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Blockade of HERG channels by the class III antiarrhythmic azimilide: mode of action

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1 The class III antiarrhythmic azimilide has previously been shown to inhibit I_{Ks} and I_{Kr} in guinea-pig cardiac myocytes and I_{Ks} (minK) channels expressed in *Xenopus* oocytes. Because HERG channels underly the conductance I_{Kr} in human heart, the effects of azimilide on HERG channels expressed in *Xenopus* oocytes were the focus of the present study.

2 In contrast to other well characterized HERG channel blockers, azimilide blockade was reverse use-dependent, i.e., the relative block and apparent affinity of azimilide decreased with an increase in channel activation frequency. Azimilide blocked HERG channels at 0.1 and 1 Hz with IC_{50} s of 1.4 μM and 5.2 μM respectively.

3 In an envelope of tail test, HERG channel blockade increased with increasing channel activation, indicating binding of azimilide to open channels.

4 Azimilide blockade of HERG channels expressed in *Xenopus* oocytes and I_{Kr} in mouse AT-1 cells was decreased under conditions of high $[K^+]_e$, whereas block of slowly activating I_{Ks} channels was not affected by changes in $[K^+]_e$.

5 In summary, azimilide is a blocker of cardiac delayed rectifier channels, I_{Ks} and HERG. Because of the distinct effects of stimulation frequency and $[K^+]_e$ on azimilide block of I_{Kr} and I_{Ks} channels, we conclude that the relative contribution of block of each of these cardiac delayed rectifier channels depends on heart frequency. $[K^+]_e$ and regulatory status of the respective channels.

Keywords: Azimilide; HERG; KvLQT1; I_{sK} (minK); I_{Kr}; I_{Ks}; K^+ channel; arrhythmia; torsades de pointes

Introduction

I_{Kr} channels encoded by human ether-a-gogo related gene (HERG) and I_{Ks} channels (which are heteropolymers of I_{sK}/KvLQT1 protein subunits) are both targets for selective class III antiarrhythmic drugs (Colatsky *et al*., 1990; Curran *et al*., 1995; Sanguinetti *et al*., 1995; 1996; Sanguinetti & Salata, 1996; Barhanin *et al*., 1996). Whereas many specific HERG channel blockers have been identified and developed (Sanguinetti & Salata, 1996), few compounds are known to block specifically I_{Ks} channels (Suessbrich *et al*., 1996; Busch *et al*., 1996a,b; Salata *et al*., 1996). The class III antiarrhythmic azimilide (NE-10064) was first shown to block I_{sK} channels expressed in *Xenopus* oocytes and the slowly activating I_{Ks} conductance in guinea-pig cardiac myocytes (Busch *et al*., 1994a,b). However, azimilide does not appear to be a specific I_{Ks} blocker as recent studies have shown a more potent block of the HERG channel related conductance I_{Kr} than I_{Ks} in guinea-pig cardiac myocytes (Fermini *et al*., 1995). In the heart, regulation of the K^+ conductances I_{Kr} and I_{Ks} by changes in heart rate, $[K^+]_e$ and stimulation of β-receptors determines the relative contribution of each to the action potential duration. This, at least partially, explains the reduced efficacy of I_{Kr} blockers at elevated heart rates and/or β-adrenoceptor-induced tone (Sanguinetti *et al*., 1991; Jurkiewicz & Sanguinetti, 1993). Whereas azimilide block of I_{sK} and I_{Ks} has been characterized in great detail (Busch *et al*., 1994a, b; Herzer *et al*., 1995), nothing is known about the putative block of HERG channels by azimilide. Therefore, we examined the effects of azimilide on HERG channels expressed in *Xenopus* oocytes in an effort to understand better the mechanism and relative role of I_{Kr} (HERG) block by this antiarrhythmic drug.

Methods

Handling and injection of *Xenopus* oocytes and synthesis of cRNA have previously been described in detail (Busch *et al*., 1996a). The two-microelectrode voltage-clamp configuration was used to record currents from *Xenopus laevis* oocytes. In several sets of experiments, oocytes were individually injected with cRNA encoding the K^+ channels HERG (Warmke & Ganetzky, 1994). HERG S631A (Schönherr & Heinemann, 1996), or human I_{sK} (Murai *et al*., 1989). Recordings were performed at 22°C with a Geneclamp amplifier (Axon Instruments, Foster City, U.S.A.) and MacLab D/A converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). To estimate deactivation kinetics (τ_{deact}) of HERG channels, a single exponential function was fitted to the tail currents at −85 mV after depolarizations to −15 mV. The control solution contained (mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (titrated with NaOH to pH 7.4). In some experiments KCl was raised to 10 mM by substituting 8 mM NaCl. The microelectrodes were filled with 3 M KCl solution and had resistances ranging from 0.5 to 0.9 MΩ.

AT-1 cell preparation and culture

AT-1 cells propagation *in vivo* by subcutaneous injection into syngenic host mice (female 2–3 Mo. B6D2F1/J, Charles River,

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Wilmington, MA) and the isolation and culture of AT-1 cells were conducted as described previously (Delcarpio *et al*., 1991; Yang *et al*., 1994; Jurkiewicz *et al*., 1996). The original AT-1 mouse colonies were established by Dr Loren Field (Krannert Institute of Cardiology, Indianapolis, IN) and the lineage at Merck Research Laboratories was established from tumour cells kindly provided by Dr Dan Roden (Vanderbilt University, Nashville, TN). Before the voltage-clamp studies, AT-1 cells were treated with trypsin to dislodge them from the culture dishes and were stored in PC-1 culture medium (22–24°C). AT-1 cells were studied between days 6 and 18 of culture and within 14 h of isolation.

Voltage-clamp of AT-1 cells

Voltage-clamp studies of outward K^+ currents (I_{Kr}) were performed in the whole-cell recording mode by means of a List EPC-7 amplifier. AT-1 cells were placed in a 0.5 ml chamber and were superfused with normal HEPES-buffered saline (HBS) containing (mM): NaCl 132, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1.2, HEPES 10 and glucose 10; pH = 7.2, at 24–26°C at a rate of 2 ml min^{-1}. Microelectrodes were made from square bore (1.0 mm, o.d.) borosilicate capillary tubing (Glass Co. of America, Bargaintown, NJ) and were filled with a solution containing (mM): KCl 110, K-BAPTA 5, K_2ATP 5, $MgCl_2$ 1 and HEPES 10; pH 7.2. Pipettes had tip resistances ranging from 3 to 7 MΩ (averaging 5.5±0.3 3MΩ). Series resistance was compensated 40–70%. Currents were low-pass filtered (−3 dB at 1 kHz) before digitization at 5 kHz. All cells were round in appearance, had large outward tail currents and resting membrane potentials (RMP) negative to −35 mV and did not contract spontaneously. Inward sodium (I_{Na}) and T-type calcium (I_{Ca}) currents were inactivated by voltage-clamping the cells to a holding potential (V_h) of −40 mV. L-type I_{Ca} was blocked with 0.4–1 μmol^{-1} nisoldipine. Data acquisition and analysis were performed with pClamp software (Axon Instruments, Foster City, CA) and an IBM compatible 486 computer. Concentration-response relationships were determined by measuring currents in each cell under control conditions and during superfusion with successively increasing concentrations of drug.

Data are presented as mean values ± s.e.mean and n represents the number of experiments performed. Concentration-blockade relationships were calculated with the Hill equation. Student's t test was used to test for statistical significance, which was assumed to be obtained when $P<0.05$.

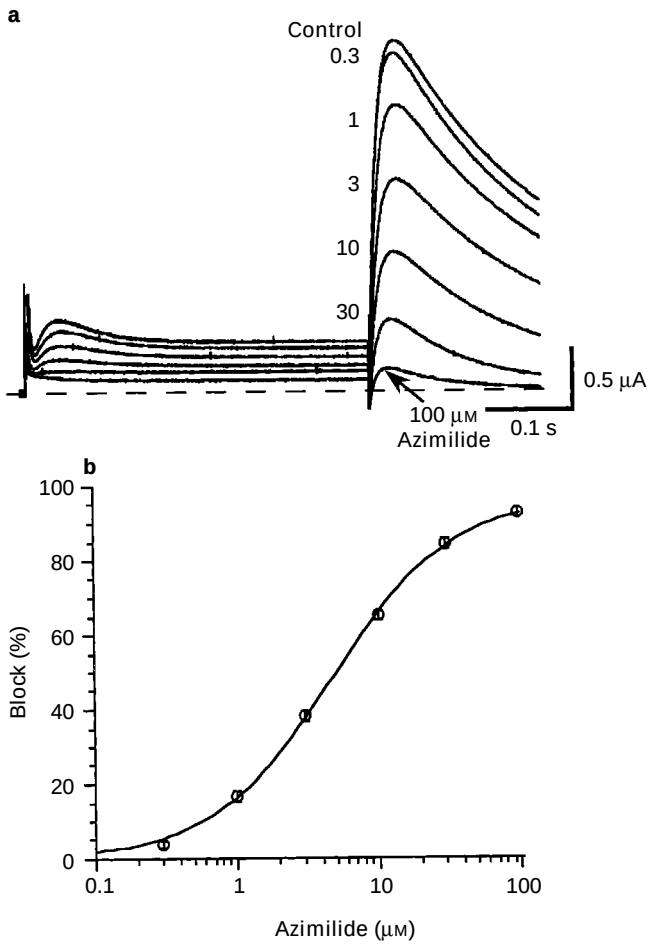

Figure 1 Concentration-dependent blockade of HERG channels by azimilide. (a) HERG currents were evoked with 0.4 s depolarizing pulses to 40 mV from a holding potential of −95 mV at 1 Hz. Tail currents were recorded at −80 mV (filtered at 0.5 kHz). The dashed line corresponds to 0 current. (b) Concentration-dependent blockade by azimilide of HERG tail current. The Hill equation was fitted to the data; 100% blockade was taken as a fixed maximal effect. Data are given as arithmetic means (with vertical lines showing s.e.mean). For most data the s.e.mean were smaller than the size of the symbols.

Results

Injection of oocytes with cRNA encoding for HERG channels resulted in the induction of a K^+ conductance with activation and rectification properties that have been previously described (Sanguinetti *et al*., 1995). HERG channels were

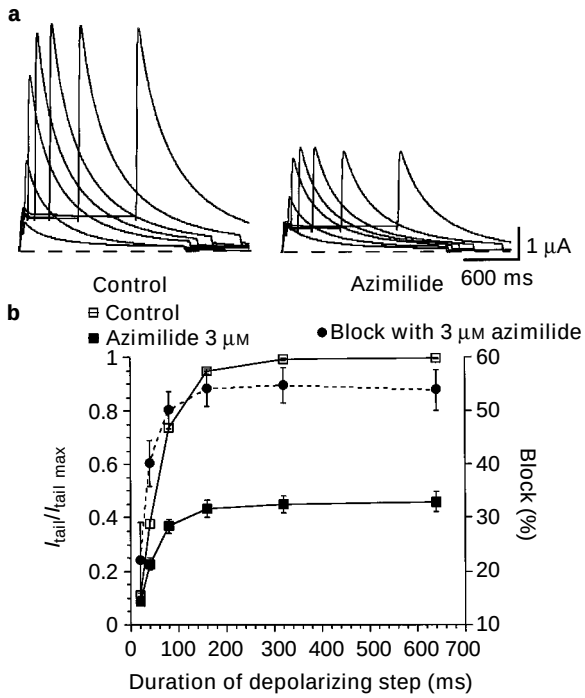

Figure 2 Envelope of tails test for HERG channels under control conditions and with 3 μM azimilide. (a) HERG channels were activated with 20, 40, 80, 160, 320 and 640 ms depolarizing steps to 20 mV at an interpulse interval of 3 s. Currents were measured under control condition (left) and after steady-state block had been achieved (right). (b) The peak tail currents under control conditions and with 3 μM azimilide were normalized to the maximal tail currents of the respective experiments. Relative HERG channel blockade is also plotted against the test pulse duration. Data are given as arithmetic means with vertical lines showing s.e.mean.

activated by depolarization, but because C-type inactivation of these channels occurs more rapidly than activation, especially at depolarized potentials, activating outward currents at voltages $\geqslant$ 0 mV were relatively small. However, the deactivating tail currents obtained during repolarizing step to -80 mV were large as a result of rapid recovery from inactivation combined with slow deactivation. Azimilide blocked both the relatively small outward currents during 0.4 s depolarizing pulses from a holding potential of -80 mV to a test potential of 40 mV and the large tail outward currents at -80 mV (interpulse interval = 1 s). Figure 1a shows original current traces illustrating the concentration-dependent blockade of HERG channels by azimilide. The concentration-dependence of the blockade of HERG tail currents was fitted with the Hill equation and yielded an IC_{50} value of 5.2 $\pm$ 0.2 μM and a Hill coefficient $\approx$ 1 (Figure 1b: $n=6$). The block was completely reversible within 15 min of washout. The apparent affinity of azimilide for HERG channels in *Xenopus* oocytes was approximately 10 fold lower than that obtained previously for I_{Kr} in guinea-pig cardiac myocytes (Fermini *et al*., 1995). This reduced potency or affinity for HERG channels expressed in *Xenopus* oocytes compared with I_{Kr} in guinea-pig myocytes has been commonly observed with other agents that block this channel (Salata *et al*., 1995; Spector *et al*., 1996; Suessbrich *et al*., 1996). Nevertheless, we examined the mechanism of HERG channel block by azimilide to determine possible factors affecting its apparent affinity.

For an open channel blocker it is expected that block increases with an increase of the activated channel population. This was analysed in an envelope of tails test for azimilide (3 μM). As shown in Figure 2a and b, the inhibition of HERG channels indeed increased with an increase in channel activation. Once maximal channel activation was obtained (after approximately 120 ms at 20 mV, reflected by maximal tail currents), an increase in the depolarization duration did not further increase azimilide-mediated HERG channel inhibition ($n=6$). During the envelope of tails test the time course of tail deactivation did not change. At control the tail current deactivation time constants (τ_{deact}) were 299 $\pm$ 27 ms and 332 $\pm$ 35 ms after 80 and 640 ms depolarizing steps, respectively. In the presence of azimilide (3 μM) τ_{deact} was 294 $\pm$ 34 ms and 314 $\pm$ 32 ms after 80 and 640 ms depolarizing steps, respectively.

As a consequence of unbinding of a blocker during channel deactivation, the time constant of deactivation increases. However, we found no effect of azimilide on the rate of HERG channel deactivation. Time constants of deactivation at -80 mV were 0.33 $\pm$ 0.02 s and 0.31 $\pm$ 0.02 s in control

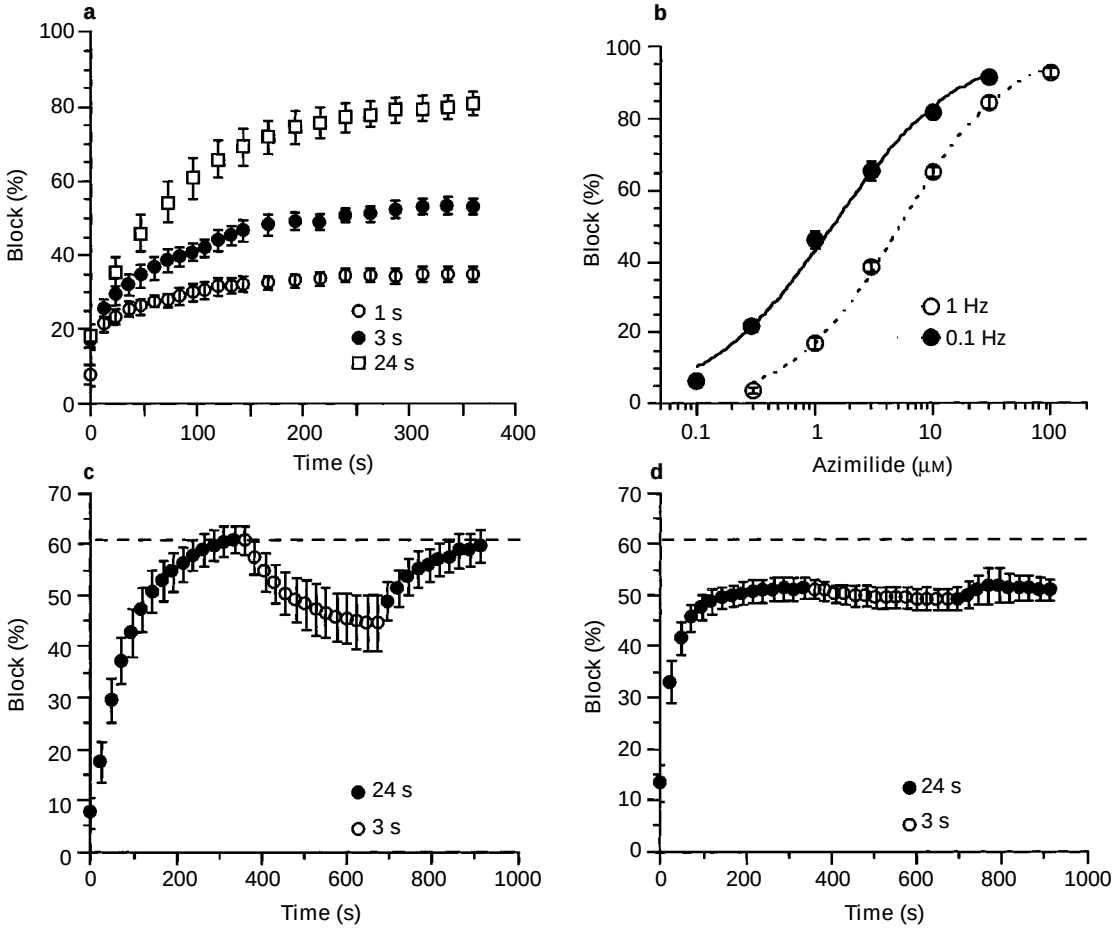

Figure 3 (a) Use-dependence of HERG channel blockade by azimilide. Tail currents were recorded at -85 mV after a 0.5 s depolarizing pre-pulse to $+40$ mV from a holding potential of -80 mV at intervals of 1 s, 3 s and 24 s, respectively. Before the first depolarizing pulse was applied (time = 0), the oocytes were superfused for 30 s with the drug-containing solution to ensure complete exchange of the bath. (b) Azimilide concentration-dependent blockade of HERG channels at 0.1 Hz and 1 Hz. The 1 Hz values represent the data from Figure 1b. Frequency-dependence of HERG channel block in the presence of 2 mM (c) and 10 mM (d) $[K^+]_e$. HERG channels were activated at 24 s and 3 s intervals. At 2 mM (c) HERG channel block decreased at an increased frequency, whereas with 10 mM $[K^+]_e$ HERG channel blockade remained constant (d) during the same protocol at a decreased maximal inhibition. Data are given as arithmetic means with vertical lines showing s.e.mean. For clarity not all data at 1 s and 3 s intervals are given in the graphs. The dashed lines in (c) and (d) correspond to the maximal HERG inhibition with 2 mM $[K^+]_e$.

solution and 2 μM azimilide, respectively ($n=5$). This indicates that azimilide unbinding occurs very slowly and cannot be resolved during the period of channel deactivation.

Previously, we have shown a rate- and voltage-dependence of HERG channel block by the histamine receptor antagonists astemizole and terfenadine and the antipsychotic durg haloperidol (Suessbrich *et al*., 1996; 1997b). Therefore, we analysed the azimilide blockade of HERG channels at distinct voltages and activation rates. In contrast to other HERG channel blockers, the block of HERG channels by azimilide was voltage-independent. Analysis of tail currents following voltage steps from -20 to 40 mV (at increments of 15 mV; 3 s interval between depolarizations) showed similar steady-state inhibition of approximately 50% at all voltages by 3 μM azimilide. For example, the block of HERG channels was 48.5 $\pm$ 2.3% and 51.6 $\pm$ 2.4% when the membrane was depolarized to -20 and 40 mV, respectively.

Analysis of the rate-dependence of azimilide block exerted an untypical behaviour. As illustrated in Figure 3a, azimilide block of HERG channels was smaller at an increased stimulation frequency ($n=5$). To test in detail whether the channel activation frequency affects the apparent affinity for azimilide we performed complete concentration-blockade at low frequency (at 10 s intervals = 0.1 Hz). At this lower channel activation frequency the concentration-blockade relationship for azimilide was shifted significantly to lower concentrations. At 0.1 Hz the interpolated IC_{50} for azimilide was 1.4 $\pm$ 0.2 μM (Figure 3b: $n=5$). Because extracellular K^+ affects HERG channel activation, inactivation and deactivation and possibly channel conformation, we analysed the frequency-dependence of azimilide block at different $[K^+]_e$. As illustrated in Figure 3c, after reaching a steady-state block of HERG channels at a low stimulation frequency (24 s interval between depolarizations), an increase in the stimulation frequency (to 0.33 Hz) decreased HERG channel blockade significantly ($n=5$). At high $[K^+]_e$, steady state block of HERG channels was decreased. However, this block did not reveal a significant frequency-dependence (Figure 3d; $n=5$).

Subsequently, we also analysed the effects of $[K^+]_e$ on the apparent azimilide affinity in more detail. As shown in Figure 4a and b, an increase in $[K^+]_e$ decreased azimilide affinity. Determination of the concentration blockade relationship revealed interpolated IC_{50} values of 1.2 $\pm$ 0.2 μM and 12.6 $\pm$ 0.8 μM under 2 and 10 mM $[K^+]_e$, respectively (Figure 4c; $n=6$).

To confirm the above results in a more physiological model, we tested the effects of azimilide on native I_{Kr} currents in mouse AT-1 cells. Phenotypical I_{Kr} currents recorded from a representative AT-1 cell during 1 s depolarizing voltage steps from a holding potential (V_h) of -40 mV to a test potential of 20 mV are shown in Figure 5a. In the presence of 4 mM $[K^+]_e$, azimilide blocked I_{Kr} in these cells in a concentration-dependent manner with an IC_{50} of 1 μM which is very similar to that observed for HERG channels described above at low stimulation frequency (Figure 5c; $n=5$). After normalization of the steady-state currents (Figure 5b) it is obvious that under azimilide the rise of I_{Kr} appeared to be faster and at 3 μM I_{Kr} displayed even a small relaxation. Such an apparent faster rise of current and current relaxation could reflect binding of the drug after channel opening, as it has been described for other channel blocker (Snyders *et al*., 1992; Yang *et al*., 1995). As described above for HERG channels expressed in oocytes, an elevation of $[K^+]_e$ to 10 mM resulted in a significant decrease in the degree of inhibition of I_{Kr} by azimilide. Azimilide (3 μM) blocked I_{Kr} by 67 $\pm$ 7% under 4 mM $[K^+]_e$, but the block was significantly reduced to 47 $\pm$ 7% under 10 mM $[K^+]_e$ (Figure 5d).

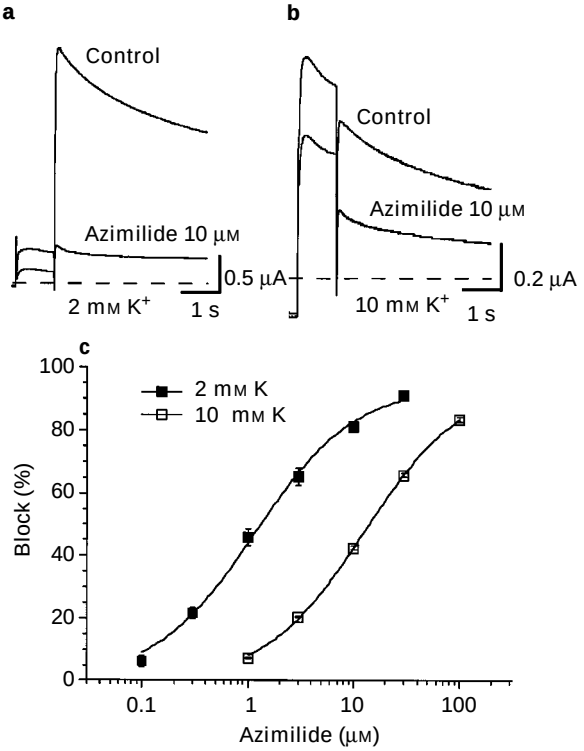

Figure 4 Effects of $[K^+]_e$ on azimilide-mediated HERG channel block. HERG channels were activated with 1 s depolarizing pulses to 30 mV from a holding potential of -85 mV in the presence of (a) 2 mM and (b) 10 mM $[K^+]_e$. Tail currents were measured at -55 mV. Azimilide 10 μM blocked HERG tail currents by approximately 80% and 50% with 2 mM and 10 mM $[K^+]_e$ respectively. The dashed line corresponds to 0 current. (c) Complete concentration-response relationship for block of HERG tail currents in the presence of 2 mM and 10 mM $[K^+]_e$. Channels were activated at 0.2 Hz.

Azimilide has been shown to block the I_{Ks} channel, another important cardiac delayed rectifier K^+ channel (Busch *et al*., 1994b). Here, we tested whether changes in $[K^+]_e$ had an effect on the azimilide block of I_{Ks} channels induced in *Xenopus* oocytes by the expression of the human I_{sK} protein. As described previously (Busch *et al*., 1994b), I_{Ks} channels were inhibited with an IC_{50} of 2.6 $\pm$ 0.1 μM (Figure 6a,b; $n=5$). In contrast to HERG channels, the azimilide block of I_{Ks} channels was not significantly altered by an increase in $[K^+]_e$ to 10 mM and an IC_{50} of 3.1 $\pm$ 0.2 μM was obtained (Figure 6b; $n=4$).

The HERG channel blockers terfenadine, astemizole and haloperidol have recently been shown to accelerate channel inactivation (Suessbrich *et al*., 1996; 1997b). Further, these compounds exerted a lower apparent affinity to the mutant HERG S631 (Suessbrich *et al*., 1997a,b; unpublished results), a channel which inactivates to a lesser extent (Schönherr & Heinemann, 1996). Here, azimilide did not alter the rate of channel inactivation at -15 mV significantly, which was 14.0 $\pm$ 0.6 ms and 12.5 $\pm$ 0.4 ms ($n=4$; $P=0.08$) at control and 3 μM azimilide, respectively. Moreover, the channel mutant HERG S631 displayed a similar sensitivity to azimilide blockade (IC_{50} of 2.6 $\pm$ 0.4 μM; $n=5$) as HERG wild-type channels (Figure 7a,b).

Discussion

HERG channels underly the cardiac K^+ conductance I_{Kr} (Sanguinetti *et al*., 1995) and both over-expressed HERG channels and the native I_{Kr} conductance have been shown to be

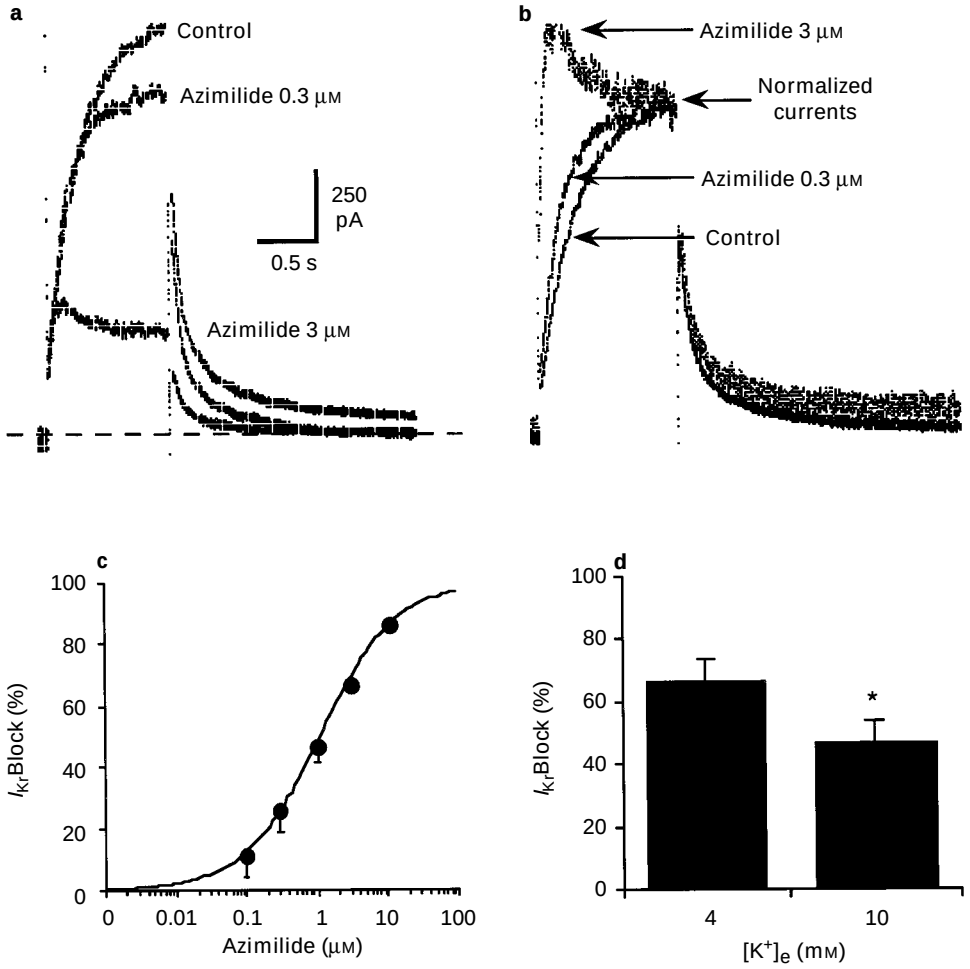

Figure 5 Azimilide block of I_{Kr} in AT-1 cells. Concentration-dependence and modulation of block by external potassium $[K^+]_e$. (a) Representative I_{Kr} currents elicited by 1 s depolarizing test pulses to +20 mV from a holding potential of −40 mV at an interpulse interval of 10 s during control conditions and after 0.3 and 3 μM azimilide. Tail currents were measured at −40 mV and the peak amplitude was measured relative to the holding current. The dashed line corresponds to 0 current. (b) Currents from (a) were normalized to the individual steady-state currents. (c) Concentration-dependence of azimilide block of I_{Kr} in the presence of normal (4 mM) $[K^+]_e$. Tail current amplitudes are expressed as a % of the control amplitude by use of the pulse protocol described in (a). The Hill equation was fitted to the data, with 100% blockade as a fixed maximum. (d) Effect of $[K^+]_e$ on block of I_{Kr} by 3 μM azimilide. Data are given as arithmetic means with vertical lines showing s.e.mean ($n \geqslant 5$).

specifically inhibited by methanesulphonanilide class III antiarrhythmics (Sanguinetti & Jurkiewicz. 1991; Lynch *et al*., 1994; Trudeau *et al*., 1995; Jurkiewicz *et al*., 1996; Kiehn *et al*., 1996; Snyders & Chaudhary, 1996; Spector *et al*., 1996). There are two main disadvantages with the use of these methanesulphonanilide antiarrhythmics: (1) they can cause proarrhythmic effects, especially during bradyarrhythmias and hypokalaemia (reviewed by Roden, 1996 and Sanguinetti & Salata. 1996): (2) their efficacy is decreased at high heart rates and after β-adrenoceptor stimulation, mainly as a consequence of strong up-regulation of the slowly activating K^+ conductance I_{Ks} (Sanguinetti *et al*., 1991; Jurkiewicz & Sanguinetti, 1993). For these reasons, strong efforts have been focused on the identification of novel blockers for the slowly activating conductance I_{Ks}, which may not have these disadvantages. So far, three compounds chemically unrelated to methanesulphonanilides have been shown to block I_{Ks} in heart myocytes and I_{Ks} channels expressed in *Xenopus* oocytes; the imidazolidinedione azimilide, the chromanol 293B and the 1,4 benzodiazepine, L735,821 (Busch *et al*., 1994b; 1996b; Salata *et al*., 1996). However, whereas the chromanol 293B and L-735,821 display a high selectivity for I_{Ks} over I_{Kr}, azimilide appears to block both conductances with little specificity (Fermini *et al*., 1995). Azimilide has since been demonstrated to produce pronounced antiarrhythmic efficacy in numerous arrhythmia models (Black *et al*., 1993; Restivo *et al*., 1996; Brooks *et al*., 1996). Whereas the mechanism of azimilide block of I_{Ks} channels has been analysed in great detail (Busch *et al*., 1994a; Herzer *et al*., 1995), little is known about the characteristics of azimilide block of I_{Kr} and the underlying HERG channels. The present study was designed to analyse the mechanism of HERG channel block by azimilide in order to understand better the antiarrhythmic properties of this compound.

Azimilide blocked HERG channels with an affinity similar to that of I_{Ks} channels, but with some unique features which differentiate this drug from other HERG channel blockers and may influence its antiarrhythmic properties. Azimilide blocks HERG in a reverse use-dependent and voltage-independent manner, which contrasts with that of all other known HERG channel blockers. For example, the HERG channel blockers dofetelide, E-4031, terfenadine, astemizol, haloperidol and clofilium all block with positive use- and voltage-dependence (Trudeau *et al*., 1995; Snyders & Chaudhary, 1996; Spector *et al*., 1996; Suessbrich *et al*., 1996; 1997a,b). Moreover, whereas haloperiodol, astemizole, terfenadine and clofilium were shown to accelerate apparently

HERG channel inactivation and had reduced inhibitory potency for the lesser inactivating HERG S631A mutant channels (Suessbrich *et al*., 1996; 1997a,b), azimilide did not affect HERG inactivation and had a similar inhibitory potency for the mutant channels. This suggests a different binding mechanism for azimilide. The reason for the reverse use-dependence of azimilide block is not completely clear, but may involve an interaction of azimilide with K^+ close to its binding site for the following reasons: (a) at high frequencies the K^+ concentration close to the pore is known to increase and (b) under conditions of high $[K^+]_e$ the reverse use-dependence was largely abolished. Furthermore, under high $[K^+]_e$ the apparent affinity of azimilide was reduced 10 fold. Modulation of dofetelide mediated block of HERG channels by $[K^+]_e$ has also recently been described (Yang & Roden, 1996). The mechanism of this modulation of block by $[K^+]_e$ is unclear, but may involve conformational changes of HERG channels which could affect the drug binding site(s).

In contrast to HERG channels, changes in $[K^+]_e$ did not affect the azimilide block of I_{Ks} channels. Further contrasting with its effect on HERG channels, I_{Ks} channels have previously been shown to be blocked by azimilide in a use-dependent manner. It is difficult define clearly the contribution of I_{Kr} versus I_{Ks} blockade by azimilide for its antiarrhythmic action because, apart from the distinct effects of azimilide on these conductances, one must consider that frequency and $[K^+]_e$ regulate the relative contribution of these conductances to cardiac action potential repolarization. It was shown that the relative contribution of I_{Ks} to action potential repolarization increases at an increased heart rate or after β-adrenoceptor stimulation (Sanguinetti *et al*., 1991; Jurkiewicz & Sanguinetti, 1993). Taking into account the fact that I_{Ks} blockade by

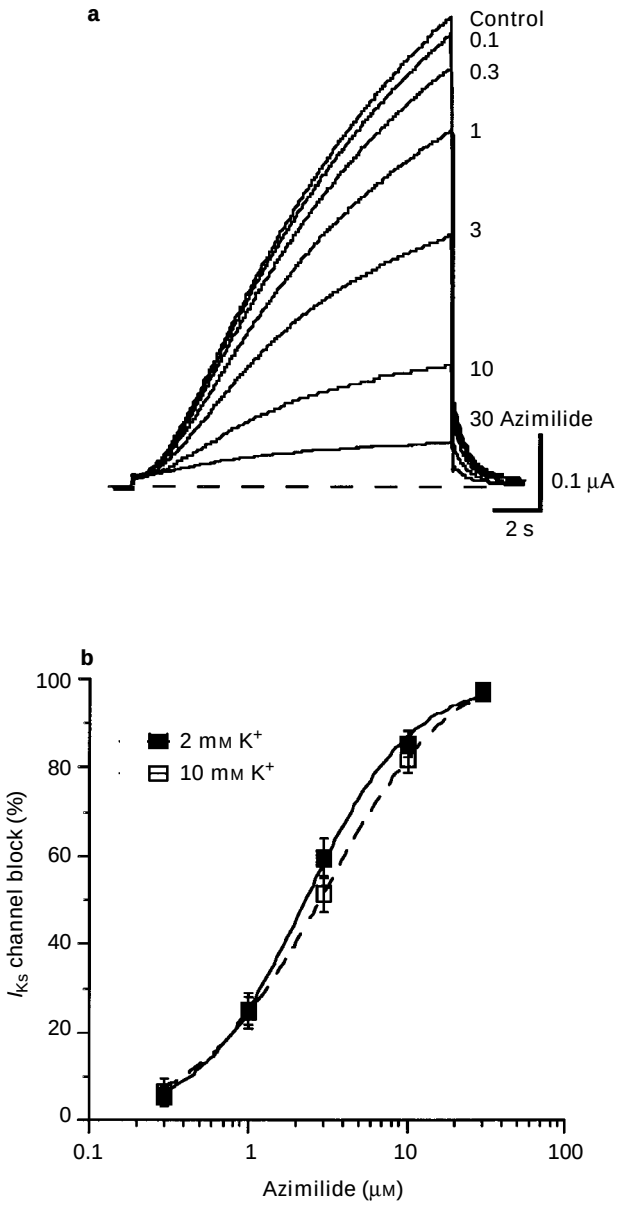

Figure 6 I_{Ks} channel blockade by azimilide. (a) Concentration-dependence of I_{Ks} channel block in the presence of 2 mM $[K^+]_e$. I_{Ks} channels were activated with 15 s depolarizing voltage steps from −80 to −10 mV at 45 s intervals. The dashed line corresponds to 0 current. (b) Concentration-response relationship for azimilide block of I_{Ks} channels with 2 mM and 10 mM $[K^+]_e$. Data are given as arithmetic means with vertical lines showing s.e.mean.

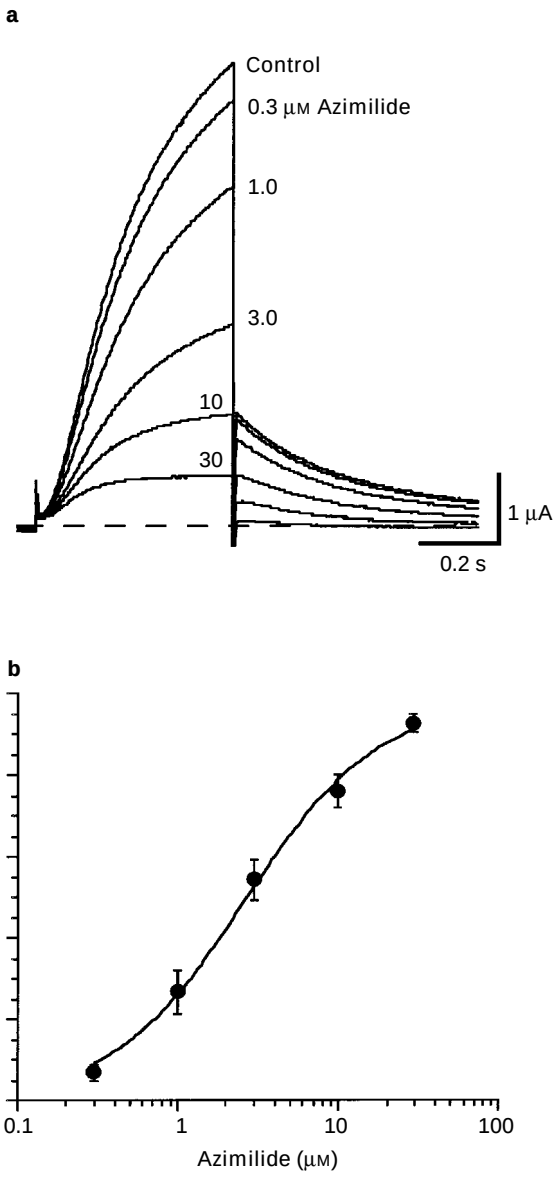

Figure 7 Concentration-dependence of HERG S631A blockade by azimilide. (a) Original current traces: the currents were evoked with 0.5 s depolarizing pulses to 0 mV from a holding potential of −80 mV every 3 s. Tail currents were recorded at −70 mV. The dashed line corresponds to 0 current. (b) Concentration-response relationship for azimilide block of HERG S631A. The data were fitted with a Hill equation with 100% blockade as a fixed maximum. Data are given as arithmetic means and vertical lines show s.e.mean.

azmilide is not altered after β-receptor stimulation and is positively use-dependent (Herzer *et al*., 1995), the present data suggest that I_{Ks} blockade by azimilide becomes more important for its action at elevated heart rates and/or β-adrenoceptor-mediated tone. In contrast, at low heart rates azimilide is expected to prolong action potenial duration principally by blocking I_{Kr}. Since high $[K^+]_e$ upregulates I_{Kr} but decreases the azimilide block, the relative contribution of I_{Kr} block by azimilide under these conditions remains unclear.

In summary, azimilide is a potent blocker of both I_{Ks} and HERG channels. The relative contribution of the respective channel blockade to the antiarrhythmic effects of azimilide is expected to depend on conditions such as heart frequency, $[K^+]_e$ and β-adrenoceptor-mediated tonus.

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References

BARHANIN, J., LESAGE, F., GUILLEMARE, E., FINK, M., LAZDUNSKI, M. & ROMEY, G. (1996). K_vLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature*, **384,** 78–80.

BLACK, S.C., BUTTERFIELD, J.L. & LUCCHESI, B.R. (1993). Protection against programmed electrical stimulation-induced ventricular tachycardia and sudden cardiac death by NE-10064, a class III antiarrhythmic drug. *J. Cardiovasc. Pharmacol*., **22,** 810–818.

BROOKS, R.R., CARPENTER, J.F., MILLER, K.E. & MAYNARD, A.E. (1996). Efficacy of the class III antiarrhythmic agent azimilide in rodent models of ventricular arrhythmia. *Proc. Soc. Exp. Biol. Med*., **212,** 84–93.

BUSCH, A.E., HERZER, T., TAKUMI, T., KRIPPEIT-DREWS, P., WALDEGGER, S. & LANG, F. (1994a). Blockade of human I_{sK} channels expressed in *Xenopus* oocytes by the novel class III antiarrhythmic NE-10064. *Eur. J. Pharmacol*., **264,** 33–37.

BUSCH, A.E., KOPP, H.-G., WALDEGGER, S., SAMARZIJA, I., SUESSBRICH, H., RABER, G., KUNZELMANN, K., RUPPERSBERG, J.P. & LANG, F. (1996a). Effect of isosorbiddinitrate on exogenously expressed slowly activating K^+ channels and endogenous K^+ channels in *Xenopus* oocytes. *J. Physiol*., **491,** 735–774.

BUSCH, A.E., MALLOY, K.J., GROH, W.J., VARNUM, M.D., ADELMAN, J.P. & MAYLIE, J.G. (1994b). The novel class III antiarrhythmics NE-10064 and NE-100133 inhibit I_{sK} channels expressed in *Xenopus* oocytes and I_{Ks} in guinea pig cardiac myocytes. *Biochem. Biophys. Res. Commun*., **202,** 265–270.

BUSCH, A.E., SUESSBRICH, H., GREGER, R., SAILER, E., LANG, H.-J., LANG, F., GIBSON, K.J. & MAYLIE, J.G. (1996b). Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by the chromanol 293B. *Pfügers Arch*., **432,** 1094–1096.

COLATSKY, T.J., FOLLMER, C.H. & STARMER, C.F. (1990). Channel specificity in antiarrhythmic drug action. *Circulation*, **82,** 2235–2242.

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

DELCARPIO, J.B., LANSON, N.A. Jr., FIELD, L.J. & CLAYCOMB, W.C. (1991). Morphological characterization of cardiomyocytes isolated from a transplantable cardiac tumor derived from transgenic mouse atria (AT-1 cells). *Circ. Res*., **69,** 1591–1600.

HERZER, T., WAGNER, C.A., WALDEGGER, S., LANG, F. & BUSCH, A.E. (1995). Effects of channel modulation and pH on I_{sK} inhibition by the novel class III antiarrhythmic Azimilide (NE-10064). *Eur. J. Pharmacol*., **291,** 205–208.

FERMINI, B., JURKIEWICZ, N.K., JOW, B., GUINOSSO, P.J. JR., BASKIN, E.P., LYNCH, J.J. JR. & SALATA, J.J. (1995). Use-dependent effects of the class III antiarrhythmic agent NE-10064 (azimilide) on cardiac repolarization: block of delayed rectifier potassium and L-type calcium currents. *J. Cardiovasc. Pharmacol*., **26,** 259–271.

JURKIEWICZ, N.K., WANG, J., FERMINI, B., SANGUINETTI, M.C. & SALATA, J.J. (1996). Mechanism of action potential prolongation by RP 58866 and its active enantiomer, terikalant. Block of the rapidly activating delayed rectifier K^+ current, I_{Kr}. *Circulation*, **94,** 2938–2946.

JURKIEWICZ, N. & SANGUINETTI, M.C. (1993). Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class III antiarrhythmic agent. *Circ. Res*., **72,** 75–83.

KIEHN, J., LACERDA, A.E., WIBLE, B. & BROWN, A.M. (1996). Molecular physiology and pharmacology of HERG. Single-channel currents and block by dofetilide. *Circulation*, **94,** 2572–2579.

LYNCH, J.J. JR., WALLACE, A.A., STUPIENSKI, R.F. III, BASKIN, E.P., BEARE, C.M., APPLEBY, S.D., SALATA, J.J., JURKIEWICZ, N.K., SANGUINETTI, M.C., STEIN, R.B., GEHRET, J.R., KOTHSTEIN, T., CLAREMON, D.A., ELLIOTT, J.M., BUTCHER, J.W., REMY, D.C. & BALDWIN, J.J. (1994). Cardiac electrophysiologic and antiarrhythmic actions of two long-acting spirobenzopyran piperdine class III agents, L-702,958 and L-706,000 [MK-499]. *J. Pharmacol. Exp. Ther*., **269,** 541–554.

MURAI, T., KAKIZUKA, A., TAKUMI, T., OHKUBO, H. & NAKANISHI, S. (1989). Molecular cloning and sequence analysis of human genomic DNA encoding a novel membrane protein which exhibits a slowly activating potassium channel activity. *Biochem. Biophys. Res. Commun*., **161,** 176–181.

RESTIVO, M., HEGAZY, M., CAREF, E.B., AVITABLE, M.J., ASSADI, M.A., EL-HAMAMI, M., YIN, H., PIRACHA, M., BROOKS, R.R. & EL-SHERIF, N. (1996). Effects of azimilide dihydrochloride on circus movement atrial flutter in the canine sterile pericarditis model. *J. Cardiovasc. Electrophysiol*., **7,** 612–624.

RODEN, D.M. (1996). Ionic mechanisms for prolongation of refractoriness and their proarrhythmic and antiarrhythmic correlates. *Am. J. Cardiol*., **78,** 12–16.

SALATA, J.J., JURKIEWICZ, N.K., WALLACE, A.A., STUPIENSKI, R.F. III, GUINOSSO, P.J. JR. & LYNCH, J.J. JR. (1995). Cardiac electrophysiologic actions of the histamine H_1-receptor antagonists astemizole and terfenadine compared with chlorpheniramine and pyrilamine. *Circ. Res*., **76,** 110–119.

SALATA, J.J., JURKIEWICZ, N.K., SANGUINETTI, M.C., SIEGL, P.K., CLAREMON, D.C., REMY, D.C., ELLIOTT, J.M. & LIBBY, B.E. (1996). The novel class III antiarrhythmic agent L-735,821 is a potent and selective blocker of I_{Ks} in guinea pig ventricular myocytes. *Circulation*, **94,** I–529, abstract.

SANGUINETTI, M.C., CURRAN, M.E., ZOU, A., SHEN, J., SPECTOR, P.S., ATKINSON, D.L. & KEATING, M.T. (1996). Coassembly of K_VLQT1 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 arrhythmic: HERG encodes the 1_{Kr} potassium channel. *Cell*, **81,** 299–307.

SANGUINETTI, M.C., JURKIEWICZ, N.C., SCOTT, A. & SIEGL, P.K.S. (1991). Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent, E-4031, in guinea-pig myocytes: mechanism of action. *Circ. Res*., **68,** 77–84.

SANGUINETTI, M.C. & SALATA, J.J. (1996). Cardiac potassium channel modulators: Potential for antiarrhythmic therapy. In *Potassium Channels and Their Modulators: From Synthesis to Clinical Experience*. ed. Evans, J.M., Hamilton, T.C., Longman, S.D. & Stemp, G. London: Taylor and Francis.

SCHÖNHERR, R. & HEINEMANN, S.H. (1996). Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. *J. Physiol*., **493,** 635–642.

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

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.

SPECTOR, P.S., CURRAN, M.E., KEATING, M.T. & SANGUINETTI, M.C. (1996). Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K^+ channel. Open-channel block by methanesulfonanilides. *Circ. Res.*, **78,** 499 – 503.

SUESSBRICH, H., SCHÖNHERR, R., HEINEMANN, S.H., LANG, F. & BUSCH, A.E. (1997a). Specific block of HERG channels by clofilium and its tertiary analog LY97241. *FEBS Lett.*, (in press).

SUESSBRICH, H., SCHÖENHERR, R., HEINEMANN, U., LANG, F., ATTALI, B. & BUSCH, A.E. (1997b). Molecular basis for proarrhythmogenic effects of haloperidol - inhibition of HERG channels. *Br J. Pharmacol.*, **120,** 968 – 974.

SUESSBRICH, H., WALDEGGER, S., LANG, F. & BUSCH, A.E. (1996). Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole. *FEBS Lett.*, **385,** 77 – 80.

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

WARMKE, J.E. & GANETZKY, B. (1994). A family of potassium channel genes related to eag in Drosophila and mammals. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 3438 – 3442.

YANG, T., PRAKASH, C., RODEN, D.M. & SNYDERS, D.J. (1995). Mechanism of block of a human cardiac potassium channel by terfenadine racemate and enantiomers. *Br J. Pharmacol.*, **115,** 267 – 274.

YANG, T. & RODEN, D.M. (1996). Extracellular potassium modulation of drug block of I_{Kr}. *Circulation*, **93,** 407 – 411.

YANG, T., WATHEN, M.S., FELIPE, A., TAMKUN, M.M., SNYDERS, D.J. & RODEN, D.M. (1994). K^+ currents and K^+ channel mRNA in cultured atrial cardiac myocytes (AT-1 cells). *Circ. Res.*, **75,** 870 – 878.

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