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Comparison of HERG channel blocking effects of various β-blockers – implication for clinical strategy

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1 β-Blockers are widely used in the treatment of cardiovascular diseases. However, their effects on HERG channels at comparable conditions remain to be defined. We investigated the direct acute effects of β-blockers on HERG current and the molecular basis of drug binding to HERG channels with mutations of putative common binding site (Y652A and F656C).

2 β-Blockers were selected based on the receptor subtype. Wild-type, Y652A and F656C mutants of HERG channel were stably expressed in HEK293 cells, and the current was recorded by using whole-cell patch-clamp technique (23°C).

3 Carvedilol (nonselective), propranolol (nonselective) and ICI 118551 (β_2-selective) inhibited HERG current in a concentration-dependent manner (IC_{50} 0.51, 3.9 and 9.2 μM, respectively). The IC_{50} value for carvedilol was a clinically relevant concentration. High metoprolol (β_1-selective) concentrations were required for blockade (IC_{50} 145 μM), and atenolol (β_1-selective) did not inhibit the HERG current.

4 Inhibition of HERG current by carvedilol, propranolol and ICI 118551 was partially but significantly attenuated in Y652A and F656C mutant channels. Affinities of metoprolol to Y652A and F656C mutant channels were not different compared with the wild-type.

5 HERG current block by all β-blockers was not frequency-dependent.

6 Drug affinities to HERG channels were different in β-blockers. Our results provide additional strategies for clinical usage of β-blockers. Atenolol and metoprolol may be preferable for patients with type 1 and 2 long QT syndrome. Carvedilol has a class III antiarrhythmic effect, which may provide the rationale for a favourable clinical outcome compared with other β-blockers as suggested in the recent COMET (Carvedilol Or Metoprolol European Trial) substudy.
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Keywords: β-Blockers; arrhythmia; ion channels; K^+ channel; membrane currents; long QT syndrome; heart failure; HEK293 cells

Abbreviations: COMET, Carvedilol Or Metoprolol European Trial; DMSO, dimethyl sulphoxide; HEK, human embryonic kidney; HERG, human *ether-a-go-go*-related gene; I_{Ca}, calcium current; I_{Kr}, rapidly activating component of the delayed rectifier K^+ current; I_{Ks}, slowly activating component of the delayed rectifier K^+ current; I_{to}, transient outward current; MiRP1, MinK-related peptide 1

Introduction

In human cardiac ventricular cells, the principal repolarising currents activated during the action potential plateau are the rapidly (I_{Kr}) and slowly (I_{Ks}) activating components of the delayed rectifier K^+ current (Sanguinetti & Jurkiewicz, 1990; Carmeliet, 1993), with I_{Kr} encoded by human *ether-a-go-go*-related gene (*HERG* or *KCNH2*) (Curran *et al*., 1995; Sanguinetti *et al*., 1995; Trudeau *et al*., 1995). Pharmacological blockade of HERG currents exhibits antiarrhythmic effect by lengthening of the cardiac action potential duration such as the effects of class III antiarrhythmic drugs, but excessive prolongation of the action potential duration sometimes leads to life-threatening ventricular tachyarrhythmia, *torsades de pointes*.

β-Blockers are widely used in the treatment of cardiovascular diseases such as hypertension, coronary heart diseases, chronic heart failure and congenital long QT syndrome (LQT). In patients with congenital LQT, β-blockers are reported to be effective in suppressing life-threatening arrhythmia especially in LQT1 and LQT2 patients (Dorostkar *et al*., 1999; Moss *et al*., 2000; Schwartz *et al*., 2001; Shimizu *et al*., 2002). However, nonselective β-receptor antagonist, carvedilol, inhibits the HERG potassium current by direct action on the channels and prolongs the QT interval (Cheng *et al*., 1999; Karle *et al*., 2001). Propranolol overdose has also been reported to cause prolongation of QT interval (Farhangi & Sansone, 2003). Question arises as which type of β-blockers is the most suitable for the treatment of LQT. On the other hand, the recent COMET (Carvedilol Or Metoprolol European Trial) study suggested that carvedilol reduced the

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rate of sudden cardiac death compared with β_1-receptor selective antagonist, metoprolol, in patients with severe heart failure (Poole-Wilson *et al*., 2003). Since the life-threatening arrhythmias are major cause of sudden cardiac death, differences in electrophysiological actions of carvedilol and metoprolol might be involved in the outcome of the study.

Although the electrophysiological effects of β-blockers have been studied in different species and expression systems, it is important to compare the electrophysiological activities of these drugs at comparable conditions (Dupuis *et al*., 2005). Interactions of drugs with HERG channels have been so far most often studied in human embryonic kidney 293 (HEK293) cells or *Xenopus* oocytes. However, HEK293 cells are more suitable for studying the drug affinity to HERG channels because the results correlate well with those observed in native cells and in clinical practice (Redfern *et al*., 2003).

In addition to class III antiarrhythmic drugs, a remarkable array of structurally diverse therapeutic agents that cause acquired LQT are known to block HERG or I_{Kr} channels (Viskin, 1999; Roden, 2004; Tamargo *et al*., 2004). One possible explanation for this is that lack of a highly conserved amino-acid motif (proline-X-proline) in HERG S6 domains compared with other potassium channels may cause the HERG channel pore and its vestibule to be uniquely large. Another important explanation for this is that these drugs bind to a common drug receptor within the pore of HERG channels. Using alanine scanning mutagenesis of the pore-S6 region of HERG channel, aromatic amino-acid residues (Y652 and particularly F656) were reported to be key determinants of drug binding to HERG channels (Mitcheson *et al*., 2000). Therefore, it is useful to use these mutant channels to examine the direct action of drugs on HERG channels.

The aim of the present study was to investigate the direct effect of various β-blockers on cloned HERG potassium channels expressed heterologously in HEK293 cells and compare the relative potencies of HERG channels blockade. In addition, we investigated the molecular basis of drug binding to HERG channels with the S6 domain mutations (Y652A and F656C).

Methods

DNA constructs and transfection of HEK293 cells

HERG cDNA (GenBank Accession Number: U04270) was subcloned into *Bam*HI/*Eco*RI sites of the pCDNA3 vector (Invitrogen, San Diego, CA, U.S.A.). HERG wild-type channels were stably expressed in HEK293 cell line as described previously (Zhou *et al*., 1998). HERG Y652A (tyrosine to alanine at position 652) and F656C (phenylalanine to cysteine at position 656) mutations were generated by site-directed mutagenesis of wild-type HERG cDNA as described previously (Kikuchi *et al*., 2005). HEK293 cells were transfected with this construct using the lipofectamine method (Invitrogen). Stably transfected cells generated through G418 antibiotic selection were subcloned to achieve a uniform HERG expression level. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM: Life Technologies, Gaithersburg, MD, U.S.A.) supplemented with 10% foetal bovine serum, 100 U ml^{-1} penicillin and 100 μg streptomycin. For electrophysiological analysis, the cells were harvested from the culture dish by trypsinisation, washed with D-MEM, and stored in this medium at room temperature for later use. The cells in culture dishes were easily detached by trypsinisation and studied within 8 h of harvest.

Electrophysiological recordings

HERG channel current was recorded using the whole-cell patch-clamp technique at room temperature (23±1°C). The transfected cells were transferred to a bath mounted on the stage of an inverted microscope (Diaphot, Nikon, Japan). The bath was perfused with 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid (HEPES)-buffered Tyrode solution containing (in mM) 137 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). The volume of the bath was 0.6 ml and the external solution in the bath was almost completely exchanged within 1 min at a perfusion rate of 1.25 ml min^{-1}. The internal pipette solution contained (in mM) 130 KCl, 1 $MgCl_2$, 5 ethylene glycol bis (beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 5 Mg-ATP, and 10 HEPES (pH 7.2). The electrodes were constructed from borosilicate glass using a micropipette puller (P-87, Sutter Instrument Co., Novato, CA, U.S.A.) and heat-polished with a microforge (MF-83, Narishige, Tokyo, Japan). The final resistance of the electrode was 3–5 MΩ when filled with the pipette solution. Membrane currents were recorded with an Axopatch 200B amplifier (Axon Instruments, Union City, CA, U.S.A.) and digitised at 2 kHz with an analogue-to-digital converter (DigiData 1200B; Axon Instruments). The computer software (pCLAMP Ver. 8.1, Axon Instruments) was used to generate voltage clamp protocols, acquire data and analyse current traces.

Chemicals

β-Blockers were selected based on their receptor subtype. Propranolol (nonselective β-blocker), atenolol (selective β-blocker) and ICI 118551 (selective β_2-blocker) were purchased from Sigma-Aldrich Company (St Louis, MO, U.S.A.). Metoprolol (a selective β-blocker) was purchased from MP

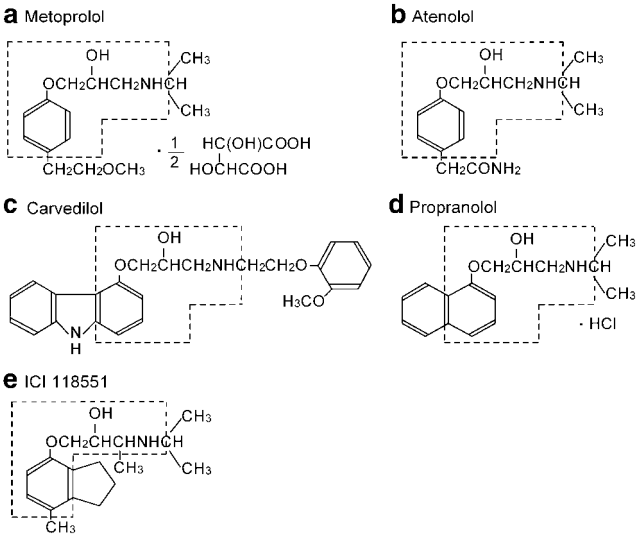

Figure 1 Structure of β-blockers.

Table 1 Molecular weight, β-selectivity and therapeutic plasma concentrations of β-blockers

β-*Blockers*	*Molecular weight*	*IC_{50} for β_1* (nM)[a]	*IC_{50} for β_2* (nM)[a]	*Selectivity (β_1/β_2)*[a]	*Therapeutic concentration* (μM)
Metoprolol	342.4	204±24	1227±270	6.0	0.40±0.07[b]
Atenolol	266.3	1520±110	8600±1360	5.7	1.64±0.14[b]
Carvedilol	406.5	0.32±0.06	0.18±0.04	0.6	0.1–0.6[c]
Propranolol	295.8	3.6±0.3	1.1±0.2	0.3	0.145±0.07[b]
ICI 118551	313.9	148±1–9	1.48±1–2	0.01	—

[a]Smith & Teitler (1999).
[b]Data based on the information provided by manufacturer.
[c]McPhillips *et al*. (1988).

Biomedicals (Germany). These drugs were dissolved in distilled water to prepare a stock solution. Carvedilol, a nonselective β-blocker with α-blocking action, was kindly provided by Daiichi Pharmaceutical Co. (Tokyo, Japan) and was dissolved in dimethyl sulphoxide (DMSO) to prepare a stock solution (100 mM). The final concentration of the drug was prepared by diluting the stock solution with Tyrode solution. The highest concentration of DMSO used in the present study was 0.1%. In preliminary experiments, the amplitudes of HERG tail current expressed in HEK293 cells did not change significantly after 3 min of DMSO application at 0.1% ($n=4$). The chemical structures of β-blockers used in the present study are shown in Figure 1. The molecular weight, β-receptor selectivity (Smith & Teitler, 1999) and therapeutic plasma concentrations of β-blockers used in the present study are summarised in Table 1.

Statistical analysis

Data are expressed as mean±s.e.m., unless otherwise noted. Where applicable, n represents the number of cells studied. Statistical significance was analysed using a two-tailed Student's t-test. P-values <0.05 were considered statistically significant. Curve fitting was performed using multiple nonlinear least-squares regression analysis (pCLAMP Ver. 8.1, Axon Instruments or Sigma Plot Ver. 7.0, SPSS Science, Chicago, IL, U.S.A.).

Results

Effects of β-blockers on wild-type HERG channels expressed in HEK293 cells

Representative current recordings in control and 3 min after application of β-blockers in the same cell are shown in Figure 2. HERG current was elicited from a holding potential of −80 mV by 4-s long depolarising steps to between −70 and 60 mV applied in 10 mV increments every 15 s. Tail current was recorded with a step pulse to −50 mV for 6 s. Carvedilol, propranolol, and ICI 118551 reduced HERG current amplitude during the depolarising step as well as tail current at relatively low concentrations. High concentrations of metoprolol were required to reduce HERG current. However, atenolol did not produce noticeable inhibition of HERG current when used at concentrations up to 1 mM.

The effects of metoprolol, carvedilol, propranolol and ICI 118551 on the HERG current–voltage (I–V) relationship are shown in Figure 3. The averaged normalised I–V relationships for HERG current measured at the end of the depolarising steps and for tail current are shown. The control HERG current during the depolarising steps was maximal at about 0–10 mV, with tail current fully activated following steps to 10–20 mV. Metoprolol (100 μM), carvedilol (1 μM), propranolol (10 μM) and ICI 118551 (10 μM) reduced the steady-state current amplitude to 48.9±10% ($n=5$), 34.6±8.6% ($n=4$), 32.1±6.4% ($n=5$) and 55.0±3.5% ($n=7$) of the control at 0 mV, respectively, and the peak tail current amplitude to 54.3±6.1% ($n=5$), 41.1±8.3% ($n=4$), 44.3±2.1% ($n=5$) and 61.6±3.8% ($n=7$) of the control at 20 mV, respectively. Voltage-dependence of activation (Figure 3) was also evaluated by plotting normalised tail current as a function of voltage. Normalised data were fitted with a Boltzmann function:

$$I = I_{\max} \times [1 + \exp(V_{1/2} - V)/\kappa]^{-1}$$

where $I_{\max}$ is maximum amplitude, $V_{1/2}$ and κ are half-activation voltage and the slope factor, respectively. After application of the test drugs, the half-maximal activation voltages significantly shifted in a negative direction compared with the control (metoprolol −13.2±1.5 to −17.0±3.8 mV, $P<0.05$, $n=5$; carvedilol −11.0±1.3 to −21.3±1.8 mV, $P<0.05$, $n=4$; propranolol −15.1±2.4 to −29.5±4.9 mV, $P<0.05$, $n=5$; ICI 118551 −9.1±1.7 to −12.1±2.1 mV, $P<0.05$, $n=7$).

Figure 4 shows the effects of β-blockers on the HERG peak tail current during a drug wash-in protocol at various drug concentrations. HERG current was elicited from a holding potential of −80 mV by a 4-s depolarising step to 20 mV followed by a repolarising step to −50 mV for 6 s, and the protocol was applied every 15 s. After obtaining the control record, β-blockers were applied to the bath. Application of metoprolol, carvedilol, propranolol and ICI 118551 resulted in a rapid HERG channel blockade to reach a nearly steady-state level within 5 min. The blocking effects were reversed gradually during washout (45–88%). Atenolol at 1 mM did not induce marked reduction of the HERG tail current.

The concentration–response relationship of drug block was assessed by changes in the HERG peak tail current amplitude at 5 min after application of the drug. Currents in the presence of varying concentrations of β-blockers were normalised to the control current amplitude and plotted as a function of drug concentration. The concentration-response curves were obtained by using the Hill equation:

$$I_{\text{drug}}/I_{\text{control}} = 1/[1 + (D/\text{IC}_{50})^n]$$

where D is the drug concentration, IC_{50} is the drug concentration for 50% block, and n is the Hill coefficient.

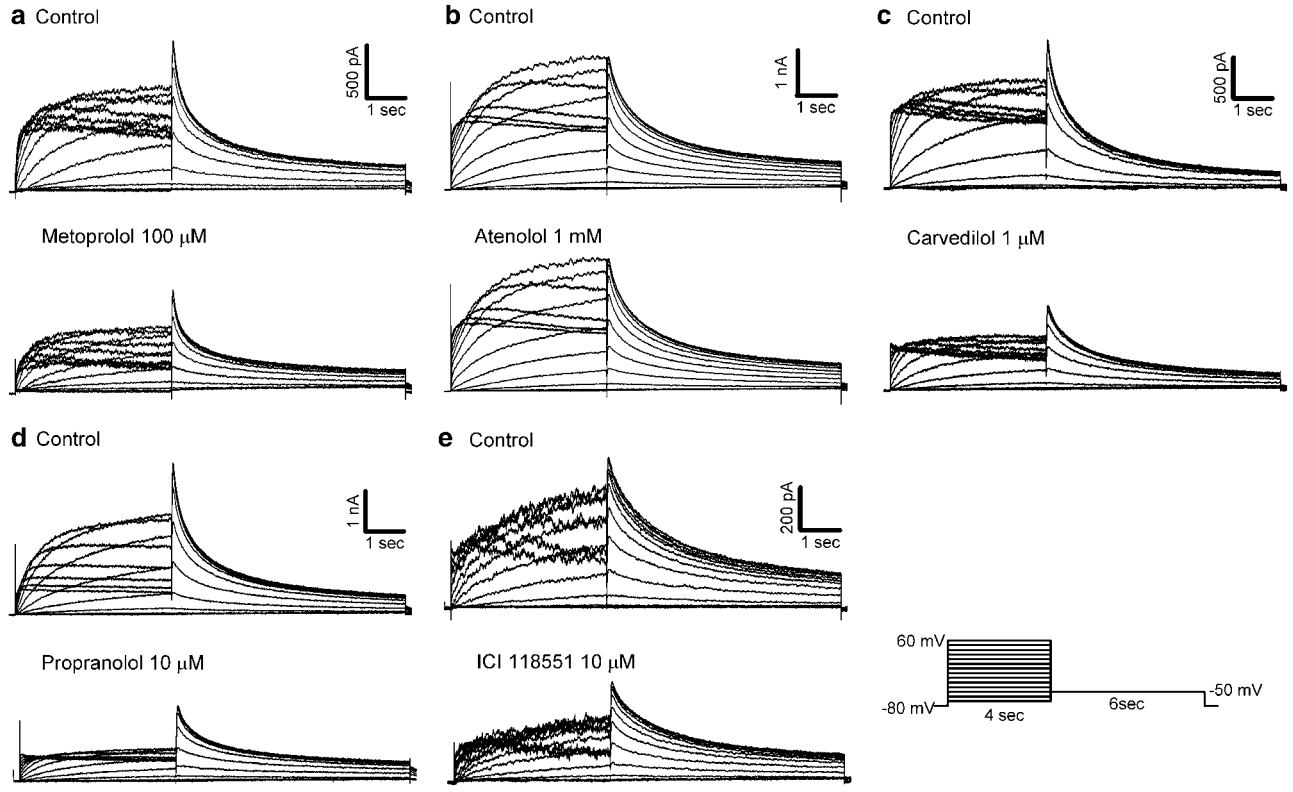

Figure 2 Effects of β-blockers on HERG current. HERG currents in control (top traces) and in the presence of β-blockers (bottom traces) at indicated concentrations were recorded using the pulse protocol shown at bottom right: (a) 100 μM metoprolol, (b) 1 mM atenolol, (c) 1 μM carvedilol, (d) 10 μM propranolol and (e) 10 μM ICI 118551.

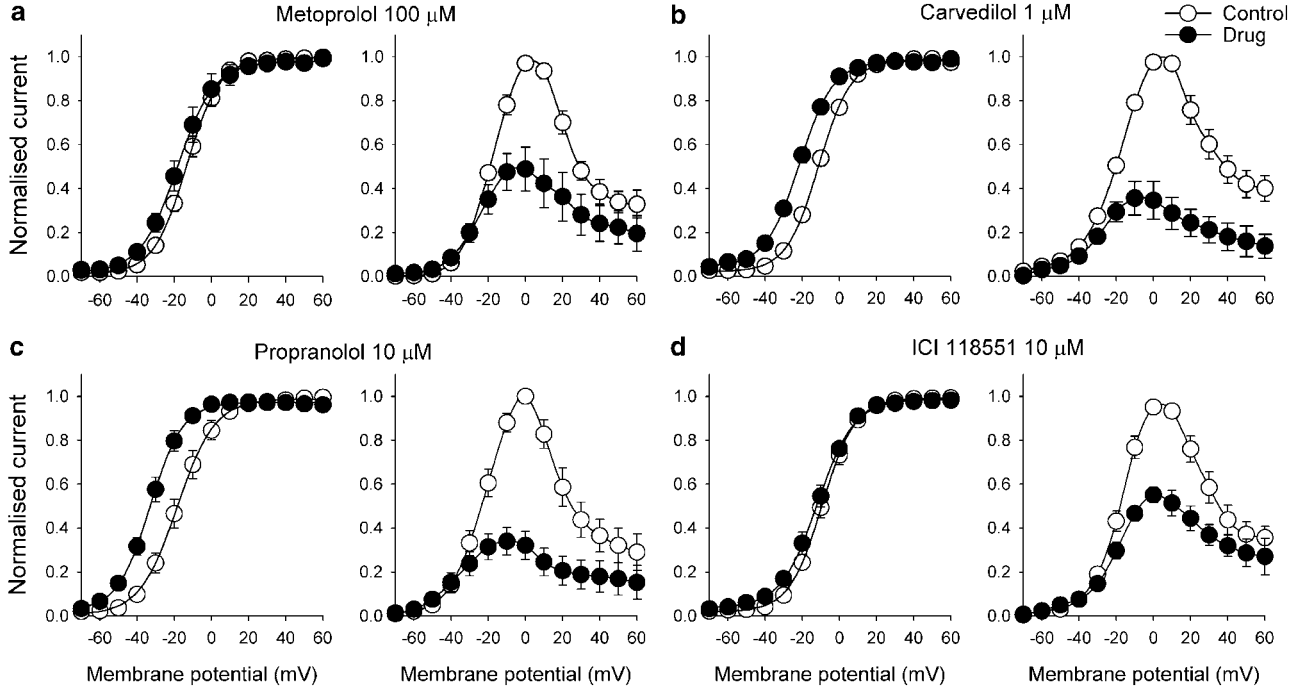

Figure 3 Current–voltage relationship for HERG channels and blockade by β-blockers. Normalised *I–V* relationships for peak tail currents (left) and currents measured at the end of depolarising steps (right) in the control and presence of β-blockers. Peak tail currents were normalised to their respective maximum current amplitude (control and drugs) to illustrate changes in half-maximal activation voltages: (a) 100 μM metoprolol ($n=5$), (b) 1 μM carvedilol ($n=4$), (c) 10 μM propranolol ($n=5$), (d) 10 μM ICI 118551 ($n=7$). Data are mean ± s.e.m.

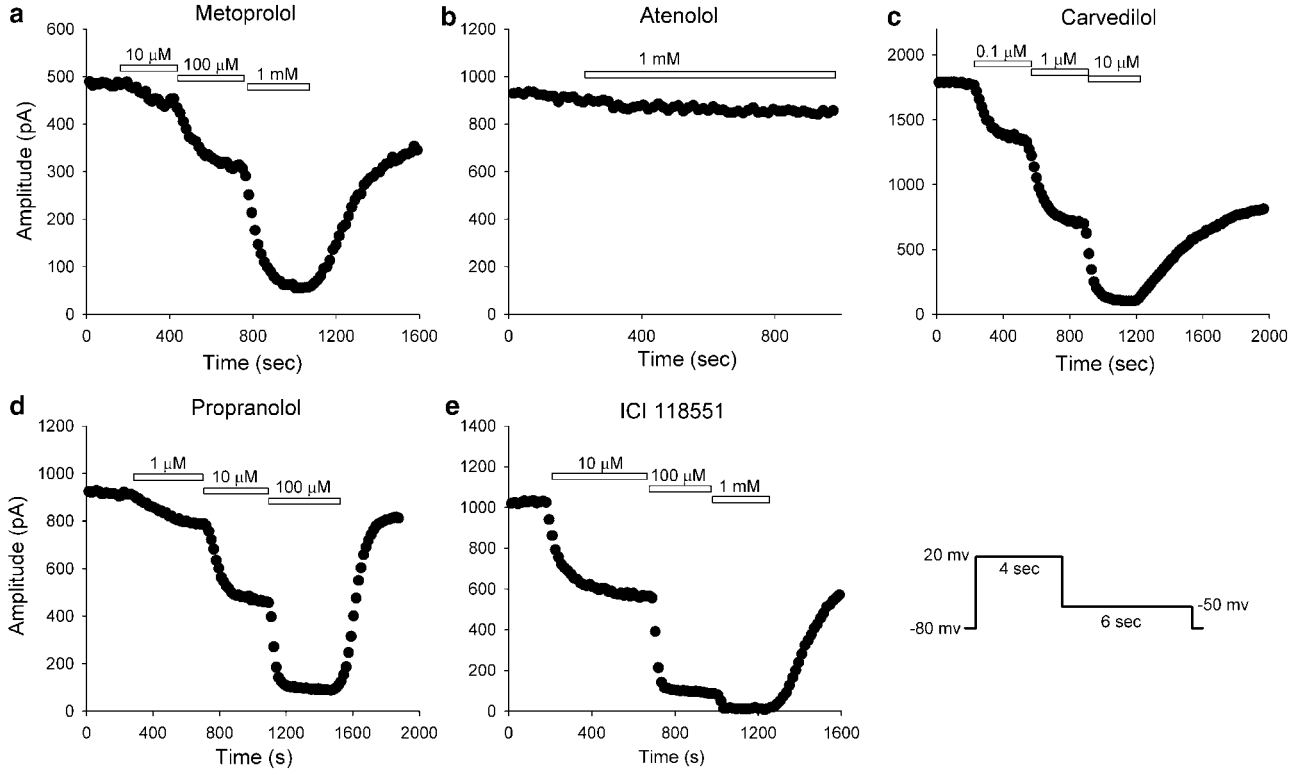

Figure 4 Time course of β-blocker-induced HERG tail current inhibition. HERG currents were elicited every 15 s by the pulse protocol shown at bottom right. Metoprolol (a), carvedilol (c), propranolol (d) and ICI 118551 (e) inhibited HERG tail current in a concentration-dependent manner and reached nearly a steady-state level within 5 min at each concentration. A partial recovery of current was observed after drug washout. (b) Atenolol at 1 mM did not significantly inhibit HERG tail current.

Metoprolol, carvedilol, propranolol and ICI 118551 reduced HERG current amplitude in a concentration-dependent manner but atenolol did not inhibit HERG channels for up to 1 mM (Figure 5). The IC_{50} value and Hill coefficient are summarised in Table 2.

Attenuation of HERG current blockade by carvedilol, propranolol and ICI 118551 in Y652A and F656C mutant channels

Next, we assessed the molecular basis of drug binding to HERG channels using the HERG Y652A and F656C mutant channels. Figure 6a shows representative current recordings in control conditions and 5 min after application of β-blockers in Y652A and F656C mutant channels. Compared with the wild-type, HERG channels blockade by carvedilol, propranolol and ICI 118551, but not metoprolol, was partially but significantly attenuated in both the Y652A and the F656C mutant channels (Figure 6b). We also assessed the concentration–response relationships of the drug block by measuring the peak tail current amplitude of Y652A and F656C mutant current. Current reduction was evaluated at 5 min after application of the drug, similar to the protocol used in the wild-type HERG channels. The results of the concentration-response relationships were plotted simultaneously with those of wild-type channels (Figure 5). The IC_{50} values and Hill coefficients for Y652A and F656C mutant channels, and the relative IC_{50} values compared with wild-type (ΔIC_{50}) are summarised in Table 2.

Frequency dependence of HERG current block

Finally, we investigated the frequency dependence of HERG current block by applying 35 repetitive pulses at 0.2 and 1 Hz, after holding the cell at −80 mV for 5 min during wash-in of 100 μM metoprolol, 1 μM carvedilol, 10 μM propranolol and 10 μM ICI 118551. HERG current was first rapidly activated by a 100 ms step to 60 mV, which was followed by a 400 ms step to 20 mV and a 200 ms step to −50 mV (see bottom of Figure 7). HERG current was measured as the peak tail current amplitude during the step pulse at −50 mV, and was normalised to the control current measured prior to drug exposure. For control conditions, the HERG current amplitude during the pulse train did not change significantly (<5%). Following exposure to drugs, application of the pulse train at either 0.2 or 1.0 Hz decreased current amplitude. The time course of HERG current block was fit with double exponential function:

$$A_f \exp -t/\tau_f + A_s \exp -t/\tau_s + \text{base}$$

where t is the pulse number; τ_f and τ_s are time constants of fast and slow components; A_f and A_s are fractional amplitudes of fast and slow components. The time constants of the fast and slow components of the time course of block, and the amount of steady-state block were not significantly different between the 0.2 and 1.0 Hz frequencies (Table 3). The lack of frequency-dependent block can be interpreted as the result of fast onset of open channel block and slow unblocking.

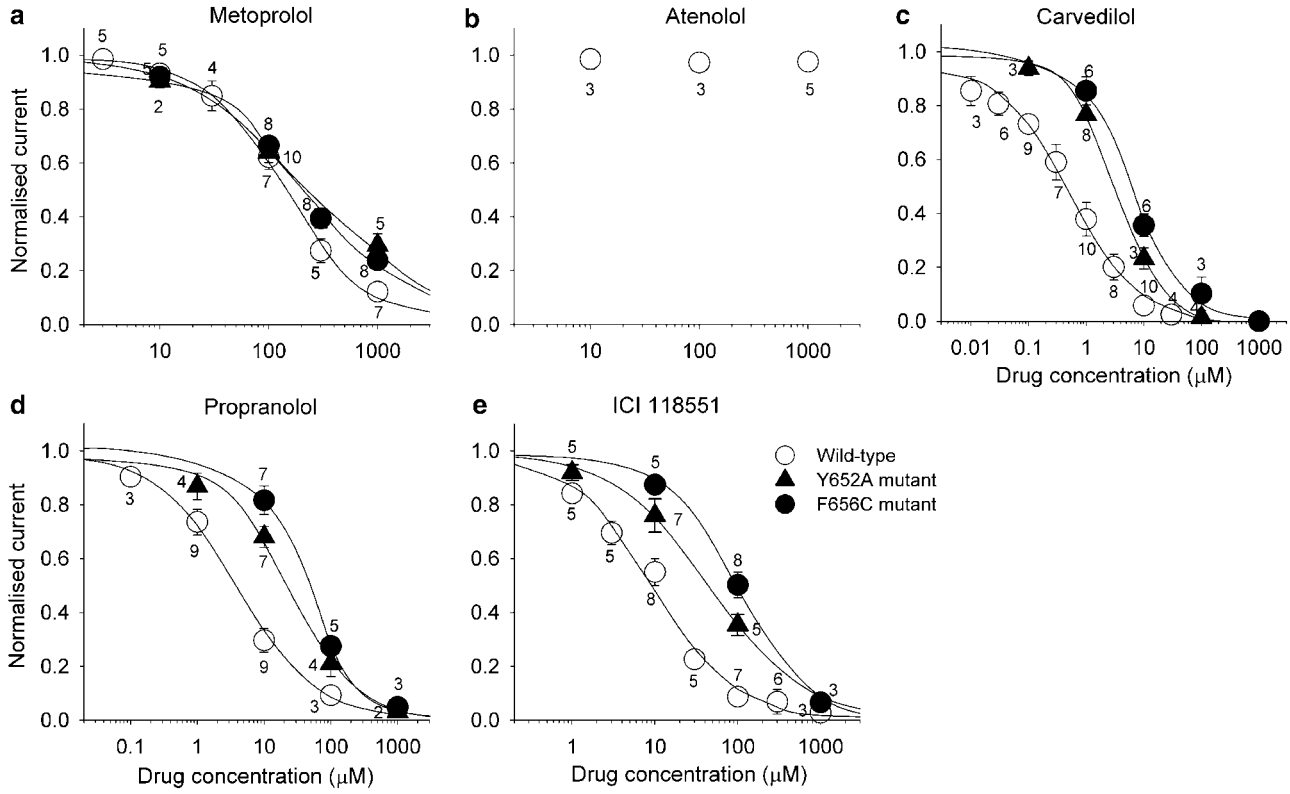

Figure 5 Concentration–response relationships for peak tail current in HERG wild-type, Y652A and F656C mutant channels. Currents in the presence of varying concentrations of β-blockers were normalised to the control amplitude and plotted as a function of drug concentration: (a) metoprolol, (b) atenolol, (c) carvedilol, (d) propranolol, (e) ICI 118551. Data are mean ± s.e.m. Numbers shown near the data points indicate the number of cells tested. The effects of atenolol on Y652A and F656C mutant channels were not tested. Solid lines represent fits with a Hill equation (see text for detail).

Table 2 Drug affinities to wild type, Y652A and F656C HERG channels

β-*Blockers*	*Wild-type (WT)*		*Y652A*			*F656C*		
	IC_{50} (μM)	*Hill Co.*	IC_{50} (μM)	*Hill Co.*	$\Delta IC_{50\ Y652A}$	IC_{50} (μM)	*Hill Co.*	$\Delta IC_{50\ F656C}$
Metoprolol	145	1.1	266	0.7	1.8	207	0.8	1.4
Carvedilol	0.51	0.8	3.4	1.1	6.7	5.5	0.9	10.8
Propranolol	3.9	0.8	24.3	0.8	6.2	40.4	1.0	10.3
ICI 118551	9.2	0.9	45.6	0.8	5.0	98.1	1.0	10.7

Hill Co. = Hill coefficient, $\Delta IC_{50\ Y652A} = IC_{50\ Y652A}/IC_{50\ WT}$, $\Delta IC_{50\ F656C} = IC_{50\ F656C}/IC_{50\ WT}$.

Discussion

In the present study, we examined the direct effect of β-blockers on HERG channels and the molecular basis of drug binding to the channel using HERG Y652A and F656C mutant channels. Our major findings were: (1) propranolol, carvedilol and ICI 118551 inhibited HERG current in a concentration-dependent manner. (2) High concentrations of metoprolol were required for blockade, and atenolol did not significantly inhibit HERG current. (3) Y652A and F656C mutations partially attenuated the affinity for propranolol, carvedilol and ICI 118551 but did not alter the affinity for metoprolol.

Effects of β*-blockers on HERG channels*

Carvedilol has been reported to inhibit HERG or I_{Kr} current. Cheng *et al*. (1999) reported that carvedilol inhibited I_{Kr}, L-type I_{Ca}, I_{to} and I_{Ks} in rabbit ventricular myocytes with IC_{50} values of 0.35, 3.59, 3.34 and 12.54 μM, respectively. It was important that I_{Kr} was the most sensitive to carvedilol and that the action potential duration was actually prolonged. The IC_{50} value for I_{Kr} blockade was very close to that determined in the present study (0.51 μM). Carvedilol has been reported also to inhibit HERG channels expressed in *Xenopus* oocytes by acting directly on the channels (Karle *et al*., 2001). Although the IC_{50} value in the oocyte expression system (10.4 μM) was much higher than those in native cardiac myocytes and HEK293 cells, the difference was attributed to the viteline membrane and yolk of oocytes, which required up to 30-fold higher concentrations for drug effects (Madeja *et al*., 1997). Taken together, carvedilol blocks HERG current at the range of therapeutic concentrations in clinical use (0.1–0.6 μM) (McPhillips *et al*., 1988), and has class III antiarrhythmic effect.

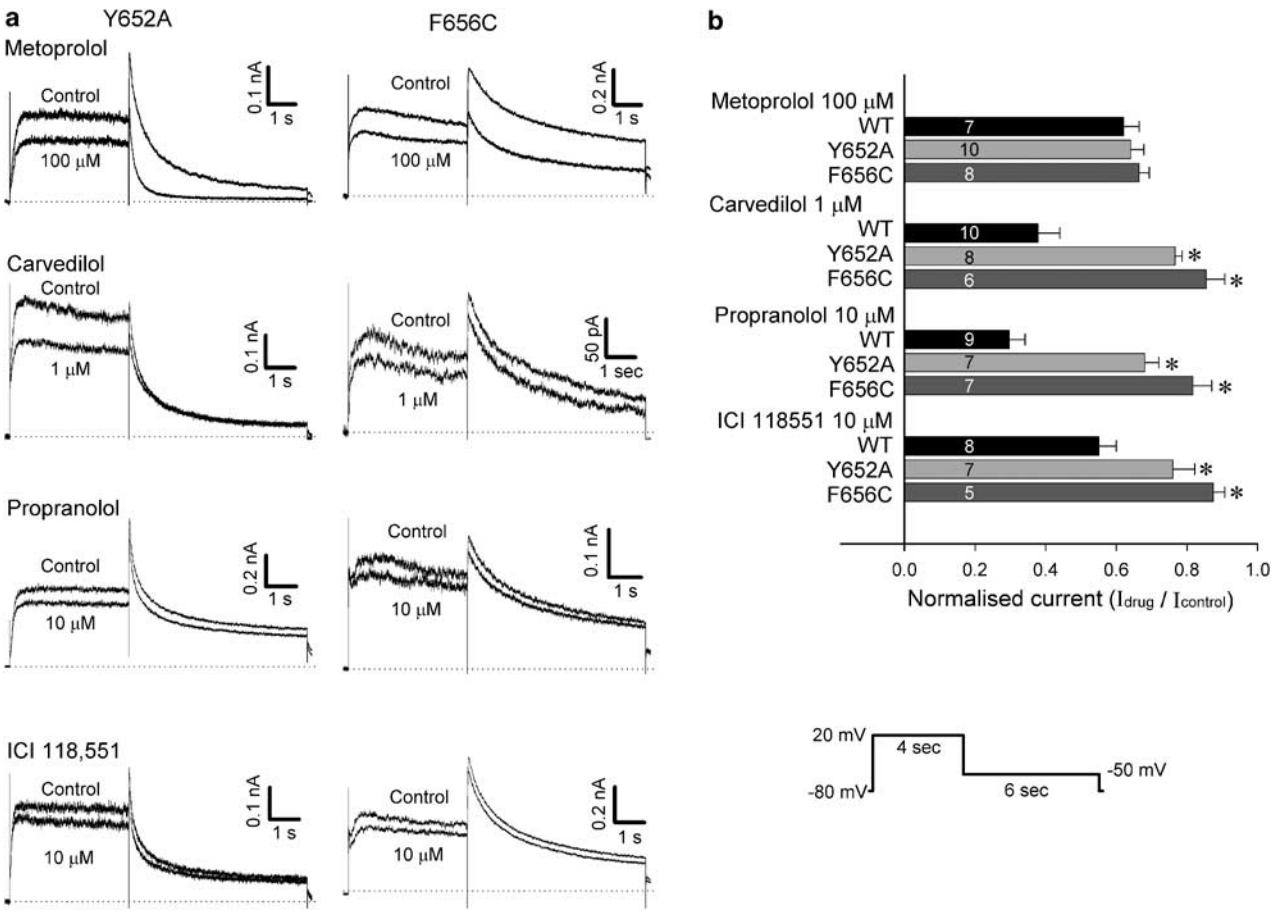

Figure 6 Y652A and F656C mutant channels attenuated HERG channel blockade by carvedilol, propranolol and ICI 118551. (a) HERG currents in control and in the presence of β-blockers at the indicated concentrations elicited by using the pulse protocol shown at the bottom right. (b) Comparison of HERG current inhibition by β-blockers with wild-type (WT), Y652A and F656C mutant channels. HERG currents evaluated at peak tail current amplitude in the presence of β-blockers were normalised to respective control values. Data are mean ± s.e.m. Numbers shown in parenthesis indicate the number of cells tested. $^*P<0.05$, compared with the wild-type.

At high dose, propranolol was reported to prolong QT interval (Farhangi & Sansone, 2003). More recently, propranolol has been reported to inhibit HERG channels expressed in Chinese hamster ovary cells with an IC_{50} value of $9.9 \pm 1.3\,\mu M$ (Yao *et al*., 2005). The present study also suggested that propranolol has an inhibitory effect on HERG current by binding to the putative common binding site. However, the IC_{50} value ($3.9\,\mu M$) was higher than the therapeutic concentration (C_{max}: $0.145 \pm 0.07\,\mu M/20\,mg$ p.o., mean ± s.d., $n = 10$, based on the information provided by the manufacturer). Thus, propranolol might have a less powerful effect on QT interval at a clinically relevant concentration as reported previously (Linker *et al*., 1992; Shimizu & Antzelevitch, 1998; Shimizu *et al*., 2002).

In contrast to carvedilol, atenolol and metoprolol did not show significant effects on HERG currents at least at therapeutic concentrations (C_{max}: atenolol: $1.64 \pm 0.14\,\mu M/50\,mg$ p.o., $n = 12$; metoprolol $0.40 \pm 0.07\,\mu M/120\,mg$ p.o., $n = 12$, based on the information provided by the manufacturer). The β_1-selective antagonists have a single ring compound in the common structure of β-blockers, while nonselective and β_2-receptor selective antagonists have other ring compounds in addition to the common structure (Figure 1). These structural differences may contribute to the drug affinity to HERG channels.

The structural requirements for the drug binding site in HERG channels have been studied recently in detail (Mitcheson *et al*., 2000; Kamiya *et al*., 2001; Milnes *et al*., 2003; Sanchez-Chapula *et al*., 2003; Scholz *et al*., 2003; Perry *et al*., 2004). It was demonstrated that aromatic amino-acid residues (Y652 and particularly F656) located in the S6 domain are the most important molecular determinants of drug binding (Mitcheson *et al*., 2000). In the present study, Y652A and F656C mutant channels attenuated HERG current blockade by carvedilol, propranolol and ICI 118551, suggesting that the binding of these β-blockers involves a putative drug receptor within the pore-S6 region of the HERG channel. However, inhibition of HERG channels by most drugs tested to date was abolished or markedly attenuated by F656 mutant channels except fluvoxamine and dronedarone (Milnes *et al*., 2003; Ridley *et al*., 2004). In the present study, the drug affinities of carvedilol, propranolol and ICI 118551 were about five- to 11-fold less to the Y652A and F656C mutant channels than to the wild-type channels. Considered together, in addition to Y652 and F656, other amino-acid residues might be also involved in the molecular determinant of drug binding. Metoprolol decreased

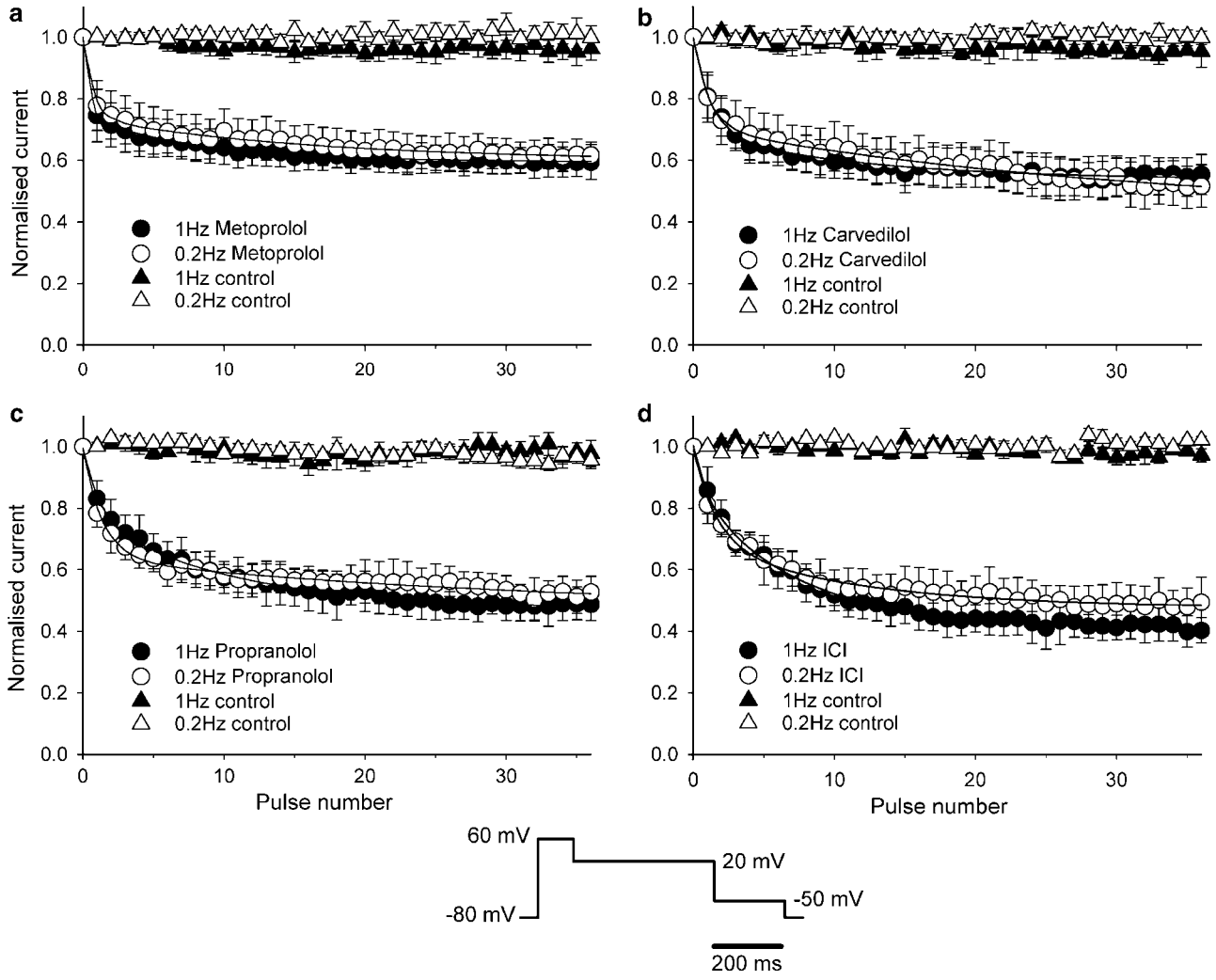

Figure 7 Inhibition of HERG tail current by β-blockers was not frequency-dependent. After the cell was held at −80 mV for 5 min during exposure to 100 μM metoprolol (a), 1 μM carvedilol (b), 10 μM propranolol (c) and 10 μM ICI 11855 (d), 35 repetitive pulses (see protocol at centre bottom of the figure) were applied at 1 and 0.2 Hz. Solid lines represent fits to averaged data with double exponential function (see text for detail). Fitting parameters are summarised in Table 3.

Table 3 Parameters of the time course of HERG current block by β-blockers

β-*Blockers*	*0.2* Hz				*1* Hz			
	τ_f (pulses^{-1})	τ_s (pulses^{-1})	*Base*	n	τ_f (pulses^{-1})	τ_s (pulses^{-1})	*Base*	n
Metoprolol	0.55±0.14	13.7±2.4	0.56±0.05	4	0.35±0.11	9.8±1.8	0.59±0.04	4
Carvedilol	1.00±0.10	17.7±5.1	0.52±0.07	4	1.18±0.13	14.4±2.6	0.54±0.06	4
Propranolol	0.92±0.16	9.5±3.4	0.52±0.04	4	1.64±0.55	10.8±1.2	0.47±0.04	4
ICI 118551	0.61±0.09	9.2±2.8	0.48±0.06	4	0.61±0.07	7.5±0.5	0.41±0.04	4

τ_f and τ_s, time constants of fast and slow components.

HERG current in a dose-dependent manner, but Y652A and F656C mutant channels did not significantly attenuate the HERG current blockade. Since high dose of metoprolol was required to block HERG current, the drug-channel interaction might be considered nonspecific although the involvement of other amino-acid residues could not be excluded.

Clinical implications

In the COMET study (Poole-Wilson *et al*., 2003) and its subanalysis (Remme *et al*., 2004), carvedilol reduced the mortality rate and sudden cardiac death rate more effectively than metoprolol. Although the mechanisms for this favourable clinical outcome for patients treated with carvedilol are still unknown, it is likely to be multifactorial involving the blockade of β- and α-adrenergic receptors, a greater anti-ischemic effect, inhibition of apoptosis, an antioxidant action, free-radical scavenging, and/or electrophysiological effect. In the present study, we compared the class III antiarrhythmic effects of multiple β-blockers by estimating HERG channel blocking activity. The class III antiarrhythmic effects were significantly potent for carvedilol compared with other

β-blockers. Carvedilol might provide favourable outcome *via* class III antiarrhythmic effects, in the context of β-blockade, in chronic heart failure patients. Thus, our results seem to provide a hypothetical molecular explanation for the favourable outcome in carvedilol treated patients in the COMET study.

Mutations in *KCNQ1* (or *KVLQT1*) are responsible for defects in the slow component of the delayed rectifier potassium current (I_{Ks}) underlying the LQT1, whereas mutations in *KCNH2* (or *HERG*) result in defects in the rapid component of the delayed rectifier current (I_{Kr}) responsible for the LQT2. Life-threatening arrhythmias including *torsades de pointes* tachyarrhythmia and sudden cardiac death tend to occur with physical or emotional stress in patients with LQT1 and LQT2 syndrome (Schwartz *et al*., 2001). Since adrenergic stimulation results in QT prolongation on the surface ECG, β-blockers have been considered to be effective in preventing cardiac events especially in patients with LQT1 (Dorostkar *et al*., 1999; Moss *et al*., 2000; Schwartz *et al*., 2001; Shimizu *et al*., 2002). Since the HERG channel function is defective in LQT2 patients and HERG channel dependency is increased in LQT1 patients, it is reasonable to consider that β-blockers without HERG channel blocking activity are more preferable for the treatment of these patients. In the present study, atenolol and metoprolol did not inhibit HERG currents significantly at least in clinically relevant concentrations. Thus, these drugs are suitable for treatment of LQT1 and LQT2 patients. High concentrations of propranolol also blocked HERG channels in the present study. Since overdose application of propranolol actually prolongs QT interval (Farhangi & Sansone, 2003), propranolol may not be the best choice for the treatment of patients with LQT1 and LTQ2. In contrast, carvedilol directly inhibited HERG channels at clinically relevant concentrations. Thus, carvedilol might not be recommended in the treatment of patients with LQT1 and LQT2.

Study limitations

The present study has certain limitations. We used HERG channels heterologously expressed in a human cell line. The possible effects of β-blockers on other ion channels as well as receptors *in vivo* should be considered. Although the direct inhibition of HERG channels by binding to a common binding site presented in this study is one of the mechanisms that regulate HERG channels, the existence of other pathways that regulate HERG channels has been reported. Recent studies revealed that increased intracellular levels of cAMP regulated HERG channels directly by binding to the cyclic nucleotide binding domain and indirectly through cAMP-dependent protein kinase (PKA)-mediated phosphorylation of the channel protein, resulting in net reduction of HERG current (Thomas *et al*., 1999; Cui *et al*., 2000; 2001). These effects were, at least in part, mediated by the activation of β_1-adrenergic receptors (Karle *et al*., 2002). According to these results, β-blockers without HERG channel blocking activity competitively attenuate the β-receptor-mediated HERG current inhibition. On the other hand, β-blockers with HERG channel blocking activity have dual pathways to regulate cardiac HERG channels by both direct binding to the HERG channels and competitive antagonism with β-adrenergic agonists on their receptor site. Further studies are needed to identify the most potent regulator of HERG channel at various concentrations.

Secondly, β-blockers, especially carvedilol and propranolol, caused a negative shift in activation curves (Figure 3). One previous study reported that carvedilol did not shift the activation curve in an oocyte expression system (Karle *et al*., 2001). In our preliminary experiments, we confirmed using Western blot analysis that β_1-receptors are expressed on the surface of HEK293 cells (data not shown). Since β-adrenergic activation causes a positive shift in the activation curve (Cui *et al*., 2000), it is possible that inhibition of baseline activity of endogenous β-receptors caused the negative shift of activation curve and offset the current reduction induced by direct HERG channels block. The negative shift of the activation curve may cause a net increase of current if other biophysical parameters remain unchanged. One of the reasons for the lack of current increase may be due to pronounced pharmacological blockade of HERG channels.

Finally, minK-related peptide 1 (MiRP1), encoded by *KCNE*2, coassembles with the pore-forming HERG subunit and probably reconstitutes native I_{Kr} (Abbott *et al*., 1999). Compared to channels formed by the HERG subunit alone, HERG/MiRP1 complexes show altered channel properties and increase the potency of channel block by E-4031. Moreover, the mutant forms of MiRP1 demonstrated diminished potassium currents or increased channel blockade by drugs (Sesti *et al*., 2000). Therefore, it is possible that drug sensitivity might be different between HERG and HERG/MiRP1 channels, although wild-type MiRP1 does not markedly influence the drug sensitivity of the channels (Numaguchi *et al*., 2000; Kamiya *et al*., 2001; Scherer *et al*., 2002; Friederich *et al*., 2004).

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