Increased Expression of the Cardiac L-type Calcium Channel in Estrogen Receptor–deficient Mice

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ABSTRACT Steroid hormones control the expression of many cellular regulators, and a role for estrogen in cardiovascular function and disease has been well documented. To address whether the activity of the L-type Ca^{2+} channel, a critical element in cardiac excitability and contractility, is altered by estrogen and its nuclear receptor, we examined cardiac myocytes from male mice in which the estrogen receptor gene had been disrupted (ERKO mice). Binding of dihydropyridine Ca^{2+} channel antagonist isradipine (PN200-110) was increased 45.6% in cardiac membranes from the ERKO mice compared to controls, suggesting that a lack of estrogen receptors in the heart increased the number of Ca^{2+} channels. Whole-cell patch clamp of acutely dissociated adult cardiac ventricular myocytes indicated that Ca^{2+} channel current was increased by 49% and action potential duration was increased by 75%. Examination of electrocardiogram parameters in ERKO mice showed a 70% increase in the QT interval without significant changes in PQ or QRS intervals. These results show that the membrane density of the cardiac L-type Ca^{2+} channel is regulated by the estrogen receptor and suggest that decreased estrogen may lead to an increase in the number of cardiac L-type Ca^{2+} channels, abnormalities in cardiac excitability, and increased risk of arrhythmia and cardiovascular disease.

KEY WORDS: heart • ion channels • action potential • electrocardiogram

INTRODUCTION

Calcium channels are important regulators of cell function, but there is little information on the factors which influence their level of expression in cells. Steroid hormones are likely regulators of calcium channel expression as they control the expression of many cellular regulators and are implicated in regulation of cardiac excitability which is critically dependent on calcium channels. Premenopausal women have a lower incidence of cardiovascular disease than men and continue to have a lower risk of heart disease after menopause when receiving estrogen replacement (Collins et al., 1993). Estrogen replacement is associated with a reduction in cardiac arrhythmia in postmenopausal women (Cagnacci et al., 1992), and cyclical increases in estrogen in premenopausal women are associated with a reduction in paroxysmal supraventricular tachycardia (Rosano et al., 1996). Some of the benefits of estrogen on the cardiovascular system may derive from a reduction in cholesterol deposition on arterial walls (Chow, 1995), but estrogen may also have direct effects on arterial smooth muscle and cardiomyocytes. Estrogen in vitro reduces L-type Ca^{2+} channel activity and causes relaxation in both types of muscle (Jiang et al., 1992; Shan et al., 1994; Grohé et al., 1996; Ogata et al., 1996), but the effective estrogen concentrations in these experiments were in excess of those normally present in vivo. To search for effects of estrogen on L-type Ca^{2+} channels in the heart in vivo, we examined Ca^{2+} channel number and function in ventricular myocytes from mice with a disrupted estrogen receptor gene (ERKO mice) (Lubahn et al., 1993). We report here that disruption of estrogen action in vivo causes increased density of L-type Ca^{2+} channels in the heart as assessed from ligand binding and whole-cell voltage clamp experiments. The results demonstrate an important influence of estrogen on calcium channel expression in the heart and implicate this as one factor which may contribute to the reduced incidence of cardiovascular disease associated with estrogen action in vivo.

MATERIALS AND METHODS

Dihydropyridine Binding

The hearts from eight control (age-matched male C57B1/6J) and eight male ERKO mice were isolated quickly after anesthesia with CO_2. The whole heart was minced with scissors and homogenized in 5 ml ice-cold Tris buffer (50 mM, pH 7.4) by two bursts in a Polytron (Brinkman Instruments, Inc., Westbury, NY), followed by 10 passes of a motor-driven glass-Teflon homogenizer (TRI-R Instruments Inc., Rockville Center, NY). The homogenate was filtered through four layers of cheesecloth and centri-

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fuged at 45,000 *g* for 45 min at 4°C. The resultant pellet was suspended in 5 ml Tris buffer. The protein concentration was determined by the bicinchoninic acid method using a protein assay kit (Pierce, Rockford, IL). The saturation binding assay was performed in test tubes containing 2.5 ml Tris buffer (50 mM, pH 7.4). Membrane protein (0.15–0.25 mg) was incubated with 0.01–0.6 nM $^3H(+)$-PN-200-110 for 2 h at 37°C. Samples were then filtered over Whatman GF/B filters and washed twice with 5 ml ice-cold 50 mM Tris buffer, using a cell harvester (Brandel Instruments, Gaithersburg, MD). The radioactivity on the filters was measured by liquid scintillation counting at an efficiency of ~50%. Nonspecific binding was determined by addition of 1 μM unlabeled PN200-110. The binding data were calculated by Scatchard plot using the program LIGAND (Munson and Rodbard, 1980). The results were analyzed by a standard set of pharmacological programs (Tallarida and Murray, 1981). Significance of difference was accepted at the 0.05 level. $^3H(+)$-PN-200-110 (81.5 Ci/mmol) was purchased from DuPont NEN (Boston, MA). All other reagents were ordered from Sigma Chemical Co. (St. Louis, MO).

Cell Preparation and Electrophysiological Recording

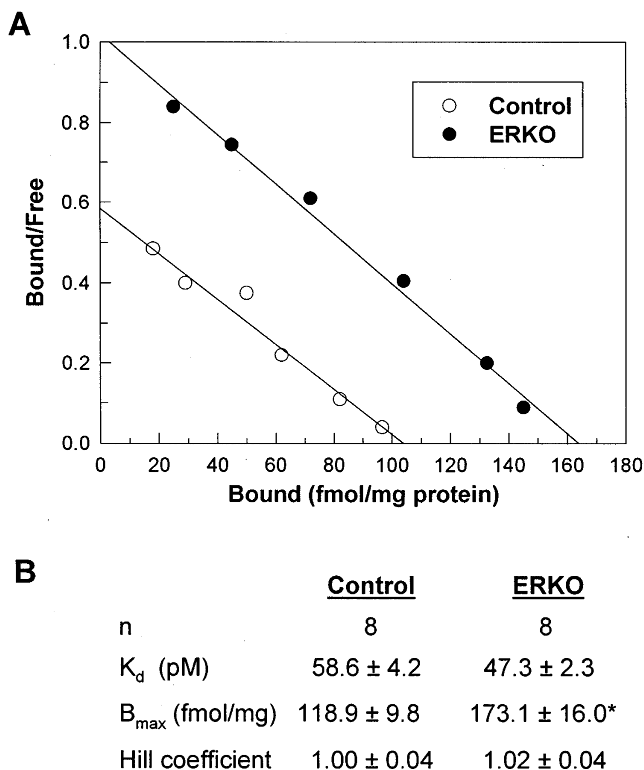
Ventricular myocytes from six control and six ERKO adult mice (24–28 wk old) were dissociated as described (Benndorf, 1993). Briefly, after the animals were anaesthetized with pentobarbital (50 mg/kg, i.p.), the hearts were excised and perfused through the aorta for 5 min (~10 ml) with 37°C, oxygenated Ca^{2+}- and Mg^{2+}-free Hanks' Balanced Salt Solution (CMF HBSS, Sigma Chemical Co.) containing 10 mM HEPES (pH 7.3), then for 30 min (~10 ml, recirculated) with 37°C, oxygenated CMF HBSS to which 1 mg/ml type I collagenase (Worthington Biochemical Corp., Freehold, NJ) and 100 μM Ca^{2+} had been added, then for 3 min (~7 ml) with relaxing solution containing (mM units): 30 KCl, 30 KH_2PO_4, 50 glutamate, 20 taurine, 20 HEPES, 10 glucose, 0.5 EGTA (pH 7.3 with KOH). The ventricles were minced and triturated in relaxing solution then centrifuged and plated in fresh relaxing solution. Myocytes were stored in this solution at room temperature until use (<6 h). Four control mice were age-matched C57BL/6 mice and two were wild-type littermates (ER +/+) of the ERKO mice. Ca^{2+} channel current was recorded using the whole-cell configuration of the patch clamp technique. Patch pipettes were pulled from VWR micropipettes and fire-polished to produce an inner tip diameter of 4–6 μm. Currents were recorded using a List EPC-7 patch clamp amplifier and filtered at 2 kHz (8-pole Bessel filter, −3 dB). Data was acquired using Fastlab software (Indec Systems). Voltage-dependent currents have been corrected for leak using an on-line P/4 subtraction paradigm. The extracellular saline (bath) contained (mM units) $BaCl_2$ (2), $CaCl_2$ (2), or NaCl (20) as the permeant cation, Tris (150), $MgCl_2$ (1), and methanesulfonic acid (used to adjust pH to 7.3). Patch pipette saline (intracellular) contained *N*-methyl-D-glucamine (130), EGTA (10), HEPES (60), MgATP (2), $MgCl_2$ (1), and methanesulfonic acid (used to adjust pH to 7.3). Experiments were performed at room temperature (20–23°C). Only L-type Ca^{2+} channels were activated from the −60 mV holding potential as verified by complete channel block by 1 μM isradipine (PN200-110). T-type Ca^{2+} channel current was recorded at −40 mV from a holding potential of −80 mV. Na^+ channel current was recorded from a holding potential of −80 mV. Action potentials were recorded using the whole-cell configuration of the patch clamp technique (current-clamp mode) using an Axoprobe 1A amplifier (Axon Instruments, Foster City, CA). The extracellular bath contained Hanks' Balanced Salt Solution (HBSS) with 10 mM HEPES (pH 7.3), and the patch pipette (intracellular) contained (in mM) aspartic acid (140), NaCl (5), $MgCl_2$ (3), HEPES (10), EGTA (2.5), KOH (used to adjust pH to 7.4). Experiments were performed at room temperature (20–23°C). Electrocardiograms were recorded in the Lead I configuration from mice under pentobarbital anesthesia (50 mg/kg, i.p.). ECG intervals were measured between the points of deviation from the baseline (Bayes de Luna, 1993). The pulse-corrected QT interval (QTc) was calculated according to Bazett's formula ($QTc = QT \times RR^{-1/2}$) (Bayes de Luna, 1993).

RESULTS

The estrogen receptor is a ligand-activated transcription factor which regulates gene expression in many cell types both directly by binding to *cis*-acting elements in estrogen-responsive genes and indirectly by interacting with other members of the ligand-activated transcription factor family and by influencing the expression of other transcription factors (Katzenellenbogen, 1996). We chose to examine expression of cardiac L-type Ca^{2+} channels in male rather than female ERKO mice since circulating levels of estradiol and progesterone have been found to change dramatically in female ERKO mice but remain constant in male mice (control males, 11.8 ± 3.4 pg/ml; ERKO males 12.9 ± 3.4 pg/ml; Couse et al., 1995). Controlled circulating hormone levels were important in this study given possible direct pharmacological effects of estradiol on Ca^{2+} and K^+ channels (Rusko et al., 1995; Ogata et al., 1996; Shan et al., 1994; Grohé et al., 1996; Jiang et al., 1992), and the recent discovery of a second estrogen receptor, named ERβ (Kuiper et al., 1996).

The number of L-type Ca^{2+} channels in the hearts of control and ERKO mice was first measured directly by binding of the dihydropyridine Ca^{2+} channel antagonist PN200-110. Dihydropyridines bind specifically and with high affinity to L-type Ca^{2+} channels with a 1:1 stoichiometry. The number of specific binding sites for PN200-110 (B_{max}) in cardiac membranes was increased 45.6% in ERKO mice without a change in the affinity (K_d) of the channel for the dihydropyridine (Fig. 1). This increase in the number of Ca^{2+} channels was not due to cardiac hypertrophy in ERKO mice since neither the weight of the heart nor amount of protein in each heart was changed.

Whole-cell patch clamp of dissociated ventricular myocytes from control and ERKO mice revealed a similar increase in L-type Ca^{2+} channel activity. Fig. 2 *A* shows the mean Ca^{2+} channel current density (with Ba^{2+} as the permeant ion) in 15 myocytes of each type recorded at 0 mV. The mean current-voltage relations for control and ERKO myocytes (Fig. 2 *B*) showed a 17% increase at 0 mV and a 49% increase at +30 mV. The larger percentage increase in Ba^{2+} current at 30 mV implies a shift in the current voltage relationship to more positive membrane potentials. This shift can be seen in the superimposed data in Fig. 2 *B*. It is caused by a positive shift in the voltage dependence of activation (see



	Control	ERKO
n	8	8
K_d (pM)	58.6 ± 4.2	47.3 ± 2.3
B_{max} (fmol/mg)	118.9 ± 9.8	173.1 ± 16.0*
Hill coefficient	1.00 ± 0.04	1.02 ± 0.04

FIGURE 1. Dihydropyridine binding to cardiac membranes. Saturation binding of the L-type Ca^{2+}-channel dihydropyridine ligand $^3H(+)$-PN200-110 to cardiac membranes was analyzed in hearts isolated from eight control and eight ERKO male mice. (*A*) Comparison of Scatchard plots of $^3H(+)$-PN200-110 binding to cardiac membranes from one control and one ERKO mouse shows an elevated number of binding sites (B_{max}: intersection of plot with the x-axis) but similar binding affinity (K_d: slope of plot) in ERKO hearts. (*B*) Mean values (± SEM) of eight experiments reveal statistically significant increase of B_{max} (by 45.6%) (*, $p < 0.05$), but no significant change in K_d or Hill-coefficient of $^3H(+)$-PN200-110 binding to cardiac membranes from ERKO hearts.

below). The increased density of Ca^{2+} channel current was independent of the ion carrying the current and was observed for Ca^{2+} current as well as Ba^{2+} current (16% increase at 0 mV, $n = 5$). The kinetics of activation and inactivation of Ca^{2+} channel current were not changed (Fig. 2 *C*), indicating that the increased current in the ERKO ventricular myocytes was not due either to a reduction in Ca^{2+}-dependent or voltage-dependent inactivation or to acceleration of activation.

To yield a more accurate measure of Ca^{2+} channel activity at a broad range of membrane potentials, the current-voltage relations shown in Fig. 2 *B* were corrected for the effect of electrical driving force using the Goldman-Hodgkin-Katz current equation (Bargas et al., 1994; Hille, 1992) (Fig. 2 *D*). Apparent reversal potentials for the two groups were not different (control, 44 ± 3 mV, $n = 23$; ERKO, 46 ± 5 mV, $n = 21$). However, the $V_{1/2}$ for activation was shifted from −13.3 ± 1.5 mV for controls to −8.4 ± 1.4 mV for ERKO mice ($p < 0.05$). Fits to these permeability vs. voltage curves using the Boltzmann equation (Hille, 1992) show a 49 ± 5% increase in the mean membrane permeability to Ba^{2+} (control, $17.6 \pm 2.1 \times 10^{-6}$ cm/s, $n = 23$; ERKO, $26.2 \pm 3.0 \times 10^{-6}$ cm/s, $n = 21$; $p < 0.05$). As with previous measures of cardiac hypertrophy, the mean membrane surface area of dissociated ventricular myocytes as estimated from cell capacitance was not changed in the ERKO mice (control, 17,489 ± 1,083 μm^2; ERKO, 16,778 ± 1,188 μm^2; using a conversion factor of 0.01 pF/μm^2). In addition, the other voltage-dependent Ca^{2+} channel in ventricular myocytes, the T-type Ca^{2+} channel, did not exhibit an increase in activity (control, 4.2 ± 1.4 $\mu A/cm^2$, $n = 7$; ERKO, 3.8 ± 0.6 $\mu A/cm^2$, $n = 12$). The voltage-dependent Na^+ channel showed a decrease in current density (control, 87.3 ± 23.9 $\mu A/cm^2$, $n = 7$; ERKO, 57.9 ± 10.9 $\mu A/cm^2$, $n = 9$), as expected from previous evidence that block of the L-type Ca^{2+} channel causes an increase in cardiac Na^+ channel expression (Duff et al., 1992). Thus, these data show that disruption of the estrogen receptor gene specifically increases expression of the L-type Ca^{2+} channel in ventricular myocytes.

An increase in Ca^{2+} channel current would be predicted to lengthen the ventricular action potential in the absence of other modulatory effects (Rardon and Fisch, 1994). Action potentials were recorded in control and ERKO ventricular myocytes in the whole cell recording configuration (Fig. 3, *A* and *B*). Action potential duration measured at half repolarization (APD_{50}) was increased 75 ± 11% in ERKO myocytes compared to controls (Fig. 3 *A*), and action potential duration measured at 90% repolarization (APD_{90}) was increased 53 ± 9% (Fig. 3 *B*). Except for action potential amplitude, which was reduced by 9% (control, 109.3 ± 3.6 mV, $n = 6$; ERKO, 99.6 ± 2.7 mV, $n = 14$; $p < 0.05$), other cell membrane parameters including action potential threshold (control, −66 ± 3 mV; ERKO, −61 ± 2 mV) and membrane resistance at threshold (control, 11.9 ± 2.5 MΩ; ERKO, 12.0 ± 1.3 MΩ) were not significantly changed.

Electrocardiogram (ECG) parameters were measured in anesthetized control and ERKO mice. Cardiac electrical activity recorded from electrodes placed on the skin of humans and mice consists of three waveforms, designated P, QRS, and T. The P-wave represents atrial contraction, the QRS-wave represents ventricular depolarization, and the T-wave represents ventricular repolarization (Bayes de Luna, 1993). Agents which lengthen the ventricular action potential lengthen the time interval between Q and T. A greatly prolonged ventricular action potential and QT interval leads to cardiac arrhythmia since the heart is not sufficiently repolarized between contractions (Tan et al., 1995). Examples of ECGs from control and ERKO mice are shown in Fig. 3 *C*. Disruption of the estrogen receptor was not found to

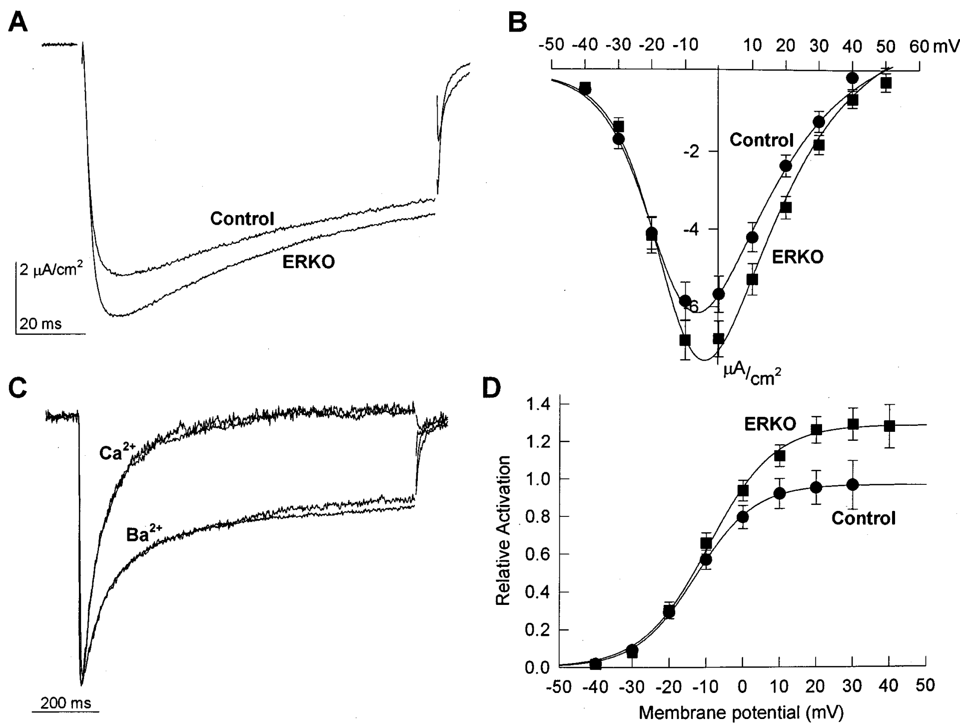


FIGURE 2. Increased Ca^{2+} channel current in ERKO mice. Ca^{2+} channel current (carried by Ba^{2+}) was recorded in acutely dissociated ventricular myocytes from control and ERKO mice during a 100-ms depolarization from -60 mV to 0 mV. Current amplitude (normalized to membrane surface area) measured in 15 myocytes from each group was averaged to give the traces shown (*A*). Membrane surface area was calculated from cell capacitance as 1 $\mu F/cm^2$. Mean capacitance was not different between cell types. (*B*) Mean current density-voltage relations (±SEM) for control ($n = 23$) and ERKO mice ($n = 21$) were calculated from measurements of peak current during 100-ms depolarizations from -60 mV to the potentials indicated. Solid lines represent fits of the Boltzmann and Goldman-Hodgkin-Katz current equations to the mean data with the following values: control, $\alpha = 17 \times 10^{-6}$ cm/s, $V_{1/2} = -13.6$ mV, $k = 7.4$; ERKO, $\alpha = 24 \times 10^{-6}$ cm/s, $V_{1/2} = -11.1$ mV, $k = 7.3$, where $V_{1/2}$ represents the voltage for half-maximal activation, k the exponential slope factor from the Boltzmann equation, and α the maximum permeability. E_{rev} was fixed to 47 mV for both. (*C*) Inactivation properties of Ca^{2+} and Ba^{2+} current in control and ERKO myocytes are compared during 1-s depolarizations to 0 (Ba^{2+}) or +10 mV (Ca^{2+}). Traces represent means from 10 cells each normalized to the same peak amplitude for comparison of time course. (*D*) Current density-voltage relations from the same cells shown in *B* were converted to absolute membrane permeability using the Goldman-Hodgkin-Katz current equation. Mean apparent reversal potentials were not different between the two groups. Permeabilities, representing Ca^{2+} channel activation, were then averaged and displayed (±SEM) on a scale relative to the mean of control (21×10^{-6} cm/s). Solid lines represent fits of the Boltzmann equation to the mean data with the following values: control, $\alpha = 21 \times 10^{-6}$ cm/s, $V_{1/2} = -12.9 \pm 0.3$ mV, $k = 8.0$; ERKO, $\alpha = 28 \times 10^{-6}$ cm/s, $V_{1/2} = -9.4 \pm 0.7$ mV, $k = 8.9$.

alter the PQ interval (a measure of action potential conduction in the AV node, Bundle of His and Purkinje fibers) or the duration of the QRS complex (a measure of the rate of rise of the ventricular action potential). In contrast, disruption of the estrogen receptor prolonged the QT interval by 70% ($p < 0.05$, Fig. 3 *D*). Since the pulse rate of anesthetized ERKO mice was slightly faster than control mice (Fig. 3 *B*), correction of the QT interval for the pulse rate (QTc) increased the difference between control and ERKO mice (Fig. 3 *D*). The QT interval was more variable in ERKO mice, consistent with our observation of less uniform repolarization time for action potentials in ERKO mice compared to wild-type. This variability in repolarization was seen in the ECG records as a T-wave with fast and slow components (Fig. 3 *C*).

DISCUSSION

Our results provide the first evidence to our knowledge for regulation of expression of calcium channels by a steroid hormone in vivo. Previous results have implicated glucocorticoids (Takimoto and Levitan, 1994) and sex steroids (White et al., 1995; Drici et al., 1996) in regulation of cardiac K^+ channels. Together, the effects of the steroid hormones on these two prominent classes of cardiovascular ion channels are likely to have important influence on the excitability of the heart.

17β-estradiol has been found to act as an L-type Ca^{2+} channel antagonist when acutely applied to both vascular smooth muscle and cardiac myocytes in vitro, where Ca^{2+} channel current is reduced at concentrations in the range of 1–30 μM (Jiang et al., 1992; Collins et al., 1993; Shan et al., 1994; Farhat et al., 1996; Grohé et al., 1996; Ogata et al., 1996). These concentrations substantially exceed the levels of circulating estrogen of approximately 40–60 pM in males (Contoreggi et al., 1990; Couse et al., 1995) and 90 pM to 8 nM in females (Collins et al., 1993; Couse et al., 1995; Samaan and Crawford, 1995; Volterrani et al., 1995; Rosano et al., 1996). In addition, Mermelstein et al. (1996) reported acute, membrane-receptor–mediated inhibition of L-type Ca^{2+} currents in neostriatal neurons. This effect was observed at physiological concentrations of 17 β-estra-

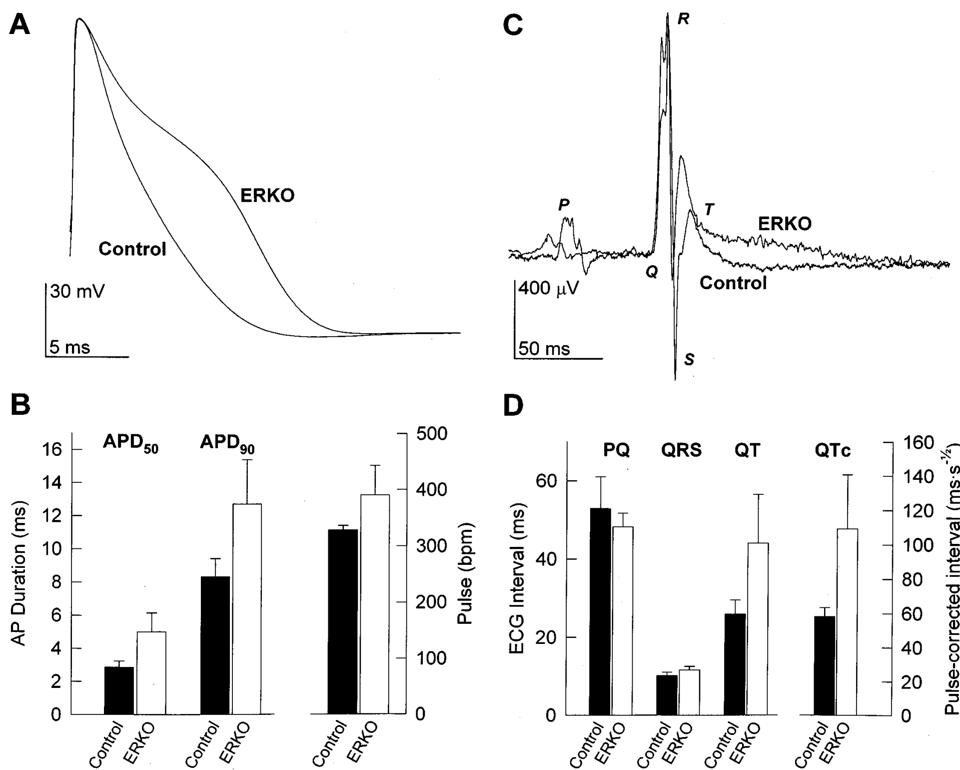


FIGURE 3. Comparison of cardiac action potentials and electrocardiograms from control and ERKO mice. (*A*) Examples of action potentials recorded from acutely dissociated control and ERKO ventricular myocytes. The ERKO trace has been scaled to the amplitude of control for comparison of time course. (*B*) Mean action potential durations (±SEM) at 50% repolarization (APD_{50}) and 90% repolarization (APD_{90}) in control (n = 6) and ERKO mice (n = 14). Action potentials were recorded in myocytes held at −90 mV and paced at 5 Hz. Mean pulse rate (±SEM) is also shown. (*C*) Examples of electrocardiograms recorded from control and ERKO mice. The ERKO trace has been scaled to the amplitude of control for comparison of time course. (*D*) Mean electrocardiogram intervals (±SEM) measured in six control and six ERKO mice.

diol but was rapidly reversible and therefore unlikely to influence our measurements on dissociated cardiac myocytes in the absence of added estrogen. Our results suggest that physiological levels of estrogen may reduce the number of cardiac Ca^{2+} channels through the actions of the estrogen receptor. Evidently, the estrogen receptor normally suppresses Ca^{2+} channel expression in the heart, and disruption of this receptor relieves this suppression. This effect may occur through a direct action of the estrogen receptor on expression of the cardiac Ca^{2+} channel gene or through indirect influences on other transcriptional regulators or second messenger pathways (Katzenellenbogen, 1996).

The increased Ca^{2+} current in cardiomyocytes of ERKO mice is accompanied by prolonged action potentials in dissociated cells and a prolonged QT interval in the heart in situ. The increased Ca^{2+} current would contribute to both of these effects, but other unidentified effects of estrogen receptor deficiency may also play a role. For example, reduction in the level of any of the several K^+ channels involved in the repolarization phase of the cardiac action potential would also contribute to the prolonged action potentials and prolonged QT interval that we have observed (White et al., 1995; Drici et al., 1996).

This new role for estrogen in modulation of cardiac contractility may complement its role as a modulator of lipid metabolism. In both cardiac contractility and lipid metabolism, estrogen appears to maintain a favorable balance which is disrupted as estrogen levels decline with age (Farhat et al., 1996). Both men and women exhibit an inverse correlation between estrogen levels and cardiovascular disease (Contoreggi et al., 1990; Chow, 1995), and estrogen replacement in women has been found to reduce cardiac arrhythmia (Cagnacci et al., 1992) and angina pectoris (Sarrel, 1996). As treatment with blockers of L-type Ca^{2+} channels is an effective therapy for both atrial arrhythmias and angina pectoris (Braunwald, 1982), it is plausible that an increase in L-type Ca^{2+} channel activity may contribute to these conditions. In the present study, we found that disruption of the estrogen receptor in mice leads to an increase in the expression of the L-type Ca^{2+} channel in ventricular myocytes and a corresponding delay in cardiac repolarization. If similar changes occur in humans during a decline in estrogen and/or estrogen receptor levels, they could contribute to the increased incidence of arrhythmia and angina pectoris after menopause. Further examination of the role the L-type Ca^{2+} channel plays in estrogen-induced protection against cardiovascular disease may aid in the development of more specific therapies.

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REFERENCES

Bargas, J., A. Howe, J. Eberwine, Y. Cao, and D.J. Surmeier. 1994. Cellular and molecular characterization of Ca^{2+} currents in acutely isolated, adult rat neostriatal neurons. *J. Neurosci.* 14: 6667–6686.

Bayes de Luna, A. 1993. Clinical Electrocardiography: A Textbook. Futura, Mount Kisco, NY. 500 pp.

Benndorf, K. 1993. Multiple levels of native cardiac Na^{+} channels at elevated temperature measured with high-bandwidth/low-noise patch clamp. *Pflüg. Archiv.* 422:506–515.

Braunwald, E. 1982. Mechanism of action of calcium-channel-blocking agents. *N. Engl. J. Med.* 307:1618–1626.

Cagnacci, A., R. Soldani, E. Puccini, P. Fioretti, and G.B. Melis. 1992. Lipid-independent therapeutic properties of transdermal 17-β-estradiol on cardiovascular diseases. *Acta Obstet. Gynecol. Scand.* 71:639–641.

Chow, M.S. 1995. Benefit/risk of estrogen therapy in cardiovascular disease: current knowledge and future challenges. *J. Clin. Pharmacol.* 35:11S–17S.

Collins, P., G.M. Rosano, C. Jiang, D. Lindsay, P.M. Sarrel, and P.A. Poole Wilson. 1993. Cardiovascular protection by oestrogen: a calcium antagonist effect? *Lancet.* 341:1264–1265.

Contoreggi, C.S., M.R. Blackman, R. Andres, D.C. Muller, E.G. Lakatta, J.L. Fleg, and S.M. Harman. 1990. Plasma levels of estradiol, testosterone, and DHEAS do not predict risk of coronary artery disease in men. *J. Androl.* 11:460–470.

Couse, J.F., S.W. Curtis, T.F. Washburn, J. Lindzey, T.S. Golding, D.B. Lubahn, O. Smithies, and K.S. Korach. 1995. Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. *Mol. Endocrinol.* 9:1441–1454.

Drici, M.D., T.R. Burklow, V. Haridasse, R.I. Glazer, and R.L. Woosley. 1996. Sex hormones prolong the QT interval and downregulate potassium channel expression in the rabbit heart. *Circulation.* 94:1471–1474.

Duff, H.J., J. Offord, J. West, and W.A. Catterall. 1992. Class I and IV antiarrhythmic drugs and cytosolic calcium regulate mRNA encoding the sodium channel alpha subunit in rat cardiac muscle. *Mol. Pharmacol.* 42:570–574.

Farhat, M.Y., M.C. Lavigne, and P.W. Ramwell. 1996. The vascular protective effects of estrogen. *FASEB J.* 10:615–624.

Grohé, C., S. Kahlert, K. Lobbert, R. Meyer, K.W. Linz, R.H. Karas, and H. Vetter. 1996. Modulation of hypertensive heart disease by estrogen. *Steroids.* 61:201–204.

Hille, B. 1992. Ionic Channels of Excitable Membranes. 2nd ed. Sinauer Associates, Sunderland, MA. 607 pp.

Jiang, C., P.A. Poole Wilson, P.M. Sarrel, S. Mochizuki, P. Collins, and K.T. MacLeod. 1992. Effect of 17 β-oestradiol on contraction, Ca^{2+} current and intracellular free Ca^{2+} in guinea-pig isolated cardiac myocytes. *Br. J. Pharmacol.* 106:739–745.

Katzenellenbogen, B.S. 1996. Estrogen receptors: bioactivities and interactions with cell signaling pathways. *Biol. Reprod.* 54:287–293.

Kuiper, G.G., E. Enmark, M. Pelto-Huikko, S. Nilsson, and J.A. Gustafsson. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. USA.* 93:5925–5930.

Lubahn, D.B., J.S. Moyer, T.S. Golding, J.F. Couse, K.S. Korach, and O. Smithies. 1993. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc. Natl. Acad. Sci. USA.* 90: 11162–11166.

Mermelstein, P.G., J.B. Becker, and D.J. Surmeier. 1996. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci.* 16:595–604.

Munson, P.J., and D. Rodbard. 1980. Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107:220–239.

Ogata, R., Y. Inoue, H. Nakano, Y. Ito, and K. Kitamura. 1996. Oestradiol-induced relaxation of rabbit basilar artery by inhibition of voltage-dependent Ca channels through GTP-binding protein. *Br. J. Pharmacol.* 117:351–359.

Rardon, D., and C. Fisch. 1994. Electrolytes and the heart. *In* The Heart, Arteries and Veins. R.C. Schlant, W. Alexander, R.A. O'Rourke, R. Roberts, and E.H. Sonnenblick, editors. McGraw-Hill, New York. 759–774.

Rosano, G.M., F. Leonardo, P.M. Sarrel, C.M. Beale, F. De Luca, and P. Collins. 1996. Cyclical variation in paroxysmal supraventricular tachycardia in women. *Lancet.* 347:786–788.

Rusko, J., L. Li, and C. van Breemen. 1995. 17 β-Estradiol stimulation of endothelial K^{+} channels. *Biochem. Biophys. Res. Commun.* 214:367–372.

Samaan, S.A., and M.H. Crawford. 1995. Estrogen and cardiovascular function after menopause. *J. Am. Coll. Cardiol.* 26:1403–1410.

Sarrel, P.M. 1996. Cardiovascular disease in women: implications of hormone replacement therapy. *Int. J. Fertil. Menopausal Stud.* 41: 90–93.

Shan, J., L.M. Resnick, Q.Y. Liu, X.C. Wu, M. Barbagallo, and P.K. Pang. 1994. Vascular effects of 17 β-estradiol in male Sprague-Dawley rats. *Am. J. Physiol.* 266:H967–H973.

Takimoto, K., and E.S. Levitan. 1994. Glucocorticoid induction of Kv1.5 K^{+} channel gene expression in ventricle of rat heart. *Circ. Res.* 75: 1006–1013.

Tallarida, R.J., and R.B. Murray. 1981. Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York. 150 pp.

Tan, H.L., C.J. Hou, M.R. Lauer, and R.J. Sung. 1995. Electrophysiologic mechanisms of the long QT interval syndromes and torsade de pointes. *Ann. Int. Med.* 122:701–714.

Volterrani, M., G. Rosano, A. Coats, C. Beale, and P. Collins. 1995. Estrogen acutely increases peripheral blood flow in postmenopausal women. *Am. J. Med.* 99:119–122.

White, R.E., D.J. Darkow, and J.L. Lang. 1995. Estrogen relaxes coronary arteries by opening BKCa channels through a cGMP-dependent mechanism. *Circ. Res.* 77:936–942.