British Journal of Pharmacology (2002) 136, 717–729

www.nature.com/bjp

Inhibition of the current of heterologously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine

[1]Ashok A. Paul, [1]Harry J. Witchel & *,[1]Jules C. Hancox

[1]Department of Physiology and Cardiovascular Research Laboratories, School of Medical Sciences, University Walk, Bristol, BS8 1TD

1 The inhibition of the cardiac 'rapid' delayed rectifier current (I_{Kr}) and its cloned equivalent HERG mediate QT interval prolonging effects of a wide range of clinically used drugs. In this study, we investigated the effects of the Class Ic antiarrhythmic agent flecainide (FLEC) on ionic current (I_{HERG}) mediated by cloned HERG channels at 37°C . We also compared the inhibitory potency of FLEC with other Class I agents: quinidine (QUIN, Class Ia); lignocaine (LIG, Class Ib) and propafenone (PROPAF, Class Ic).

2 Whole cell voltage clamp recordings of I_{HERG} were made from an HEK293 cell line stably expressing HERG. FLEC inhibited I_{HERG} 'tails' following test pulses to +30 mV with an IC_{50} of 3.91 ± 0.68 μM (mean ± s.e.mean) and a Hill co-efficient close to 1 (0.76 ± 0.09).

3 In experiments in which I_{HERG} tails were monitored following voltage commands to a range of test potentials, I_{HERG} inhibition by FLEC was observed to be voltage-dependent and to be associated with a ~ -5 mV shift of the activation curve for the current. Voltage-dependence of inhibition was greatest over the range of potentials corresponding to the steep portion of the I_{HERG} activation curve. The time-course of I_{HERG} tail deactivation was not significantly altered by FLEC.

4 In experiments in which 10 s depolarizing pulses were applied from −80 to 0 mV, the level of current inhibition by FLEC did not increase between 1 and 10 s. Some time-dependence of inhibition was observed during the first 200–300 ms of depolarization. This observation and the voltage-dependence of inhibition are collectively consistent with FLEC exerting a rapid open channel state inhibition of I_{HERG}.

5 Under similar recording conditions QUIN inhibited I_{HERG} with an IC_{50} of 0.41 ± 0.04 μM and PROPAF inhibited I_{HERG} with an IC_{50} of 0.44 ± 0.07 μM. Similar to FLEC, both QUIN and PROPAF showed voltage-dependence of inhibition and blockade developed rapidly during a sustained depolarization.

6 LIG showed little effect on I_{HERG} at low micromolar concentrations, but could inhibit the current at higher concentrations; the observed IC_{50} was 262.90 ± 22.40 μM.

7 Our data are consistent with FLEC, PROPAF and QUIN exerting I_{HERG} blockade at clinically relevant concentrations. The rank potency as HERG blockers of the Class I drugs tested in this study was QUIN = PROPAF > FLEC > > LIG.

British Journal of Pharmacology (2002) **136,** 717–729

Keywords: Antiarrhythmic; Class Ia; Class Ib; Class Ic; flecainide; HERG; I_{Kr}; QT interval; QT prolongation; quinidine

Abbreviations: ANOVA, analysis of variance; δ, fractional distance in the transmembrane field sensed at drug receptor site; DISO, disopyramide; FLEC, flecainide; HERG, Human *ether-a-go-go* related gene; IC_{50}, half maximal inhibitory drug concentration; $I_{Ca,L}$, L-type calcium current; I_{HERG}, current mediated by the HERG channel; I_K, delayed rectifier potassium current; I_{Kr} 'rapid' delayed rectifier potassium current; I_{Ks} 'slow' delayed rectifier potassium current; I_{to}, transient outward potassium current; k, slope factor describing voltage dependent activation; LIG, lignocaine; PROPAF, propafenone; QT interval, QT interval of the electrocardiogram; QUIN, quinidine; τ_f, fast time constant of deactivation; τ_s slow time constant of deactivation; s.e.mean, standard error of the mean; $V_{0.5}$ half maximal activation voltage

Introduction

A number of different cardiac potassium (K^+) currents contribute to ventricular action potential repolarization (Carmeliet, 1993). Of these, delayed rectifier K^+ current regulates repolarization over plateau voltages and hence modulates action potential duration (APD). Composite cardiac I_K is comprised of two distinct subtypes: 'rapidly activating' and 'slowly activating' I_K (I_{Kr} and I_{Ks} respectively) separable on the basis of distinct kinetics, pharmacology and molecular origin (e.g. Sanguinetti & Jurkiewicz, 1990; Chinn, 1993; Sanguinetti *et al*., 1995; Trudeau *et al*., 1995; Barhanin *et al*., 1996; Heath & Terrar, 1996; Sanguinetti *et al*., 1996b). The measured profile of I_{Kr} during the ventricular action potential (Hancox *et al*., 1998; Zhou *et*

*Author for correspondence; E-mail: jules.hancox@bristol.ac.uk

al., 1998; Mitcheson & Hancox, 1999a; Rocchetti *et al*., 2001) supports a major role for this current in mediating plateau repolarization.

The '*Human ether-a-go-go-related Gene*' *(HERG)* is now widely accepted as encoding the α-subunit of channels carrying I_{Kr}; polymorphisms in *HERG* can impair channel function and thereby lead to chromosome-7-linked long QT syndrome (LQTS – LQT2; Curran *et al*., 1995; Sanguinetti *et al*., 1996a). Moreover, when HERG was first identified as encoding an I_{Kr}-like channel, it was suggested that it may also mediate the 'acquired' (drug-induced) form of the syndrome (Sanguinetti *et al*., 1995). Indeed, it is now known that structurally diverse drugs associated with LQTS (e.g. Class III antiarrhythmics, macrolide antibiotics, prokinetic drugs, antihistamines, antidepressants and antipyschotic agents) share in common an ability to block the HERG/I_{Kr} K^+ channel (for recent reviews see Witchel & Hancox, 2000; Vandenberg *et al*., 2001). Recent evidence suggests that the pharmacological 'promiscuity' of the HERG/I_{Kr} channel is associated (a) with an unusually large vestibule in the channel pore (Mitcheson *et al*., 2000b) and (b) with the presence of particular aromatic amino-acid residues in the pore and pore helix that provide a substrate for interactions with drugs that themselves contain aromatic rings (Lees-Miller *et al*., 2000; Mitcheson *et al*., 2000a; Kamiya *et al*., 2001; Vandenberg *et al*., 2001).

Aside from the well known QT-prolonging effects of Class III antiarrhythmic agents (Nattel & Singh, 1999; Viskin, 1999; Witchel & Hancox, 2000), Class Ia antiarrhythmic agents are also known normally to prolong repolarization and QT interval to a moderate extent, whilst also carrying a risk of LQTS and associated pro-arrhythmia (Viskin, 1999). Quinidine is perhaps the best known example, having been associated with syncope for many years (Lewis, 1922) and more recently with blockade of both native I_{Kr} (Yang & Roden, 1996; Yang *et al*., 1997) and HERG (Po *et al*., 1998; Lees-Miller *et al*., 2000; Ishii *et al*., 2001). Disopyramide can also induce QT prolongation (e.g. Lima & Boudoulas, 1987), and block HERG/I_{Kr} (Virag *et al*., 1998; Paul *et al*., 2001). Interestingly, intravenous administration of the Class Ic drug propafenone can also produce QT interval prolongation and *torsade de pointes* (e.g. Rehnqvist *et al*., 1984; Hii *et al*., 1991) and, experimentally, propafenone can block native I_{Kr} (Duan *et al*., 1993; Delpon *et al*., 1995) and HERG current (Mergenthaler *et al*., 2001).

Less is known in this regard about the Class Ic drug flecainide. Flecainide is effective against a variety of supraventricular arrhythmias (Falk & Fogel, 1994) and exhibits a low incidence of QT interval prolongation and *torsade de pointes* (Lui *et al*., 1982; Wickers *et al*., 1988; Sarubbi *et al*., 1996). Experiments performed on feline ventricular myocytes have shown blockade by flecainide of delayed rectifier tail currents consistent with identity as I_{Kr} (Follmer & Colatsky, 1990; Follmer *et al*., 1992). Limited data from guinea-pig ventricular myocytes also suggest that flecainide can inhibit I_{Kr} (Wang *et al*., 1996). Little detailed information exists regarding this effect under conditions that are entirely selective for I_{Kr}/HERG. Therefore, a major aim of the present study was to investigate the actions of flecainide on recombinant HERG channels expressed in a mammalian (Human Embryonic Kidney; HEK293) cell line. Furthermore, there has been no systematic comparison of the relative potencies and characteristics of HERG blockade by drugs drawn from the different Class I subtypes (a – c) under a standardized set of recording conditions. Thus, a second aim of the present study was for effects of flecainide on HERG current (I_{HERG}) to be compared with those of other representative Class Ia, Ib and Ic agents.

Methods

Maintenance of mammalian cell line stably expressing HERG

All experiments were performed on a cell line (Human embryonic kidney; HEK 293) stably expressing HERG, and generously donated by Professor Craig January, University of Wisconsin (Zhou *et al*., 1998). Cells were passaged using a non-enzymatic agent (Splittix, AutogenBioclear) and plated out onto small sterilized glass coverslips in 30 mm petri dishes containing a modification of Dulbecco's Minimum Essential Medium with Glutamax-1 (DMEM; Gibco), supplemented with 10% foetal calf serum, 400 μg ml^{-1} gentamycin (Gibco) and 400 μg ml^{-1} geneticin (G418; Gibco). The cells were incubated at 37°C for a minimum of 2 – 4 days prior to any electrophysiological study.

Electrophysiological recording

The glass coverslips were placed in a bath (0.5 ml volume) mounted on an inverted microscope (Nikon Diaphot) and the cells were superfused with Normal Tyrode's solution which contained (in mM): NaCl 130, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Glucose 10, HEPES 5, (titrated to a pH of 7.4 with NaOH). Drugs were also added to this to make up test solutions at the final concentrations mentioned in Results. Patch-pipettes (Corning 7052 glass, AM Systems Inc.) were pulled to resistances of 2 – 3 MΩ (Narashige PP83) and fire-polished to 4 – 5 MΩ (Narishige, MF83). The internal dialysis solution contained (in mM): KCl 130, $MgCl_2$ 1, EGTA 5, MgATP 5, HEPES 10 (titrated to a pH of 7.2 with KOH). The 'pipette-to-bath' liquid junction potential was measured for this filling solution and was −3.2 mV. Since this value was small, no corrections of membrane potential were made. Whole-cell patch clamp recordings of membrane currents were made using an Axopatch 1D amplifier (Axon Instruments) and a CV-4 1/100 headstage. Between 75 and 80% of the electrode series resistance could be compensated. Voltage clamp commands were generated using 'WinWCP', a program written and supplied free of charge by John Dempster of Strathclyde University. Data were recorded *via* a Digidata interface and stored on the hard-disk of a Viglen EX computer for analysis. All experiments were carried out at 37 ± 1°C.

Data analysis and presentation

Data were analysed using WinWCP, Excel 5.0 and 'FigP for Windows' (Biosoft) and Instat (Graphpad Inc). Data are presented as mean ± standard error of the mean (s.e.mean) and statistical comparisons were made using Student's *t*-test or analysis of variance (ANOVA) with *post-hoc* Bonferroni correction (Instat, Graphpad Inc.). *P* values of less than 0.05

were taken as significant. Quantitative fits to data were made using the equations listed below:

Fractional block of the I_{HERG} tail was determined using the equation:

$$\text{Fractional block} = 1 - (I_{HERG-drug}/I_{HERG-control}) \quad (1)$$

where $I_{HERG\text{-}drug}$ and $I_{HERG\text{-}control}$ represent 'tail' current amplitudes in control and drug containing solution respectively.

Concentration-response data were fitted using the equation:

$$\text{Fractional block} = 1/(1 + (IC_{50}/[FLEC])^{h}) \quad (2)$$

where IC_{50} is [FLEC] producing half-maximal inhibition of the I_{HERG} tail and h is the Hill coefficient for the fit.

Current-voltage (I–V) relations were fitted with a modified Boltzmann equation of the form:

$$I = I_{max}/(1 + \exp((V_{0.5} - V_m)/k)) \quad (3)$$

where $I = I_{HERG}$ tail amplitude following test potential V_m, I_{max} is the maximal I_{HERG} tail current observed, $V_{0.5}$ is the potential at which I_{HERG} was maximally activated, and k is the slope factor describing I_{HERG} activation.

Voltage-dependent activation curves for I_{HERG} were obtained by calculating activation variables at 2 mV intervals between −80 and +40 mV. To do this, values for $V_{0.5}$ and k derived from fits to experimental I–V data with equation 3were inserted into the following equation:

$$\text{Activation parameter} = 1/(1 + \exp[(V_{0.5} - V_m)/k]) \quad (4)$$

where 'activation parameter' at any test potential, V_m, occurs within the range 0–1 and $V_{0.5}$ and k have similar meanings to those in equation 3.

Deactivation of I_{HERG} tails at −40 mV, following a test potential of +30 mV was described by a bi-exponential equation of the form:

$$y = A1 * \exp(-x/Tau1) + A2 * \exp(-x/Tau2) + C \quad (5)$$

where Tau1 and Tau2 represent the fast (τ_f) and slow (τ_s) components of the deactivating tail current and A1 and A2 represent the total current fitted by each component respectively. C represents any residual current component not described by τ_f or τ_s (C was equal to or close to zero for fits to I_{HERG} tail deactivation).

Drugs

Quinidine, lignocaine and propafenone were obtained from Sigma and flecainide was obtained in solution from '3M' (Tambocor Injection; 10 mg ml^{-1}). Ten mM stock solutions of each agent were made according to the solubility profiles given in the Merck index (The Merck Index, 12th Edition). Lignocaine and flecainide were dissolved in deionized water (Milli-Q, Millipore) while quinidine and propafenone were dissolved in ethanol (BDH). These stock solutions were further serially diluted so that similar volumes of the dissolved agent were added from stock solution to extracellular solution to make up the concentrations mentioned in Results. The maximum concentration of ethanol used was never in excess of 1% by volume and during preliminary experiments, this concentration was found to have no effect on I_{HERG} ($n = 4$). All drugs were applied to the cells under study using a warmed, multi-barrelled solution application device (Levi *et al*., 1996) which was capable of changing the bathing solution surrounding a cell in <1 s.

Results

Concentration-dependent inhibition of I_{HERG} by FLEC

At 35–37°C, I_{HERG} is known to activate rapidly upon depolarization to a positive membrane potential (Hancox *et al*., 1998; Zhou *et al*., 1998). Thus, in order to determine the effects of FLEC, I_{HERG} was elicited by 500 ms duration test pulses to +30 mV from a holding potential of −40 mV (pulse frequency of 0.2 Hz). Currents were measured at

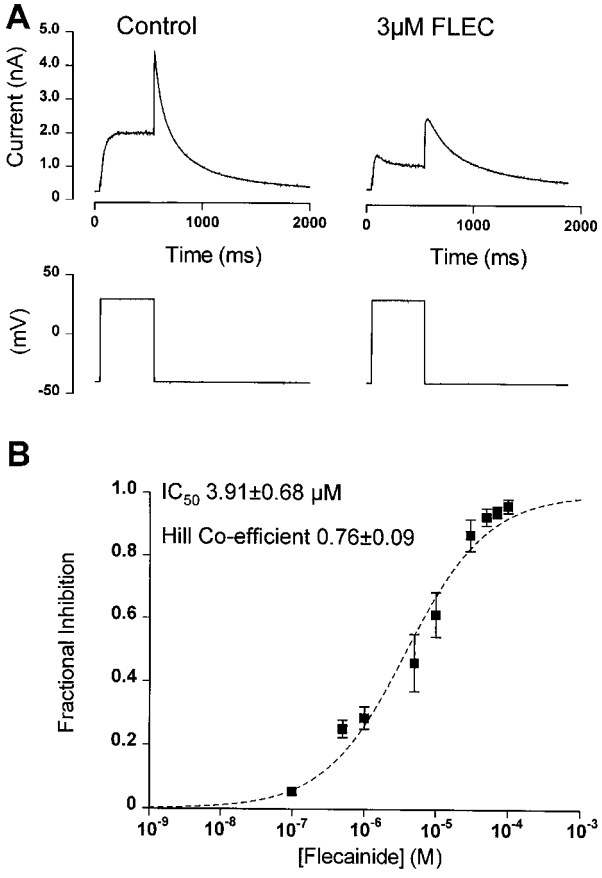

Figure 1 Inhibitory effect of flecainide on I_{HERG}. (A) Representative currents (upper traces) elicited by 500 ms duration pulses from −40 to +30 mV. Left panel shows current in control solution and right panel shows current in the presence of 3 μM flecainide (FLEC), which produced a marked inhibition of both the current during the pulse and of the outward tail current on repolarization. (B) Dose response relation for the inhibitory effect of flecainide on I_{HERG} tails. Between 4 and 19 cells were used for each of the different drug concentrations tested. Mean data-points were fitted by equation 2in Methods.

steady-state firstly in normal Tyrode's solution and then in the presence of FLEC. Figure 1A shows representative current traces before and during exposure to 3 μM FLEC. The 'control' record shows a characteristic I_{HERG} profile: outward current developed rapidly during the applied depolarization, followed by a large amplitude current 'tail' on repolarization to -40 mV. The I_{HERG} tail amplitude exceeds current magnitude during voltage commands to positive voltages due to the particularly rapid voltage-dependent inactivation kinetics of HERG channels (Sanguinetti *et al*., 1995; Trudeau *et al*., 1995; Smith *et al*., 1996; Zhou *et al*., 1998). Both the current during the pulse and the ensuing I_{HERG} tail on repolarization to -40 mV were inhibited by the drug. There was little change in I_{HERG} amplitude after washout, indicating poor reversibility of blockade. A total of nine different concentrations of FLEC between 100 nM and 100 μM were tested on I_{HERG} (using between four and 19 cells for each concentration); this revealed a concentration-dependent blockade by the agent. For each drug concentration and for each cell tested, I_{HERG} blockade was quantified by measuring peak I_{HERG} tail amplitudes in control and FLEC-containing solutions. Fractional block of the I_{HERG} tail was determined using equation 1(see Methods). Data from individual cells were pooled to obtain mean ($\pm$s.e. mean) fractional block values, which were then plotted against the corresponding FLEC concentration ([FLEC]) as shown in Figure 1B. The mean data-points were then fitted with a Hill-plot (equation 2in Methods); the observed IC_{50} was 3.91 ± 0.68 μM and the Hill-coefficient for the fit was close to 1 (0.76 ± 0.09).

Voltage dependence of I_{HERG} *inhibition by FLEC*

The effects of FLEC on I_{HERG} were investigated further by applying voltage-commands of 5 s duration from a holding membrane potential of -80 mV to a range of test-potentials. I_{HERG} tails were elicited on repolarization to -40 mV at the end of each test pulse, before returning to the holding potential of -80 mV. Representative current traces are shown in the upper panel of Figure 2A, with the corresponding voltage protocols in the lower panel. At more negative test potentials, 3 μM FLEC exerted a comparatively smaller effect on I_{HERG} than it did at more positive potentials. Figure 2B shows the normalized mean current-voltage (I–V) relations for I_{HERG} tails in control solution and in the presence of FLEC. For each of 16 cells, I_{HERG} tail amplitudes at each potential were normalized to the maximal I_{HERG} tail in control conditions, as described recently (Paul *et al*., 2001). I–V plots were constructed for each cell, and data from the different cells were also pooled in order to obtain the mean I–V relations in control and drug. I–V relations showed a progressive divergence between control and FLEC up to ~ -10 mV, consistent with voltage-dependence of inhibition. For each cell, and for the mean data, the I–V relations were fitted by equation 3(see Methods). From the fits to each cell, the mean values for $V_{0.5}$ in control and drug were -20.67 ± 0.18 and -25.71 ± 0.31 mV respectively. The mean values for k in control and drug were 7.37 ± 0.17 and 8.68 ± 0.30 mV respectively. The left-ward shift in $V_{0.5}$ for I_{HERG} activation was statistically significant ($P < 0.02$). However, the values of k were not significantly different from one another ($P > 0.7$).

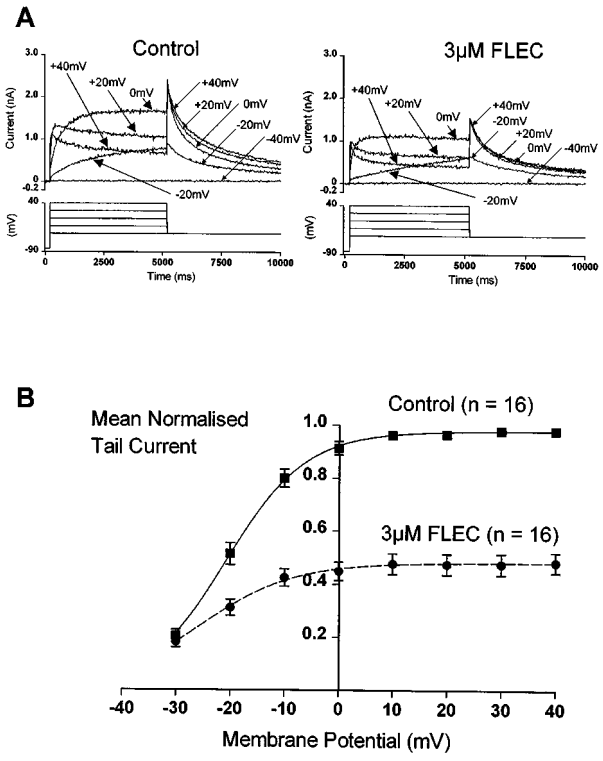

Figure 2 Inhibitory effect of flecainide on I_{HERG} at different test potentials. (A) Representative currents (upper traces) elicited by 5 s test pulses (lower traces) to the potentials marked. I_{HERG} tail amplitudes on repolarization to -40 mV were monitored. Left panel shows control currents, and right panel shows those in the presence of 3 μM flecainide. (B) Current-voltage plot for effect of flecainide on I_{HERG} tails following test pulses to potentials between -30 and $+40$ mV. The pooled mean$\pm$s.e.mean data-points were fitted by equation 3in a similar fashion as for individual cells, and as described in Methods. For 'control': $V_{0.5} = -21.57 \pm 1.70$ mV and $k = 7.53 \pm 0.51$. For FLEC, $V_{0.5} = -25.92 \pm 1.75$ mV and $k = 10.83 \pm 3.13$ mV.

The voltage-dependence of I_{HERG} blockade by FLEC was quantified by plotting mean fractional block (calculated using equation 1) against test potential, as shown in Figure 3. The level of I_{HERG} blockade produced by 3 μM FLEC increased progressively up to -20 mV and levelled out between -10 and $+40$ mV; the dependence of fractional block on test potential was found to be statistically significant ($P < 0.0001$; ANOVA). Also plotted in Figure 3 are the activation curves for I_{HERG} in control and flecainide, (constructed using the experimentally derived $V_{0.5}$ and k values and equation 4in Methods). Voltage-dependent blockade coincided with the voltage range over the steep phase of the activation curve (i.e. the membrane potential range over which channel opening occurs). This is consistent with the drug's blocking action being activation/open-state dependent (Snyders *et al*., 1992; Mergenthaler *et al*., 2001).

Deactivation of I_{HERG} *tails*

The effect of FLEC on I_{HERG} tails was further studied by investigating whether or not their deactivation time-course was altered by the action of FLEC, I_{HERG} tails which were recorded on repolarization to -40 mV from a test potential of $+30$ mV were fitted with equation 5 (see Methods). We

observed some variation between between cells in deactivation time-course under control conditions. Values for τ_f ranged from 175 to 520 ms and for τ_s ranged from 1140 to 4390 ms. The mean values for both time constants that we observed under control conditions (see Figure 4) were slower than those reported by Zhou *et al.* (1998). Using data from 16 cells, we compared mean values for each of τ_f and τ_s between control solution and FLEC-containing solution. The relative proportions of current deactivation described by the fast and slow time-constants were also determined. None of these values was significantly different between control and FLEC. Similarly, measurements of I_{HERG} deactivation time-course from nine cells with a higher FLEC concentration of 5 μM showed no significant alteration to parameters describing deactivation time-course.

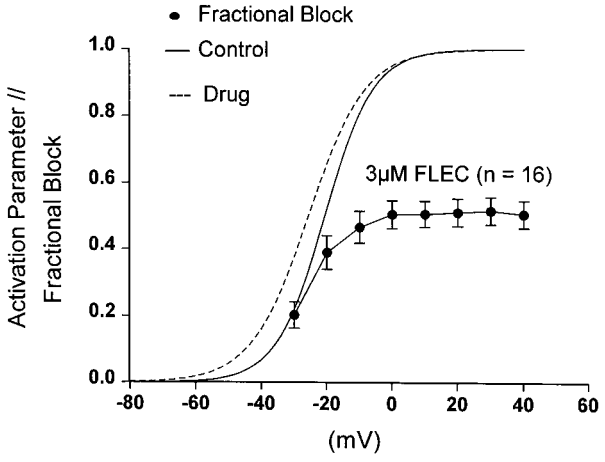

Figure 3 Voltage-dependence of I_{HERG} inhibition by flecainide. Filled circles show mean level of fractional block of I_{HERG} at potentials between −30 and +40 mV ($n=16$ cells). Superimposed are plots of the voltage-dependent activation curve for I_{HERG} in control solution (continuous line) and in 3 μM flecainide (broken line). Activation curves were produced using a Boltzmann relation (equation 4,in Methods) and mean values for half-maximal activation voltage and slope factor derived from the data-sets in Figure 2B. The voltage-dependence of blockade was greatest over the steep portion of the I_{HERG} activation relation.

Development of blockade during a sustained depolarization

I_{HERG} blockade by high affinity methanesulphonanilides during a sustained depolarization shows a characteristic profile; it begins with little blockade initially and this progressively develops and increases over seconds during the maintained depolarization (e.g. Snyders & Chaudhary, 1996; Zhou *et al.*, 1998). Some other HERG-blocking agents show a different profile, with blockade evident early during depolarization (Teschemacher *et al.*, 1999; Walker *et al.*, 2000; Mergenthaler *et al.*, 2001; Witchel *et al.*, 2002). In order to study the development of I_{HERG} blockade by FLEC, long-duration (10 s) depolarizing pulses were applied from a holding potential of −80 to 0 mV to elicit I_{HERG} in control solution, as shown in Figure 5A. Following repolarization to −80 mV, the external solution surrounding the cell under study was then rapidly (<1 s) exchanged for one containing 3 μM FLEC. The cell was then equilibrated at −80 mV in the

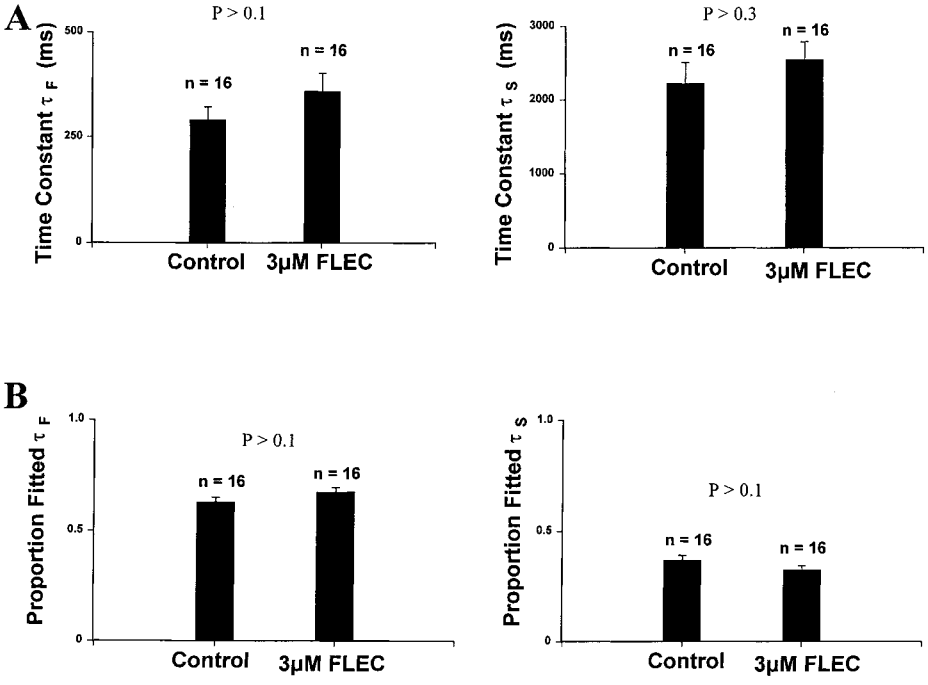

Figure 4 Effect of flecainide on deactivation time-course of I_{HERG}. The deactivation time-course of I_{HERG} tails at −40 mV following test-pulses to +30 mV was fitted by equation 5in Methods. Histograms show plots of mean±s.e.mean values for fast (τ_f, left panel) and slow (τ_s, right panel) deactivation time constants in control solution and in the presence of 3 μM flecainide. Histograms show the mean±s.e.mean proportion of current deactivation described by fast (left panel) and slow (right panel) deactivation processes.

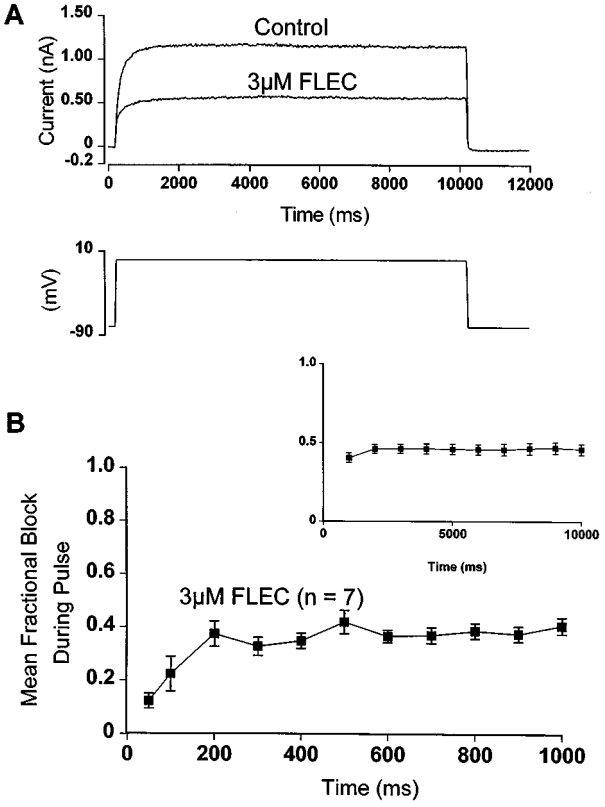

Figure 5 Development of I_{HERG} blockade during a sustained depolarization. (A) Upper traces show I_{HERG} in control and 3 μM flecainide activated by a 10 s test pulse (lower trace) from -80 to 0 mV. Blockade developed rapidly on depolarization. (B) Plots of mean level of fractional block ($n=7$ cells) by flecainide of I_{HERG} over the first 1000 ms of a sustained depolarization (main panel) and between 1000 and 10,000 ms (insert). Some time-dependence of inhibition was observed during the first $\sim$200 ms of the pulse. Between 1000 and 10,000 ms there was no significant increase in the developed level of current inhibition.

FLEC-containing solution for between 3–4 min, in the absence of applied pulses, before a second identical long-duration pulse was applied. Current during the pulse in the presence of drug was compared with that in control. Figure 5A shows representative current records from such an experiment. Current in the presence of FLEC was attenuated throughout the applied pulse. Figure 5B shows mean data from seven cells, for which the mean level of fractional block of current by 3 μM FLEC was plotted over the first second of the pulse (main panel) and also at 1 s intervals during the pulse (inset). Blockade of I_{HERG} did show some time-dependence ($P<0.001$), with blockade developing over the first 200–300 ms of the pulse. Between 1 and 10 s, however, there was no statistically significant difference in the level of current blockade ($P>0.05$; ANOVA). When considered collectively, the data in Figures 2–5 are consistent with FLEC exerting an open-state dependent inhibitory action on I_{HERG} that developed rapidly on membrane potential depolarization. The lack of statistically significant alteration to current deactivation by FLEC, together with poor reversibility of blockade are consistent with flecainide being retained ('trapped') in the channel once it has reached its site of action.

Comparison with QUIN

Blockade of I_{HERG} by pharmacological agents can differ depending on expression system and experimental conditions (e.g. Po *et al*., 1998; Paul *et al*., 2001; Yang *et al*., 2001). Therefore, in order to contextualize our findings with flecainide against those of other Class I agents, additional experiments with QUIN were performed; this Class Ia agent is known to block both native I_{Kr} and heterologously expressed HERG (Po *et al*., 1998; Lees-Miller *et al*., 2000; Ishii *et al*., 2001). A total of eight different QUIN concentrations were tested and the mean fractional block for each concentration was determined using equation 1. As for FLEC a concentration-response relation was plotted; this is shown in Figure 6A,B. Under our recording conditions, QUIN produced a blockade of the I_{HERG} tail that was more potent than that observed with FLEC. The IC_{50} was 0.41 ± 0.04 μM and the Hill co-efficient for the fit was again close to 1 (0.76 ± 0.05).

QUIN was also applied in experiments again utilizing voltage-commands to a range of test potentials. I–V relations for the I_{HERG} tail in control and drug were constructed (Figure 6B) and voltage-dependent activation parameters determined using equation 3. For seven cells, the mean $V_{0.5}$ values in control and drug were -19.10 ± 1.48 and -23.31 ± 1.55 respectively ($0.07> P>0.05$), whilst the k values were 6.31 ± 1.48 and 8.11 ± 1.18 respectively ($P>0.2$). Mean levels of fractional blockade at each test potential are plotted in Figure 6D together with the activation curves for I_{HERG} in control and drug (calculated using equation 4). As for FLEC, the voltage-dependence of blockade was greatest over the steep range of voltage-dependent activation.

The development of blockade during a sustained depolarization was also determined for QUIN (Figure 6E,F). Similarly to FLEC, there was some time-dependence of blockade over the first 100–200 ms of the pulse ($P<0.001$), but the level block did not significantly alter between 1 and 10 s under our conditions ($P>0.05$).

Comparison with PROPAF

For further comparison, we tested the effects of a second Class Ic antiarrhythmic agent, PROPAF, on I_{HERG}. PROPAF has been reported to block native cardiac I_K (Duan *et al*., 1993; Delpon *et al*., 1995) and, recently, to inhibit HERG channels expressed in *Xenopus* oocytes (Mergenthaler *et al*., 2001). However, the recently reported potency for inhibition I_{HERG} by PROPAF (Mergenthaler *et al*., 2001) is somewhat less than that obtained in experiments on cardiomyocytes (Duan *et al*., 1993). We reasoned that an IC_{50} value obtained from I_{HERG} recordings from a mammalian cell line might more accurately reflect the true blocking potency of the drug.

Eight different PROPAF concentrations were tested and a minimum of five cells used to test each concentration. As for FLEC and QUIN, the mean fractional block level for each concentration was determined, and a concentration-response relation was constructed (Figure 7A,B). Under our conditions, PROPAF produced a blockade of the I_{HERG} tail that was more potent than that observed with FLEC, and comparable to that observed with QUIN; the IC_{50} value for inhibition was 0.44 ± 0.07 μM and the Hill co-efficient for

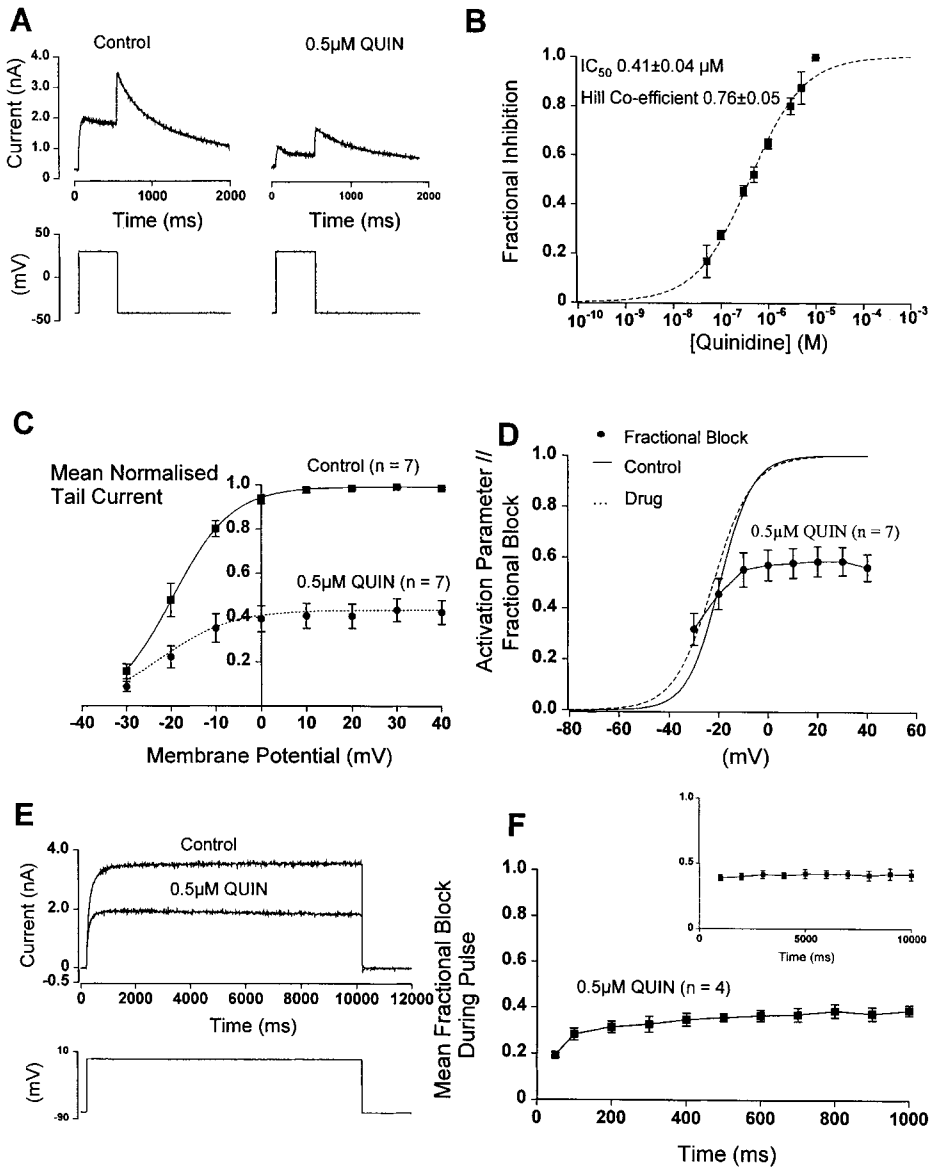

Figure 6 Effects of quinidine on I_{HERG}. (A) Upper traces show representative currents elicited by depolarizing test pulses (lower traces) in the presence and absence of 0.5 μM quinidine (QUIN). (B) Concentration-response relationship for inhibition of I_{HERG} tail (same protocol as A) by QUIN. A minimum of eight cells were used to test each concentration of QUIN. (C) Current-voltage relation for action of QUIN on I_{HERG} tails at -40 mV following pulses to test potentials between -30 and $+40$ mV. Protocol similar to that shown in Figure 2A. The pooled mean$\pm$s.e.mean data-points were fitted by equation 3. In control, mean $V_{0.5}$ was $-19.48\pm$ 0.12 mV and k was 6.56 ± 0.11 mV. In QUIN, $V_{0.5}$ was -22.19 ± 0.68 mV and k was 7.91 ± 0.87 mV. (D) Mean level of fractional block of I_{HERG} at potentials between -30 and $+40$ mV (solid circles). Overlaid on this are activation curves for I_{HERG} in control solution and in the presence of quinidine (calculated using equation 4). (E) Sample current records (upper traces) elicited by a long duration test pulse (lower trace) from -80 to 0 mV, in control and in the presence of 0.5 μM quinidine. (F) Mean data ($n=4$ cells) showing level of blockade during the first 1000 ms of the long duration pulse (main panel) and between 1000 and 10,000 ms during sustained depolarization (inset).

the fit was close to 1 (0.79 ± 0.10). This compares with an IC_{50} value of ~14 μM obtained from *Xenopus* oocytes (Mergenthaler *et al*., 2001).

In order to determine any voltage dependence of blockade under our conditions, PROPAF was also applied in experiments utilizing voltage-commands to a range of test potentials. I–V relations for the I_{HERG} tail in control and drug were constructed (Figure 7B) and voltage-dependent activation parameters determined using equation 3.

For seven cells, the mean $V_{0.5}$ values in control and drug were -22.18 ± 0.15 and -27.17 ± 2.51 respectively ($P<0.04$), whilst the k values were 5.99 ± 0.15 and 5.62 ± 2.24 respectively ($P>0.2$). Mean levels of fractional blockade at each test potential are plotted in Figure 6D, together with the activation curves for I_{HERG} in control and drug (calculated using equation 4).

As for FLEC and QUIN, the voltage-dependence of blockade was greatest over the steep portion of the

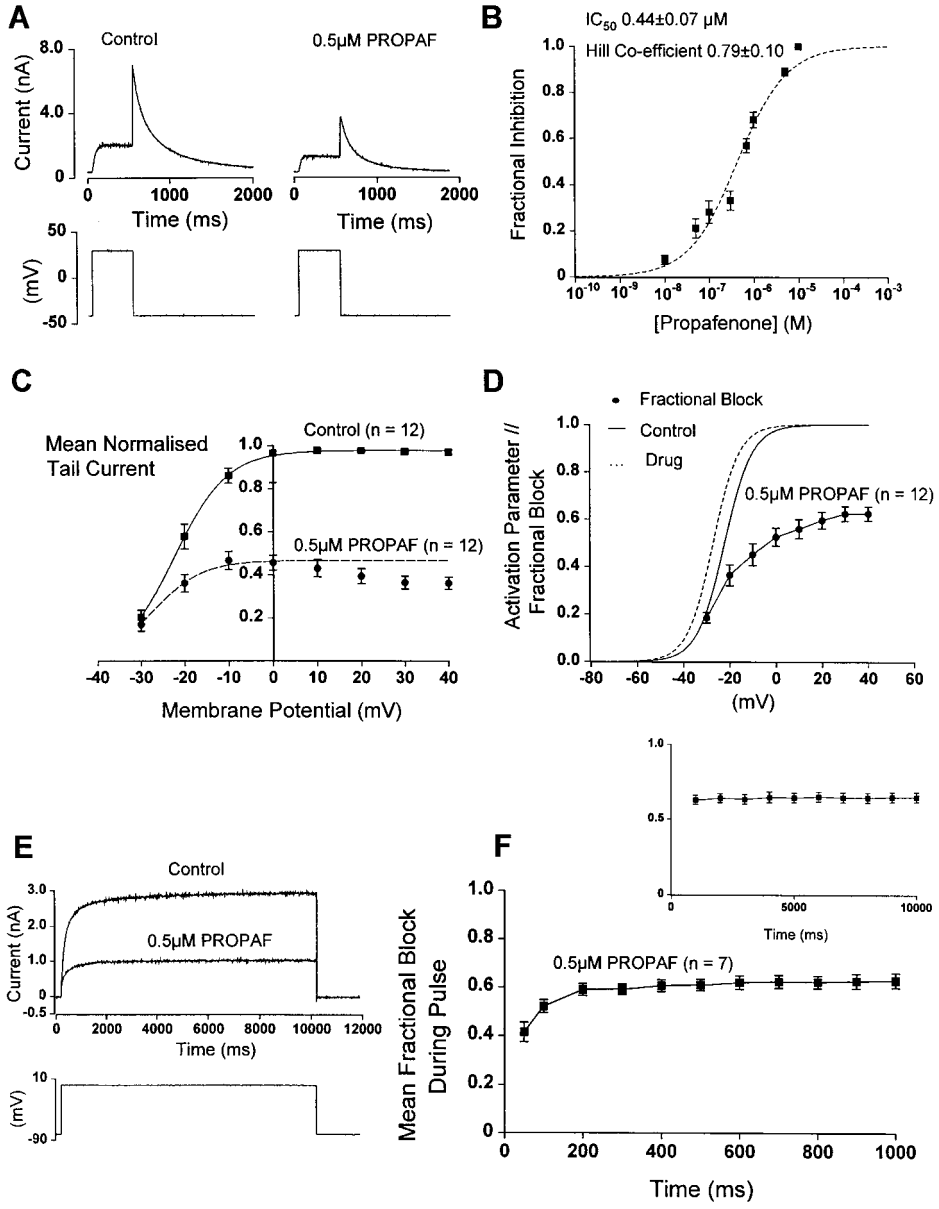

Figure 7 Effects of propafenone on I_{HERG}. (A) Upper traces show representative currents elicited by depolarizing test pulses (lower traces) in the presence and absence of 0.5 μM propafenone (PROPAF). (B) Concentration-response relationship for inhibition of I_{HERG} tail (same protocol as A) by PROPAF. (C) Current-voltage relation for action of PROPAF on I_{HERG} tails at -40 mV following pulses to test potentials between -30 and $+40$ mV. Protocol similar to that shown in Figure 2A. The pooled mean$\pm$s.e.mean data-points were fitted by equation 3. In control, $V_{0.5}$ was -19.10 ± 1.48 mV and k was 6.31 ± 0.87 mV. In PROPAF $V_{0.5}$ was -17.59 ± 1.74 mV and k was 10.71 ± 2.24 mV. (D) Mean level of fractional block of I_{HERG} at potentials between -30 and $+40$ mV (solid circles). Overlaid on this are activation curves for I_{HERG} in control solution and in the presence of PROPAF (calculated using equation 4). (E) Sample current records (upper traces) elicited by a long duration test pulse (lower trace) from -80 to 0 mV, in control and in the presence of 0.5 μM propafenone. (F) Mean data ($n=4$ cells) showing level of blockade during the first 1000 ms of the long duration pulse (main panel) and between 1000 and 10,000 ms during sustained depolarization (inset).

activation curve. However, the overall voltage-dependence of blockade was more marked for PROPAF than for either of the other two agents. Fractional block continued to increase at potentials at which I_{HERG} would be expected to be maximally activated, whereas for FLEC and QUIN, the extent of blockade levelled out between 0 and $+40$ mV (compare Figure 7D with Figures 3 and 6D). This observation regarding voltage-dependent inhibition by PROPAF corresponds well to that recently obtained with I_{HERG} measurements from *Xenopus* oocytes (Mergenthaler *et al*., 2001), which allowed an estimate to be made of the fractional distance (δ) in the transmembrane field sensed at the receptor site for PROPAF. We applied a similar methology to estimate from mean fractional block data the K_D values for PROPAF inhibition at $+40$ mV and a reference voltage of 0 mV, and then estimated δ, by substituting these values into the equation:

$$K_{D+40\,mV} = K_{D0\,mV} \cdot \mathrm{Exp}(-z\delta FV/RT) \quad (6)$$

Where $K_{D+40\,mV}$ and $K_{D0\,mV}$ represent half maximal blocking concentrations at +40 and 0 mV respectively, V = membrane test potential (+40 mV in this instance) and z, R, F and T have their usual meanings (*cf.* Snyders *et al.*, 1992; Mergenthaler *et al.*, 2001). This yielded an estimate for δ of 0.27 (i.e. that the propafenone binding site sensed 27% of the transmembrane potential field, relative to the inside). This value is close to that recently reported by Mergenthaler and colleagues ($\delta = 0.2$; Mergenthaler *et al.*, 2001).

As for FLEC and QUIN, the development of blockade during a sustained depolarization was also determined for PROPAF (Figure 7E,F). Similarly to FLEC, some time-dependence of blockade was observed over the first 200 ms of the pulse ($P < 0.001$). However, the level of block did not significantly alter between 1 and 10 s under our conditions ($P > 0.05$). This observation is in qualitative agreement with observations made for PROPAF on I_{HERG} from *Xenopus* oocytes (Mergenthaler *et al.*, 2001).

Comparison with LIG

Class Ib antiarrhythmic agents are typically associated with action potential shortening rather than lengthening (Campbell *et al.*, 1991). Having compared the effects of the Class Ic FLEC with a Class Ia drug (QUIN) and a second Class Ic drug (PROPAF), we therefore considered it to be useful to determine whether or not a Class Ib agent (LIG) could block I_{HERG} under the same set of experimental conditions used to test the other agents. Once again, eight different drug concencentrations were tested. In contrast to the other agents tested, LIG concentrations in the low micromolar range exerted comparatively little effect on I_{HERG}, and concentrations of 100 μM or greater were required to produce marked I_{HERG} tail blockade (Figure 8A). A concentration-response plot for LIG (Figure 8B) gave an estimated IC_{50} value of 262.90 ± 22.40 μM and a Hill co-efficient close to 1 (0.83 ± 0.07). These experiments demonstrated that I_{HERG} is not entirely resistant to blockade by LIG, but that this drug is a blocker of rather low potency. Our data suggest that little I_{HERG} blockade would be anticipated to occur at therapeutic lignocaine concentrations (~7–15 μM; Lui *et al.*, 1986). For this reason, the effects of LIG on I_{HERG} were not investigated in further detail.

Comparative potencies of the 4 Class I agents

Having determined the IC_{50} values for each of the four agents on I_{HERG}, these were resubstituted into the Hill equation 2) and simulated dose response relationships for each agent were plotted on a single set of axes as shown in Figure 9. To summarise: I_{HERG} exhibited similar sensitivity to both QUIN and PROPAF; both of these, in turn, were more potent blockers than FLEC and LIG, the latter being by far the weakest inhibitor.

Discussion

Measurements of action potential upstroke velocity and of current carried by cardiac Na channels expressed in *Xenopus* oocytes suggest IC_{50} values of ~14–41 μM for FLEC (Ranger *et al.*, 1993; Krafte *et al.*, 1994). In addition to the Na channel blocking action that underlies its 'Class I' antiarrhythmic activity (Cowan & Williams, 1981; Campbell, 1983), FLEC has been reported to block L-type calcium current ($I_{Ca,L}$; IC_{50} in frog myocytes of 20 μM; Scamps *et al.*, 1989), transient outward current potassium current (I_{to}; e.g. Wang *et al.*, 1993; Yamashita *et al.*, 1995; Mitcheson & Hancox, 1999b; with an IC_{50} of 17 μM for rabbit I_{to} Yamashita *et al.*, 1995) and to show selectivity for recombinant Kv 4.2 over Kv 1.4 channels (Yeola & Snyders, 1997). FLEC has also been reported to inhibit delayed rectifier current from feline ventricular myocytes with an IC_{50} of 2.1 μM (Follmer *et al.*, 1992), that from rabbit atrial myocytes 'slightly' (Yamashita *et al.*, 1995), and at 10 and 30 μM to be associated with drug sensitive currents from guinea-pig ventricular myocytes that suggest a preferential action on I_{Kr} over I_{Ks} (Wang *et al.*, 1996). The drug appears to be without significant effect on inwardly rectifying K^+ current (Follmer *et al.*, 1992; Yamashita *et al.*, 1995).

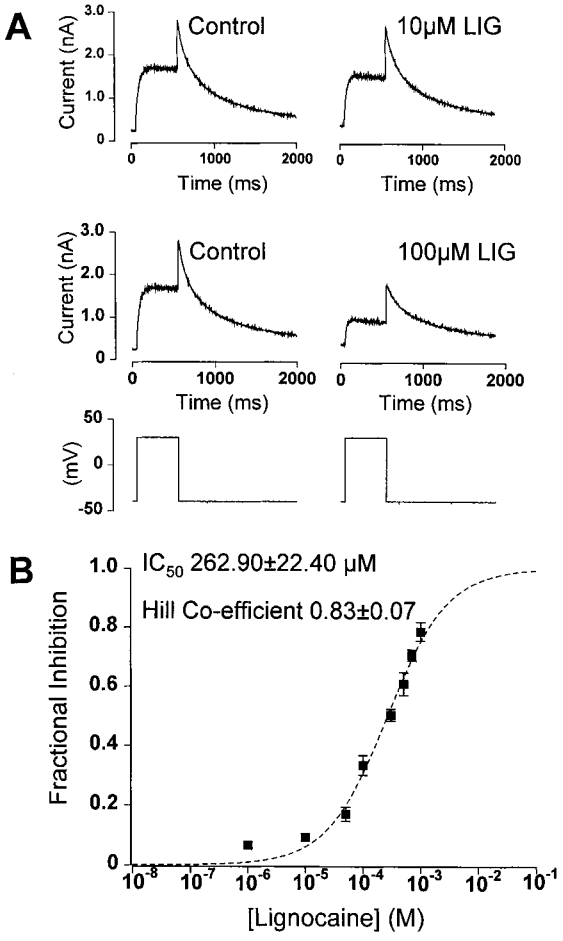

Figure 8 Effects of lignocaine on I_{HERG}. Representative currents (upper and middle traces) elicited by 2 s duration pulses from −40 to +30 mV (lower traces). For each pair of records, left panel shows current in control solution and right panel shows current in the presence of lignocaine (LIG). Top traces show effect of 10 μM LIG and middle traces show effect of 100 μM LIG. (B) Dose response relation for inhibitory effect of LIG on I_{HERG} tails. Mean data-points were fitted by equation 2 in Methods.

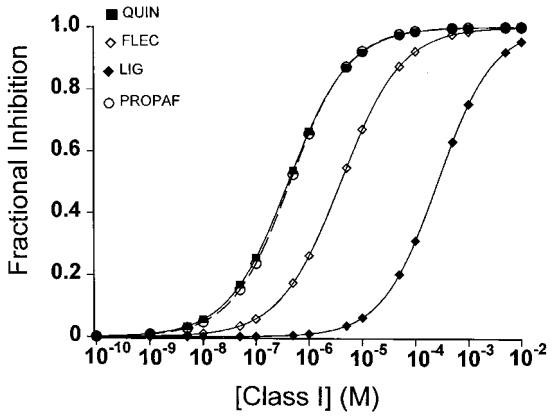

Figure 9 Comparative effects of Class 1a–c agents on I_{HERG}. Experimentally derived IC_{50} values for actions on I_{HERG} of each of quinidine (QUIN), flecainide (FLEC), lignocaine (LIG) and propafenone (PROPAF) were used with a Hill equation (equation 2in Methods), in order to simulate and display on the same axes the concentration-response relations for the different agents studied. The rank potency of the compounds as I_{HERG} inhibitors was QUIN = PROPAF > FLEC > > LIG.

I*HERG* blockade by flecainide

This is the first study in which the actions of FLEC on recombinant HERG channels have been examined. A major advantage conferred by such a preparation over recordings from native cardiomyocytes is the lack of overlapping and potentially contaminating currents; this facilitates the accurate characterization of drug actions. For example, whilst I_K tails from feline myocytes were reported to be sensitive to both E-4031 and FLEC (Follmer & Colatsky, 1990), time-dependent outward current during applied depolarizations was only sensitive to blockade by FLEC in some preparations (Follmer *et al*., 1992). In subsequent experiments on guinea-pig myocytes (Wang *et al*., 1996), the presence of overlapping I_{Ks} precluded detailed analysis of the FLEC effect on I_{Kr}. In spite of different experimental preparations and conditions, the IC_{50} for inhibition of I_{HERG} by FLEC in the present study (3.91 μM) is close to that reported for native feline I_K tails and, collectively, such data suggest that I_K/HERG is more sensitive to FLEC than are I_{to} and $I_{Ca,L}$. Therapeutic serum levels of FLEC lie approximately in the range of 0.5–2.4 μM (Breindahl, 2000). Thus, with the *caveat* that data from *in vitro* experiments must be extrapolated to the clinical situation with caution, the inhibitory actions of FLEC on I_{HERG} that we observed occurred at clinically relevant concentrations.

Comparative effects of FLEC, QUIN and PROPAF

Although the incidence of QT prolongation and *torsade de pointes* is comparatively low with FLEC, effects of the agent on HERG/I_{Kr} may underlie or significantly contribute to those cases that have been reported (Lui *et al*., 1982; Wickers *et al*., 1988; Sarubbi *et al*., 1996). Comparative data on QUIN obtained under identical experimental conditions suggest that FLEC is ~9 fold less potent as a HERG blocker than is QUIN. The IC_{50} for QUIN in the present study (0.41 μM) compares well with submicromolar IC_{50} values reported for blockade of I_{Kr} from AT-1 cells (Yang *et al*.,1997, 2001) and HERG expressed in Ltk cells (Yang *et al*., 2001) or inferred from a high level of blockade (~76%) by 1 μM quinidine of HERG expressed in tsA201 cells (Po *et al*., 1998). QUIN blockade of I_{HERG} in experiments on *Xenopus* oocytes is somewhat less potent (IC_{50} of ~8–10 μM; Lees-Miller *et al*., 2000; Ishii *et al*., 2001), even when drug effects on mammalian cells and oocytes have been compared within a single laboratory (Po *et al*., 1998). Altered drug diffusion at the cell membrane or, possibly, yolk sac absorption (as postulated by Zhou *et al*., 1998) might account for lower drug potency in oocyte experiments. It is likely that IC_{50} values for blockade of I_{HERG} recorded from mammalian cell lines are of more relevance to therapeutic concentrations than are values obtained from oocytes. Plasma levels of QUIN are in the range of ~6–18 μM (Halkin *et al*., 1979). Thus, on the basis of our data, the degree of I_{HERG} blockade at plasma drug levels might be expected to be much higher for QUIN than for FLEC, and this may account for the greater association of QT prolongation with QUIN than with FLEC use.

Intriguingly, PROPAF showed a similar I_{HERG} blocking potency to QUIN under our conditions (with an IC_{50} of 0.44 μM). The value we obtained is similar to that observed for I_K tails from rabbit atrial myocytes (IC_{50} of 0.76 μM; Duan *et al*., 1993), and is consistent with a prediction by Mergenthaler and colleagues that their data from *Xenopus* oocytes are likely to have underestimated the true potency of PROPAF on HERG/I_{Kr} from cardiomyocytes (Mergenthaler *et al*., 2001). Duan *et al*. (1993) estimated therapeutic PROPAF levels to range between 2 and 6 μM and, taking into account likely binding to plasma protein, suggested that *in vitro* concentrations of 0.2 and 0.6 μM most likely correspond to clinical 'free' drug concentrations. The IC_{50} for I_{HERG} blockade by PROPAF that we observed falls within this concentration range. Arguably, it is therefore perhaps surprising that QT prolongation and *torsade de pointes* are not more commonly observed with PROPAF. However, PROPAF is also an effective blocker of$I_{Ca,L}$ (IC_{50} 1.5–1.7 μM; Fei *et al*., 1993; Hancox & Mitcheson, 1997) and this effect would be anticipated to mitigate effects on the ventricular action potential that otherwise result from the drug's I_{HERG} blocking action. QUIN is less potent than PROPAF as an $I_{Ca,L}$ blocker (IC_{50} of 10 μM; Scamps *et al*., 1989). Thus, at any given drug concentration, this additional channel blocking action is less likely offset I_{HERG} blockade for QUIN than for PROPAF.

I*HERG* and LIG

In contrast to the other agents tested, LIG showed little effect on I_{HERG} at clinically relevant concentrations and our data are therefore consistent with a proposition that I_{HERG} blockade is unlikely to occur during clinical use of LIG. However, the fact that LIG can inhibit I_{HERG} (albeit at high concentrations), support the notion that it is necessary to test wide-ranging drug concentrations in order to classify agents as active or inactive against I_{HERG}.

On the mechanism of I*HERG inhibition by FLEC, QUIN and PROPAF*

The results of this study provide insight into the nature of I_{HERG} blockade by FLEC and the other agents tested. Our

data show both similarities and differences to those obtained previously for FLEC and native feline I_K (Follmer *et al*., 1992). In studying a relatively narrow concentration range of FLEC between 1.1 and 10 μM, Follmer *et al*. (1992) reported I_K tail inhibition to show a voltage-dependence consistent with open channel blockade. Our data with I_{HERG} are consistent with an activation/open channel blocking mechanism, both because voltage-dependent inhibition occurs and because the level of blockade increases over the first 200–300 ms of a sustained depolarization. Associated with this action, we observed a significant left-ward shift in voltage-dependent I_{HERG} activation. This contrasts with the observations by Follmer *et al*. (1992) for feline I_K, as they reported no significant alteration to $V_{0.5}$ or k values for activation. The reason for this discrepancy between the two sets of experiments is not entirely clear. However, although FLEC-sensitive I_K tails were reported to be also sensitive to the I_{Kr} blocker E-4031 (Follmer & Colatsky, 1990; Follmer *et al*., 1992), the measured activation $V_{0.5}$ values (between $\sim +3$ and $+7$ mV) were somewhat more positive than would be expected for pure I_{Kr}, for which $V_{0.5}$ usually lies somewhat negative to 0 mV (e.g. Sanguinetti & Jurkiewicz, 1990; 1991). A second difference between the two sets of experiments is that Follmer *et al*. (1992) observed FLEC to slow I_K tail deactivation (consistent with open channel unblock on repolarization), whereas we saw no significant drug effects on I_{HERG} tail deactivation time course. Direct comparison between the two studies in this respect is difficult: to enable quantitative analysis, Follmer *et al*. (1992) performed these particular experiments in the presence of Cd^{2+} to accelerate tail current deactivation and described deactivation as a mono-exponential rather than bi-exponential process. These differences aside, our observations regarding I_{HERG} tail deactivation did not provide evidence for unblock of HERG channels on repolarization. The poor reversibility of blockade by FLEC that we observed is consistent with known propensity towards drug 'trapping' by the HERG channel (Mitcheson *et al*., 2000b; Vandenberg *et al*., 2001).

In this study, QUIN and PROPAF showed similar blocking characteristics to FLEC (with the voltage-dependence of blockade by the three agents being the most pronounced for PROPAF). Our observations regarding voltage-dependent inhibition of I_{HERG} by PROPAF agree well with those made for I_{HERG} from *Xenopus* oocytes (Mergenthaler *et al*., 2001) and the two sets of results are also in qualitative agreement regarding time-dependent development of inhibition on depolarization, the time-dependence being more pronounced in the oocyte experiments which were performed at ambient temperature (Mergenthaler *et al*., 2001). Blockade of rabbit atrial I_K by PROPAF also showed a clear time-dependent profile, consistent with open channel blockade (Duan *et al*., 1993). This occurred without a significant alteration to I_K tail deactivation kinetics. A recent study from this laboratory also reported rapidly developing, time- and voltage-dependent blockade of I_{HERG} by the Class Ia agent disopyramide (DISO; Paul *et al*., 2001). Thus, it seems likely that across Class I sub-divisions agents active against I_{HERG} share a core similarity in producing a rapid, activation-dependent, open channel blockade. For all the agents tested, the Hill co-efficient for fits to concentration-dependent blockade was close to 1, consistent with one drug molecule exerting its effect by binding to one target (HERG) molecule. Although we did not specifically examine any role for voltage-dependent inactivation in I_{HERG} blockade by the agents under study in this report, some information on this issue is available. For both DISO and QUIN, a mutation to the HERG channel that vastly attenuates current inactivation (S631A) does not remove I_{HERG} blockade (Lees-Miller *et al*., 2000; Paul *et al*., 2001). Channel inactivation is therefore not obligatory for I_{HERG} blockade by two major Class Ia antiarrhythmic drugs. It is notable that, in the present study, the profile of I_{HERG} blockade during a sustained depolarization for each of FLEC, QUIN and PROPAF differed from that characteristic for methanesulphonanilide agents (e.g. Snyders & Chaudhary, 1996; Zhou *et al*., 1998; Witchel *et al*., 2002). This suggests that the likely site(s) of action of these Class I agents and the methanesulphonanilides is/are likely to differ in some respects. This notion is supported by comparison of QUIN and dofetilide in the effects on potency of I_{HERG} inhibition of the mutation S631A: whilst blockade by QUIN was not impaired, the potency of dofetilide inhibition was reduced (Lees-Miller *et al*., 2000), implicating a differential role of channel inactivation in channel inhibition by the two agents. Since QUIN and the Class Ic agents FLEC and PROPAF showed very similar blocking characteristics in the present study, it is possible that channel inactivation is not mandatory for any of these agents to bind to the HERG channel, although this possibility remains to be confirmed by mutagenesis experiments.

The authors are grateful to Professor Craig January, University of Wisconsin, for providing the HERG-expressing HEK 293 cell line. They also thank Lesley Arberry and Terri Harding for technical assistance and Dr Andrew James for useful discussion. J.C. Hancox and H.J. Witchel gratefully acknowledge financial support by the British Heart Foundation (PG 98081 and PG 2001104). J.C. Hancox was supported by a Research Fellowship from the Wellcome Trust.

References

BARHANIN, J., LESAGE, F., GUILLEMARE, E., FINK, M., LAZDUNSKI, M. & ROMEY, G. (1996). KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature,* **384,** 78–80.

BREINDAHL, T. (2000). Therapeutic drug monitoring of flecainide in serum using high-performance liquid chromatography and electrospray mass spectrometry. *J. Chromatogr. B. Biomed. Sci. Appl.,* **746,** 249–254.

CAMPBELL, T.J. (1983). Kinetics of onset of rate-dependent effects of class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. *Cardiovasc. Res.,* **17,** 344–352.

CAMPBELL, T.J., WYSE, K.R. & PALLANDI, R. (1991). Differential effects on action potential duration of class IA, B, and C antiarrhythmic drugs: modulation by stimulation rate and extracellular K + concentration. *Clin. Exp. Pharmacol. Physiol.,* **18,** 533 – 541.

CARMELIET, E. (1993). K^+ channels and control of ventricular repolarisation in the heart. *Fundam. Clin. Pharmacol.,* **7,** 19 – 28.

CHINN, K. (1993). Two delayed rectifiers in guinea pig ventricular myocytes distinguished by tail current kinetics. *J. Pharm. Exp. Ther.,* **264,** 553 – 560.

COWAN, J.C. & WILLIAMS, E.M. (1981). Characterization of a new oral antiarrhythmic drug, flecainide (R-818). *Eur. J. Pharmacol.,* **73,** 333 – 342.

CURRAN, M.E., SPLAWSKI, I., TIMOTHY, K.W., VINCENT, G.M., GREEN, E.D. & KEATING, M.T. (1995). A molecular basis for cardiac arrhythmia: HERG mutations cause Long QT syndrome. *Cell,* **80,** 795 – 803.

DELPON, E., VALENZUELA, C., PEREZ, O., CASIS, O. & TAMARGO, J. (1995). Propafenone preferentially blocks the rapidly activating component of delayed rectifier K current in guinea pig ventricular myocytes. *Circ. Res.,* **76,** 223 – 235.

DUAN, D., FERMINI, B. & NATTEL, S. (1993). Potassium channel blocking properties of propafenone in rabbit atrial myocytes. *J. Pharmacol., Exp. Ther.,* **264,** 1113 – 1123.

FALK, R.H. & FOGEL, R.I. (1994). Flecainide. *J. Cardiovas. Electrophysiol.* **5,** 964 – 981.

FEI, L., GILL, J.S., MCKENNA, W.J. & CAMM, A.J. (1993). Effects of propafenone on calcium currents in single ventricular myocytes of guinea-pig. *Br. J. Pharmacol.,* **109,** 178 – 182.

FOLLMER, C.H. & COLATSKY, T.J. (1990). Blockade of delayed rectifier potassium current, I_K, by flecainide and E-4031 in cat ventricular myocytes. *Circulation,* **82,** 289 – 293.

FOLLMER, C.H., CULLINAN, C.A. & COLATSKY, T.J. (1992). Differential block of cardiac delayed rectifier current by class lc antiarrhythmics: evidence for open channel block and unblock. *Cardiovasc. Res.,* **26,** 1121 – 1130.

HALKIN, H., VERED, Z., MILLMAN, P., RABINOWITZ, B. & NEUFELD, H.N. (1979). Steady-state quinidine concentration: role in prophylactic therapy following acute myocardial infarction. *Isr. J. Med. Sci.,* **15,** 583 – 587.

HANCOX, J.C., LEVI, A.J. & WITCHEL, H.J. (1998). Time course and voltage dependence of expressed HERG current compared with native 'rapid' delayed rectifier K current during the cardiac ventricular action potential. *Pflugers Arch.,* **436,** 843 – 853.

HANCOX, J.C. & MITCHESON, J.S. (1997). Inhibition of L-type calcium current by propafenone in single myocytes isolated from the rabbit atrioventricular node. *Br. J. Pharmacol.,* **121,** 7 – 14.

HEATH, B.M. & TERRAR, D.A. (1996). Separation of the components of the delayed rectifier K current using selective blockers of I_{Kr} and I_{Ks} in guinea-pig isolated ventricular myocytes. *Exp. Physiol.,* **81,** 587 – 603.

HII, J.T.Y., WYSE, G., GILLIS, A.M., COHEN, J.M. & MITCHELL, L.B. (1991). Propafenone-induced Torsades de Pointes: Cross-reactivity with quinidine. *Pacing Clin. Electrophysiol.,* **14,** 1568 – 1570.

KAMIYA, K., MITCHESON, J.S., YASUI, K., KODAMA, I. & SANGUINETTI, M.C. (2001). Open channel block of HERG K^+ channels by vesnarinone. *Mol. Pharmacol.,* **60,** 244 – 253.

ISHII, K., KONDO, K., TAKAHASHI, M., KIMURA, M. & ENDOH, M. (2001). An amino acid residue whose change by mutation affects drug binding to the HERG channel. *FEBS Lett.,* **506,** 191 – 195.

KRAFTE, D.S., DAVISON, K., DUGRENIER, N., ESTEP, K., JOSEF, K., BARCHI, R.L., KALLEN, R.G., SILVER, P.J. & EZRIN, A.M. (1994). Pharmacological modulation of human cardiac Na^+ channels. *Eur. J. Pharmacol.,* **266,** 245 – 254.

LEES-MILLER, J.P., DUAN, Y., TENG, G.Q. & DUFF, H.J. (2000). Molecular determinant of high affinity dofetilide binding to HERG1 expressed in *Xenopus* oocytes: involvement of S6 sites. *Mol. Pharmacol.,* **57,** 367 – 374.

LEVI, A.J., HANCOX, J.C., HOWARTH, F.C., CROKER, J. & VINNICOMBE, J. (1996). A method for making rapid changes of superfusate whilst maintaining temperature at 37°C. *Pflugers Arch.,* **432,** 930 – 937.

LEWIS, T. (1922). The value of quinidine in cases of auricular fibrillation and methods of studying the clinical reaction. *Am. J. Med. Sci.,* **163,** 781 – 794.

LIMA, J.J. & BOUDOULAS, H. (1987). Stereoselective effects of disopyramide enantiomers in humans. *J. Cardiovasc. Pharmacol.,* **9,** 594 – 600.

LUI, H.K., LEE, G., DIETRICH, P., LOW, R.I. & MASON, D.T. (1982). Flecainide-induced QT prolongation and ventricular tachycardia. *Am. Heart. J.,* **103,** 567 – 569.

LUI, H.K., HARRIS, F.J., CHAN, M.C., LEE, G. & MASON, D.T. (1986). Comparison of intravenous mexiletine and lidocaine for the treatment of ventricular arrhythmias. *Am. Heart. J.,* **112,** 1153 – 1158.

MERGENTHALER, J., HAVERKAMP, W., HUTTENHOFER, A., SKRYABIN, B.V., MUBHOFF, U., BORGGREFE, M., SPECKMANN, E.J., BREITHARDT, G. & MADEJA, M. (2001). Blocking effects of the antiarrhythmic drug propafenone on the HERG potassium channel. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 472 – 480.

MITCHESON, J.S., CHEN, J., LIN, M., CULBERSON, C. & SANGUINETTI, M.C. (2000a). A structural basis for drug-induced long QT syndrome. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 12329 – 12333.

MITCHESON, J.S., CHEN, J. & SANGUINETTI, M.C. (2000b). Trapping of a methanesufonanlide by closure of the HERG potassium channel activation gate. *J. Gen. Physiol.,* **115,** 229 – 240.

MITCHESON, J.S. & HANCOX, J.C. (1999a). An investigation of the role played by the E-4031-sensitive (rapid delayed rectifier) potassium current in isolated rabbit atrioventricular nodal and ventricular myocytes. *Pflügers Arch.,* **438,** 843 – 850.

MITCHESON, J.S. & HANCOX, J.C. (1999b). Characteristics of a transient outward current (sensitive to 4-aminopyridine) in Ca-tolerant myocytes isolated from the rabbit atrioventricular node. *Pflügers Arch.,* **438,** 68 – 78.

NATTEL, S. & SINGH, B.N. (1999). Evolution, mechanisms and classification of antiarrhythmic drugs: focus on class III actions. *Am. J. Cardiol.,* **84,** 11R – 19R.

PAUL, A.A., WITCHEL, H.J. & HANCOX, J.C. (2001). Inhibition of HERG potassium channel current by the Class 1a antiarrhythmic agent disopyramide. *Biochem. Biophys. Res. Comm.,* **280,** 1243 – 1250.

PO, S.S., WANG, D.W., YANG, I.C.H., JOHNSON, J.P., NIE, L. & BENNETT, P.B. (1998). Modulation of HERG potassium channels by extracellular magnesium and quinidine. *J. Cardiovasc. Pharmacol.,* **33,** 181 – 185.

RANGER, S., SHELDON, R., FERMINI, B. & NATTEL, S. (1993). Modulation of flecainide's cardiac sodium channel blocking actions by extracellular sodium: a possible cellular mechanism for the action of sodium salts in flecainide cardiotoxicity. *J. Pharmacol. Exp.,* **264,** 1160 – 1167.

REHNQVIST, N., ERICSSON, C.G., ERIKSSON, S., OLSSON, G. & SVENSSON, G. (1984). Comparative investigation of the antiarrhythmic effect of propafenone (Rytmonorm) and lidocaine in patients with ventricular arrhythmias during acute myocardial infarction. *Acta Med. Scandinavica,* **216,** 525 – 530.

ROCCHETTI, M., BESANA, A., GURROLA, G.B., POSSANI, L.D. & ZAZA, A. (2001). Rate dependency of delayed rectifier currents during the guinea-pig ventricular action potential. *J. Physiol.,* **534.3,** 721 – 732.

SANGUINETTI, M.C., CURRAN, M.E., SPECTOR, P.S. & KEATING, M.S. (1996a). Spectrum of HERG K channel dysfunction in an inherited cardiac arrhythmia. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 2208 – 2212.

SANGUINETTI, M.C., CURRAN, M.E., ZOU, A., SHEN, J., SPECTOR, P.S., ATKINSON, D.L. & KEATING, M.T. (1996b). Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. *Nature,* **384,** 80 – 83.

SANGUINETTI, M.C., JIANG, C., CURRAN, M.E. & KEATING, M.T. (1995). A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell,* **81,** 299 – 307.

SANGUINETTI, M.C. & JURKIEWICZ, N.K. (1990). Two components of cardiac delayed rectifier K^+ current. *J. Gen. Physiol.,* **96,** 195–215.

SANGUINETTI, M.C. & JURKIEWICZ, N.K. (1991). Delayed rectifier outward K^+ current is composed of two currents in guinea pig atrial cells. *Am. J. Physiol.,* **260,** H393–H399.

SARUBBI, B., DUCCESCHI, V., BRIGLIA, N., ESPOSITO, R., MAYER, M.S., SCIALDONE, A., SANTANGELO, L. & IACONO, A. (1996). Sotalol, propafenone and flecainide: compared multiparametric analysis of ventricular repolarization in subjects without organic cardiopathy. *Cardiologia,* **41,** 645–651.

SCAMPS, F., UNDROVINAS, A. & VASSORT, G. (1989). Inhibition of I_{Ca} in single frog cardiac cells by quinidine, flecainide, ethmozin and ethazin. *Am. J. Physiol.,* **256,** C549–C559.

SMITH, P.L., BAUKROWITZ, T. & YELLEN, G. (1996). The inward rectification mechanism of the HERG cardiac potassium channel. *Nature,* **379,** 833–836.

SNYDERS, D.J. & CHAUDHARY, A. (1996). High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol. Pharmacol.,* **49,** 949–955.

SNYDERS, D.J., KNOTH, K.M., ROBERDS, S.L. & TAMKUN, M.M. (1992). Time-, voltage-, and state-dependent block by quinidine of a cloned human cardiac potassium channel. *Mol. Pharmacol.,* **41,** 322–330.

TESCHEMACHER, A.G., SEWARD, E.P., HANCOX, J.C. & WITCHEL, H.J. (1999). Inhibition of the current of heterologously expressed HERG potassium channels by imipramine and amitriptyline. *Br. J. Pharmacol.,* **128,** 479–485.

TRUDEAU, M.C., WARMKE, J.W., GANETZKY, B. & ROBERTSON, G.A. (1995). HERG, an inward rectifier in the voltage-gated potassium channel family. *Science,* **269,** 92–95.

VANDENBERG, J.I., WALKER, B.D. & CAMPBELL, T.J. (2001). HERG K^+ channels: friend and foe. *TIPS,* **22,** 240–246.

VIRAG, L., VARRO, A. & PAPP, J.G. (1998). Effect of disopyramide on potassium currents in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **357,** 268–275.

VISKIN, S. (1999). Long QT syndromes and torsade de pointes. *Lancet,* **354,** 1625–1633.

WALKER, B.D., SINGLETON, C.B., TIE, H., BURSILL, J.A., WYSE, K.R., VALENZUELA, S.M., BREIT, S.N. & CAMPBELL, T.J. (2000). Comparative effects of azimilide and ambisilide on the human ether-a-go-go related gene (HERG) potassium channel. *Cardiovasc. Res.,* **48,** 44–58.

WANG, D.W., KIYOSUE, T., SATO, T. & ARITA, M. (1996). Comparison of the effects of Class-I antiarrhythmic drugs, cibenzoline, mexiletine and flecainide, on the delayed rectifier K current of guinea-pig ventricular myocytes. *J. Mol. Cell. Cardiol.,* **28,** 893–903.

WANG, Z., FERMINI, B. & NATTEL, S. (1993). Mechanism of flecainide's rate-dependent actions on action potential duration in canine atrial tissue. *J. Pharm. Exp. Ther.,* **267,** 575–581.

WICKERS, F., HAISSAGUERE, M. & PALUSSIERE, J. (1988). QT prolongation and induction of torsade de pointe by flecainide. Apropos of a case. *Archives des Maladies du Coeur et des Vaisseaux* **81,** 1283–1285.

WITCHEL, H.J. & HANCOX, J.C. (2000). Congenital and acquired long QT syndrome and the cardiac rapid delayed rectifier potassium channel. *Clin. Exp. Pharmacol. Physiol.,* **27,** 753–766.

WITCHEL, H.J., PABBATHI, V.K., HOFMANN, G., PAUL, A.A. & HANCOX, J.C. (2002). Inhibitory actions of the selective serotonin re-uptake inhibitor citalopram on HERG and ventricular L-type calcium currents. *FEBS Lett.,* **512,** 59–66.

YAMASHITA, T., NAKAJIMA, T., HAMADA, E., HAZAMA, H., OMATA, M. & KURACHI, Y. (1995). Flecainide inhibits the transient outward current in atrial myocytes isolated from the rabbit heart. *J. Pharm. Exp. Ther.,* **274,** 315–321.

YANG, T. & RODEN, D.M. (1996). Extracellular potassium modulation of drug block of I_{Kr}. Implications for torsade de pointes and reverse use-dependence. *Circulation,* **93,** 407–411.

YANG, T., SNYDERS, D.J. & RODEN, D.M. (1997). Rapid inactivation determines the rectification and $[K]_o$ dependence of the rapid component of the delayed rectifier K current in cardiac cells. *Circ. Res.,* **80,** 782–789.

YANG, T., SNYDERS, D.J. & RODEN, D.M. (2001). Drug block of I_{Kr}: model systems and relevance to human arrhythmias. *J. Cardiovasc. Pharmacol.,* **38,** 737–744.

YEOLA, S.W. & SNYDERS, D.J. (1997). Electrophysiological and pharmacological correspondence between Kv4.2 current and rat cardiac transient outward current. *Cardiovasc. Res.,* **33,** 540–547.

ZHOU, Z., GONG, Q., YE, B., FAN, Z., MAKIELSKI, J.C., ROBERTSON, G.A. & JANUARY, C.T. (1998). Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. *Biophys. J.,* **74,** 230–241.

(Received February 21, 2002
Revised April 17, 2002
Accepted April 26, 2002)