Effects of estrogen on action potential and membrane currents in guinea pig ventricular myocytes

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Effects of estrogen on action potential and membrane currents in guinea pig ventricular myocytes, Am. J. Physiol. Heart Circ. Physiol. 46: H826–H833, 1999.—To explore a possible ionic basis for the prolonged Q-T interval in women compared with that in men, we investigated the electrophysiological effects of estrogen in isolated guinea pig ventricular myocytes. Action potentials and membrane currents were recorded using the whole cell configuration of the patch-clamp technique. Application of 17β-estradiol (10–30 µM) significantly prolonged the action potential duration (APD) at 20% (APD20) and 90% repolarization (APD90) at stimulation rates of 0.1–2.0 Hz. In the presence of 30 µM 17β-estradiol, APD20 and APD90 at 0.1 Hz were prolonged by 46.2 ± 17.1 and 63.4 ± 11.7% of the control (n = 5), respectively. In the presence of 30 µM 17β-estradiol the peak inward Ca2+ current (I_{Ca,L}) was decreased to 80.1 ± 2.5% of the control (n = 4) without a shift in its voltage dependence. Application of 30 µM 17β-estradiol decreased the rapidly activating component of the delayed outward K+ current (I_{K1}) to 63.4 ± 8% and the slowly activating component (I_{K4}) to 65.8 ± 8.7% with respect to the control; the inward rectifier K+ current was barely affected. The results suggest that 17β-estradiol prolonged APD mainly by inhibiting the I_{K4} components I_{K1} and I_{K4}.

17β-estradiol; Q-T interval; torsades de pointes; action potential duration; delayed outward potassium current

IT IS A WELL-KNOWN CLINICAL observation that the Q-T interval of the electrocardiogram is generally longer in women than in men (1, 6, 11, 21) and that there is also a greater risk of women developing drug-related torsades de pointes with a prolonged Q-T interval (11). Furthermore, it has been shown that sex hormones prolong the Q-T interval and downregulate K+ channel expression (6). These facts suggest that sex hormones may have a direct and an indirect effect on cardiac repolarization. Although 17β-estradiol is known to affect cardiovascular function (19, 27, 30), its effects on cardiac membrane potentials have not been fully elucidated.

The repolarization phase of the cardiac action potential is formed by several ionic currents, including inward Ca2+ current (I_{Ca,L}), transient outward K+ current (I_{to}), and delayed outward K+ current (I_{K}), which overlap each other with similar time courses (4, 16). Previous studies demonstrated that 17β-estradiol inhibited I_{Ca,L} and shortened the action potential duration (APD) in guinea pig ventricular muscles and myocytes (7, 9, 10). Although these findings are in line with the reported negative inotropic effect of 17β-estradiol in cardiac preparations (20, 26), they do not explain the clinical observations of prolonged Q-T interval and high prevalence of torsades de pointes in women. Recently, 17β-estradiol was found to prolong the APD due to inhibition of I_{to} in rat ventricular myocytes (2). Because guinea pig and rat ventricular myocytes have different components of repolarizing K+ currents with a less developed I_{to} in the former and a prominent I_{to} in the latter, different effects of estrogen may arise from different components of the repolarizing currents. We thus investigated the effects of 17β-estradiol on action potential and ionic currents responsible for the repolarization phase of the action potential in guinea pig ventricular myocytes.

METHODS

Preparation of guinea pig ventricular myocytes. Single ventricular myocytes from guinea pig hearts were prepared by a previously described enzymatic dissociation procedure (8). We used mainly female guinea pigs weighing 300–400 g, unless otherwise stated. Briefly, animals were anesthetized with pentobarbital sodium (15–20 mg/kg ip). The chest was opened under artificial respiration, and the aorta was cannulated in situ before the heart was removed. By use of a Langendorff apparatus, the excited heart was first perfused with normal Tyrode solution and then with nominally Ca2+-free Tyrode solution for 5 min. Subsequently, Ca2+-free Tyrode solution with collagenase (0.6 mg/ml, type II; Worthington Biochemical, Lakewood, NJ) was perfused through the heart for ~20 min. The temperature of all perfusates was kept constant at 36–37°C. Single cells were obtained by gentle agitamtion of small pieces of ventricular tissue in high-K+, low-Cl− solution. Harvested cells were stored in the high-K+, low-Cl− solution, kept for 60 min at 4°C, and then transferred to normal Tyrode solution at room temperature before use.

Solutions. The normal Tyrode solution contained (in mM) 144 NaCl, 4.0 KCl, 4.0 CaCl2, 0.53 MgCl2, 0.33 NaH2PO4, 5.5 glucose, and 5.0 HEPES, and the pH was adjusted to 7.4 with NaOH. The nominally Ca2+-free Tyrode solution was prepared by omitting CaCl2 from the normal Tyrode solution. The high-K+, low-Cl− solution contained (in mM) 70 glutamic acid, 15 taurine, 30 KCl, 10 NaH2PO4, 10 HEPES, 0.5 MgCl2, 11 glucose, and 0.5 EGTA, and the pH was adjusted to 7.3 with KOH. The standard external bath solution was normal Tyrode solution. The internal solution contained (in mM) 100 potassium aspartate, 20 KCl, 0.02 CaCl2, 5.0 MgCl2 ATP, 5.0 potassium creatine phosphate, 0.05 EGTA, and 5.0 HEPES, and the pH was adjusted to 7.25 with KOH. For measurement of I_{K1}, 2 µM nisoldipine was added to the standard bath external solution to block I_{Ca,L}. To record the isolated I_{Ca,L}, the external bath solution contained (in mM) 140 tetraethylammonium chloride, 2.0 CaCl2, 0.53 MgCl2, 10 glucose, and 10...
HEPES, and the pH was adjusted to 7.4 with tetraethylammonium hydroxide. The internal solution contained (in mM) 130 CsCl, 2.0 MgCl2, 5.0 Na2-ATP, 20 tetraethylammonium chloride, 10 EGTA, and 10 HEPES, and the pH was adjusted to 7.25 with CsOH. All experiments were carried out at 35–36°C.

Drugs. 17β-Estradiol (Sigma Chemical, St. Louis, MO) was dissolved in ethanol to give a stock solution of 50 mM. The final concentration of 17β-estradiol was obtained by diluting the stock solution into the bath solution. The same amount of ethanol (1:2,000 vol/vol) was also added to normal Tyrode solution for use as the control. Nisoldipine (a gift from Bayer Pharmaceutical, Osaka, Japan) was dissolved in DMSO to give a stock solution of 10 mM.

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The stability of current amplitudes in the control state was checked 5 min before drug application. The analog signals were digitized using an analog-to-digital converter (Digidata 1200, Axon Instruments, Foster City, CA). To make the suction pipettes, borosilicate glass capillaries with inner filaments (Clark Electromedical Instruments, Pangbourne, UK) were heated and pulled by two steps with a microelectrode puller (model PA-91, Narishige, Tokyo, Japan). Resistance of a typical electrode was 2–4 MΩ when the pipette was filled with the internal solution. At the start of each experiment the junction potential was adjusted to zero by adjusting the compensation circuit in the external bath solution; it was also checked at the end of each experiment. If the difference between the two measurements was >2 mV, the values were corrected accordingly. Membrane potential and current signals were monitored by a storage oscilloscope (model VC10, Nihon Koden, Tokyo, Japan) and a storage oscilloscope (model VC10, Nihon Koden, Tokyo, Japan). The stability of current amplitude in the control state was checked 5 min before drug application. The analog signals were digitized using an analog-to-digital converter (Digidata 1200, Axon Instruments, Foster City, CA). The analog signals were digitized using an analog-to-digital converter (Digidata 1200, Axon Instruments, Foster City, CA).

RESULTS

Effects of 17β-estradiol on action potential parameters of ventricular myocytes. Effects of 17β-estradiol (3–30 μM) on action potentials were examined by a current-clamp mode (Fig. 1, A and B). After exposure to 3 μM 17β-estradiol, no significant changes in action potential characteristics were observed for 15 min. Application of 10 μM 17β-estradiol caused a significant prolongation of the APD at 20% (APD20) and 90% repolarization (APD90). Estradiol prolonged APD20 by 16.4 ± 4.8% of the control and APD90 by 25.2 ± 5.8% (n = 5, P < 0.05). Application of 30 μM 17β-estradiol prolonged APD20 by 46.2 ± 17.1% of the control and APD90 by 63.4 ± 11.7% (n = 5, P < 0.05). The prolongation induced by 17β-estradiol developed rapidly and reached a steady state 5 min after start of estradiol superfusion. Effects of estrogen were almost reversible during 10 min of washout with estrogen-free solution. After washout, APD20 recovered to 102.3 ± 9.6% and APD90 to 106.9 ± 7.8% of the control. The resting potential and the amplitude of the action potential were unaffected even at the highest concentration (30 μM) of 17β-estradiol.

We studied the frequency dependence of 17β-estradiol (30 μM)-induced action potential prolongation at 0.1, 0.5, 1.0, and 2.0 Hz in five myocytes. Under control condition (hormone free), APD was shortened with
increasing stimulation frequency (Fig. 1, C and D). Estradiol prolonged APD<sub>90</sub>, expressed as a percentage of the respective control, to 147 ± 8% (P < 0.05) at 0.1 Hz, 131 ± 12% (P < 0.05) at 0.5 Hz, 124 ± 8% (P < 0.05) at 1.0 Hz, and 119 ± 8% (P < 0.05) at 2.0 Hz. A tendency for a prolonged APD<sub>20</sub> was also shown after estradiol at every stimulation rate (Fig. 1, C and D).

We also examined the effect of 17β-estradiol on action potentials of myocytes derived from male guinea pigs. Application of 30 µM 17β-estradiol prolonged the APD<sub>20</sub> to 146.9 ± 3.6% (n = 4, P < 0.05) of the control and APD<sub>90</sub> to 149.2 ± 6.6% (n = 4, P < 0.05). Therefore, APD prolongation was similarly seen in myocytes from male guinea pigs.

Effects of 17β-estradiol on membrane currents. To examine the effects of 17β-estradiol on membrane currents, 1-s test pulses to voltages between -2100 and -150 mV were applied from a holding potential of -240 mV. Figure 2 shows the results of a typical experiment. Application of 30 µM 17β-estradiol had little effect on membrane currents at voltages negative to -50 mV and induced a slight decrease at -40 mV. At potentials positive to -30 mV, 17β-estradiol mildly suppressed initial inward current on depolarization and decreased the late outward currents at test voltages positive to 20 mV. Results similar to those shown in Fig. 2 were confirmed in five myocytes. At -100 mV the current was -6.46 ± 0.8 pA/pF in the control and -6.33 ± 1.0 pA/pF (P = NS) after application of 30 µM 17β-estradiol. At 0 mV, initial inward current was -7.10 ± 0.8 pA/pF in the control and -5.60 ± 0.9 pA/pF after 17β-estradiol (P < 0.05). At the test potential of 50 mV, the late currents were 6.77 ± 1.3 pA/pF in the control and 5.32 ± 1.1 pA/pF after estradiol (P < 0.05).

Effect of 17β-estradiol on I<sup>CaL</sup>. Suppression of the initial inward current on depolarization suggested a

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**Figure 2.** Effects of 30 µM 17β-estradiol on membrane currents. Insets: protocol for A and B and for C and D. A and B: current traces induced by 1-s test pulses between -30 and +50 mV with 10-mV steps from a holding potential of -40 mV at a rate of 0.1 Hz. C and D: current traces induced by test voltages between -100 and -50 mV by 10-mV steps. E: current-voltage (I-V) relationships. Outward currents were measured at end of a 1-s test pulse. Inward currents were measured at initial peak relative to holding current level during test voltages. *†Significantly different from respective controls (P < 0.05).
decrease in \( I_{\text{Cal}} \) after application of estradiol. Therefore, using the solutions described in METHODS, we examined the effects of 17\( \beta \)-estradiol on isolated \( I_{\text{Cal}} \). The currents were recorded during 200-ms test pulses between \(-30\) and \(+50\) mV in 10-mV steps applied at 10-s intervals after 200-ms conditioning steps to \(-40\) mV from a holding potential of \(-80\) mV. Figure 3A demonstrates that 30 \( \mu M \) 17\( \beta \)-estradiol decreased the peak amplitude of \( I_{\text{Cal}} \). The inhibition occurred rather quickly to reach a steady level within 3–5 min of 17\( \beta \)-estradiol application. The inhibition of \( I_{\text{Cal}} \) was reversible after 2–5 min of washout. The peak \( I_{\text{Cal}} \) was decreased by 30 \( \mu M \) 17\( \beta \)-estradiol to 80.1 \( \pm \) 2.5\% of the control (measured at 0 mV current; \( n = 5 \), \( P < 0.05 \)). After washout, \( I_{\text{Cal}} \) recovered to 93.6 \( \pm \) 2.2\% of the control (\( n = 5 \), \( P < 0.05 \) vs. estradiol). Figure 3B shows the current-voltage (I-V) relationships of \( I_{\text{Cal}} \) in the absence and presence of 30 \( \mu M \) 17\( \beta \)-estradiol and after washout. The shape of the I-V curve was not affected by estradiol.

Effects of 17\( \beta \)-estradiol on \( I_{\text{K}} \). \( I_{\text{K}} \) is mainly composed of two components: the rapidly activating component (\( I_{\text{Kr}} \)) and the slowly activating component (\( I_{\text{Kr}} \)) (25). The estrogen-induced block development of \( I_{\text{K}} \) was examined by the envelope of tails test. Membrane potential was held at \(-40\) mV and pulsed to \(+40\) mV for a variable time, from 50 to 3,000 ms. Sufficient time (\( >12\) s) between test pulses was allowed for full deactivation of tail currents before the application of another depolarizing pulse. In six experiments the envelope of tails test was performed in the same cell before and after application of 30 \( \mu M \) 17\( \beta \)-estradiol. In the presence of 30 \( \mu M \) 17\( \beta \)-estradiol, tail currents were suppressed at variable test durations (Fig. 4A). At the short pulse (50 ms), tail current was decreased by 30 \( \mu M \) 17\( \beta \)-estradiol to 71.8 \( \pm \) 6\% (\( n = 6 \), \( P < 0.05 \)) of the control, whereas at the long pulse (3,000 ms), the current was decreased to 53.3 \( \pm \) 4\% (\( n = 6 \), \( P < 0.05 \)). To ensure inhibition of the two \( I_{\text{K}} \) components, we used 5 \( \mu M \) E-4031, which specifically blocked \( I_{\text{Kr}} \). In the presence of 5 \( \mu M \) E-4031, 30 \( \mu M \) 17\( \beta \)-estradiol also decreased the tail current. At the long pulse (3,000 ms), 17\( \beta \)-estradiol inhibited the tail current to 60.4 \( \pm \) 8\% (\( n = 5 \), \( P < 0.05 \)) of the control. After washout of 17\( \beta \)-estradiol, tail \( I_{\text{K}} \) (\( I_{\text{Ktail}} \)) recovered to 81.8\% (Fig. 4B). Thus 17\( \beta \)-estradiol reversibly inhibited \( I_{\text{Ks}} \).

We further examined the fully activated I-V relationship for \( I_{\text{Ks}} \). Figure 5A shows the results of a typical experiment. Application of 30 \( \mu M \) 17\( \beta \)-estradiol decreased tail currents recorded on repolarization (7 s) to a range of potentials after an activating pulse (3 s) to +60 mV, a voltage sufficient to fully activate \( I_{\text{Ks}} \). Figure 5B is a plot of the fully activated \( I_{\text{Ks}} \)-voltage relationship. This relationship was linear at voltages negative to \(-20\) mV and had a slope conductance of 33.5 pS in the control and 11.7 pS after 30 \( \mu M \) 17\( \beta \)-estradiol. The reversal potential (\( E_{\text{rev}} \)) of \( I_{\text{Ks}} \) was \(-68.2 \pm 1.7\) mV in the control and \(-72.2 \pm 2.9\) mV after 30 \( \mu M \) 17\( \beta \)-estradiol (\( n = 5 \)). Thus 17\( \beta \)-estradiol decreased slope conductance without apparent changes in the \( E_{\text{rev}} \) of \( I_{\text{Ks}} \).

The envelope of tails test predicts that if \( I_{\text{K}} \) results from the conductance of a single type of channel, then the magnitude of tail currents after a given depolarizing pulse of variable duration should increase in parallel to the time course of activation of the outward current during the pulse. In other words, the ratio of tail current to time-dependent current (\( \Delta I_{\text{Ktail}}/\Delta I_{\text{K}} \)) should be constant, regardless of the pulse duration (25). In untreated cells, tail currents were larger than time-dependent currents for very short pulses (<250 ms), but as the pulse duration was lengthened the time-dependent current slowly increased in magnitude, such that for a 3,000-ms pulse a ratio of 0.4 \( \pm \) 0.01 was
attained. Application of 30 µM 17β-estradiol shifted the curve upward (Fig. 6A). In cells treated with 5 µM E-4031 to block I_{Kr}, ΔI_{Ktail}/ΔI_K was constant (0.29 ± 0.03, n = 5), as reported previously (25). Figure 6B shows the ΔI_{Ktail}/ΔI_K of the 17β-estradiol-sensitive current, which was obtained by subtracting the currents in the presence of 30 µM 17β-estradiol from the currents in the absence of 17β-estradiol. The presence of large ΔI_{Ktail}/ΔI_K values at the shorter pulses may indicate that 30 µM 17β-estradiol partially blocks I_{Kr} and I_{Ks}, and this possibility was tested by the following experiments.

We performed the envelope of tails test at four different conditions in the same cells (Fig. 7A). In these experiments, we assume that time-dependent tail current components in the control represent I_K (I_{K,con}) and are exclusively composed of I_{Kr} and I_{Ks}. In the presence of 17β-estradiol, tail current components (I_{KE2}) were composed of estradiol-resistant I_{Kr} and I_{Ks}. After addition of 17β-estradiol plus E-4031, tail currents [I_{K(E2+E-4031)}] represented estradiol-resistant I_{Ks}. Finally, estradiol was washed out, leaving E-4031 in the test solution, where the tail current (I_{K(E-4031)}) was exclusively composed of I_{Ks}. We selected this order of treatments for the calculation, since the effects of E-4031 on I_{Kr} were not readily reversible. For this order of treatments, we calculated percent inhibition of I_{Kr} and I_{Ks} by 17β-estradiol. The percent inhibition of I_{Kr} by...
estradiol was calculated as follows

\[
\text{\% inhibition} = \left[1 - \frac{I_{KE2} - I_{KE(E2+E-4031)}}{I_{KE4031}}\right] \times 100
\]

where tail currents were measured after short depolarizations (50 and 100 ms; Fig. 7B). The percent inhibition of \(I_{KS}\) by estradiol was calculated as follows

\[
\text{\% inhibition} = \left[1 - \frac{I_{KE(E2+E-4031)}}{I_{KE4031}}\right] \times 100
\]

where tail currents were measured after long depolarizations (2,000 and 3,000 ms; Fig. 7C). 17\(\beta\)-estradiol inhibited \(I_{KR}\) to 63.4 \(\pm\) 6.3% of the control (\(n = 5\), \(P < 0.05\)) and \(I_{KS}\) to 65.8 \(\pm\) 8.7% of the control (\(n = 5\), \(P < 0.05\)). According to these results, 17\(\beta\)-estradiol inhibited \(I_{KR}\) and \(I_{KS}\) to a similar extent.

DISCUSSION

In the present study we demonstrated that application of \(\approx 10 \mu M\) 17\(\beta\)-estradiol prolonged the APD in guinea pig ventricular myocytes. This hormone was also shown to inhibit three important currents forming the repolarization of the ventricular action potential: \(I_{CaL}\), \(I_{KR}\), and \(I_{KS}\). It is thus likely that the prolongation of APD by 17\(\beta\)-estradiol is mainly caused by the inhibition of \(I_{KR}\) and \(I_{KS}\).

Electrophysiological studies exploring the direct actions of estrogen on cardiac membrane have not offered any explanation for the clinical observations of the prolonged Q-T interval observed in women compared with that in men (1, 6, 11, 21) but, rather, have presented conflicting results. Action potentials were shortened by 17\(\beta\)-estradiol in guinea pig ventricular muscles and myocytes at a frequency of 1.0 Hz, with inhibition of \(I_{CaL}\) (7, 9, 10). In contrast, Berger et al. (2) observed that 17\(\beta\)-estradiol caused prolonged APD at a frequency of 0.05–5.0 Hz by mainly inhibiting \(I_{to}\) in rat ventricular myocytes. Our results in guinea pig preparations agree with the observed changes in action potential in rat myocytes demonstrating APD prolongation in a reverse use dependent manner at a wide range of stimulation rates, although different currents were affected. Therefore, different results in the previous reports could not be explained by the species difference or different stimulation rates. We are unable to explain why in previous studies opposite effects on action potentials were observed in guinea pig preparations.
With regard to the effects of estrogen on membrane currents, most studies demonstrated the inhibition of \( I_{\text{cal}} \) in various cardiac preparations (2, 7, 9, 10, 15, 18). These results correspond to reports of a negative inotropic effect by estrogen (20, 26). The inhibition of \( I_{\text{cal}} \) has also been noted in other tissue preparations, including GH3 cells (29), neurons (12), myometrial cells (31), and vascular smooth muscle cells (5, 15, 18). In the present experiments, application of 30 \( \mu \)M \( 17\beta \)-estradiol caused a reduction of \( I_{\text{cal}} \) by 20% with respect to the control without a shift in voltage dependence. Other studies also reported a 60–70% reduction of \( I_{\text{cal}} \) compared with the control after application of 30 \( \mu \)M \( 17\beta \)-estradiol (7, 9, 10). Because \( I_{\text{cal}} \) has a tendency to decrease with time during the whole cell recording (rundown), the reduction after treatment may be somewhat overestimated. Our measurements of \( I_{\text{cal}} \) returned to 94% of the control value on washout of \( 17\beta \)-estradiol. This may indicate that the 20% reduction with respect to the control represents a real effect caused by 30 \( \mu \)M \( 17\beta \)-estradiol.

The hormone affected two components of \( I_{Kr} \), \( I_{Kr} \) and \( I_{Ks} \), without reduction of the inward rectifier \( K^+ \) current, while it was mildly inhibited in rat myocytes with 30 \( \mu \)M \( 17\beta \)-estradiol (2). Although the late currents at potentials negative to −50 mV were not affected, the current at −40 mV was significantly decreased by \( 17\beta \)-estradiol (Fig. 2). Because the inward currents at negative voltages to the \( E_{\text{rev}} \) were not depressed, we judged that the depression of the current at −40 mV was not due to the \( I_{K1} \) inhibition but to the effect on \( I_{Kr} \). The differential effects on different components of \( K^+ \) currents exclude a nonspecific action of estrogen but indicate channel-specific action. Its effects on \( I_{Kr} \) and \( I_{Ks} \) were demonstrated by the envelope of tails test. The degree of inhibition of \( I_{Kr} \), and \( I_{Ks} \) was nearly equal, i.e., a 30–40% decrease with respect to the control. The voltage-dependent activation of both components was unaffected. The fully activated \( I-V \) relationship of \( I_{Ks} \) was depressed by \( 17\beta \)-estradiol, indicating that the number of functional channels was decreased or the single-channel current amplitude was reduced. At 30 \( \mu \)M \( 17\beta \)-estradiol, the maximum amplitudes of the fast and slow components of \( I_{Kr} \) in rat myocytes were decreased to 50 and 43%, respectively (2). Therefore, the degrees of inhibition of \( I_{to} \) and \( I_{K} \) appear to be similar. In previous studies the effects of \( 17\beta \)-estradiol on \( K^+ \) channels seemed to depend on the tissues. For example, estradiol had no significant effect on the outward \( K^+ \) current in vascular smooth muscle cells (15, 18), whereas \( 17\beta \)-estradiol stimulated the \( Ca^{2+} \)- and voltage-activated \( K^+ \) channels in aortic endothelial cells and coronary myocytes (24, 30).

The inward \( I_{\text{cal}} \) and outward \( I_{Kr} \) and \( I_{Ks} \) are in delicate equilibrium during the plateau, and their net effects determine the repolarization phase of the action potential (4, 16). It is difficult to quantify the contribution of each current component to the action potential prolongation induced by \( 17\beta \)-estradiol. Despite this uncertainty, the reduction of the two outward current components by estrogen was comparable to or higher than that of \( I_{\text{cal}} \). Therefore, it seems reasonable to assume that the former effects can overcome the shortening effect by the latter to prolong APD.

The concentration of \( 17\beta \)-estradiol (30 \( \mu \)M) that was found to cause inhibition of \( I_{\text{cal}} \), \( I_{Kr} \), and \( I_{Ks} \) is much higher than the in vivo concentration of \( 17\beta \)-estradiol. Normal plasma concentrations of \( 17\beta \)-estradiol have been shown to be <10 nM in various species, including the guinea pig (14). Maximal plasma concentrations in humans are 0.14 nM in men and 1.4 nM in women during the preovulatory period. During pregnancy the \( 17\beta \)-estradiol concentration increases up to a maximum of 0.1 µM by the end of the third trimester (23). Recent evidence has indicated that the acute effective concentration of steroid hormone accumulated by target cells may far exceed plasma levels (17). Nearly all circulating estrogen (95–98%) is bound to plasma proteins, i.e., albumin and sex hormone-binding globulin. Interestingly, accumulation of estrogen in target cells is greater in the presence of sex hormone-binding globulin than in the presence of free hormone alone. Although the physiological solutions used in the present and previous experiments did not contain plasma proteins, micromolar range concentrations of estrogen are often required to produce consistent and maximal responses of target cells in vitro. Further experiments are necessary to determine the effective steroid concentrations accumulated by target cells, including cardiac cells in vivo.

Action potential prolongation by estrogen was observed in myocytes from males as well as from females. Although the nuclear estrogen receptor was not expressed in rat ventricle of either gender (28), estrogen affected membrane currents of ventricular myocytes (2). Therefore, \( 17\beta \)-estradiol seems to exert its effect in rat ventricle via a nongenomic pathway. In other species, including the guinea pig, the estrogen membrane receptor has not been identified in cardiac tissue. In our study, \( 17\beta \)-estradiol prolonged APD and inhibited \( I_{\text{cal}} \), \( I_{Kr} \), and \( I_{Ks} \) within 5 min. This rather rapid effect of \( 17\beta \)-estradiol and the above features are inconsistent with its action being mediated via conventional slow-acting nuclear receptors. Recently, Meyer et al. (13) reported that the reduction of \( I_{\text{cal}} \) by \( 17\beta \)-estradiol developed with a time constant of 3–4 s. This is consistent with the presence of a cell surface receptor that could also affect \( I_{K} \). It is not clear from the present study how the inhibitory effects of \( 17\beta \)-estradiol are exerted on the three channels, and further studies are necessary to prove the mechanism of action on the different membrane currents.

Our results show that \( 17\beta \)-estradiol prolonged APD by inhibition of \( I_{K} \), which may contribute to the high prevalence of the incidence of torsades de pointes with QT interval prolongation. This result may not necessarily indicate that estrogen always exhibits proarrhythmic potential. In the previous as well as the present studies, estrogen has been shown to reduce L-type \( Ca^{2+} \) channel activity in vitro and to cause relaxation in arterial smooth muscles and cardiac myocytes (7, 9, 15, 18). Clinical observations have demonstrated that estro-
Estrogen replacement therapy is associated with a reduced incidence of cardiac arrhythmias in postmenopausal women (3), and cyclic increases in estrogen at the premenopausal stage abolish the appearance of supraventricular tachycardia (22). Therefore, estrogen may exhibit proarrhythmic as well as antiarrhythmic effects, depending on the clinical situation. Large-scale prospective studies to investigate the clinical effects of estrogen are necessary to clarify this problem.

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