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Frequency-dependent acceleration of cardiac repolarization by progesterone underlying its cardiac protection against drug-induced proarrhythmic effects in female rabbits

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ABSTRACT

Concurrent supplement of estradiol and progesterone has been shown to reduce the cardiac sensitivity to class III antiarrhythmic agent-induced arrhythmias in ovariectomized rabbits. To understand the underlying cardiac electrophysiological mechanisms of the hormones, present study explored the modulation of progesterone and estradiol on repolarization and its frequency dependence in papillary muscles of female rabbit right ventricles by glass microelectrode technique. Results showed that progesterone shortened action potential duration for 90% repolarization (APD_{90}) whereas estradiol prolonged APD_{90} and those actions on APD_{90} were concentration-dependent for both hormones at 1.0–30 μM ($P<0.05$ or $P<0.01$). Further, the action of both hormones on APD_{90} was found to be dependent on stimulation frequencies (0.2–3.3 Hz). The shortening of APD_{90} by progesterone (10 μM) was enhanced with the increase in frequencies reaching a statistic significance at frequencies ≥ 1.0 Hz, whereas the prolongation of APD_{90} by estradiol (3 μM) was weakened with the increase in frequencies and the significant change was observed at frequencies ≤ 2.0 Hz ($P<0.05$ or $P<0.01$). More interestingly, the relative change of APD_{90} and the incidence of early afterdepolarization induced after by dofetilide (0.1 μM), a class III antiarrhythmic agent, were significantly less or lower in the papillary muscles pretreated with progesterone than in those pretreated with estradiol ($P<0.01$ or $P<0.05$). In conclusion, progesterone has a reverse modulating affect on cardiac repolarization to that of estradiol. By acceleration of ventricular repolarization, progesterone may reduce the susceptibility of females to class III antiarrhythmic agents-induced proarrhythmic affection.

1. Introduction

Gender difference in cardiac repolarization has been demonstrated in various animal species, such as guinea pig, rat, rabbit and dog, etc. (James et al., 2007; Cheng, 2006). Among many experimental animals, rabbit has been of considerable value in the investigation of cardiac electrophysiology, because of its similarity in the cardiac repolarization currents to human heart, particularly in respect to the drug-induced QT prolongation. In female rabbits, significantly longer QT, JT intervals and T wave length, and significantly longer action potential durations (i.e. APD_{30}, APD_{50} and APD_{90}: the durations for 20%, 50% and 90% repolarization of the action potential, respectively) were documented (Lu et al., 2000; Valverde et al., 2003; Liu et al., 1998), predisposing female rabbits to an enhanced sensitivity to class III antiarrhythmic agents induced arrhythmias. However, there are controversial evidences. Several reports showed that QT interval has no gender difference (Johansson and Carlsson, 2001; Lu et al., 2001; Philp et al., 2007) and one report showed that the QT interval in the female was even shorter than that in the male (Spear and Moore, 2000). More concerning, much of the research in this field has focused on the gender-related effects of estrogen and androgen, little is known about the effects of progesterone on cardiac repolarization and on the QT interval prolongation secondary to drugs, despite that progesterone is of an abundant sex hormone in the female body.

Our previous study had shown that concurrent supplement of estradiol and progesterone to ovariectomized rabbits reduced cardiac sensitivity to class III antiarrhythmic agent-induced arrhythmias (Cheng et al., 2012a). More recently, our preliminary electrophysiological study in spontaneously beating Langendorff-perfused rabbit

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heart showed that estradiol prolonged the epicardial monophasic action potential durations ($MAPD_{30}$ and $MAPD_{90}$, the durations for 30% and 90% repolarization of the monophasic action potential, respectively) in a concentration-dependent manner, whereas the effects of progesterone were biphasic: it prolonged $MAPD_{30}$ and $MAPD_{90}$ at lower concentrations but shortened $MAPD_{30}$ and $MAPD_{90}$ at higher concentrations (Cheng et al., 2012b). In order to elucidate the possible electrophysiological basis underlying the diverse results observed in the aforementioned studies and to clarify the differences in the effects of progesterone and estradiol on cardiac repolarization, the acute effects of estradiol and progesterone on action potentials were investigated in papillary muscles of female rabbit right ventricles. The concentration dependence, frequency dependence of their effects on action potentials as well as the modulation of class III antiarrhythmic agent sensitivity by progesterone and estradiol were comparatively explored.

2. Materials and methods

The experimental protocol was approved by the Ethical Committee for Biological and Medical Research in our university and conforms to International Guiding Principles for Biomedical Research Involving Animals.

2.1. Recording of action potentials in papillary muscles

Female rabbits weighing 2.0–2.5 kg were anesthetized intravenously with sodium pentobarbital (30 mg/kg) after being heparinized (500 IU/kg, i.v.), and the heart were rapidly excised after thoracotomy and the right ventricular papillary muscles (0.4–0.6 mm in diameter and 3–4 mm in length) were dissected from the hearts. The papillary muscle was mounted in a tissue bath (0.5 ml) and superfused with modified Tyrode's solution (gassed with 100% O_2 at 34 °C) at a speed of 6 ml/min, which was maintained by a peristaltic pump (Peri-star, WPI, USA). The preparation was stimulated at 1 Hz by a pair of 1.0 mm platinum wire electrodes placed 1.0 apart from each side of the muscle. Pulses used for stimulation were 3 ms in width and 150% of the threshold voltage. After an initial stimulation period (1–1.5 h), the action potentials were recorded from the papillary muscle using the standard microelectrode recording technique. The signals were amplified (Neurolog NL 900D, Digitimer Ltd., UK) and recorded through a PowerLab device (2/26, AD Instruments, Australia) connected to a microcomputer for off-line analysis. In the study of frequency dependence, stimulation frequency was changed in steps from 0.2–3.3 Hz, and the steady-state action potentials were recorded 3–5 min after pacing at each frequency. The main parameters analyzed were resting potential, the action potential amplitude, the maximum rate of depolarization, the action potential durations at 20% and 90% repolarizations (APD_{20} and APD_{90}), respectively.

2.2. Solutions and drugs

The modified Tyrode's solution used for the recording of action potential was composed of (in mM): NaCl, 143; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 0.25; HEPES, 5.0; and glucose, 5.6, pH adjusted to 7.4 with NaOH. Progesterone and estradiol (Sigma) were dissolved in ethanol to make 50 mM stock solutions, respectively; and dofetilide (Sigma) was dissolved in DMSO to make 1 mM stock solution. The agents were diluted in perfusates to the desired final concentrations immediately before each application.

2.3. Statistical analysis

Data were expressed as mean ± S.E.M. Analysis of variance (ANOVA) was used for critical differences among multiple means, and Student's *t*-test was used for comparison between two means. Fisher's exact test was used to analyze the incidence of

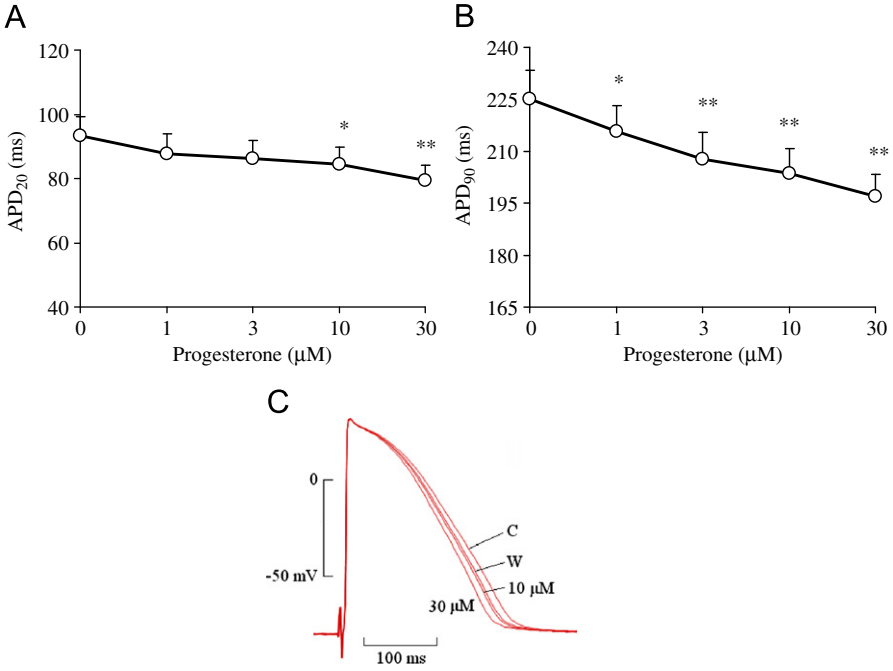

Fig. 1. Concentration-dependent effects of progesterone on APD in papillary muscles of female rabbit right ventricles stimulated at 1 Hz. A and B: Concentration-response curves of progesterone on APD_{20} and APD_{90}, respectively. APD_{20} and APD_{90} refer to the duration for 20% and 90% repolarization of the action potentials, respectively. The data were expressed as mean ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the corresponding control, respectively. $n=7$. C: Examples of action potentials recorded before (C), after the exposure to 10 and 30 μM of progesterone, and after washout (W).

dofetilide-induced proarrhythmic events. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Concentration-dependent effects of female hormones on APD in rabbit papillary muscles

The concentration-dependent effects of progesterone (1–30 μM, $n=7$) and estradiol (1–30 μM, $n=6$) on APD_{20} and APD_{90} were observed at 1 Hz and the results were illustrated in Figs. 1 and 2 respectively. Within the concentration range applied, progesterone produced a progressive shortening of APD_{20} and APD_{90} with the increase in its concentration ($P<0.05$ or $P<0.01$). In contrast, estradiol caused a concentration-dependent prolongation of APD_{20} and APD_{90} ($P<0.05$ or $P<0.01$). For other parameters of action potentials, such as the resting potential, action potential amplitude and the maximum rate of depolarization, no significant influence was observed with progesterone or estradiol (Table 1).

3.2. Frequency-dependent effects of female hormones on APD in rabbit papillary muscles

The rabbit papillary muscles underwent stimulation with frequencies ranging from 0.2 to 3.3 Hz (Figs. 3 and 4). When the stimulation frequency was changed in steps from 0.2 to 3.3 Hz, the APD in control condition initially increased and then decreased, showing a bell-shaped frequency-response curve with the longest APD at 1.0 Hz. Progesterone (10 μM) produced a significant shortening of APD_{90} at frequencies of 1.0 to 3.3 Hz ($P<0.05$ or $P<0.01$, $n=9$) (Fig. 3A). To further analyze the frequency dependence of the APD shortening by progesterone, the percentage shortenings at each frequency were compared. The percentage shortening of APD_{90} induced by progesterone at 3.3 Hz was significantly larger compared with that at 0.2 Hz ($P<0.01$) (Fig. 3B). On the other hand, estradiol (3 μM) prolonged APD_{90} significantly at frequencies of 0.1–2 Hz ($P<0.05$ or $P<0.01$, $n=8$) (Fig. 4A). The percentage prolongations induced by estradiol at 0.2 and 0.5 Hz were significantly larger than that obtained at 3.3 Hz ($P<0.05$ or $P<0.01$) (Fig. 4B). These results

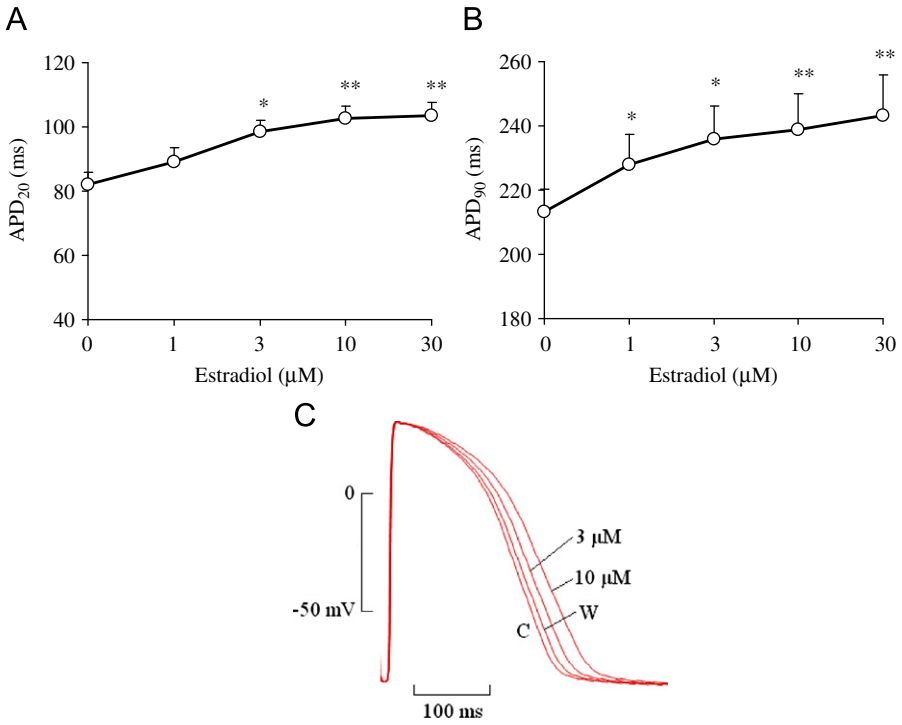

Fig. 2. Concentration-dependent effects of estradiol on APD in papillary muscles of female rabbit right ventricles stimulated at 1 Hz. A and B: Concentration-response curves of estradiol on APD_{20} and APD_{90}, respectively. APD_{20} and APD_{90} refer to the duration for 20% and 90% repolarization of the action potentials, respectively. The data were expressed as mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared with the corresponding control, respectively. $n=6$. C: Examples of action potentials recorded before (C), after the exposure to 3 and 10 μM of estradiol, and after washout (W).

Table 1
Effects of progesterone and estradiol on RP, APA and V_{max} in papillary muscles of female rabbit right ventricles stimulated at 1 Hz.

Progesterone (μM)	RP (mV)	APA (mV)	V_{max} (V/s)	Estradiol (μM)	RP (mV)	APA (mV)	V_{max} (V/s)
Control	−83 ± 5	104 ± 2	201 ± 13	Control	−80 ± 4	106 ± 3	206 ± 10
1	−81 ± 5	103 ± 2	195 ± 10	1	−79 ± 4	108 ± 3	193 ± 4
3	−80 ± 5	104 ± 4	209 ± 11	3	−80 ± 3	107 ± 3	191 ± 6
10	−80 ± 4	103 ± 3	205 ± 7	10	−78 ± 3	105 ± 7	192 ± 19
30	−77 ± 4	104 ± 2	188 ± 10	30	−77 ± 4	102 ± 2	189 ± 16

The data are expressed as mean ± S.E.M. n equals seven or six in progesterone or estradiol group, respectively. RP: resting potential; APA: action potential amplitude; V_{max}: the maximum rate of depolarization.

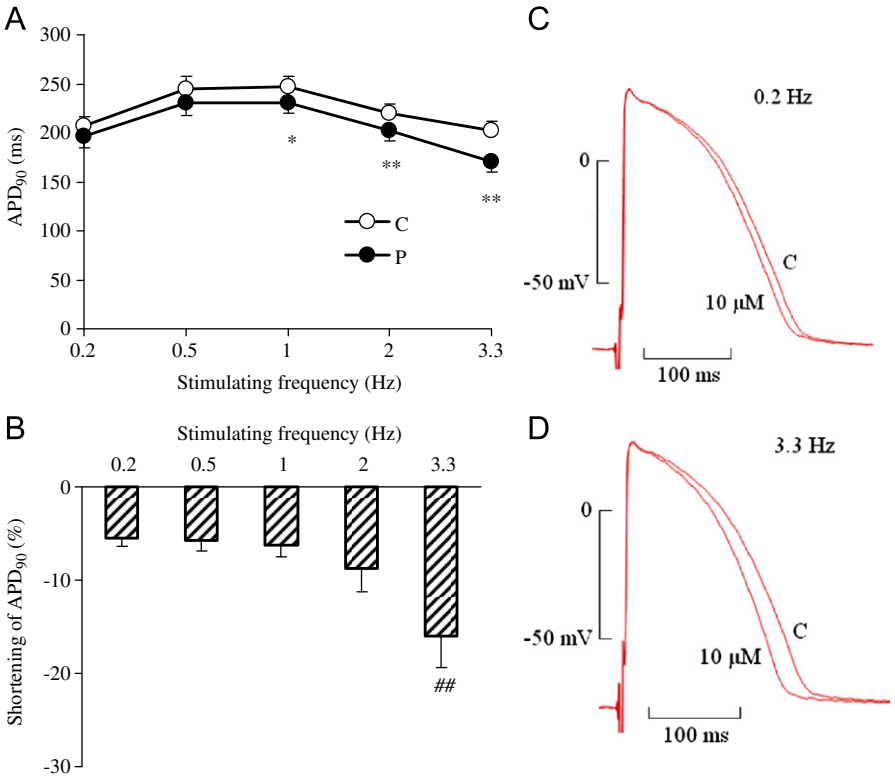

Fig. 3. Frequency-dependent effects of progesterone on APD in female rabbit right ventricular papillary muscles. A: Frequency-response curves of progesterone (10 μM) on APD_{90}. APD_{90} refer to the duration for 90% repolarization of the action potentials. The data were expressed as mean ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the corresponding control, respectively. $n = 9$. B: Comparison of the percentage shortening of APD induced by progesterone at different frequencies. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the value at 0.2 Hz, respectively. C and D: Examples of action potentials recorded before and after the exposure to 10 μM of progesterone at stimulation frequencies of 0.2 and 3.3 Hz, respectively.

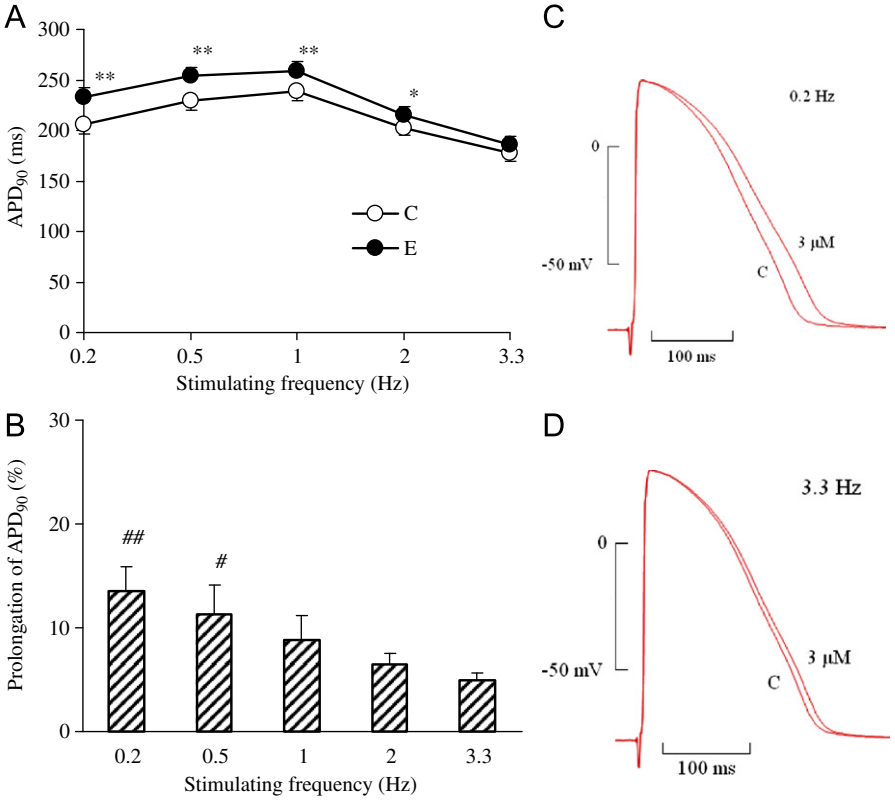

Fig. 4. Frequency-dependent effects of estradiol on APD in female rabbit right ventricular papillary muscles. A: Frequency-response curves of estradiol (3 μM) on APD_{90}. APD_{90} refer to the duration for 90% repolarization of the action potentials. The data were expressed as mean ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the corresponding control, respectively. $n = 8$. B: Comparison of the percentage prolongation of APD induced by estradiol at different frequencies. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with the value at 3.3 Hz, respectively. C and D: Examples of action potentials recorded before and after the exposure to 3 μM of estradiol at stimulation frequencies of 0.2 and 3.3 Hz, respectively.

suggest that these two female hormones modulate the repolarization process in different ways: progesterone selectively shortens APD_{90} at higher stimulating frequencies, whereas estradiol mainly prolongs APD_{90} at lower stimulating frequencies.

3.3. Modulation of female hormones on the susceptibility of papillary muscles to dofetilide

Since progesterone and estradiol have diverse effects on APD, it is speculated that pretreatment with progesterone or estradiol may differently modulate the sensitivity of the papillary muscles to dofetilide, a class III antiarrhythmic agent. APD_{20}, APD_{90} were recorded in papillary muscles pretreated with solvent (as control, group C, $n=5$), estradiol (group E, $n=7$) or progesterone (group P $n=9$), respectively. As showed in Fig. 5, pretreatment with estradiol (3 μM) prolonged APD, whereas that with progesterone (10 μM) shortened APD significantly ($P<0.05$ or $P<0.01$). Then, dofetilide (0.1 μM) was applied to each of the three groups, respectively. As expected, dofetilide significantly prolonged both APD_{20} ($P<0.05$ or $P<0.01$) and APD_{90} ($P<0.01$) in control group, as well as in estradiol or in progesterone pretreated group (Fig. 5). Typical recordings of the action potentials obtained in drug-free state, after the pretreatment of solvent, estradiol or progesterone, respectively; and then after further application of dofetilide were illustrated in Fig. 6. The relative changes in APD_{90} after the application of dofetilide in relation to the drug-free state were calculated in the three differently pretreated groups for a better comparison of the drugs' effects (Table 2). The total change in APD_{90} was significantly larger in group E (pretreated with estradiol) compared with that in group C (control), while that in group P (pretreated with progesterone) was significantly less than those in group C ($P<0.05$) and group E ($P<0.01$), respectively. As for the rate of dofetilide-induced early afterdepolarization (EAD), it was slightly increased in group E and slightly decreased in group P compared with that in group C, respectively (Table 2). However, there is a significant difference between the group E and group P: the rate of dofetilide-induced EAD was much lower with progesterone pretreatment than that with estradiol ($P<0.05$). Fig. 7 demonstrated a typical recording of EAD induced by dofetilide in the presence of estradiol.

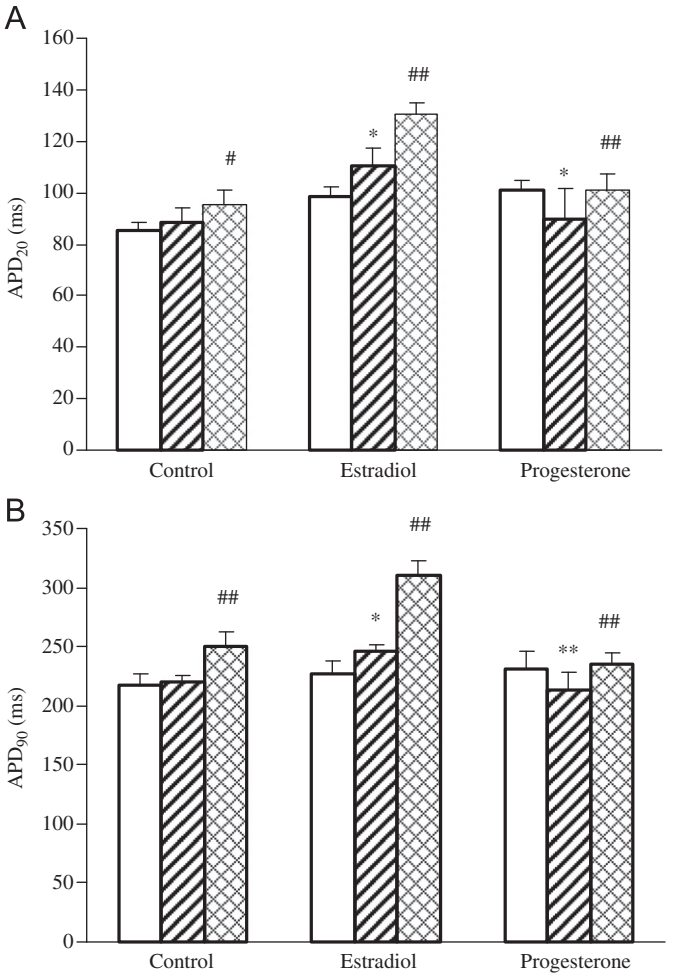

Fig. 5. Modulation of estradiol (3 μM) or progesterone (10 μM) on dofetilide (0.1 μM) induced prolongation of APD in female rabbit right ventricular papillary muscles stimulated at 1 Hz. APD_{20} and APD_{90} refer to the duration for 20% and 90% repolarization of the action potentials, respectively. The data were expressed as mean ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the corresponding value in drug-free state (empty bars), respectively; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ compared with the corresponding values in the presence of solvent, estradiol or progesterone (shaded bars), respectively. $n=5$, 7, 9 in control, estradiol, or progesterone group, respectively.

4. Discussion

Progesterone shortened APD with concentration-dependence in the present study and that action was more significant at higher frequencies, and thus may protect the female from drug-induced proarrhythmic effects; whereas estradiol showed an action to prolong APD with concentration-dependence, and that action was more significant at lower frequencies, and thus increased the susceptibility of the cardiac preparation to dofetilide-induced proarrhythmic effects. It is clearly demonstrated that progesterone and estradiol modulate APD in papillary muscles of female rabbit right ventricles with contrary actions and those actions are of frequency dependence.

It seems that clear gender difference in cardiac repolarization was demonstrated usually in vitro at stimulation rates much lower than the physiological heart rates of rabbits. Indeed, our results alert that stimulation frequency should be a concern when comparing literature data. For example, the APD_{90} of the Purkinje fibers isolated from female rabbit hearts was not significantly different from that of the male preparation when it was stimulated at 1.0 Hz, but the female APD_{90} was significantly longer than that of the male preparation at 0.2 Hz (Lu et al., 2000). A clear gender difference was observed for the QT intervals when rabbit hearts were paced at a cycle length of 2300 ms (≈ 0.4 Hz) but disappeared at a cycle length of 400 ms (2.5 Hz) (Liu et al., 1998). Similarly, no difference in APD of endocardium between adult male and female rabbit hearts was found when the cardiac preparations were paced at a cycle length of 300 ms (3.3 Hz), although significant difference could be seen when the preparation were paced with longer cycle lengths of 5000, 1000 and 500 ms (i.e. at slower frequencies of 0.2, 1 and 2 Hz) (Valverde et al., 2003). In the same article, Valverde et al. compared the QT, QTc, JT_{end} and JT_{endC} between the sexes in in vivo situation, only JT_{endC} showed a clear sex difference (Valverde et al., 2003). Another in vivo study also showed that the QTc interval was not significantly different between the female and male rabbits (Johansson and Carlsson, 2001). Thus, a lower pacing rate seems to be necessary for the reveal of the difference in repolarization between sexes in rabbit, as gender-based difference in QT intervals or APD could be obscure at physiological heart rates. In the present study, it is found that the APD prolonging effects of estradiol is enhanced at slow stimulation rates, whereas the ADP shortening effects of progesterone is more prominent at fast stimulation rates. These findings may suggest that the repolarization process speeds up due to the concurrent deterioration of the estradiol's effects and exaggeration of the progesterone's effects

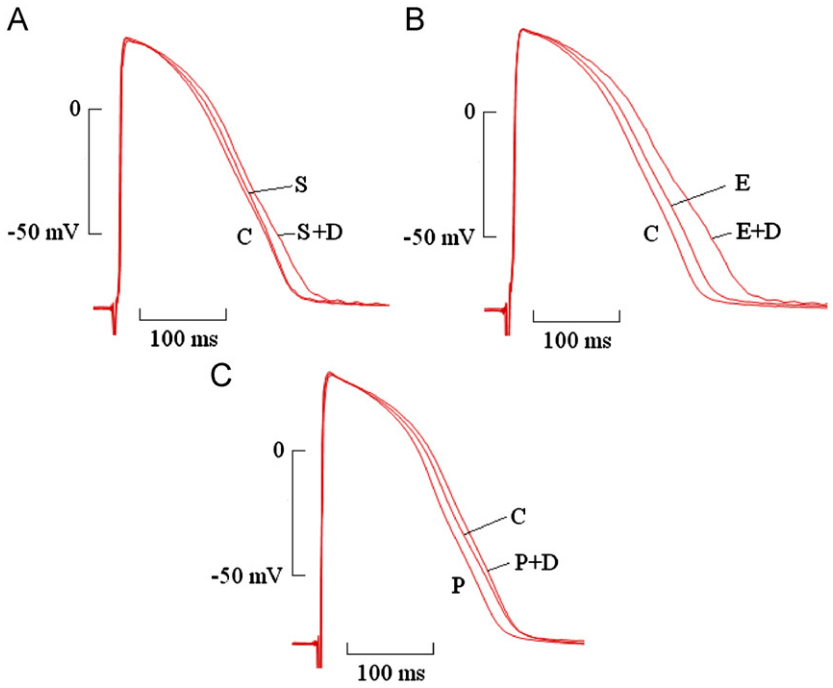

Fig. 6. Typical recordings of the effects of dofetilide (0.1 μM) on action potentials in female rabbit right ventricular papillary muscles in the presence of estradiol (3 μM) or progesterone (10 μM). The stimulating frequency was 1 Hz. C, D, E, P and S in the figure indicate control, dofetilide, estradiol, progesterone or solvent, respectively.

Table 2
Relative change in APD_{90} and the incidence of early afterdepolarization (EAD) after the application of dofetilide in rabbit right ventricular papillary muscles pretreated with estradiol or progesterone.

	Control	Estradiol (3 μM)	Progesterone (10 μM)
ΔAPD_{90} (ms)	33 ± 7	82 ± 14[a]	4 ± 5[a,c]
EAD	20% (1)	43% (3)	0% (0)[b]

The stimulating frequency was 1 Hz. ΔAPD_{90} was obtained by subtracting the value of the drug-free state from those measured after dofetilide (0.1 μM) in the presence of solvent, estradiol, or progesterone, respectively; and was expressed as mean ± S.E.M. The numbers in the parenthesis indicate the actual cases of EAD. $n=5, 7, 9$ in control, estradiol, or progesterone group, respectively.

[a] $P<0.05$ compared with the corresponding value in control.
[b] $P<0.05$ compared with the corresponding value in estradiol group.
[c] $P<0.01$ compared with the corresponding value in estradiol group.

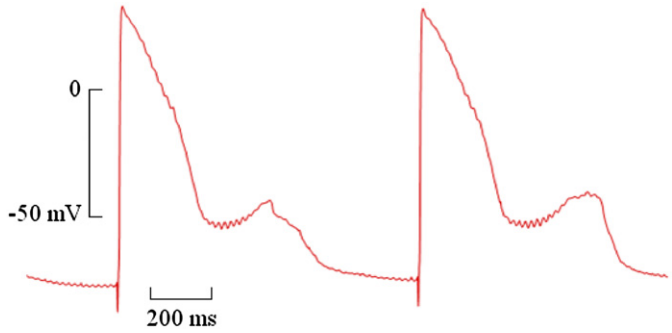

Fig. 7. Induction of early afterdepolarization by dofetilide (0.1 μM) in rabbit right ventricular papillary muscles in the presence of estradiol (3 μM) at 1 Hz stimulation.

on repolarization, thus resulting in a lack of gender difference between the female and the male at physiological heart rates in rabbits.

The enhancement of estradiol-induced repolarization prolonging effect together with the concurrent decrease of the repolarization shortening effect of progesterone at slow heart rates may predispose the female to an increased susceptibility to drug-induced proarrhythmic effects. In the present study, the female hormones-induced modulation of the cardiac proarrhythmic susceptibility to dofetilide was studied at 1 Hz, at which both progesterone and estradiol exerted significant effects on APD. Estradiol significantly enhanced the prolongation of APD_{90} induced by dofetilide, whereas progesterone significantly decreased the prolongation of APD_{90} induced by dofetilide. The rate of dofetilide-induced EAD in papillary muscles pretreated with progesterone was significantly lower than that in preparations pretreated with estradiol, although both of them were not significantly different from that of the control. In the literature, the studies in which a significant gender-based difference in the rate or the severity of the drug-induced proarrhythmic events (i.e. Torsades de Pointes, TdP) was observed, were performed either in isolated hearts with the destruction of the atrio-ventricular node or in tissues, both paced at long cycle lengths. For instance, at a slow stimulation rate of 0.2 Hz female Purkinje fibers tended to have a larger increase in APD_{90} and a higher incidence of EAD in response to dofetilide than male Purkinje fibers (Lu et al., 2000). However, in most in vivo studies with the rabbits in anesthetized states sensitized with α-adrenoceptor agonists, the gender-related difference in drug-induced proarrhythmic effects disappeared: QT intervals were similar at baseline and no differences in the incidence of drug-induced TdP between the sexes were observed (Johansson and Carlsson, 2001; Philp et al., 2007). Thus, it is possible that the low pacing rate may also be necessary for revealing of the sex difference in drug-induced proarrhythmic effects.

The modulation of exogenous progesterone and estradiol on cardiac repolarization has been studied in vitro/in vivo preparations. Acute infusion of 17β-estradiol had only minor effects on QT intervals and epicardial monophasic APD in anaesthetized rabbits stimulated with α_1-adrenoceptor, but it did increased dose-dependently the total duration of TdP induced by clofilium (Philp et al., 2007). Chronic exposure to estradiol accentuates the effects of quinidine on ventricular $MAPD_{90}$, whereas chronic progesterone exposure protects against quinidine-induced prolongation of ventricular $MAPD_{90}$ and

increase in ventricular action potential triangulation in Langendorff-perfused hearts isolated from ovariectomized rabbits (Tisdale et al., 2011). Our previous study showed that acute application of progesterone protects against *d*,*l*-sotalol-induced proarrhythmic effects in spontaneously beating Langendorff-perfused rabbit hearts, as evidenced by significantly lower rates of *d*,*l*-sotalol-induced EAD and premature ventricular excitations in hearts pretreated with progesterone than those with estradiol (Cheng et al., 2012b). Since progesterone modulates cardiac repolarization and drug sensitivity in a way different from that of estradiol, it may counteract the effect of estradiol and protect the female from drug-induced excessive repolarization prolongation and the resulted proarrhythmic risk. Our speculation is supported by the Rodriguez' study conducted in women that the increase in rate-corrected QT intervals after ibutilide was greater for women during menses and the ovulatory phase compared with women during the luteal phase, and that both the progesterone levels and the progesterone-to-estradiol ratio were inversely correlated with mean QTc and the ibutilide-induced QT interval prolongation (Rodriguez et al., 2001). Our previous study in ovariectomized rabbits with hormone replacement therapy showed that progesterone plus estradiol reduced *d*,*l*-sotalol-induced prolongation of QTc, the rate and the severity of arrhythmias in comparing with estradiol alone or control (Cheng et al., 2012a). However, in Tisdale's study, despite the protective effects exerted by progesterone alone, progesterone did not attenuate the effects of estradiol on prolongation of the ventricular $MAPD_{90}$ or triangulation associated with quinidine when progesterone was supplemented in combination with estradiol (Tisdale et al., 2011). One possible explanation for those diverse results may be the difference in the balance or the ratio between the concentrations of estradiol and progesterone employed. Further research is necessary to clarify the concentration relevance of these hormones to achieve a close resemblance to the physiological conditions during the various phases of the menstrual cycle and during pregnancy.

In conclusion, our study strongly suggest that progesterone has a reverse modulating effect on cardiac repolarization to that of estradiol, with a possible role in the acceleration of ventricular repolarization, which may give rise to a decreased drug-induced effect on cardiac repolarization in the female when the blood progesterone is at a high level. Since there is a physiological variation of both estrogen and progesterone levels during the menstrual cycle in the female, with the progesterone level being significantly higher in the luteal than in the follicular phase, it implies that the risk of drug-induced proarrhythmias may be lower during the luteal phase of the menstrual cycle. This speculation is supported by several previous studies on female patients with congenital long QT syndrome that the arrhythmic events frequently occurred during the perimenses and the postpartum periods, when blood progesterone was both at a very low level (Nakazato et al., 1992; Rashba et al., 1998; Zhou et al. 1992). Moreover, the concentrations of progesterone and estradiol used in our study were at pharmacological rather than physiological levels in human bodies, therefore the clinical implications of our findings and their relation to circulating hormone levels need to be further investigated.

Conflict of interest

None

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