Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 563 (2007) 61–68
www.elsevier.com/locate/ejphar

Raloxifene inhibits transient outward and ultra-rapid delayed rectifier potassium currents in human atrial myocytes

Hui Liu [a,c], Man-Wen Jin [c], Ji-Zhou Xiang [c], Yu Huang [d], Hai-Ying Sun [a], Shui-Wha Chiu [e], Chu-Pak Lau [a], Gui-Rong Li [a,b,*]

[a] Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
[b] Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
[c] Department of Pharmacology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[d] Department of Physiology, Chinese University of Hong Kong, Hong Kong SAR, China
[e] Cardiothoracic Unit, Grantham Hospital, University of Hong Kong, Hong Kong SAR, China

Received 21 September 2006; received in revised form 22 January 2007; accepted 24 January 2007
Available online 8 February 2007

Abstract

The selective estrogen receptor modulator raloxifene is widely used in the treatment of postmenopausal osteoporosis, and has cardioprotective properties. However, effects of raloxifene on cardiac ion channels are unclear. The present study was designed to investigate the effects of raloxifene and β-estradiol on transient outward and ultra-rapid delayed rectifier potassium currents (I_{to1} and I_{Kur}) in human atrial myocytes with a whole cell patch-clamp technique. I_{to1} was inhibited by raloxifene in a concentration-dependent manner with an IC_{50} of 0.9 μM. Raloxifene at 1 μM decreased I_{to1} by 40.2±1.9% (at +50 mV, $n=14$, $P<0.01$ vs control). Time-dependent recovery from inactivation was slowed, and time to peak and time-dependent inactivation of I_{to1} were significantly accelerated, while steady-state voltage dependent activation and inactivation of I_{to1} were not affected by raloxifene. In addition, raloxifene remarkably suppressed I_{Kur} ($IC_{50}=0.7$ μM). Raloxifene at 1 μM decreased I_{Kur} by 57.3± 3.3% (at +50 mV, n=10, $P<0.01$ vs control). However, β-estradiol inhibited I_{to1} ($IC_{50}=10.3$ μM) without affecting I_{Kur}. The inhibitory effects of raloxifene and β-estradiol on I_{to1} and/or I_{Kur} were unaffected by the estrogen receptor antagonist ICI 182,780. Our results indicate that raloxifene directly inhibits the human atrial repolarization potassium currents I_{to1} and I_{Kur}. Whether raloxifene is beneficial for supraventricular arrhythmias remains to be studied.

Keywords: Raloxifene; Human atrium; Transient outward potassium current; Ultra-rapid delayed rectifier potassium current

1. Introduction

It is well known that cardiac repolarization and action potential duration are dependent of the balance of inward L-type Ca^{2+} current ($I_{Ca.L}$) and outward K^+ currents. The 4-aminopyridine (4-AP)-sensitive transient outward K^+ current (I_{to1}) and ultra-rapid delayed rectifier K^+ current (I_{Kur}) are believed to play an important role in human atrial repolarization (Li et al., 1995; Shibata et al., 1989). Inhibition of I_{to1} and/or I_{Kur} prolongs the action potential duration in human atrium (Van Wagoner and Nerbonne, 2000; Courtemanche et al., 1998, 1999). In addition, I_{Kur} is present in the atrium, but not in the ventricle of human heart (Li et al., 1996), inhibition of I_{Kur} may be useful in the treatment of patients with atrial fibrillation (Van Wagoner and Nerbonne, 2000; Van Wagoner, 2000; Nattel et al., 1999). Therefore, I_{Kur} may be a viable target for developing specific anti-atrial fibrillation agents.

Raloxifene, a second-generation of selective estrogen receptor modulator, is currently used in the treatment of postmenopausal osteoporosis (Rossouw et al., 2002). Raloxifene is also found to be cardiovascular protective by direct action on vascular cells and by favorable blood lipid regulation via estrogen receptor-dependent or independent mechanisms (Wenger, 2002). Experimental studies demonstrated that intracoronary infusion of raloxifene reduced myocardial infarct size and incidence of ventricular fibrillation in a

* Corresponding author. Department of Medicine, L8-01, Laboratory Block, FMB, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China. Tel.: +852 2819 9513; fax: +852 2855 9730.
E-mail address: grli@hkucc.hku.hk (G.-R. Li).

0014-2999/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2007.01.072

canine model of ischemia/reperfusion (Ogita et al., 2002), and prevented cardiac hypertrophy and dysfunction in pressure-overloaded mice (Ogita et al., 2004b). Raloxifene (1 μM) directly decreased $I_{Ca,L}$ and shortened action potential duration in guinea pig ventricular myocytes (Liew et al., 2004). However, it is unknown whether raloxifene affects human atrial repolarization K^+ currents. The present study was therefore designed to determine the effects of raloxifene and β-estradiol on I_{to1} and I_{Kur} in human atrial myocytes using a whole cell patch-clamp technique.

2. Materials and methods

2.1. Myocytes preparation

Atrial cells were isolated from specimens of human right atrial appendage obtained from patients (50.1±5.7 years old) undergoing coronary artery bypass grafting. The procedure for obtaining the human tissue was approved by the Ethics Committee of the University of Hong Kong based on the patients' consent. All patients were free from supraventricular tachyarrythmias, and the atria were grossly normal at the time of surgery. After excision, the samples were then quickly immersed in oxygenated, normally Ca^{2+}-free cardioplegic solution (~4 °C) for transport to the laboratory. Atrial myocytes were enzymatically dissociated as described previously (Du et al., 2003). Briefly, the atrial tissue was minced, and gently agitated by continuous bubbling with 100% O_2 in a Ca^{2+}-free Tyrode solution for 15 min (5 min at a time in fresh solutions), and then incubated for 50 min in a similar solution containing 150–200 U/ml collagenase (CLS II, Worthington Biochemical, Freehold, NJ, USA), 0.2 mg/ml protease (type XXIV, Sigma-Aldrich Chemical, St Louis, MO, USA) and 1 mg/ml bovine serum albumin (Sigma-Aldrich). Afterwards, the chunks were re-incubated in a fresh enzyme solution of the same composition but no protease. The quantity and quality of isolated cells were monitored under a microscope (E200, Nikon, Japan). When the cell yield appeared optimal, the chunks were suspended in a high K^+ medium containing (mM): 10 KCl, 120 K-glutamate, 10 KH_2PO_4, 1.8 $MgSO_4$, 10 taurine, 10 HEPES, 0.5 EGTA, 20 glucose, 10 mannitol, at pH adjusted to 7.3 with KOH, and gently blown with a pipette. The isolated myocytes were kept at room temperature in the medium at least 1 h before use.

A small aliquot of the solution containing the isolated cells was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope (IX50, Olympus, Japan). Myocytes were allowed to adhere to the bottom of the chamber for 5–10 min and were then superfused at 2–3 ml/min with Tyrode solution. Only quiescent rod-shaped cells with clear cross-striations were used. The experiments were conducted at room temperature (21–22 °C) for current recording, and at 36 °C for action potential recording.

2.2. Data acquisition and analysis

The whole-cell patch-clamp technique was used for electrophysiological recording. Borosilicate glass electrodes (1.2-mm OD) were pulled with a Brown-Flamming puller (model P-97, Sutter Instrument Co, Novato, CA, USA) and had tip resistances of 2–3 MΩ when filled with pipette solution. Membrane currents were recorded in voltage-clamp mode using an EPC-9 amplifier and Pulse software (HEKA, Lambrecht, Germany). A 3-M KCl-agar salt bridge was used as reference electrode. Tip potentials were zeroed before the pipette touched the cell. After a giga-ohm seal was obtained, the cell membrane was ruptured by gentle suction to establish the whole-cell configuration. The cell membrane capacitance (C_m, 77.8±3.4 pF, n=52) was directly measured using the lock-in module of the Pulse software, and used for normalizing the current in individual cells. The series resistance (R_s, 3.9±0.4 MΩ, n=52) was compensated by 50–70% to minimize voltage errors. Current signals were low-pass filtered at 5 kHz and stored on the hard disk of an IBM compatible computer.

Nonlinear curve fitting was performed using Pulsefit (HEKA) and Sigmaplot (SPSS, Chicago, IL). Paired and/or unpaired Student's t-test was used as appropriate to evaluate the statistical significance of differences between two group means, and ANOVA was used for multiple groups. Values of $P<0.05$ were considered to indicate statistical significance. Results are presented as mean±S.E.M.

2.3. Solutions and drugs

The Ca^{2+}-free cardioplegic solution for specimen transport contained (in mM) 50 KH_2PO_4, 8 $MgSO_4$, 5 adenosine, 10 HEPES, 140 glucose, 100 mannitol, 10 taurine, pH was adjusted to 7.3 with KOH. Tyrode solution contained (in mM) 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 0.33 NaH_2PO_4, 5 HEPES, 10 glucose, pH was adjusted to 7.4 with NaOH. The pipette solution contained (in mM) 20 KCl, 110 K-aspartate, 1 $MgCl_2$, 10 HEPES, 5 EGTA, 0.1 GTP, 5 Na_2-phosphocreatine,

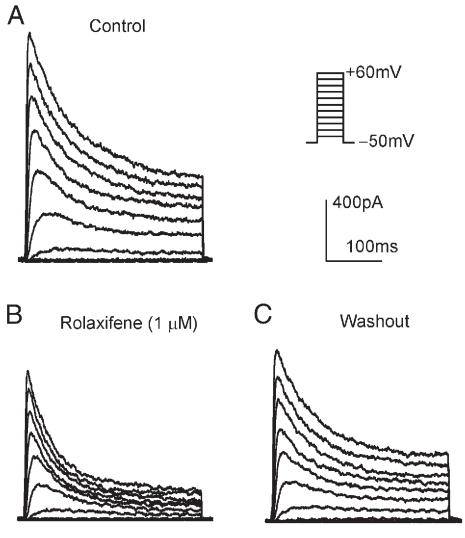

Fig. 1. Effects of raloxifene on membrane current in a representative human atrial myocyte (C_m: 65 pF). A. Membrane currents were recorded using 300-ms voltage pulses from −40 to +60 mV at a holding potential of −50 mV during control. B. Both transient outward (I_{to1}) and sustained (I_{Kur}) K^+ currents were decreased by 1 μM raloxifene. C. I_{to1} and I_{Kur} partially recovered on drug washout.

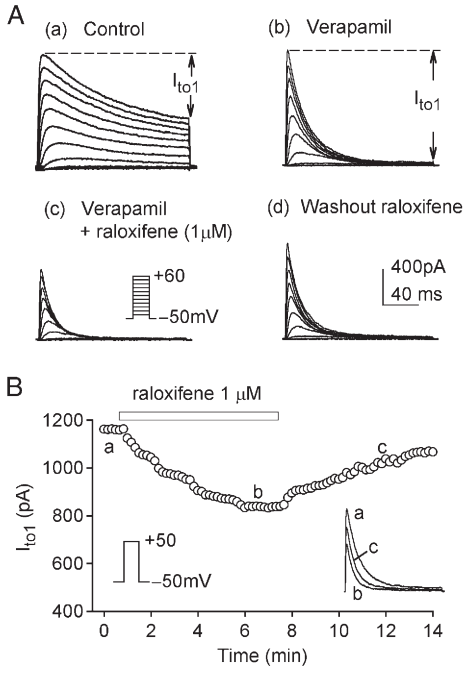

Fig. 2. Effect of raloxifene on I_{to1}. A. Membrane currents recorded in a typical experiment (C_m: 87 pF) with the voltage protocol shown in the *inset* during control (a), after application of 10 μM verapamil to inhibit I_{Kur} (b), co-application of verapamil and 1 μM raloxifene (c), and washout of raloxifene (d). B. Time course of I_{to1} recorded in a representative cell (C_m: 75 pF) pretreated with 10 μM verapamil. Raloxifene gradually inhibited I_{to1}, and reached a steady-state level within 6 min. The effect was significantly reversed by washout. The original I_{to1} traces at corresponding time points are shown in the right *inset*.

and 5 Mg_2-ATP, pH was adjusted to 7.2 with KOH. For I_{to1} and I_{Kur} recording, $BaCl_2$ (200 μM) and $CdCl_2$ (200 μM) were added to the superfusion to block I_{K1} and $I_{Ca.L}$. Atropine (1.0 μM) was used to minimize possible $I_{K,Ach}$ contamination during the current recording. Verapamil (10 μM) was added to inhibit I_{Kur} when recording I_{to1} (Gao et al., 2004). Raloxifene (Sigma-Aldrich), 17β-estradiol (Sigma-Aldrich), and ICI 182780 (7α,17b-[9[(4,4,5,5,5-Pentafluoropentyl)sulfinyl] nonyl]estra-1,3,5(10)-triene-3,17-diol) (Tocris, Bristol, UK) were dissolved in dimethyl sulphoxide (DMSO) with a stock solution of 100 mM.

3. Results

3.1. Effects of raloxifene on I_{to1}

Fig. 1 shows the voltage-dependent membrane currents (capacitance was compensated) recorded in a human atrial myocyte with the voltage protocol shown in the *inset* at 0.2 Hz in control, in the presence of raloxifene, and after raloxifene washout. The transient outward K^+ current I_{to1} was inhibited by 1 μM raloxifene, and the inhibitory effect was partially reversed by washout. The sustained current (*i.e.* I_{Kur}) was also reduced as I_{to1} was inhibited by raloxifene. Verapamil was found to inhibit I_{Kur} without reducing the I_{to1} amplitude in human atrial myocytes (Gao et al., 2004). Therefore, verapamil at 10 μM was used to separate I_{to1} from I_{Kur} as described below.

Fig. 2A displays voltage-dependent I_{to1} traces recorded with the voltage protocol shown in the *inset* during control, in the presence of 10 μM verapamil, co-presence of verapamil and 1 μM raloxifene, and washout of raloxifene. Amplitude of I_{to1} was actually increased by application of verapamil to inhibit I_{Kur} as described previously (Gao et al., 2004). Raloxifene remarkably suppressed I_{to1}, and the effect was partially reversed by washout. Fig. 2B displays the time course of I_{to1} in the presence of 10 μM verapamil. I_{to1} amplitude measured from peak to 'quasi'-steady-state level was gradually decreased by 1 μM raloxifene, and the effect reached a steady-state level within 6 min, and partially recovered upon washout. The original I_{to1} traces at corresponding time points are shown in the right *inset* of the panel. I_{to1} was inhibited by 41% with 1 μM raloxifene ($n=7$, $P<0.01$ vs control).

The effects of raloxifene on current–voltage (I–V) relationships of I_{to1} density were studied in seven cells at concentrations of 0.3, 1, 3, and 10 μM (Fig. 3A). Raloxifene inhibited I_{to1} in a concentration-dependent manner. Raloxifene at 0.3 to 10 μM suppressed I_{to1} at test potentials of 0 mV to +60 mV ($n=7$, $P<0.05$ or $P<0.01$ vs control, respectively), and the effect was partially reversed by washout. No significant voltage-dependence was observed with any concentration of raloxifene

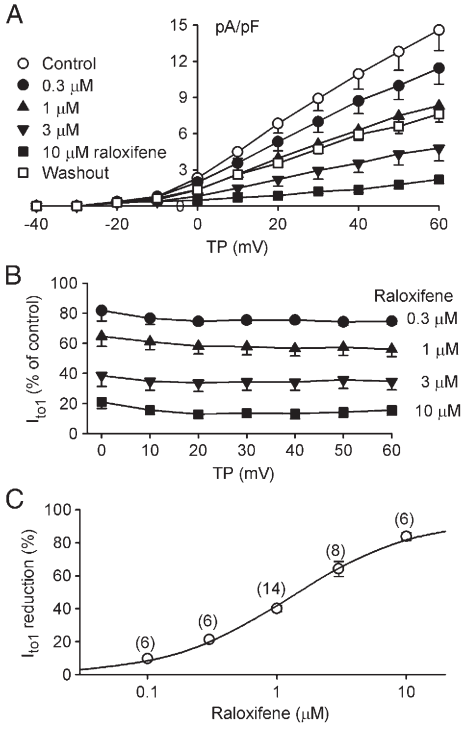

Fig. 3. Concentration-dependent effects of raloxifene on I_{to1}. A. I–V relationships of I_{to1} in cells pretreated with 10 μM verapamil (control) and in the co-presence of 0.3, 1, 3, and 10 μM raloxifene. Raloxifene inhibited I_{to1} in a concentration-dependent manner, and the effect recovered by 51% on washout (10 min). B. Percent reduction of I_{to1} at 0 to +60 mV by raloxifene at 0.3–10 μM. Raloxifene significantly inhibited I_{to1} at concentrations from 0.3 to 1, 3, and 10 μM ($n=7$, $P<0.01$ vs control). No significant voltage-dependence was observed for the drug effect at 0.3–10 μM. C. Concentration-response relationship for inhibiting I_{to1} by raloxifene. Symbols are mean experimental data at +50 mV. Solid line is the best-fit of the Hill equation.

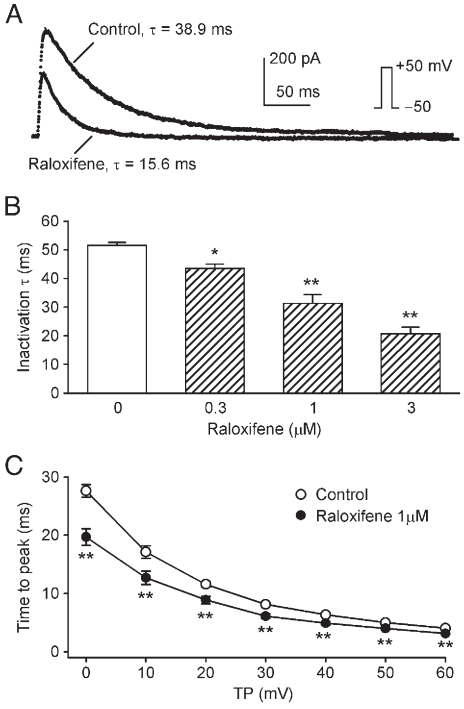

Fig. 4. Effects of raloxifene on time-dependent kinetics of I_{to1}. A. I_{to1} tracing from a representative cell (C_m: 78 pF) upon 300-ms voltage pulse from −50 to +50 mV at a holding potential of −50 mV during control (with pretreatment of 10 μM verapamil), and in the co-presence of 1 μM raloxifene. Inactivation raw data (points) during control and in the presence of raloxifene were fitted to a mono-exponential function (solid lines) with time constants shown. B. Mean values of time constants of I_{to1} inactivation in the absence and presence of 0.3, 1, and 3 μM raloxifene. Raloxifene accelerated I_{to1} inactivation as the concentration increased (n=6, *P<0.05, **P<0.01 vs control). C. The time to peak of I_{to1} activation at 0 to +60 mV under control conditions and in the presence of 1 μM raloxifene. Raloxifene reduced I_{to1} activation time course (n=6, **P<0.01 vs control). The statistical significance was analyzed by the repeated measures ANOVA.

(Fig. 3B). Concentration-response relationship for inhibiting I_{to1} by raloxifene was evaluated at +50 mV (Fig. 3C). Data were fitted to the Hill equation: $E=E_{max}/[1+(IC_{50}/C)^b]$, where E is the inhibition of I_{to1} in percentage at concentration C, E_{max} is the maximum inhibition, IC_{50} is the concentration for a half-maximum action, and b is the Hill coefficient. The IC_{50} of inhibiting I_{to1} by raloxifene was 0.9 μM, Hill coefficient was 1.2, and E_{max} was 83.8%.

Time-dependent kinetics of I_{to1} was determined in the presence of 10 μM verapamil (to inhibit I_{Kur}) plus raloxifene. Fig. 4A shows I_{to1} traces recorded in a representative cell with the voltage protocol shown in the *inset* before and after application of raloxifene. Raw data of I_{to1} (points) were well fitted to a mono-exponential function (solid line) during control, and in the presence of 1 μM raloxifene with time constants shown. The inactivation time constant was remarkably reduced by 1 μM raloxifene. The averaged time constants observed in six cells during control and after application of 0.3, 1, and 3 μM raloxifene are shown in Fig. 4B. Raloxifene significantly accelerated I_{to1} inactivation in a concentration-dependent manner. Fig. 4C shows the time to peak of I_{to1} activation determined from the onset of depolarization to the current peak. Raloxifene at 1 μM reduced the time to peak of I_{to1} at 0 to +60 mV (n=6, P<0.01 vs control).

Voltage dependence of I_{to1} activation and inactivation was evaluated in the absence and presence of 1 μM raloxifene. The variable (g) of voltage-dependent activation was determined from I–V relationships for each cell from Fig. 3A during control and in the presence of 1 μM raloxifene, based on the formulation $g=I/(V_t-V_r)$, where I is the peak current at test potential (V_t), and V_r is the measured reversal potential (around −70 mV). The variable (I/I_{max}) for steady-state inactivation of I_{to1} was determined with the protocol as illustrated in the *inset* of Fig. 5A. Mean data values of normalized activation and inactivation were fitted to the Boltzmann distribution to obtain half activation or inactivation voltage ($V_{0.5}$) and slope factor (S) of the current during control, and in the presence of 1 μM raloxifene are shown in Fig. 5B. The voltage dependence of I_{to1} activation and inactivation was not affected by the application of raloxifene.

Time-dependent recovery of I_{to1} from inactivation was studied using a paired-pulse protocol by varying P1–P2 interval

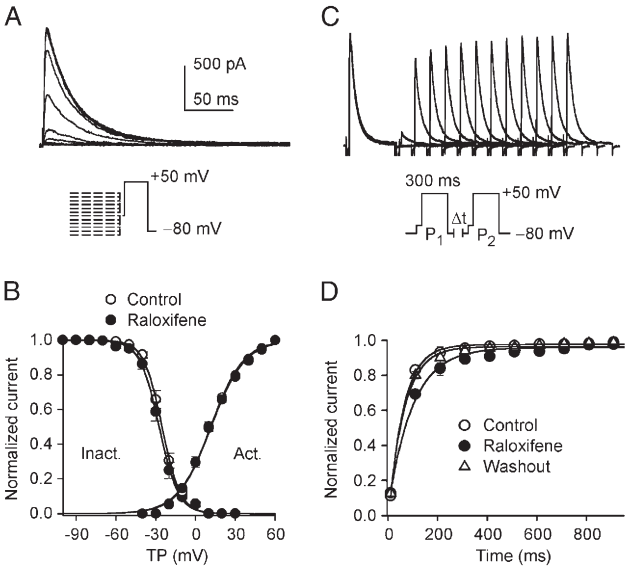

Fig. 5. Effects of raloxifene on voltage-dependence of activation and inactivation of I_{to1}, and recovery of I_{to1} from inactivation. A. Representative current traces and voltage protocol (1-s conditioning pulses from −100 to +30 mV at a holding potential of −80 mV, followed by a 300-ms test pulse to +50 mV after a 30-ms interval at −40 mV) used to evaluate steady-state inactivation of I_{to1} recorded in a human atrial cell (C_m: 67 pF) in the presence of 10 μM verapamil. B. Voltage dependent variables for I_{to1} activation (Act.) and inactivation (Inact.) were fitted to the Boltzmann distribution: $y=1/\{1+\exp[(V_m-V_{0.5})/S]$, where V_m is membrane potential, $V_{0.5}$ is the midpoint, and S is slope. For activation, $V_{0.5}$ and S were 11.1±1.2 and −12.5±0.4 mV in control, and 11.4±0.8 and −13.1±0.5 mV after 1 μM raloxifene (n=8, P=NS). For inactivation, $V_{0.5}$ and S were −25.8±1.8 and 6.6±0.2 mV during control, and −27.4±1.4 and 7.0±0.5 mV after 1 μM raloxifene (n=6, P=NS). C. Representative current traces recorded in a typical experiment (C_m: 81 pF) in the presence of 10 μM verapamil using 300-ms paired pulses to +50 mV after a 30-ms step of −40 mV (to inactivate I_{Na}) from −80 mV with varying P1 and P2 interval (*inset*), which are used for assessing time-dependent recovery of I_{to1} from inactivation. D. Mean data for time course of recovery of I_{to1} from inactivation in the absence and presence of 1 μM raloxifene in six cells. Data were best fitted to a mono-exponential function.

as shown in the *inset* of Fig. 5C. The recovery curves were fitted to a monoexponential function in the absence and presence of raloxifene (Fig. 5D). Recovery time constant was 66.4±9.5 ms in control, 105.0±12.4 ms in the presence of 1 μM raloxifene (n=6, P<0.05), and 69.3±7.5 ms after washout. The result suggests that recovery of I_{to1} from inactivation is reversibly slowed by raloxifene.

3.2. Effects of raloxifene on I_{Kur}

Fig. 6A displays voltage-dependent I_{Kur} traces recorded in a representative cell with voltage protocol shown in the *inset.* The current was rapidly activated by depolarization potentials with a small tail current at −30 mV. Raloxifene at 1 μM substantially inhibited both I_{Kur} and tail currents, and the effect partially recovered on washout. At test potential of +50 mV, I_{Kur} was reduced by 57.3±3.3% with 1 μM raloxifene (n=10, P<0.01 vs control). Fig. 6B shows the time-course of I_{Kur} recorded in a representative cell with the voltage protocol shown in the left *inset*. I_{Kur} measured from the zero level to the current at end of voltage step was gradually inhibited by raloxifene, and the effect partially recovered upon washout. The original I_{Kur} traces at corresponding time points are shown in the right *inset* of the panel.

Fig. 7A displays the *I–V* relationships of I_{Kur} in the absence and presence of 0.3, 1, 3, and 10 μM raloxifene in six cells.

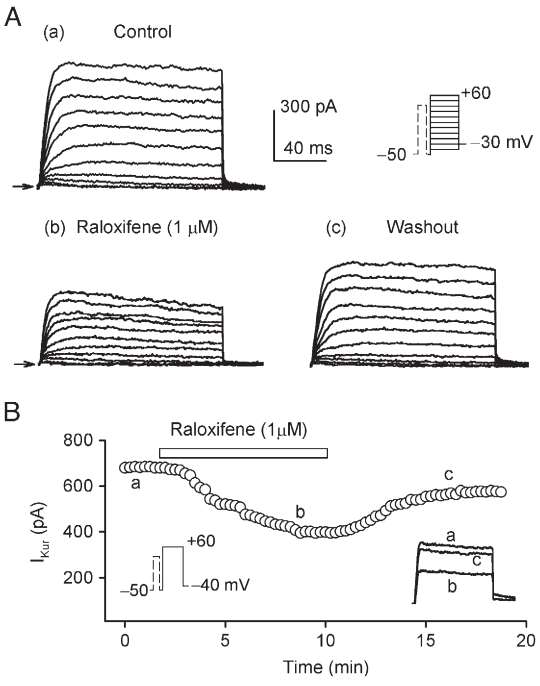

Fig. 6. Effects of raloxifene on I_{Kur}. A. Voltage-dependent I_{Kur} traces recorded in a representative cell (C_m: 85 pF) with the voltage protocol shown in the *inset*, a 100-ms prepulse to +40 mV to partially inactivate I_{to1}, followed by 150-ms test pulses from −40 to +60 mV at a holding potential of −50 mV after a 10-ms interval, then to −30 mV. I_{Kur} was reversibly inhibited by 1 μM raloxifene. B. Time-course of I_{Kur} recorded in a typical experiment (C_m: 78 pF) in the absence and presence of 1 μM raloxifene. The original I_{Kur} traces at corresponding time points are shown in the right *inset* of the panel.

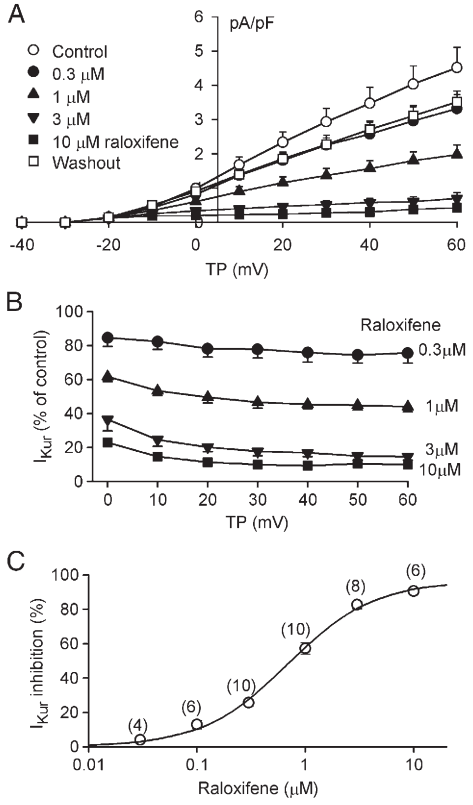

Fig. 7. Concentration-dependence of raloxifene on I_{Kur}. A. *I–V* relationships of I_{Kur} during control, in the presence of 0.3, 1, 3, and 10 μM raloxifene. Raloxifene inhibited I_{Kur} in a concentration-dependent manner, and the effect recovered by 78% (at +50 mV) on washout (10 min). B. Percent reduction of I_{Kur} at 0 to +60 mV by 0.3–10 μM raloxifene. Raloxifene significantly inhibited I_{Kur} at concentrations from 0.3 to 1, 3, and 10 μM (n=6, P<0.05 of P<0.01 vs control at all potentials). Significant voltage-dependence was observed for the drug effect at 1–10 μM, and stronger effect was observed at potentials positive to +10 and +60 mV (P<0.05 or P<0.01 vs 0 mV). C. Concentration response-relationship for inhibiting I_{Kur} (at +50 mV) by raloxifene.

Raloxifene inhibited I_{Kur} in a concentration-dependent manner, and the effect was partially reversed by washout. The inhibition showed significant voltage-dependence with 1–10 μM raloxifene (Fig. 7B), and stronger effect was observed at potentials positive to +10 mV (n=6, P<0.05 or P<0.01 vs 0 mV). Fig. 7C shows the concentration-response relationship of raloxifene. The IC_{50} (at +50 mV) of raloxifene for inhibiting I_{Kur} was 0.7 μM, Hill coefficient was 1.1, and E_{max} was 90.6%.

3.3. Effects of estrogen on I_{to1} and I_{Kur}

To study whether estrogen would have similar inhibitory effects on I_{Kur} and I_{to1}, β-estradiol was tested. β-estradiol at 100 μM showed an inhibitory effect on I_{to1}, but a little effect on I_{Kur} in a representative human atrial myocyte (Fig. 8A). We therefore analysed the effects of β-estradiol on I_{to1}. Voltage-dependent I_{to1} recorded in the presence of 10 μM verapamil was reversibly decreased by application of 10 μM β-estradiol (Fig. 8B). At test potential of +50 mV, 10 μM β-estradiol decreased I_{to1} by 29.4±3.2% (n=7, P<0.01 vs control). Fig. 8C shows time-dependent effect of β-estradiol on I_{to1}. β-estradiol decreased

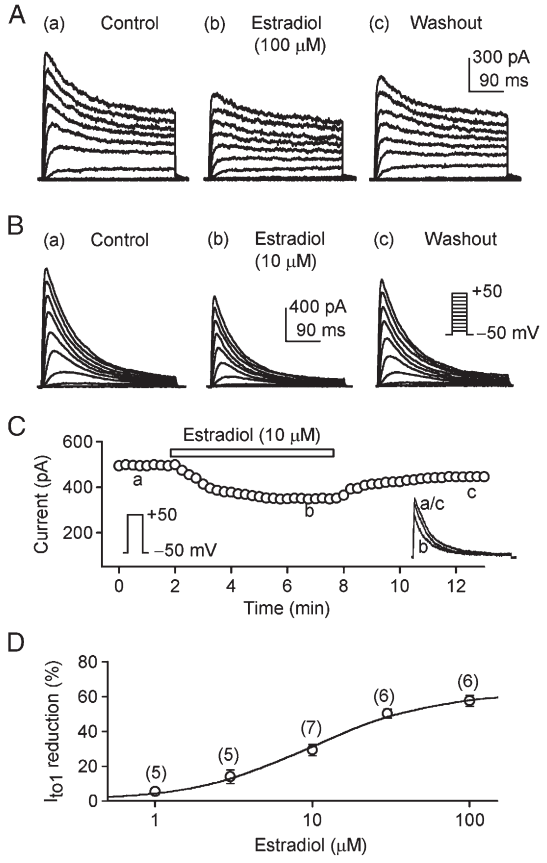

Fig. 8. Effects of β-estradiol on I_{to1} and I_{Kur}. A. Membrane currents were recorded with the voltage protocol shown in B.c during control (a), 100 μM β-estradiol (b), and washout (c). β-estradiol at 100 μM showed a slight reduction of I_{Kur}, and a substantial inhibition of I_{to1} in a typical experiment (C_m: 68 pF). B. I_{to1} recorded in a human atrial moycyte (C_m: 74 pF) in the presence of 10 μM verapamil during control (a), 10 μM β-estradiol co-administration (b), and washout of β-estradiol (c). β-estradiol reversibly inhibited I_{to1}. C. Time course of I_{to1} recorded in a human atrial cell (C_m: 81 pF) by a voltage pulse from −50 to +50 mV at a holding potential of −50 mV in the absence and presence of 10 μM β-estradiol. Original I_{to1} traces at corresponding time points are shown in the right of the panel. D. Concentration-response relationship of inhibiting I_{to1} (at +50 mV) by β-estradiol.

I_{to1} in a concentration-dependent manner (Fig. 8D), and the IC_{50} was 10.3 μM, Hill coefficient was 1.1, and E_{max} was 57.6%.

The effects of β-estradiol on voltage-dependence and recovery of I_{to1} were determined in different cells. β-estradiol at 10 μM, like raloxifene, had no effect on voltage-dependence of steady-state activation and inactivation of I_{to1}. The $V_{0.5}$ of activation was 13.2±1.9 mV in control, and 14.8±2.0 mV for β-estradiol (n=5, P=NS). The $V_{0.5}$ for inactivation of I_{to1} was −24.5±1.2 mV and −25.2±1.1 mV for control and 17β-estradiol (n=5, P=NS). In addition, the recovery time constant of I_{to1} was not altered by β-estradiol (69.8±12.3 and 71.2±10.5 ms before and after β-estradiol, n=5, P=NS). The inactivation time constant of I_{to1} (at +50 mV) was reduced by application of 10 μM β-estradiol (53.1±7.4 to 41.7±7.5 ms of control, n=5, P<0.05), but the time to peak of I_{to1} at +50 mV was not significantly affected (3.7±0.6 ms vs 3.3±0.7 ms of control, n=5, P=NS) by β-estradiol.

3.4. The effects of ICI 182,780

To test whether the raloxifene- or β-estradiol-induced inhibition of I_{Kur} and/or I_{to1} was mediated by estrogen receptors, we treated the cells with 10 μM ICI 182,780, a classical estrogen receptor antagonist. This concentration effectively blocked estrogen receptor activation and intracellular signaling (Mori-Abe et al., 2003) and raloxifene-induced endothelium-dependent relaxation in rabbit coronary arteries (Figtree et al., 1999). The cells were incubated with 10 μM ICI 182,780 for at least 1 h, and then the effects of raloxifene on I_{Kur} and/or I_{to1} were determined. After the pretreatment of ICI 182,780, I_{to1} at +50 mV was decreased by 38.8±3.5% with 1 μM raloxifene (n=5, P=NS vs raloxifene alone). The incubation with 10 μM ICI 182,780 did not affect the inhibitory effect of 1 μM raloxifene on I_{Kur}, either (n=5, 52.6±4.5%, P=NS vs raloxifene alone), suggesting that the reduction of I_{Kur} and/or I_{to1} by raloxifene is not mediated by estrogen receptor. Similarly, the inhibition of I_{to1} by β-estradiol was not affected by ICI 182,780 (data not shown).

3.5. Raloxifene on human atrial action potentials

The effect of raloxifene on cardiac action potential duration was determined in human atrial myocytes with a perforated configuration (Li et al., 2002) at 36 °C. Fig. 9A displays action potential traces recorded in a representative cells at 2 Hz. Raloxifene at 1 μM reversibly prolonged action potential duration. Action potential duration at 50% and 90% repolarization were significantly increased by the application of raloxifene (Fig. 9B, n=6, P<0.01). In addition, we measured action potential duration at 50% and 90% repolarization at

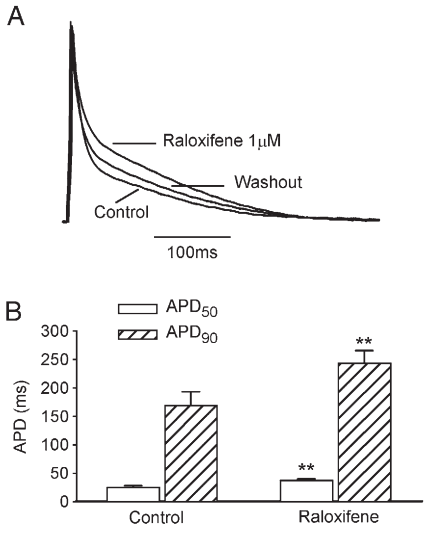

Fig. 9. Effect of raloxifene on cardiac action potential. A. Action potentials recorded in a representative human atrial myocyte with perforated patch configuration at 2 Hz in the absence and presence of 1 μM raloxifene. B. Mean values of human atrial action potential duration at 50% and 90% repolarization (APD_{50} and APD_{90}) during control and after application of 1 μM raloxifene for 5 min (n=6, P<0.01 vs control).

frequencies of 0.5, 1 and 2 Hz in four cells in the absence and presence of 1 μM raloxifene. The percent increases of action potential duration at 50% and 90% repolarization were 47.0±4.2 and 45.3±6.4%, 49.2±10.5 and 46.4±9.4%, and 46.8±6.3 and 44.8±7.4% (P=NS) respectively at 0.5, 1, and 2 Hz, suggesting that there is no reverse frequency dependence of action potential with raloxifene. Moreover, no instability of action potential was observed in all the cells studied.

4. Discussion

The present study demonstrated for the first time that the selective estrogen receptor modulator raloxifene blocks human atrial native I_{to1} and I_{Kur} in a concentration-dependent manner, while β-estradiol has no significant effect on I_{Kur}, and shows a relatively weak inhibitory effect on I_{to1}. These effects are unaffected by the estrogen receptor antagonist ICI 182,780.

Raloxifene possesses cardiovascular protective properties (Wenger, 2002). However, potential mechanisms of cardiovascular effects are not fully understood. Previous studies showed that the cardiovascular protection of raloxifene was related to the reduction of low-density lipoprotein-2 cholesterol and increase of high-density lipoprotein-2 cholesterol (Walsh et al., 1998), and the vascular dilation in estrogen receptor-dependent and independent pathways (Simoncini et al., 2002; Simoncini and Genazzani, 2000; Figtree et al., 1999). In addition, raloxifene was found to have direct cardioprotection against myocardial injury and ventricular fibrillation via intra-coronary infusion (1 μM in coronary blood) in a canine model of ischemia/reperfusion (Ogita et al., 2002, 2004a), and to prevent cardiac hypertrophy and dysfunction in pressure-overloaded mice (Ogita et al., 2004b).

A recent report demonstrated that raloxifene at 1 μM directly inhibited the contractility by blocking $I_{Ca.L}$ in guinea pig ventricular myocytes (Liew et al., 2004), and the inhibition of $I_{Ca.L}$ in cardiac myocytes is very close to that of $I_{Ca.L}$ (IC_{50}=1 μM) in rat cerebral artery cells (Tsang et al., 2004). In addition, a more recent study demonstrated that raloxifene at 0.3–10 μM exhibited a substantial relaxation of porcine coronary artery contraction induced by high KCl, prostaglandin, Bay K 8644, etc. The effect was related to the inhibition of voltage-gated and receptor-operated L-type Ca^{2+} channels, and was independent of endothelial and ICI 182,780-sensitive estrogen receptors (Moritz et al., 2006).

The present study provided additional information that raloxifene directly blocks both I_{to1} and I_{Kur} in human atrial myocytes. I_{to1} contributes importantly to repolarization in human heart (Li et al., 1995, 1998). Raloxifene inhibited I_{to1} in human atrial myocytes (Figs. 1 and 2) with an IC_{50} of 0.9 μM, slowed the recovery of I_{to1} from inactivation, but had no effect on the voltage dependence of activation and inactivation of the current (Fig. 5). The drug significantly accelerated inactivation processes, and reduced the time to peak of the current (Fig. 4), suggesting involvement of open channel blocking action (Dukes et al., 1990; Feng et al., 1997). β-estradiol, however, had a relatively weak inhibitory effect on I_{to1} in human atrial myocytes (Fig. 8). The selective estrogen receptor antagonist ICI 182,780 did not affect the inhibitory effects of I_{to1} by raloxifene or β-estradiol, suggesting these effects are non-genomic and/or independent of estrogen receptor.

I_{Kur} is found to be functionally expressed in human atrium but not in ventricle (Li et al., 1996). Therefore, the drugs that specially inhibit the unique I_{Kur} may provide a means of preventing atrial fibrillation without the risk of ventricular proarrhythmia (Nattel, 2002). In the present study, we found that raloxifene inhibited I_{Kur} with an IC_{50} of 0.7 μM. The significant effect of raloxifene on I_{Kur} started from the concentration of 0.1 μM; however, β-estradiol had no effect on I_{Kur}. The inhibitory effect was not affected by the selective estrogen receptor antagonist ICI 182,780, suggesting a direct blocking effect on I_{Kur} in human atrial myocytes. Suppression of I_{Kur} and I_{to1} is useful for anti-atrial fibrillation (Nattel, 2002). Raloxifene inhibited both I_{Kur} and I_{to1}, and prolonged action potential duration (Fig. 9) in human atrial myocytes, suggesting a possible beneficial action on supraventricular arrhythmias.

Because there is no specific I_{Kur} available to separate I_{to1} from I_{Kur}, it is always a weakness when I_{to1} and/or I_{Kur} are studied in native cells expressing the two types of channel currents. The present study used verapamil to separate I_{to1} from I_{Kur}; however, verapamil at 10 μM, although this concentration inhibited I_{Kur} without affecting I_{to1} (Gao et al., 2004), could not fully inhibit I_{Kur}. It should be noted that a small portion of remaining I_{Kur} might still contaminate the measured I_{to1}, which may underestimate the effect of raloxifene on I_{to1}.

In summary, the present study demonstrates the novel information that raloxifene directly inhibits I_{to1} and I_{Kur}, and prolongs action potential duration in human atrial myocytes. These results suggest that raloxifene may be beneficial for supraventricular arrhythmias in humans, which remains to be studied in the future.

Acknowledgement

The study was supported by a grant from Sun Chieh Yeh Heart Foundation of Hong Kong and a grant (CUHK4362/04) from Research Grant Council of Hong Kong.

References

Courtemanche, M., Ramirez, R.J., Nattel, S., 1998. Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model. Am. J. Physiol. 275, H301–H321.

Courtemanche, M., Ramirez, R.J., Nattel, S., 1999. Ionic targets for drug therapy and atrial fibrillation-induced electrical remodeling: insights from a mathematical model. Cardiovasc. Res. 42, 477–489.

Du, X.L., Lau, C.P., Chiu, S.W., Tse, H.F., Gerlach, U., Li, G.R., 2003. Effects of chromanol 293B on transient outward and ultra-rapid delayed rectifier potassium currents in human atrial myocytes. J. Mol. Cell. Cardiol. 35, 293–300.

Dukes, I.D., Cleemann, L., Morad, M., 1990. Tedisamil blocks the transient and delayed rectifier K+ currents in mammalian cardiac and glial cells. J. Pharmacol. Exp. Ther. 254, 560–569.

Feng, J., Wang, Z., Li, G.R., Nattel, S., 1997. Effects of class III antiarrhythmic drugs on transient outward and ultra-rapid delayed rectifier currents in human atrial myocytes. J. Pharmacol. Exp. Ther. 281, 384–392.

Figtree, G.A., Lu, Y., Webb, C.M., Collins, P., 1999. Raloxifene acutely relaxes rabbit coronary arteries in vitro by an estrogen receptor-dependent and nitric oxide-dependent mechanism. Circulation 100, 1095–1101.

Gao, Z., Lau, C.P., Chiu, S.W., Li, G.R., 2004. Inhibition of ultra-rapid delayed rectifier K^+ current by verapamil in human atrial myocytes. J. Mol. Cell. Cardiol. 36, 257–26.

Li, G.R., Feng, J., Wang, Z., Fermini, B., Nattel, S., 1995. Comparative mechanisms of 4-aminopyridine-resistant I_{to} in human and rabbit atrial myocytes. Am. J. Physiol. 269, H463–H472.

Li, G.R., Feng, J., Yue, L., Carrier, M., Nattel, S., 1996. Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. Circ. Res. 78, 689–696.

Li, G.R., Feng, J., Yue, L., Carrier, M., 1998. Transmural heterogeneity of action potentials and Ito1 in myocytes isolated from the human right ventricle. Am. J. Physiol. 275, H369–H377.

Li, G.R., Zhang, M., Satin, L.S., Baumgarten, C.M., 2002. Biphasic effects of cell volume on excitation-contraction coupling in rabbit ventricular myocytes. Am. J. Physiol. 282, H1270–H1277.

Liew, R., Stagg, M.A., MacLeod, K.T., Collins, P., 2004. Raloxifene acutely suppresses ventricular myocyte contractility through inhibition of the L-type calcium current. Br. J. Pharmacol. 142, 89–96.

Mori-Abe, A., Tsutsumi, S., Takahashi, K., Toya, M., Yoshida, M., Du, B., Kawagoe, J., Nakahara, K., Takahashi, T., Ohmichi, M., Kurachi, H., 2003. Estrogen and raloxifene induce apoptosis by activating p38 mitogen-activated protein kinase cascade in synthetic vascular smooth muscle cells. J. Endocrinol. 178, 417–426.

Moritz, A., Radtke, O.A., Gust, R., Glusa, E., Pertz, H.H., 2006. Characterisation of the relaxant response to raloxifene in porcine coronary arteries. Eur. J. Pharmacol. 545, 153–160.

Nattel, S., 2002. New ideas about atrial fibrillation 50 years on. Nature 415, 219–226.

Nattel, S., Yue, L., Wang, Z., 1999. Cardiac ultrarapid delayed rectifiers: a novel potassium current family of functional similarity and molecular diversity. Cell. Physiol. Biochem. 9, 217–226.

Ogita, H., Node, K., Asanuma, H., Sanada, S., Takashima, S., Asakura, M., Kitakaze, M., Hori, M., 2002. Amelioration of ischemia-and reperfusion-induced myocardial injury by the selective estrogen receptor modulator, raloxifene, in the canine heart. J. Cardiol. 39, 55–56.

Ogita, H., Node, K., Asanuma, H., Sanada, S., Kim, J., Takashima, S., Minamino, T., Hori, M., Kitakaze, M., 2004a. Raloxifene improves coronary perfusion, cardiac contractility, and myocardial metabolism in the ischemic heart: role of phosphatidylinositol 3-kinase/Akt pathway. J. Cardiovasc. Pharmacol. 43, 821–829.

Ogita, H., Node, K., Liao, Y., Ishikura, F., Beppu, S., Asanuma, H., Sanada, S., Takashima, S., Minamino, T., Hori, M., Kitakaze, M., 2004b. Raloxifene prevents cardiac hypertrophy and dysfunction in pressure-overloaded mice. Hypertension 43, 237–242.

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J., 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288, 321–333.

Shibata, E.F., Drury, T., Refsum, H., Aldrete, V., Giles, W., 1989. Contributions of a transient outward current to repolarization in human atrium. Am. J. Physiol. 257, H1773–H1781.

Simoncini, T., Genazzani, A.R., 2000. Raloxifene acutely stimulates nitric oxide release from human endothelial cells via an activation of endothelial nitric oxide synthase. J. Clin. Endocrinol. Metab. 85, 2966–2969.

Simoncini, T., Genazzani, A.R., Liao, J.K., 2002. Nongenomic mechanisms of endothelial nitric oxide synthase activation by the selective estrogen receptor modulator raloxifene. Circulation 105, 1368–1373.

Tsang, S.Y., Yao, X., Essin, K., Wong, C.M., Chan, F.L., Gollasch, M., Huang, Y., 2004. Raloxifene relaxes rat cerebral arteries in vitro and inhibits L-type voltage-sensitive Ca^{2+} channels. Stroke 35, 1709–1714.

Van Wagoner, D.R., 2000. Pharmacologic relevance of K(+) channel remodeling in atrial fibrillation. J. Mol. Cell. Cardiol. 32, 1763–1766.

Van Wagoner, D.R., Nerbonne, J.M., 2000. Molecular basis of electrical remodeling in atrial fibrillation. J. Mol. Cell. Cardiol. 32, 1101–1117.

Walsh, B.W., Kuller, L.H., Wild, R.A., Paul, S., Farmer, M., Lawrence, J.B., Shah, A.S., Anderson, P.W., 1998. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. JAMA 279, 1445–1451.

Wenger, N.K., 2002. Cardiovascular effects of raloxifene: the potential for cardiovascular protection in women. Diabetes Obes. Metab. 4, 166–176.