

Escitalopram block of hERG potassium channels

Yun Ju Chae · Ji Hyun Jeon · Hong Joon Lee ·
In-Beom Kim · Jin-Sung Choi · Ki-Wug Sung ·
Sang June Hahn

Received: 11 April 2013 / Accepted: 21 August 2013 / Published online: 18 September 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Escitalopram, a selective serotonin reuptake inhibitor, is the pharmacologically active *S*-enantiomer of the racemic mixture of *RS*-citalopram and is widely used in the treatment of depression. The effects of escitalopram and citalopram on the human ether-a-go-go-related gene (hERG) channels expressed in human embryonic kidney cells were investigated using voltage-clamp and Western blot analyses. Both drugs blocked hERG currents in a concentration-dependent manner with an IC_{50} value of 2.6 μ M for escitalopram and an IC_{50} value of 3.2 μ M for citalopram. The blocking of hERG by escitalopram was voltage-dependent, with a steep increase across the voltage range of channel activation. However, voltage independence was observed over the full range of activation. The blocking by escitalopram was frequency dependent. A rapid application of escitalopram induced a rapid and reversible blocking of the tail current of hERG. The extent of the blocking by escitalopram during the depolarizing pulse was less than that during the repolarizing pulse, suggesting that

escitalopram has a high affinity for the open state of the hERG channel, with a relatively lower affinity for the inactivated state. Both escitalopram and citalopram produced a reduction of hERG channel protein trafficking to the plasma membrane but did not affect the short-term internalization of the hERG channel. These results suggest that escitalopram blocked hERG currents at a supratherapeutic concentration and that it did so by preferentially binding to both the open and the inactivated states of the channels and by inhibiting the trafficking of hERG channel protein to the plasma membrane.

Keywords hERG · Escitalopram · Citalopram · Channel trafficking

Introduction

Citalopram is a highly selective serotonin reuptake inhibitor (SSRI) that is widely used in the treatment of major depressive disorders (Hyttel 1982). The clinically used citalopram is a racemic mixture of the *R* and *S*-enantiomers (Sanchez 2006). Escitalopram, which also is an SSRI, is the pharmacologically active *S*-enantiomer of the racemic mixture of *RS*-citalopram (Garnock-Jones and McCormack 2010). Because the SSRI activity is more potent for escitalopram than that of the *R*-enantiomer, which is practically devoid of SSRI potency (Hyttel 1982), by comparison with citalopram, escitalopram has demonstrated better efficacy in the treatment of severe depression (Waugh and Goa 2003). Although SSRIs are generally reported to have a favorable cardiac safety profile, numerous studies have demonstrated that several SSRIs have been associated with cardiovascular side effects, such as arrhythmias, prolonged QT interval, and Torsade de Pointes (TdPs) arrhythmias (Pacher and Kecskemeti 2004). In support of this line of reasoning, electrophysiological studies have shown that SSRIs inhibit several cardiac voltage-gated K^+

Y. J. Chae · S. J. Hahn (✉)
Department of Physiology, Cell Death and Disease Research Center,
College of Medicine, The Catholic University of Korea,
224 Banpo-daero, Seocho-gu, Seoul 137-701, Korea
e-mail: sjhahn@catholic.ac.kr

J. H. Jeon · I.-B. Kim
Department of Anatomy, College of Medicine, The Catholic
University of Korea, Seoul 137-701, Korea

H. J. Lee · K.-W. Sung
Department of Pharmacology, Cell Death and Disease Research
Center, College of Medicine, The Catholic University of Korea,
Seoul 137-701, Korea

J.-S. Choi
College of Pharmacy, Integrated Research Institute of
Pharmaceutical, The Catholic University of Korea, 43-1 Yeokgok
2-dong, Wonmi-gu, Bucheon, Gyeonggi-do, Korea

(Kv) channels in different tissues. For example, citalopram and fluoxetine have produced a significant block of cloned Kv1.5 in stable cell lines (Perchenet et al. 2001; Lee et al. 2010). These drugs also have blocked the human ether-a-go-go-related gene (hERG) potassium channels that underlie the rapidly activating delayed rectifier Kv currents in cardiomyocytes (Thomas et al. 2002; Witchel et al. 2002). The blockade of hERG currents typically causes a prolongation of the action potential duration on the electrophysiology and the QT interval on the ECG, which can lead to TdPs and to sudden cardiac death (Vandenberg et al. 2001). An increasing number of case reports show a dose-dependent prolongation of the QT interval in patients receiving escitalopram and citalopram overdoses (Catalano et al. 2001; Engebretsen et al. 2003; Baranchuk et al. 2008; Fayssol et al. 2011). Escitalopram has been reported to prolong the corrected QT interval even in a low dose (Tseng et al. 2012). These results suggest that the blockade of hERG by these drugs could play an important role in the prolonged QT interval. To the best of our knowledge, however, there is no report regarding the effect of escitalopram on cardiac ion channels, in particular on hERG channels. Since escitalopram is structurally similar to citalopram, the present study further elucidates the effects of both enantiomers (escitalopram and citalopram) on hERG channels and compares their potencies.

Materials and methods

Cell cultures

The hERG-HEK293 recombinant cell line (CYL3039, Millipore, Billerica, MA, USA) was used for electrophysiological measurements. Cells were maintained in an environment that consisted of 95 % humidified air and 5 % CO₂ at 37°C in D-MEM/F-12 (Invitrogen, Grand Island, NY, USA) and were supplemented with 10 % fetal bovine serum, 1 % nonessential AA, and 400 µg/ml geneticin, according to the manufacturer's instructions. The cells used for electrophysiological recordings were seeded on glass coverslips (12 mm in diameter; Fisher Scientific, Pittsburgh, PA, USA) 24 h before use.

Electrophysiology

hERG currents were recorded using the whole-cell patch-clamp technique with an Axopatch 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). All experiments were carried out at room temperature (22–24°C). No leak subtraction was done during experiments. The recording chamber (RC-13; Warner Instruments, Hamden, CT, USA) was continually

perfused with an extracellular bath solution. Patch pipettes (PG10165-4, World Precision Instruments, Sarasota, FL, USA) had a resistance of 2 to 4 MΩ when filled with an internal pipette solution. The effective series resistances were usually compensated by 80 % when the current exceeded 1 nA. For rapid drug application, escitalopram was applied with a superfusion system using a piezoelectric-driven micromanipulator (P-287.70, Physik Instrumente, Waldbronn, Germany) as described previously (Choi et al. 2003). The internal pipette solution contained (in mM) 140 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 EGTA and was adjusted to pH 7.3 using KOH. The external bath solution contained (in mM) 140 NaCl, 5 KCl, 1.3 CaCl₂, 1 MgCl₂, 20 HEPES, and 10 glucose and was adjusted to pH 7.3 using NaOH. Escitalopram and citalopram (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA). The concentration of DMSO in the final dilution was less than 0.1 %. The tail currents of hERG were not significantly changed after 5 min of 0.1 % DMSO application (94.6 ± 0.9 % of the control, $n=9$).

Western blotting

Western immunoblot analyses were performed as described previously (Yang et al. 2013). hERG-HEK293 cells were incubated for 24 h with escitalopram and citalopram and with fluoxetine as a positive control. The cells were homogenized in ice-cold RIPA buffer (50 mM Tris buffer, pH 8.0; 150 mM NaCl; 1 % NP-40; 0.5 % deoxycholate; and 0.1 % SDS). Samples from each cell corresponding to 20 µg of total protein were separated by SDS-PAGE, and the proteins were blotted on a nitrocellulose membrane and probed with the anti-hERG antibody (dilution, 1:500; Alomone labs, Jerusalem, Israel). The membrane was rinsed three times and incubated for 2 h at room temperature in a 1:1,000 dilution of the appropriate biotin-conjugated IgG antibody (Vector Laboratories, Burlingame, CA, USA). Afterwards, the membrane was rinsed three times and incubated for 1 h at room temperature in ABC solution (Vector Laboratories). The blot was washed three times and the immunoreactive bands were detected using an Enhanced Chemiluminescence Detection Kit (Amersham, Arlington Heights, IL, USA).

Immunohistochemistry

The cells were grown for 4 h on poly-lysine-coated glass coverslips under control conditions or in the presence of 1, 3, 10, and 30 µM escitalopram or citalopram. After incubation, cells were washed with PBS and fixed in ice-cold 4 % formaldehyde and PBS for 30 min. After fixation, cells were incubated in 10 % normal donkey serum and incubated with the anti-hERG antibody (dilution, 1:200; Alomone labs) for

1 day at 4 °C. The cells were washed and incubated in the presence of secondary antibody conjugated with Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 2 h. The fluorescent specimens were mounted using Vectashield mounting medium (Vector Laboratories). Digital images (1,024×1,024 pixels) were acquired using a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss Co. Ltd., Germany) and were imported into Photoshop (Adobe Systems, San Jose, CA, USA). The brightness and contrast of the final images were adjusted.

Data analysis and statistics

Analysis of the data was performed using pClamp 10.0 software (Molecular Devices) and Origin 8.0 software (Microcal Software, Inc., Northampton, MA, USA). The results are expressed as means ± S.E. A paired Student's *t*-test for comparison between two groups and an analysis of variance for comparisons of multiple groups followed by Bonferroni test were used for the statistical analyses. A value of $P < 0.05$ was considered as statistically significant.

Results

Escitalopram and citalopram block hERG in a concentration-dependent manner

Figure 1 shows whole-cell recordings of hERG currents elicited by 2-s depolarizing pulses to +20 mV from a holding potential of −80 mV every 15 s in the absence and presence of escitalopram and citalopram. The concentration–response relationships were measured from the peak tail currents of hERG that were elicited by 4-s repolarizing pulses to −40 mV after each voltage step. After obtaining the control measurement, the concentration–response relationships for the block of hERG currents by escitalopram and citalopram were determined once the block reached a steady state, usually after 1 min of perfusion with the drug. Both escitalopram and citalopram inhibited the tail current of hERG at −40 mV in a concentration-dependent manner, i.e., escitalopram at concentrations of 1, 3, 10, and 30 μM reduced the tail current by 24.9 ± 2.1, 51.4 ± 3.0, 78.4 ± 1.3, and 90.9 ± 1.5 % ($n=7$) and citalopram at concentrations of 1, 3, 10, and 30 μM reduced the tail current by 17.1 ± 2.5, 47.2 ± 1.5, 72.8 ± 2.3, and 88.3 ± 0.8 % ($n=7$), respectively. Analysis of the data using the Hill equation gave an IC_{50} value of 2.6 ± 0.2 μM and a Hill coefficient of 1.1 ± 0.1 for escitalopram ($n=7$) (Fig. 1a) and an IC_{50} value of 3.2 ± 0.3 μM and a Hill coefficient of 1.2 ± 0.1 for citalopram ($n=7$) (Fig. 1b). The potencies of

escitalopram and citalopram in blocking hERG were very similar, indicating the lack of a stereoselective block.

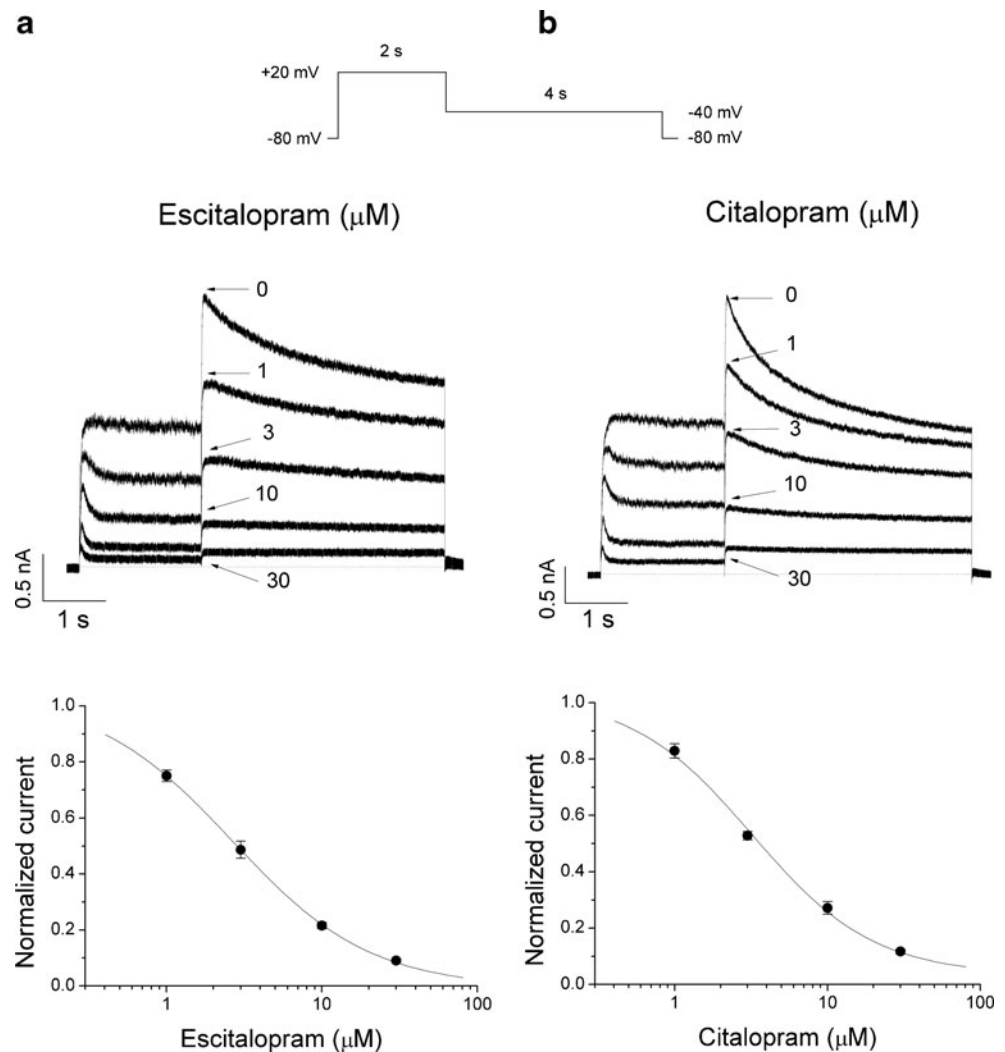
Time course for the onset of a block of hERG by escitalopram

To evaluate the time course of escitalopram effects on hERG currents, the depolarizing pulse was repeated with the application of escitalopram. Figure 2 shows the time course of a block of the tail current of hERG recorded before, during, and after the application of escitalopram. When switched to a drug-containing solution after recording control currents, the block reached a steady state within 1 min. Upon washout, the hERG currents were restored to 81.5 ± 2.9 % ($n=9$) of the control value, suggesting that the effects of escitalopram were largely reversible.

The block of hERG by escitalopram is voltage dependent

Figure 3a shows the representative hERG currents elicited after applying 2-s depolarizing pulses from a holding potential of −80 to +50 mV in 10-mV steps every 15 s in the absence and presence of escitalopram. The tail currents of hERG were recorded at −40 mV for 4 s. Current–voltage relationships were obtained by plotting the amplitudes of the hERG current at the end of 2-s depolarizing pulses against membrane potential in the absence and presence of escitalopram (Fig. 3b). Escitalopram reduced the amplitudes of the hERG current at all membrane potentials tested. To investigate the effects of escitalopram on the voltage dependency of activation curves, the peak tail currents of hERG were normalized and plotted against membrane potential before and after the application of escitalopram (Fig. 3c). The plots of normalized tail currents were fit to a Boltzmann function. Escitalopram shifted the half-activation point ($V_{1/2}$) of the activation curves to the hyperpolarizing direction but did not alter the slope factor (k) (control: $V_{1/2} = -20.8 \pm 1.4$ mV, $k = 7.2 \pm 0.3$ mV; escitalopram: $V_{1/2} = -27.1 \pm 1.0$ mV, $k = 7.1 \pm 0.3$ mV, $n=6$, $P < 0.05$). Figure 4a shows representative hERG currents under control conditions and in the presence of escitalopram at three different potentials. To evaluate the voltage dependency of the blocking by escitalopram, the fractional blocking ($I_{\text{Escitalopram}}/I_{\text{Control}}$) was plotted against the membrane potential with the normal activation curve (Fig. 4b). The blocking by escitalopram increased from -26.3 ± 3.6 % at −30 mV to -54.5 ± 2.4 % at 0 mV ($n=6$). Thus, the blocking was steeply increased in the voltage range, coinciding with the activation of hERG currents ($F_{2, 15} = 8.58$, $P < 0.05$), which suggested an interaction with the channel in the open state (Paul et al. 2002). At more depolarized potentials where the channels were fully activated (between 0 and +50 mV), the escitalopram blocking was constant, indicating that the binding of the drug was voltage independent.

Fig. 1 Concentration–response relationships for the block of hERG by escitalopram (**a**) and citalopram (**b**). Whole-cell hERG currents were elicited by a 2-s depolarizing pulse to +20 mV from a holding potential of –80 mV and repolarization to –40 mV for 4 s to generate the tail currents every 15 s. The effects of 1, 3, 10, and 30 μM of escitalopram and citalopram are shown. The dotted line marks zero current. Normalized blocks of the tail current of hERG are plotted as a function of escitalopram and citalopram concentrations. Data were fitted with the Hill equation, yielding an IC_{50} value of $2.6 \pm 0.2 \mu\text{M}$ and a Hill coefficient of 1.1 ± 0.1 ($n=7$) for escitalopram and an IC_{50} value of $3.2 \pm 0.3 \mu\text{M}$ and a Hill coefficient of 1.2 ± 0.1 ($n=7$) for citalopram. Data are expressed as means \pm S.E



The blocking of hERG by escitalopram is frequency dependent

To evaluate the frequency dependence of an escitalopram blocking of hERG, cells were depolarized from a holding potential of –80 to +80 mV for 100 ms, followed by a repolarizing pulse to +20 mV for 400 ms to record tail currents (Kikuchi et al. 2005). Trains of pulses at frequencies of 0.2 and 1 Hz were applied (Fig. 5a). The tail currents of hERG during the step pulse to +20 mV were measured and were normalized to the control current measured prior to drug application. Under control conditions, the tail current of hERG was not changed during the 30 depolarizing pulses at 0.2 Hz (Fig. 5b). The tail current progressively decreased to a steady level at 1 Hz and was reduced by $9.7 \pm 2.3\%$ ($n=6$) at the end of 30 depolarizing pulses. The time course of blocking was best fit to a double exponential function with a fast time constant of 0.23 ± 0.01 ms and a slow time constant of 10.04 ± 1.96 ms ($n=6$). In the presence of escitalopram, the tail current

of hERG was not changed at 0.2 Hz. The tail current progressively decreased to a steady level at 1 Hz ($17.0 \pm 2.1\%$, $n=6$, $P<0.05$) at the end of 30 depolarizing pulses. The time course was also best fit to a double exponential function with a fast time constant of 0.71 ± 0.18 ms ($n=6$, $P<0.05$) and a slow time constant of 13.08 ± 1.66 ms ($n=6$). These results suggest that the time course of the blocking was frequency dependent.

Escitalopram blocks hERG both in the open state and in the inactivated state

Figure 6 shows the results of the rapid drug application system that was used to study whether escitalopram would interact with the open or inactivated state of hERG channels (Ganapathi et al. 2009). The hERG currents were elicited by a 1-s depolarizing prepulse to +60 mV that fully activated the hERG channels followed by 10 s of repolarization to –40 mV, which induced a recovery from inactivation and opened the channels (Fig. 6a). Escitalopram was rapidly applied and

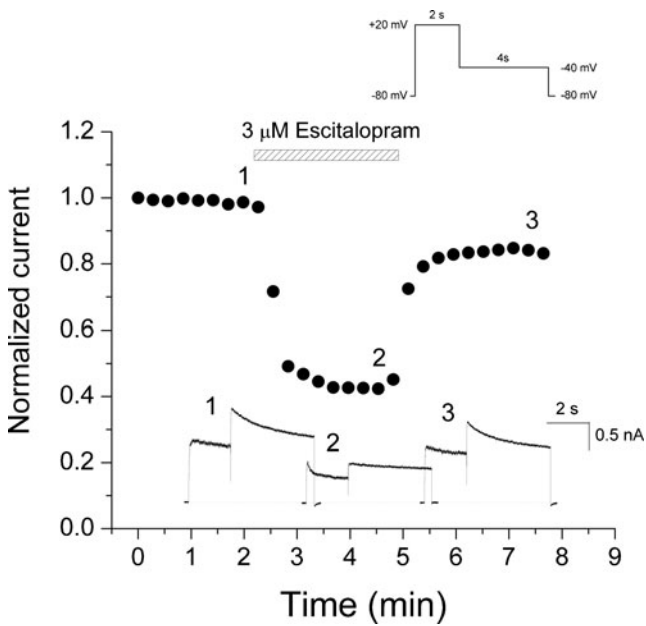


Fig. 2 Time course for the onset of the block of hERG by escitalopram. The peak tail currents of hERG in the presence of escitalopram were normalized to the first current amplitude, and the normalized data were plotted as a function of time. The insets in the graph show superimposed hERG tail currents recorded before, during, and after application of escitalopram. The dotted line marks zero current

removed during the tail currents of hERG and decreased the currents by $42.9 \pm 5.2\%$ ($n=6$), which indicated that the drug binds to the open state of the channels. The second protocol consisted of a 5-s depolarizing pulse to +60 mV to inactivate the hERG currents followed by 5 s of repolarization to -40 mV. Escitalopram was applied during the depolarizing pulse and continued during the tail currents (Fig. 6b). Escitalopram blocked the hERG currents by $34.7 \pm 1.7\%$ at the end of the depolarizing pulse to +60 mV, which increased to $51.9 \pm 2.3\%$ ($n=6$, $P<0.05$) after repolarization to -40 mV. Thus, the extent of the blocking by escitalopram during the depolarizing pulse (most channels are in an inactivated state) was less than that during the repolarizing pulse (most channels are in an open state). These results suggest that escitalopram has a high affinity for the open state of the channel, with a relatively lower affinity for the inactivated state of hERG channels.

Effects of escitalopram and citalopram on HERG channel protein trafficking

Another mechanism for drug-induced QT interval prolongation is the disruption of the normal trafficking of the hERG

Fig. 3 Effect of escitalopram on current–voltage relationships. **a** Whole-cell hERG currents were evoked by depolarizing pulses from -50 to +50 mV for 2 s in steps of 10 mV every 15 s from a holding potential of -80 mV and repolarization to -40 mV for 4 s in the absence and presence of escitalopram. The dotted line marks zero current. Current–voltage relationships of the steady-state (**b**) and peak tail (**c**) currents of hERG under control conditions and after application of escitalopram. The peak amplitudes of tail currents in the presence of escitalopram were normalized to those at each voltage in the absence of escitalopram. Data were fit to a Boltzmann function. Data are expressed as means \pm S.E

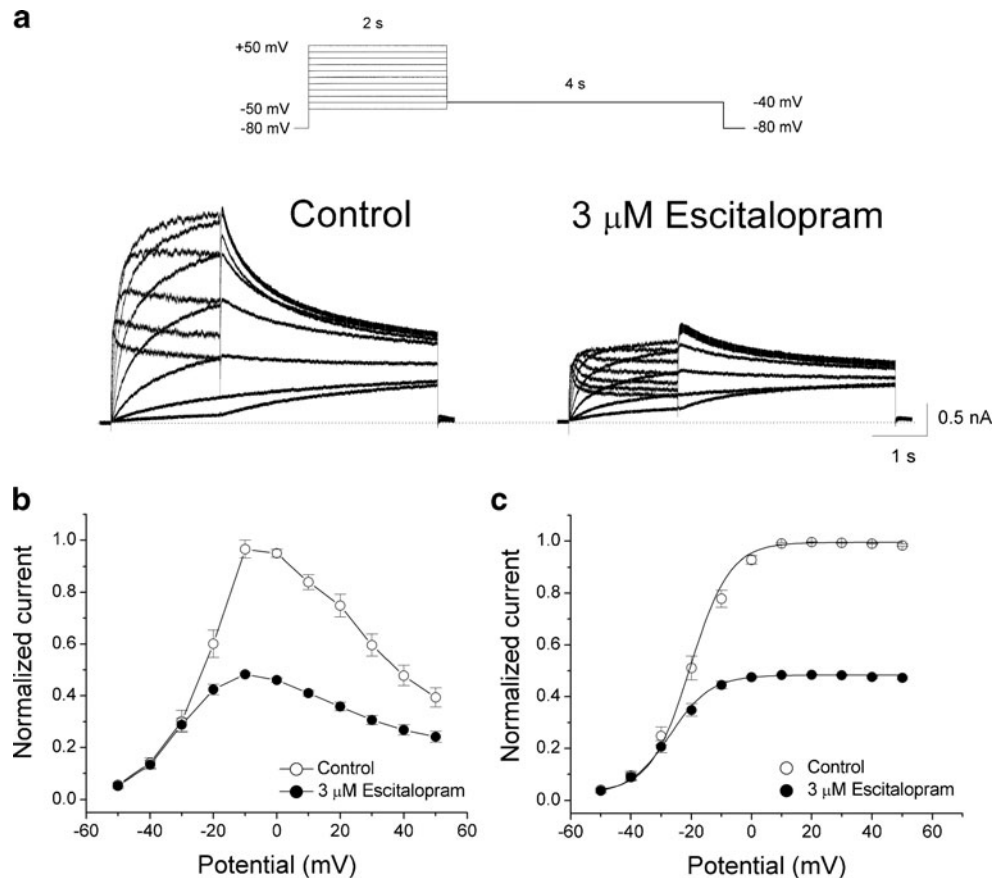
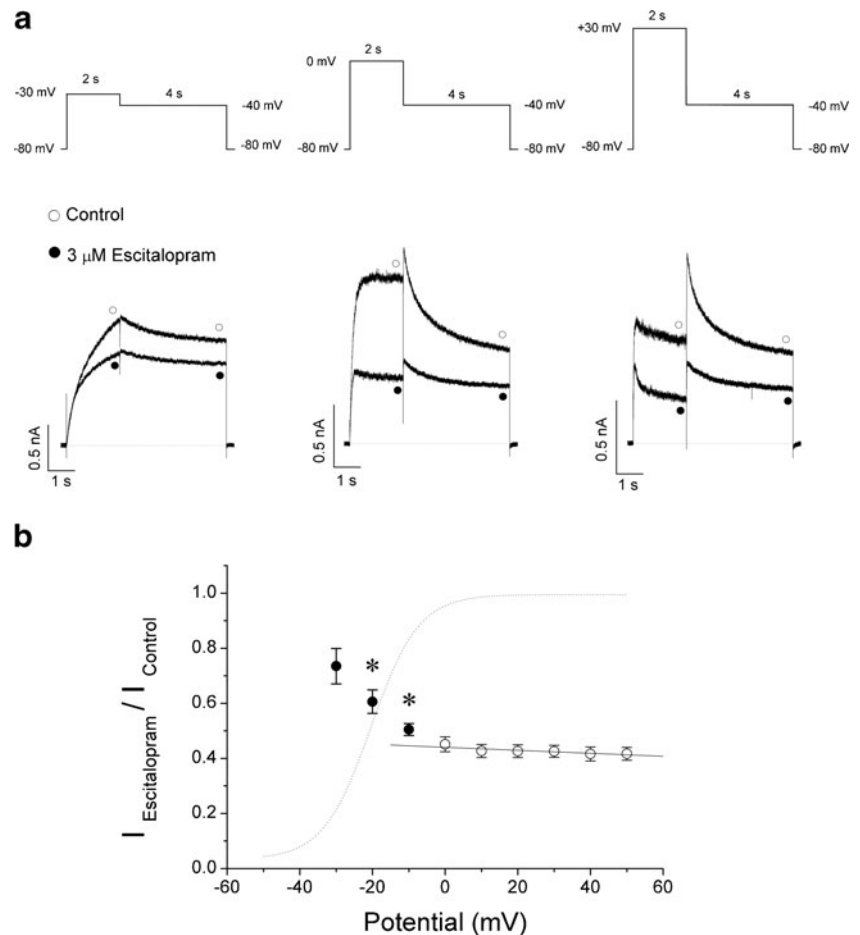


Fig. 4 Voltage-dependent block of hERG by escitalopram. **a** Representative hERG currents under control conditions and in the presence of escitalopram at three different potentials. The dotted line marks zero current. **b** Voltage-dependent block of hERG currents by escitalopram was expressed as a relative current ($I_{\text{escitalopram}}/I_{\text{control}}$). Asterisk significant difference from data obtained at -30 mV ($P < 0.05$). The dotted line represents the activation curve of hERG currents under control conditions. Data are expressed as means \pm S.E



channel to the surface membrane (Rajamani et al. 2006). Using Western blot and electrophysiology, we studied whether escitalopram and citalopram, for the sake of comparison, affected hERG channel trafficking (Fig. 7a). hERG-HEK293 cells were incubated for 24 h with escitalopram and citalopram (1, 3, 10, and 30 μ M) and with fluoxetine (30 μ M) as a positive control. Fluoxetine resulted in a significant reduction of a protein band at 155 kDa (the fully glycosylated form of the hERG channel protein) without the effects on 135 kDa (the core glycosylated form) as previously described (Rajamani et al. 2006). The intensity of the 155-kDa protein band decreased with increasing concentrations of escitalopram and citalopram. However, there was no significant difference between the 135-kDa forms of the hERG channel protein. To confirm that incubation with escitalopram resulted in a reduction of hERG channel surface expression, current density was measured electrophysiologically. Representative hERG current traces are shown in Fig. 7b for control conditions: the peak tail current of hERG was 108.4 ± 6.2 pA/pF for a voltage step to +50 mV ($n=7$). After 24 h of incubation with 30 μ M escitalopram, the peak tail current of hERG was decreased to 46.8 ± 10.1 pA/pF ($n=7$, $P < 0.05$) for a voltage step to +50 mV. Escitalopram was removed from the cell medium prior to electrophysiological recording

(following 24 h of incubation) to avoid any bias caused by acute inhibition by the drug. These results confirmed the immunological findings.

Effects of escitalopram and citalopram on the short-term internalization of the cell surface hERG channels

Few drugs, such as desipramine, have been reported to cause short-term reduction of hERG activity by increasing the internalization of cell surface hERG channels (Dennis et al. 2011). To study escitalopram and citalopram (30 μ M) effects on hERG internalization, cells were incubated for 4 h under control conditions and in the presence of both drugs to allow for hERG internalization (Fig. 8). Immunocytochemical analysis showed no difference in cell surface staining, neither under control conditions nor after 4 h of incubation with escitalopram or citalopram.

Discussion

In the present study, the effects of escitalopram and citalopram on hERG channels were examined. The main results are as follows: (1) both escitalopram and citalopram produced a

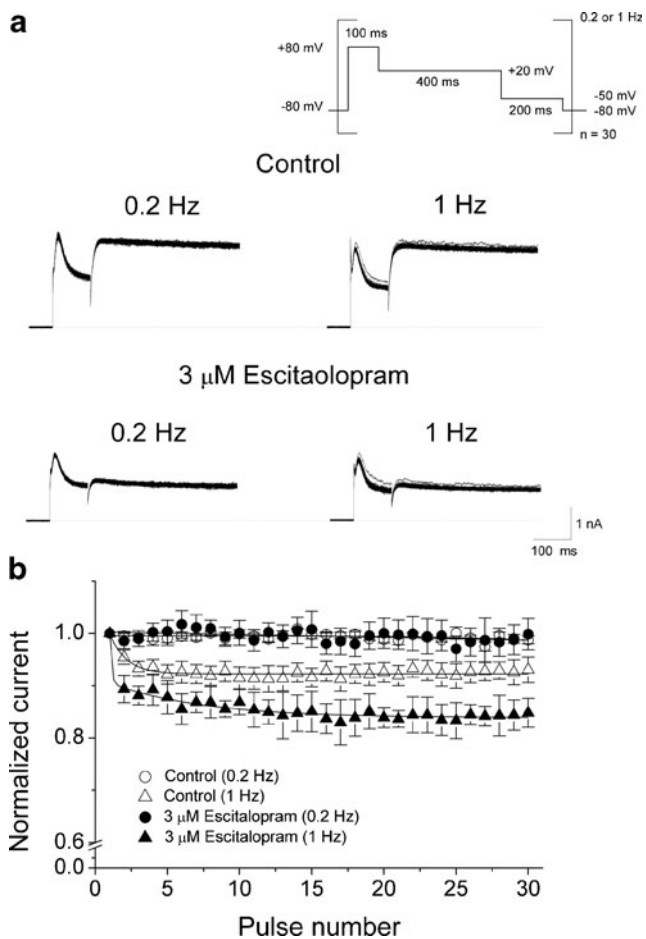


Fig. 5 Frequency-dependent block of hERG by escitalopram. **a** hERG currents obtained from depolarizing pulses from a holding potential of -80 to $+80$ mV for 100 ms, followed by a repolarizing pulse to $+20$ mV for 400 ms to record the tail current at 0.2 and 1 Hz in the absence and presence of escitalopram. **b** The tail currents during the step pulse to $+20$ mV were measured and normalized to the control current measured prior to drug application. The time course of block was best fit to a double exponential function with a fast and slow time constant. Data are expressed as means \pm S.E

reversible concentration-dependent block of hERG with IC_{50} values of 2.6 and 3.2 μ M, respectively, (2) the block by escitalopram was voltage and frequency dependent, (3) escitalopram blocked hERG by interacting with the channels in both the open and inactivated states, and (4) both drugs inhibited hERG channel protein trafficking to the plasma membrane.

Citalopram is a racemic mixture of the *R*(-)-enantiomer (*R*-citalopram) and the *S*(+)-enantiomer (escitalopram) (Sanchez 2006). The potencies of escitalopram and citalopram in blocking hERG were very similar, indicating that the channel block by these enantiomers did not display stereoselectivity. Because citalopram is a 1:1 mixture of the *R*- and *S*-enantiomers, escitalopram was equipotent to citalopram with regard to its block of hERG. The block of hERG by

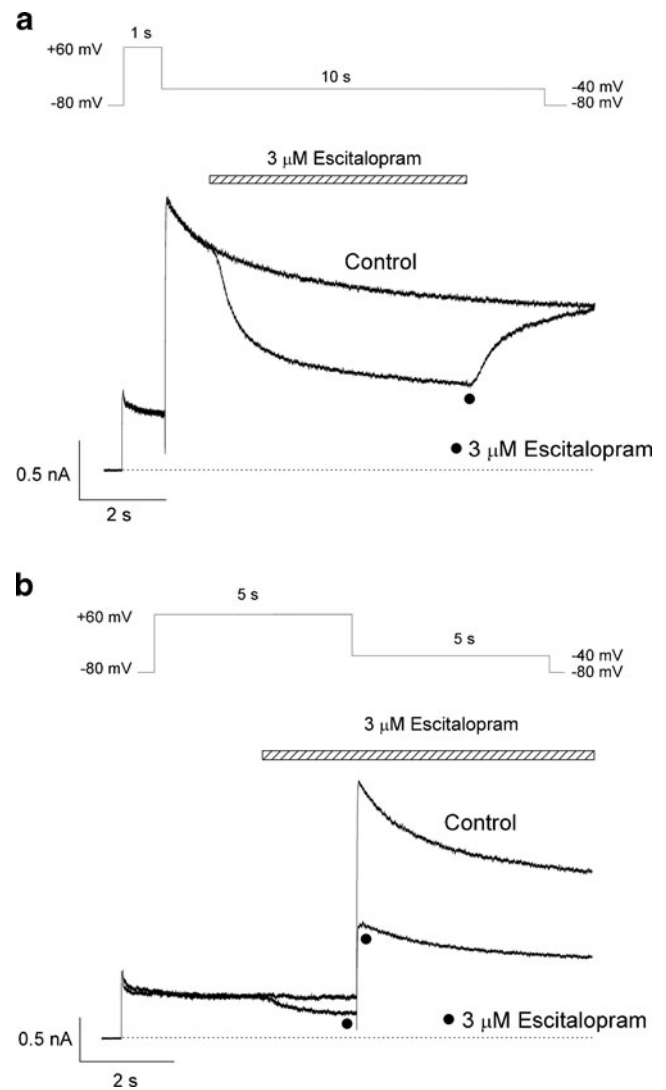
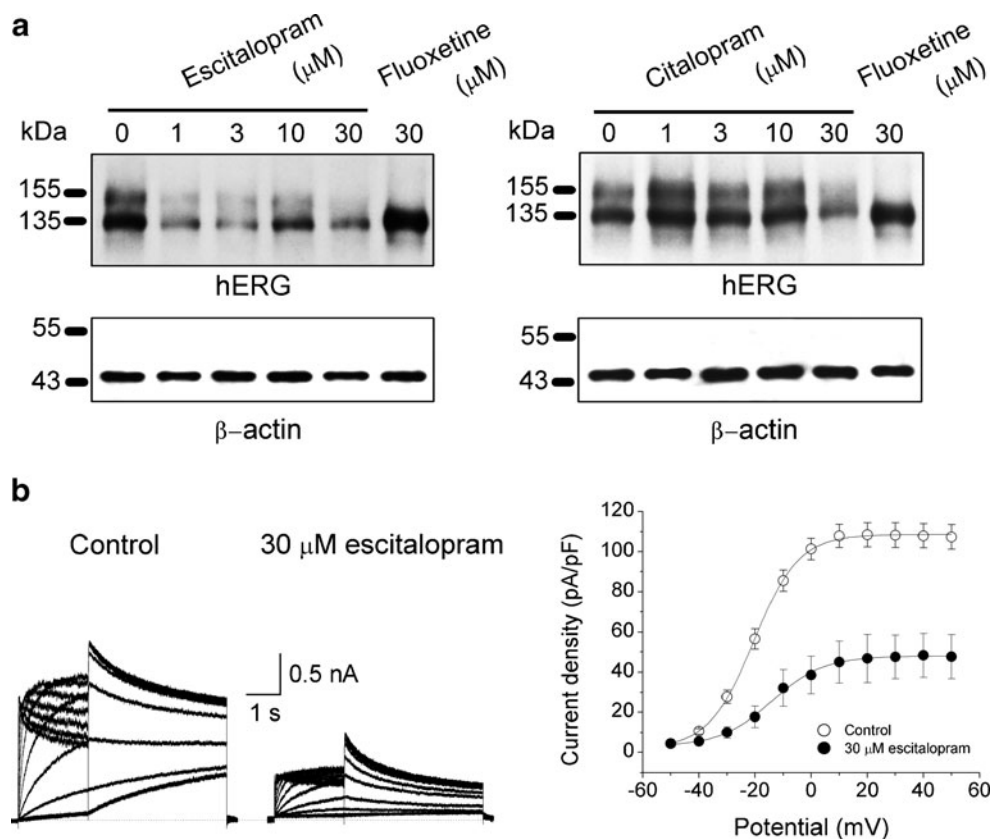


Fig. 6 Effects of the rapid application of escitalopram on hERG currents. **a** The cells were depolarized from a holding potential of -80 to $+60$ mV for 1 s, followed by a repolarizing pulse to -40 mV for 10 s to record tail currents. After a delay of 1 s, escitalopram was rapidly applied and removed during the repolarizing pulse. The bar indicates the time of application of escitalopram. The complete recovery of the currents after washout was observed during the depolarizing pulse. **b** The cells were depolarized from a holding potential of -80 to $+60$ mV for 5 s, followed by a repolarizing pulse to -40 mV for 5 s to record tail currents. Escitalopram was rapidly applied during the depolarizing pulse and continued into the repolarizing pulse as indicated by the bar. The dotted line marks zero current

escitalopram was increased at a more depolarized membrane potential where most channels are in an activated state. This voltage dependence of the escitalopram block suggests that the drug preferentially binds to the open or inactivated state of hERG channels (Paul et al. 2002). Between 0 and $+50$ mV where the channels were fully open, however, the block was uniform. Because escitalopram is a strong base with a $pK_a=9.78$ (<http://www.drugbank.ca/drugs/DB01175>), this drug exists predominantly as a cation at an intracellular pH of 7.3, which

Fig. 7 Effects of escitalopram and citalopram on hERG protein trafficking. **a** Western blot analysis of hERG channel protein under control conditions and after 24 h of incubation with increasing concentrations (1, 3, 10, and 30 μ M) of escitalopram, citalopram, and 30 μ M fluoxetine. **b** Quantitative analysis of hERG tail current densities was measured under control conditions and after 24 h of incubation with escitalopram. The pulse protocol is the same as that described in Fig. 3. The dotted line marks zero current. Data are expressed as means \pm S.E



suggests either that the drug binding with hERG channels occurred outside the electrical field or that the uncharged drug was responsible for the block. Our rapid perfusion system provided direct evidence for an activated (open and inactivated) state block: escitalopram blocked hERG currents both during depolarization, at which most channels are in inactivated state, and during repolarization, at which most channels are in open state (Ganapathi et al. 2009). The drug binding site in the intracellular pore has been well described in the hERG channel, and the aromatic pore residues (Y652 and F656) in the hERG protein have been identified as key determinants of drug binding (Mitcheson et al. 2000). Thus, further

studies are required to investigate the significance of these amino acids for escitalopram binding to hERG channel protein with mutants.

Numerous studies have demonstrated the effects of SSRIs on hERG channels. For example, fluoxetine and fluvoxamine blocked hERG currents expressed in *Xenopus* oocytes and mammalian cells with IC_{50} values of 0.7–3.8 μ M (Thomas et al. 2002; Witchel et al. 2002; Milnes et al. 2003; Rajamani et al. 2006). Citalopram also blocked hERG currents expressed in CHO cells with an IC_{50} value of 3.9 μ M (Witchel et al. 2002), which is a value that approximates the IC_{50} value we report here for hERG currents expressed in

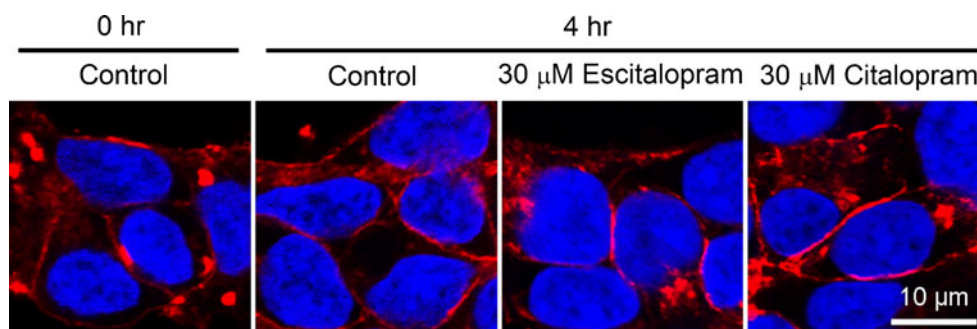


Fig. 8 Effects of escitalopram and citalopram on the short-term internalization of the cell surface hERG channels. Cells were incubated for 4 h under control conditions and in the presence of escitalopram and

citalopram (30 μ M) to allow for hERG internalization. Confocal fluorescence images showed no difference in cell surface staining, neither under control conditions nor in the presence of either escitalopram or citalopram

HEK cells. The hERG channels encode the rapidly activating delayed rectifier Kv currents that are responsible for the ventricular repolarization of action potentials in cardiomyocytes, and the blockade of hERG channels may well account for the prolongation of action potential duration and the QT interval following administration of the drugs (Vandenberg et al. 2012). The prolongation of the QT interval observed during escitalopram administration suggests that the block of hERG may be responsible for the arrhythmogenic potential of this drug. QT interval prolongation was reported after an overdose of escitalopram, but no ventricular arrhythmias or TdPs were detected (Baranchuk et al. 2008). Drugs that block hERG channels produced a prolongation of action potential duration and a lengthening of the QT interval and have proarrhythmic potential (Kass and Cabo 2000). However, citalopram blocked cardiac L-type calcium channel currents, which mediate the plateau phase of action potential in isolated rat and guinea pig ventricular cardiomyocytes (Witchel et al. 2002; Zahradnik et al. 2008). These results imply that excessive QT interval prolongation during citalopram administration may not occur and that the inhibitory effects of citalopram on an L-type calcium channel current might compensate for the proarrhythmic effects associated with hERG blockade (Bril et al. 1996). In fact, citalopram produced a concentration-dependent shortening of the action potential duration and a depression of the plateau and overshoot potential in guinea pig papillary muscle (Pacher et al. 2000). Thus, citalopram is considered to have a good safety profile regarding cardiotoxicity within the clinical range (Keller 2000; Parker and Brown 2000). Furthermore, our previous study has demonstrated that citalopram reduced cloned Kv1.5 currents as an open channel blocker in a reversible concentration-dependent manner within the therapeutic plasma concentrations (Lee et al. 2010). Kv1.5 is more abundantly expressed in atrial than in ventricular myocytes and is regarded as a therapeutic target for arrhythmia (Brendel and Peukert 2003). Although our study is the first report regarding the effects of escitalopram on hERG channels and provides new information about the potential cardiotoxicity of this drug, we did not evaluate the effects of escitalopram on other cardiac ion channels, which include L-type calcium channels and Kv1.5 channels. Thus, further studies are required to investigate the overall effects of escitalopram on these channels and cardiac action potentials.

Several drugs have been known to inhibit hERG indirectly by disruption of hERG channel protein trafficking to the plasma membrane, thereby reducing the cell surface hERG channel density (Rajamani et al. 2006), and by an increase in internalization of the cell surface channels (Dennis et al. 2011). In the present study, both escitalopram and citalopram resulted in a significant reduction in the trafficking of hERG channel protein to the plasma membrane. However, neither drug affected the internalization of the cell surface hERG channels. These results indicate that both drugs decreased

hERG currents by inhibiting hERG channel protein trafficking to the plasma membrane.

After receiving a dosage of 30 mg/day, the maximum plasma concentration of escitalopram averages about 64.4 ng/ml (0.15 μ M) (Waugh and Goa 2003). When the human plasma protein binding of escitalopram is in the range of 56 %, the effective free therapeutic plasma concentration might be approximately 66 nM (Waugh and Goa 2003). In the present study, escitalopram blocked hERG currents with an IC_{50} value of 2.6 μ M, which was well above the maximum plasma concentration. Although we do not know the concentration of escitalopram in the heart tissue, citalopram is highly lipophilic and is likely to achieve higher total concentrations in the heart and brain than in the blood serum (Melzacka et al. 1984; Pacher et al. 2000). Thus, caution is warranted during chronic administration in patients with a higher torsadogenic risk.

In summary, escitalopram and citalopram produced a concentration-dependent block of hERG currents. Similar potencies of a hERG current block were observed for escitalopram and citalopram, indicating that the channel block by these enantiomers did not display stereoselectivity. Escitalopram blocked hERG currents by preferentially binding to their open and inactivated states as well as by inhibiting the trafficking of hERG channel protein to the plasma membrane.

Acknowledgments This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2012R1A1A2008274) and by the Research Fund 2012 of The Catholic University of Korea (J.S. Choi).

References

- Baranchuk A, Simpson CS, Methot M, Gibson K, Strum D (2008) Corrected QT interval prolongation after an overdose of escitalopram, morphine, oxycodone, zopiclone and benzodiazepines. *Can J Cardiol* 24:e38–e40
- Brendel J, Peukert S (2003) Blockers of the Kv1.5 channel for the treatment of atrial arrhythmias. *Curr Med Chem Cardiovasc Hematol Agents* 1:273–287
- Bril A, Gout B, Bonhomme M, Landais L, Faivre JF, Linee P, Poyser RH, Ruffolo RR Jr (1996) Combined potassium and calcium channel blocking activities as a basis for antiarrhythmic efficacy with low proarrhythmic risk: experimental profile of BRL-32872. *J Pharmacol Exp Ther* 276:637–646
- Catalano G, Catalano MC, Epstein MA, Tsambiras PE (2001) QTc interval prolongation associated with citalopram overdose: a case report and literature review. *Clin Neuropharmacol* 24:158–162
- Choi JS, Choi BH, Ahn HS, Kim MJ, Rhie DJ, Yoon SH, do Min S, Jo YH, Kim MS, Sung KW, Hahn SJ (2003) Mechanism of block by fluoxetine of 5-hydroxytryptamine₃ (5-HT₃)-mediated currents in NCB-20 neuroblastoma cells. *Biochem Pharmacol* 66:2125–2132
- Dennis AT, Nassal D, Deschenes I, Thomas D, Ficker E (2011) Antidepressant-induced ubiquitination and degradation of the cardiac potassium channel hERG. *J Biol Chem* 286:34413–34425
- Engelbrechtsen KM, Harris CR, Wood JE (2003) Cardiotoxicity and late onset seizures with citalopram overdose. *J Emerg Med* 25:163–166

- Fayssoil A, Issi J, Guerbaa M, Raynaud JC, Herogueulle V (2011) Torsade de Pointes induced by citalopram and amiodarone. *Ann Cardiol Angeiol (Paris)* 60:165–168
- Ganapathi SB, Kester M, Elmslie KS (2009) State-dependent block of HERG potassium channels by *R*-roscovitine: implications for cancer therapy. *Am J Physiol Cell Physiol* 296:C701–C710
- Garnock-Jones KP, McCormack PL (2010) Escitalopram: a review of its use in the management of major depressive disorder in adults. *CNS Drugs* 24:769–796
- Hyttel J (1982) Citalopram—pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. *Prog Neuropsychopharmacol Biol Psychiatry* 6:277–295
- Kass RS, Cabo C (2000) Channel structure and drug-induced cardiac arrhythmias. *Proc Natl Acad Sci U S A* 97:11683–11684
- Keller MB (2000) Citalopram therapy for depression: a review of 10 years of European experience and data from U.S. clinical trials. *J Clin Psychiatry* 61:896–908
- Kikuchi K, Nagatomo T, Abe H, Kawakami K, Duff HJ, Makielski JC, January CT, Nakashima Y (2005) Blockade of HERG cardiac K⁺ current by antifungal drug miconazole. *Br J Pharmacol* 144:840–848
- Lee HM, Hahn SJ, Choi BH (2010) Open channel block of Kv1.5 currents by citalopram. *Acta Pharmacol Sin* 31:429–435
- Melzacka M, Rurak A, Adamus A, Daniel W (1984) Distribution of citalopram in the blood serum and in the central nervous system of rats after single and multiple dosage. *Pol J Pharmacol Pharm* 36:675–682
- Milnes JT, Crociani O, Arcangeli A, Hancox JC, Witchel HJ (2003) Blockade of HERG potassium currents by fluvoxamine: incomplete attenuation by S6 mutations at F656 or Y652. *Br J Pharmacol* 139:887–898
- Mitcheson JS, Chen J, Lin M, Culbertson C, Sanguinetti MC (2000) A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci U S A* 97:12329–12333
- Pacher P, Bagi Z, Lako-Futo Z, Ungvari Z, Nanasi PP, Kecskemeti V (2000) Cardiac electrophysiological effects of citalopram in guinea pig papillary muscle comparison with clomipramine. *Gen Pharmacol* 34:17–23
- Pacher P, Kecskemeti V (2004) Cardiovascular side effects of new antidepressants and antipsychotics: new drugs, old concerns? *Curr Pharm Des* 10:2463–2475
- Parker NG, Brown CS (2000) Citalopram in the treatment of depression. *Ann Pharmacother* 34:761–771
- Paul AA, Witchel HJ, Hancox JC (2002) Inhibition of the current of heterologously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine. *Br J Pharmacol* 136:717–729
- Perchenet L, Hilfiker L, Mizrahi J, Clement-Chomienne O (2001) Effects of anorexigen agents on cloned voltage-gated K⁺ channel hKv1.5. *J Pharmacol Exp Ther* 298:1108–1119
- Rajamani S, Eckhardt LL, Valdivia CR, Klemens CA, Gillman BM, Anderson CL, Holzem KM, Delisle BP, Anson BD, Makielski JC, January CT (2006) Drug-induced long QT syndrome: hERG K⁺ channel block and disruption of protein trafficking by fluoxetine and norfluoxetine. *Br J Pharmacol* 149:481–489
- Sanchez C (2006) The pharmacology of citalopram enantiomers: the antagonism by R-citalopram on the effect of S-citalopram. *Basic Clin Pharmacol Toxicol* 99:91–95
- Thomas D, Gut B, Wendt-Nordahl G, Kiehn J (2002) The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. *J Pharmacol Exp Ther* 300:543–548
- Tseng PT, Lee Y, Lin YE, Lin PY (2012) Low-dose escitalopram for 2 days associated with corrected QT interval prolongation in a middle-aged woman: a case report and literature review. *Gen Hosp Psychiatry* 34(210):e213–e215
- Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP (2012) hERG K⁺ channels: structure, function, and clinical significance. *Physiol Rev* 92:1393–1478
- Vandenberg JI, Walker BD, Campbell TJ (2001) HERG K⁺ channels: friend and foe. *Trends Pharmacol Sci* 22:240–246
- Waugh J, Goa KL (2003) Escitalopram: a review of its use in the management of major depressive and anxiety disorders. *CNS Drugs* 17:343–362
- Witchel HJ, Pabbathi VK, Hofmann G, Paul AA, Hancox JC (2002) Inhibitory actions of the selective serotonin re-uptake inhibitor citalopram on HERG and ventricular L-type calcium currents. *FEBS Lett* 512:59–66
- Yang MJ, Sim S, Jeon JH, Jeong E, Kim HC, Park YJ, Kim IB (2013) Mitral and tufted cells are potential cellular targets of nitration in the olfactory bulb of aged mice. *PLoS One* 8:e59673
- Zahradnik I, Minarovic I, Zahradnikova A (2008) Inhibition of the cardiac L-type calcium channel current by antidepressant drugs. *J Pharmacol Exp Ther* 324:977–984