quinidine blocks multiple currents: i.e., I_{Kr}, transient outward K^+ current (I_{to}), inward rectifier K^+ current (I_{K1}), the transient Na^+ current, the tetrodotoxin-sensitive persistent Na^+ current, and calcium currents [56,57].

Drici et al. [53] also demonstrated that chronic estradiol or DHT treatment downregulate message levels of potassium channels. In estradiol- and DHT-treated groups, mRNA levels of HK2 (also known as human Kv1.5, a

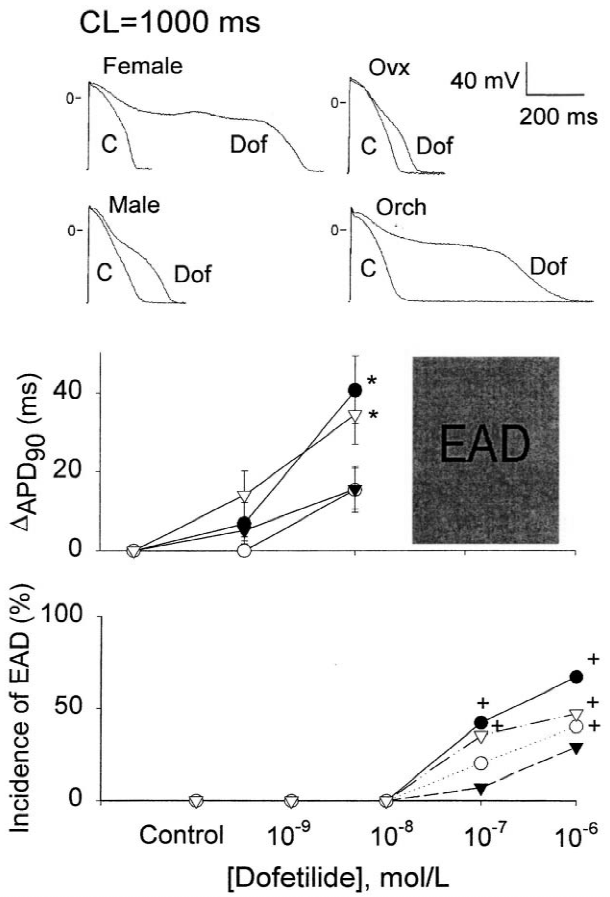

Fig. 1. Effects of increasing concentrations of dofetilide on APD and the incidence of EAD at CL=1000 ms. Upper panel: representative action potentials; C-control, Dof=10^{-6} M dofetilide. From female (●), male (○), oophorectomized female OVX (▼), and oophorectomized male ORCH (▽) rabbits, respectively. Note that dofetilide markedly prolongs repolarization than normal females and oophorectomized males and has far less effect on normal males and oophorectomized females. Middle panel: relationship of ΔAPD_{90} to increasing concentrations of dofetilide. Bottom panel: incidence of EAD induced by dofetilide. *$P<0.05$ cf. OVX and control male and $^{\dagger}P<0.05$ cf. the respective pre-drug control. (Reprinted by permission of the American Heart Association from Pham et al., Circulation 2001; 103: 2207–12.)

clone of the ultra-rapidly activating delayed rectifier, I_{Kur}) [58] were markedly reduced compared to placebo-treated rabbits. IsK (also known as minK [59], a modulatory subunit of the slowly activating delayed rectifier, I_{Ks}) transcripts of 3.4 and 0.7 kb were found in placebo-treated animals, but the 0.7-kb transcript was absent in estradiol- and DHT-treated rabbits [53]. The level of HERG (I_{Kr}) mRNA was the same among the groups.

To summarize this section, gonadal steroids are important determinants of gender differences in repolarization. The basic electrophysiological characteristics such as QT interval (increased), APD (prolonged at slow heart rate) and response to I_{Kr} blockers (exaggerated) expressed in estrogen-treated rabbits are analogous to those of female rabbits. Moreover, the electrophysiological characteristics in dihydrotestosterone-treated rabbits are analogous to those seen in control males.

8. Effects of gender and gonadal steroids on ventricular ionic currents

8.1. L-Type calcium current

There is transmural dispersion of $I_{Ca,L}$ in female but not male rabbit ventricle [52]. This gradient is due to greater whole-cell $I_{Ca,L}$ conductance in epicardium than in endocardium of female ventricle. This finding is consistent with the gender differences during early repolarization, seen electrocardiographically in the ascending and descending slopes of the T-wave [6] and cellularly in APD_{30} [52]. The $I_{Ca,L}$ transmural gradient in females may contribute to the greater transmural dispersion of APD and increased occurrence of EAD in female rabbit ventricles.

8.2. Effects of gonadectomy and hormone replacement on $I_{Ca,L}$

Gonadectomy, estradiol or DHT have no effect on $I_{Ca,L}$ in male rabbits. However, castration eliminates the transmural $I_{Ca,L}$ gradient in females [52]. Given that epicardial $I_{Ca,L}$ density is higher than endocardial in control females, the effect of oophorectomy suggests that the ovaries contribute hormonal factors important for the modulation of epicardial $I_{Ca,L}$. Importantly, both estradiol and DHT modulate $I_{Ca,L}$ properties such that chronic estradiol and DHT treatment of oophorectomized female rabbits shifted activation of epicardial $I_{Ca,L}$ to more negative potentials than endocardium and increased epicardial $I_{Ca,L}$ conductance [52]. Comparable changes did not occur in orchiectomized males. These results suggest a common pathway for gonadal steroid effects is present in female rabbits, only. The basis for the failure to identify a pathway in males is not currently known.

Because estradiol and DHT levels in normal female rabbits are similar to those of oophorectomized females [52], it is likely that in rabbits estradiol and DHT are not unique modulators of $I_{Ca,L}$ under physiological conditions. Furthermore, the effects of estradiol and DHT on $I_{Ca,L}$ properties are subtly yet importantly different from those observed in normal females. DHT increases epicardial $I_{Ca,L}$ density by shifting voltage dependence of the epicardial $I_{Ca,L}$ activation such that $I_{Ca,L}$ activates at a more negative potential than in endocardium (Table 5). Estradiol causes not only a negative shift in epicardial $I_{Ca,L}$ activation but increases epicardial more than endocardial whole cell conductance (Table 5). In contrast, in normal females, there is no difference in the voltage dependence of $I_{Ca,L}$ activation between epi- and endocardium, while epicardium has a larger whole cell $I_{Ca,L}$ conductance. These data suggest that in female rabbit other factors (e.g., progester-

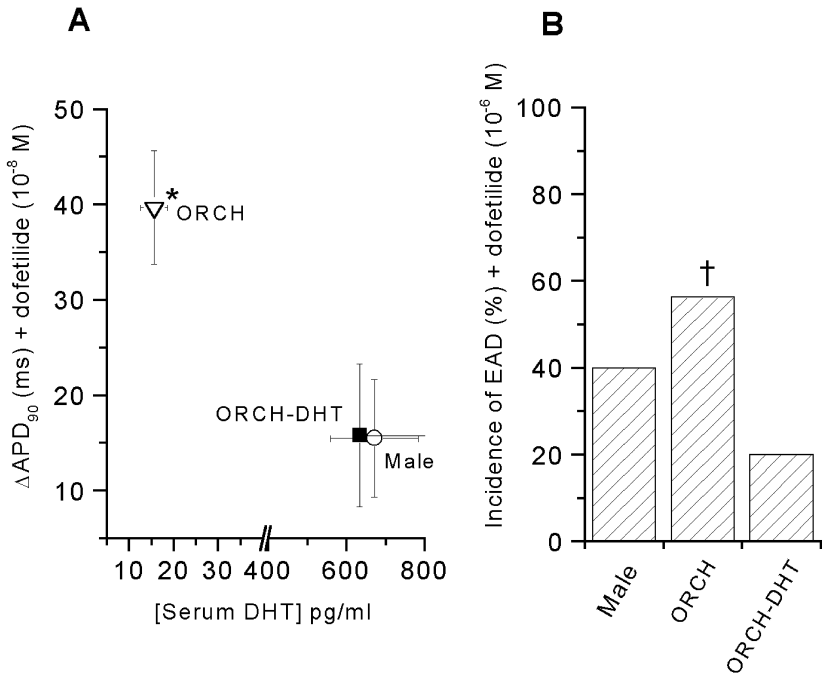

Fig. 2. (A) Orchiectomy results in a lowering of serum DHT levels and a marked increase in the effect of dofetilide to prolong APD. 5α-dihydrotestosterone replacement normalizes the DHT levels and response to dofetilide. (B) Incidence of EAD induced by dofetilide in normal males, orchiectomized males, and orchiectomized males treated with DHT. *$P<0.05$ cf. control and DHT-treated orchiectomized males. †$P<0.05$ cf. respective pre-drug control. (Modified from Pham et al., Circulation 2001; 103: 2207–12.)

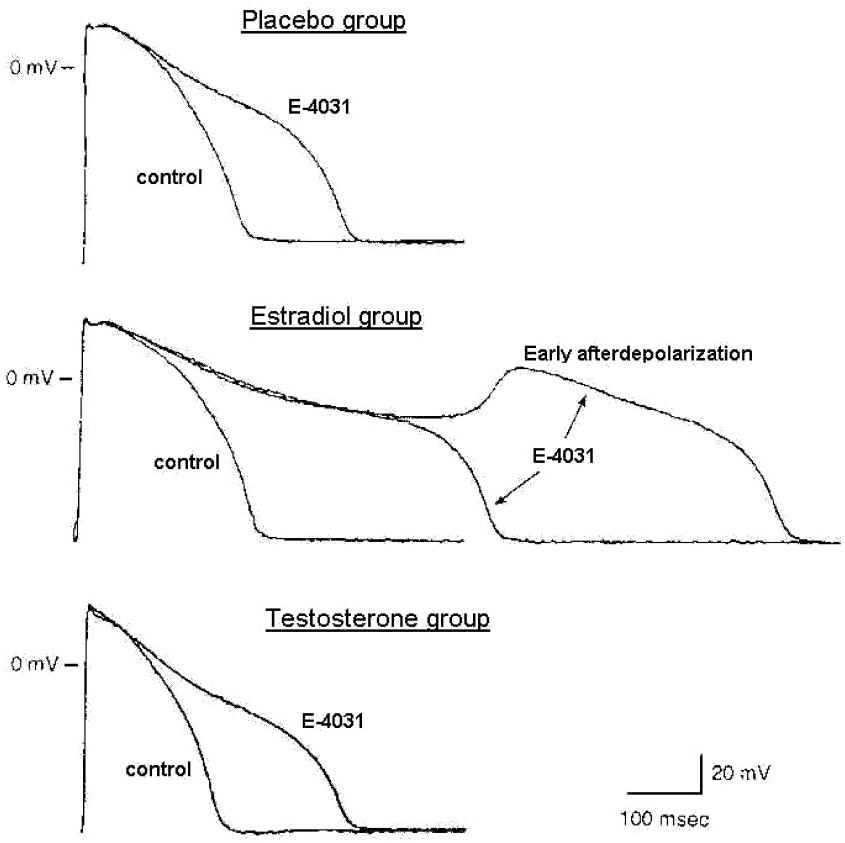

Fig. 3. Effects of the I_{Kr} blocking drug, E4031, on APD prolongation in hormone-treated oophorectomized female rabbits. I_{Kr} blockade induced the greatest APD prolongation in oophorectomized female rabbits treated chronically with 17β-estradiol. Modified from Hara et al., J Pharmacol Exp Ther 1998; 285: 1068–72.

one, GnRH, LH, or FSH) may contribute to $I_{Ca,L}$ modulation. They also suggest that the basis for the different results seen in males and females may rely on one channel subunit (e.g., the α) while the difference in activation kinetics seen with gonadal steroid treatment of oophorectomized females may reside in another subunit (e.g., β).

The epicardial conductance data for gonadectomy and gonadal steroids are consistent with results in spontaneously hypertensive rat (SHR) hearts [33]. Here, gonadectomy significantly decreased the number of [^{3}H]nitrendipine binding sites in female SHR hearts. Furthermore, the number of [^{3}H]nitrendipine binding sites in hearts from chronically estradiol-treated SHR increased compared to untreated oophorectomized female SHR. Chronic DHT treatment had no effect on [^{3}H]nitrendipine binding sites compared to oophorectomized female SHR, nor did gonadectomy and chronic hormone treatments affect [^{3}H]nitrendipine binding sites in male SHR.

The data on chronic estradiol effects on $I_{Ca,L}$ differ from previously reported studies of acutely administered estradiol, which has an $I_{Ca,L}$ antagonist effect when applied acutely to vascular smooth muscle or cardiac myocytes at concentrations of 0.27–8.1 μg/ml [60–62]. However, these concentrations substantially exceed the physiological range of circulating estrogen (20–80 pg/ml) in both male and female rabbits [63–65] and the levels achieved using hormone-impregnated pellets [52]. Hence, they represent a pharmacological effect whose relevance to normal physiology as well as pharmacological therapy is questionable.

8.3. Repolarizing potassium currents

Liu et al. [34] studied the three major repolarizing K^+ currents: they found no gender difference in I_{to} density and did not examine I_{to} kinetics. However, I_{Kr} density was 20% lower in females than males. They also reported a small but significant difference in I_{K1} 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 differences at other voltages. They suggested the smaller I_{Kr} and I_{K1} densities in female rabbits may contribute to the gender disparity in QT interval [34]. In addition, lower I_{Kr} density may explain the steeper cycle length dependence of the QT interval in females.

9. Potential ionic mechanism of gender-related differences in repolarization and proarrhythmic effects of dofetilide in rabbits

Gender-related differences in the $I_{Ca,L}$ transmural gradient [52] combined with smaller I_{Kr} and I_{K1} densities [34] could result in prolonged repolarization and greater transmural dispersion of repolarization in female compared to male rabbit ventricle. These conditions in the presence of I_{Kr}-blockade could cause higher incidences of EAD in females. Prolonged repolarization, transmural dispersion and EAD are important factors for the induction of TdP [41,42,66]. Together these factors create conditions putting females at greater risk for proarrhythmic effects of drugs.

These conclusions are consistent with action potential data demonstrating that female rabbits are at greater risk than males for dofetilide-induced excess APD prolongation, EAD and transmural dispersion of repolarization [36]. The effects of I_{Kr} blockade with dofetilide were reversed by castrating male and female rabbits; i.e., orchiectomized males were at greater risk than oophorectomized females for dofetilide-induced EAD and excessive APD prolongation. That $I_{Ca,L}$ transmural dispersion in normal females was eliminated upon gonadectomy suggests that the $I_{Ca,L}$ gradient in females may contribute to the proarrhythmic response to dofetilide. However, the parallelism does not follow in males, in that no $I_{Ca,L}$ gradient existed in normal males and gonadectomy had no effect on $I_{Ca,L}$ in orchiectomized males. Furthermore, that estradiol and DHT did not modulate $I_{Ca,L}$ in castrated males suggests there are different $I_{Ca,L}$ modulatory mechanisms in females than males. These findings indicate potential gender differences in the regulatory mechanisms of repolarization.

Although DHT protects males against the risk for drug-induced excess APD prolongation and EAD [36], DHT has no effect on $I_{Ca,L}$ in males [52]. This implies that the protective action of DHT is not through changes in $I_{Ca,L}$ but might occur via modulation of other ionic currents contributing to repolarization, e.g., persistent sodium current, I_{to}, I_{Kr} and/or I_{Ks}.

10. Mechanism and complexity of hormonal actions

This section reviews briefly the mechanisms through which estrogen and testosterone may alter cellular transcription of genes (see Beato [67] for a thorough review) thereby contributing to differences in repolarization. Estrogen and testosterone belong to a large family of endogenous signaling molecules that can modulate cell activities via gene regulation. These molecules include sex hormones (estrogen, androgens, progestin), adrenocortical hormones (glucocorticoids, mineralocorticoids), and vitamin D. Their effects are mediated via binding to specific intracellular receptors and converting them to functional transcription factors. The ligand–receptor complex then binds to hormone response elements (HRE) on DNA. Interactions of the DNA bound complex with other transcriptional components facilitate modulation of transcription of specific genes.

Several studies have demonstrated functional estrogen receptors in the heart [26,28,29]. Liu et al. [68] identified a functional HRE in the 5′-flanking sequence of the α_{1c}-subunit gene transcription start site. The α_{1c}-subunit gene is cardiac and vascular smooth muscle specific and encodes the α-subunit of the L-type Ca^{2+} channel. That

chronic estradiol treatment of oophorectomized SHR female rats induces increased [^{3}H]nitrendipine binding sites [33] is consistent with the increase in $I_{Ca,L}$ density found in estradiol-treated oophorectomized female epicardium [52]. These data suggest that the mechanisms whereby estrogen upregulates $I_{Ca,L}$ density may be via the genomic pathway described above.

Although most of the electrophysiological data from the rabbit are consistent across laboratories, there are conflicting data from the mouse. For example, single myocytes isolated from estrogen receptor knock-out male mice (ERKO) showed an increased $I_{Ca,L}$ density and a 75% increase APD [69]. In the ERKO mouse APD changes correlated with a 70% increase in QT duration on ECG. Moreover, the increase in $I_{Ca,L}$ density was associated with the lack of an estrogen receptor in ERKO mice; whereas in wild-type mice with intact estrogen receptors $I_{Ca,L}$ density was lower than in ERKO mice. Clearly, $I_{Ca,L}$ density is regulated by estrogen receptors in the mouse, and in this study estrogen receptor pathways physiologically suppress cardiac calcium channel expression. Since the magnitude of $I_{Ca,L}$ plays a major role in determining the action potential plateau and contributes to the rate of repolarization, application of this study to humans would lead to the expectation that premenopausal women would have shorter QT intervals than men. In fact, the opposite is true.

These differences in the mouse could be species-related with regard to linkages of estrogen receptors. Moreover, it is likely that we still understand too little of the complex regulatory mechanisms underlying transcriptional regulation by estrogen and therefore do not understand the result. To illustrate, a second isoform of the estrogen receptor (ERβ) has been cloned [70,71], and has a wide tissue distribution distinct from that of ERα [72]. However, some tissues such as vascular smooth muscle express both subtypes [73]. These receptor subtypes have distinct transcriptional effects, yet when co-expressed can interact to form heterodimers with the same HRE [74,75]. Perhaps, in ERKO mice, ERβ is biologically active [76] and may mediate the increase in $I_{Ca,L}$ density. This hypothesis remains to be tested. In any event, these observations add additional levels of specificity and complexity to our understanding of steroid hormone signal transduction.

11. Summary

We have explored potential cellular mechanisms underlying gender- and gonadal steroid-related differences in ventricular repolarization. Clinical data indicate that women are at greater risk for drug-induced TdP and that female gender is an independent risk factor for syncope and sudden death. These observations suggest the higher propensity toward arrhythmias in females is associated with fundamental differences in repolarization in the normal heart such that QTc intervals are longer in females than males. In addition, both clinical and experimental data implicate gonadal steroids (estradiol and testosterone) as determinants of gender-related differences in repolarization.

The findings from in vitro studies we have detailed provide only a glimpse of the subtle and intricate mechanisms underlying the impact of gender and gonadal steroids on ventricular repolarization and arrhythmias. They indicate that estradiol and DHT are not unique determinants of gender-related differences in ventricular repolarization, and non-gonadal or non-estrogenic factors may be involved. Non-estrogenic ovarian factors or pituitary–hypothalamic factors may also promote proarrhythmic responses to I_{Kr} blockers.

Although estradiol and DHT are not the only determinants of gender-related disparities in repolarization, they are important modulators of the proarrhythmic response to I_{Kr} blockade. In orchiectomized male rabbits, testosterone protected against I_{Kr} blockade-induced excessive prolongation of repolarization and high incidence of EAD. The protective mechanism provided by testosterone in males remains to be determined and the situation is even more complex in females.

At the cellular level, $I_{Ca,L}$ transmural dispersion contributes in part to a greater prolongation and transmural dispersion of repolarization in females. These conditions in the presence of decreased I_{Kr} could result in higher incidence of EAD in females than males. Taken together, these factors create a condition that increases the risk for proarrhythmic effects of drugs. Finally, $I_{Ca,L}$ data hint at possible differences in the regulatory mechanism of repolarization between males and females.

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