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Inactivation gating determines drug potency: a common mechanism for drug blockade of HERG channels

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ABSTRACT

AIM: To determine the mechanisms of interactions between different drugs and HERG channels. **METHODS:** Various antiarrhythmic (dofetilide, quinidine, azimilide, RP58866) and non-antiarrhythmic (terfenadine, nicotine) agents were used on HERG channels expressed in *Xenopus* oocyte. Whole-cell voltage-clamp techniques were used. **RESULTS:** All drugs produced concentration-dependent block of HERG current. The inhibition was markedly facilitated with voltage protocols favoring channel inactivation (eg, less negative holding potentials). Maneuvers that weakened channel inactivation (eg, elevation of external K⁺), relieved HERG blockade by all drugs. Moreover, the inhibitory potency was reduced by at least 20-300 fold with varying compounds when rapid C-type inactivation was removed by a mutation located between the transmembrane domains 5 and 6 (S631A). **CONCLUSION:** The inactivation gating of HERG channels determines the blocking potency of drugs. This mechanism might be common to drugs of various classes.

INTRODUCTION

The K⁺ channel encoded by HERG (the human ether-a-go-go related gene) is well accepted as an equivalent of the native delayed rectifier K⁺ channel I_{Kr} in cardiac myocytes. Indeed, when expressed in either mammalian cells or *Xenopus* oocytes, the HERG channel current is virtually identical to I_{Kr} in terms of the biophysical properties and pharmacological sensitivity. This channel provides a mechanistic link between the acquired and the inherited long Q-T syndrome^[1]. The pharmacological properties of HERG channels have been extensively studied with a variety of drugs belonging to

different categories, including antiarrhythmic agents^[2-5], antihistamine drugs^[6,7], and antipsychotic drugs^[8], *etc.* We have also previously investigated in detail the effects of RP58866 (a new compound with antiarrhythmic efficacy)^[9] and nicotine on HERG channels^[10]. These studies largely advanced our understanding of the ionic mechanisms underlying the antiarrhythmic action or cardiotoxicity caused by many drugs. We observed that many of the drugs tended to block HERG channels with high affinity to the inactivated state. This property bears important implications in the sense that a greater extent of inhibition is expected in the diseased states of the heart, such as myocardial ischemia, cardiac hypertrophy, and heart failure, with which channels inactivation could manifest as a consequence of abnormal membrane depolarization. Enhanced inhibition of HERG channels would result in more prolongation of action potential duration and Q-T interval which

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can in turn lead to greater alterations in cardiac electrical activity to produce either beneficial or deleterious outcomes depending on different clinical settings. However, these studies were performed under more or less different experimental conditions for different specific aims. Albeit important, direct comparison of the state-dependent block by various drugs is still lacking. This study was therefore specifically designed to investigate the inactivation-dependence of HERG channel inhibition by various compounds belonging to varying categories under the identical experimental conditions. Schonherr R reported^[11] that Mutation of S631 to A in the outer mouth of the HERG pore completely abolished channel inactivation. Since the rapid C-type inactivation was removed by a mutation located between transmembrane domain 5 and 6, the model of inactivation-deficient mutant of HERG channels (S631A) was used to test the efficiency of channel blocking on inactivation.

MATERIALS AND METHODS

***In vitro* transcription and functional expression in *Xenopus laevis* oocytes** Procedures for *in vitro* transcription and oocyte injection have been previously described in detail^[12,13]. HERG and S631A mutant of HERG were subcloned into pSP64 vector. cRNAs were prepared with the *mMESSAGE mMACHINE* kit (Ambion, Austin) using SP6 RNA polymerase after linearization of the plasmid with *EcoRI*, according to manufacturer's protocols. cRNAs were dissolved in DEPC (diethyl pyrocarbonate)-treated sterile water, stored at -80 °C, and diluted immediately prior to injection. Stage V-VI *Xenopus* oocytes were injected with 46 nL of cRNA.

Two-electrode voltage-clamp recording Whole-cell macroscopic currents were recorded with conventional two-electrode techniques^[9,14,15]. Electrodes filled with KCl 3 mol/L had resistance of approximately 1.0-1.5 M Ω when measured in the bath solution containing (mmol/L): NaCl 100, KCl 5, CaCl₂ 0.3, MgCl₂ 2, and HEPES 10 (pH 7.4). The electrodes were connected to a GeneClamp-500 amplifier (Axon Instrument, Burlingame, CA). The pClamp suite of programs was employed for data acquisition and analysis. Records were digitized at 5 kHz and filtered at 2 kHz. Experiments were conducted at room temperature (22-23 °C). Currents were measured before drugs and 10 min after drug application to the bath.

For activation, currents were elicited from holding potential of -70 mV to +50 mV. Data best fits to the Boltzmann distribution: $I/I_{\max} = 1 / \{1 + \exp[V_{1/2} - V] / k\}$,

where I is the HERG tail current amplitude at a prepulse potential V , $V_{1/2}$ is the voltage for half-maximal activation, and k is a slope factor. For inactivation, a 2-s depolarizing pulse of +20 mV was employed to inactivate the HERG channels followed by varying repolarizing pulses to potentials between -140 to +20 mV for a short period of 20-ms, followed by a test pulse to +20 mV.

The effects of drugs on the currents were determined at a test potential of 0 mV. Averaged data fits to the Hill equation: $B (\%) = 100 / [1 + (IC_{50}/D)^n]$, where $B (\%)$ is the percent changes of HERG-expressed current at a drug concentration D , IC_{50} is the concentration of a given drug that produces 50 % effects, and n is the Hill coefficient.

Voltage-dependent effects of various drugs were measured by 2-s depolarizing steps to -20 mV from two different holding potentials (-100 mV or -60 mV). Recordings made under control conditions were repeated 10 min after drug superfusion. Current amplitude was measured 400 ms after the starting point of the depolarizing pulses. Test potential-dependent effects were determined by eliciting 2-s depolarizing steps to a TP of -50 mV or 0 mV from an HP of -70 mV.

Effects of various drugs on wild type HERG channels were compared under different external K⁺ concentrations (5 or 20 mmol/L).

Drugs The drugs examined included dofetilide, RP58866, azimilide, quinidine, terfenadine, and nicotine. Dofetilide is a prototype HERG blocker with class III antiarrhythmic actions^[16], and it is actually widely used as a specific HERG blocker. Azimilide, another class III antiarrhythmic agent, was also found to be able to inhibit HERG channels with high potency^[2]. RP58866, a new compound with class III antiarrhythmic properties, is believed to block I_{Kr} and HERG^[17,18] with high potency. Quinidine is a well-known class I_A antiarrhythmic agent with K⁺ channel blocking properties. Terfenadine, an antihistamine agent used for relief of symptoms due to allergy, has been shown to cause long Q-T syndrome and HERG inhibition is thought to be the underlying mechanism^[6,8]. Finally, nicotine, a main constituent of cigarette smoking and smokeless tobacco^[19,20], has been demonstrated to be able to alter the cardiac electrophysiology and to inhibit HERG channels^[20].

All drugs were prepared as concentrated stocks right before experiments and diluted 1000 times to achieve the desired concentrations. Terfenadine was dissolved in 100 % alcohol and RP58866 in Me₂SO. The other compounds used were all dissolved in double-distilled water. The chemicals were all purchased from

Sigma Chemicals.

Data analysis Group data are expressed as mean \pm SEM. Statistical comparisons among groups were performed by analysis of variance (ANOVA). If significant effects were indicated by ANOVA, a *t*-test with Bonferroni correction or a Dunnett's test was used to evaluate the significance of differences between individual means. Otherwise, baseline and drug data were compared by *t*-test. A two-tailed $P < 0.05$ was taken to indicate a statistically significant difference. A nonlinear least-square curve-fitting program (CLAMPFIT in pCLAMP 6.0 or Graphpad Prism) was used to perform curve fitting procedures.

RESULTS

Comparisons of effects of various drugs on the wild-type and the S631A mutant HERG Expression of the HERG channels resulted in the induction of a K^+ conductance with characteristic activation and rectification properties. With standard depolarizing voltage steps (from -60 to +50 mV), time-dependent activation of outward currents was elicited, followed by large tail currents upon repolarization to -50 mV (Fig 1A). Because of the rapid C-type inactivation of HERG channels compared with their activation, outward currents at potentials positive to 0 mV became smaller^[21,22]. Expression of S631A in oocytes gave rise to delayed rectifier-like currents with a rapid activation (Fig 1B), and

the inward rectification, seen with the wild type, was absent under our experimental conditions, consistent with previous studies on the same mutation construct^[8,18,21,24].

Similar to the observations made with the wild type HERG, the drugs also caused concentration-dependent block of the S631A currents. However, the inhibition was substantially relieved in S631A relative to the wild-type HERG channels. The potency of the drugs was apparently diminished in the mutant. For example, dofetilide at 0.25 μ mol/L, which suppressed the wild type HERG channels by about 50 %, did not significantly affect the currents expressed by the mutant HERG channels. Elevation of the drug concentration up to 100 μ mol/L produced significant block of the mutated channels to the extents comparable with the blockade of the wild type HERG channels by dofetilide 0.25 μ mol/L (Fig 2).

All drugs demonstrated higher potencies, as indicated by lower IC_{50} values, for the wild type HERG channels compared to the S631A mutant channels. The ratio of IC_{50} for S631A to that for the wild type HERG channels ranged from about 10 to 350, depending on different drugs. For example, azimilide was approximately 10-times more potent for wild type than for S631A channels. The difference was most prominent for quinidine: the blockade was about 350-fold more potent for the wild type than for the mutant (Tab 1).

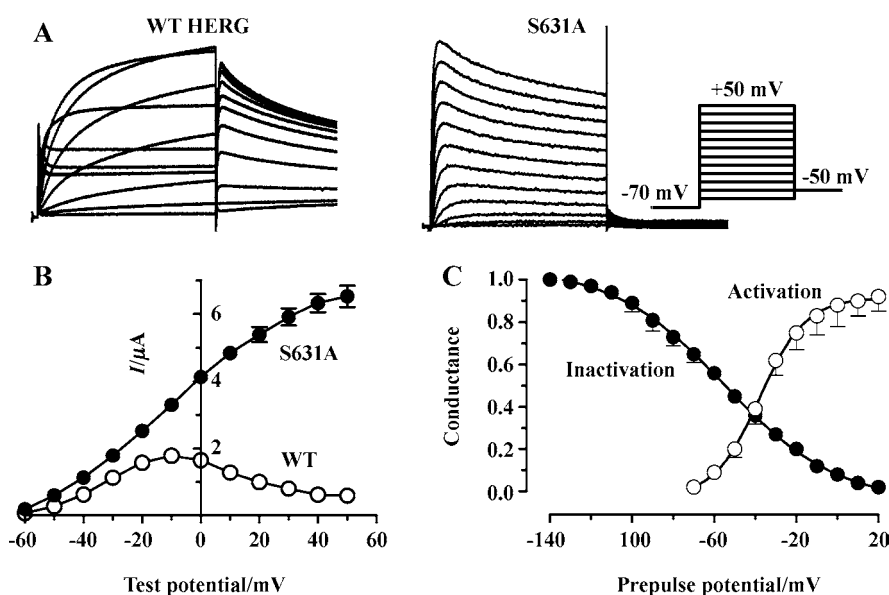


Fig 1. Voltage-dependent activation and inactivation properties of wild type and S631A mutant HERG channels expressed in *Xenopus* oocytes. (A) Typical examples of current traces. (B) *I-V* relationships. (C) Activation and inactivation conductance curves.

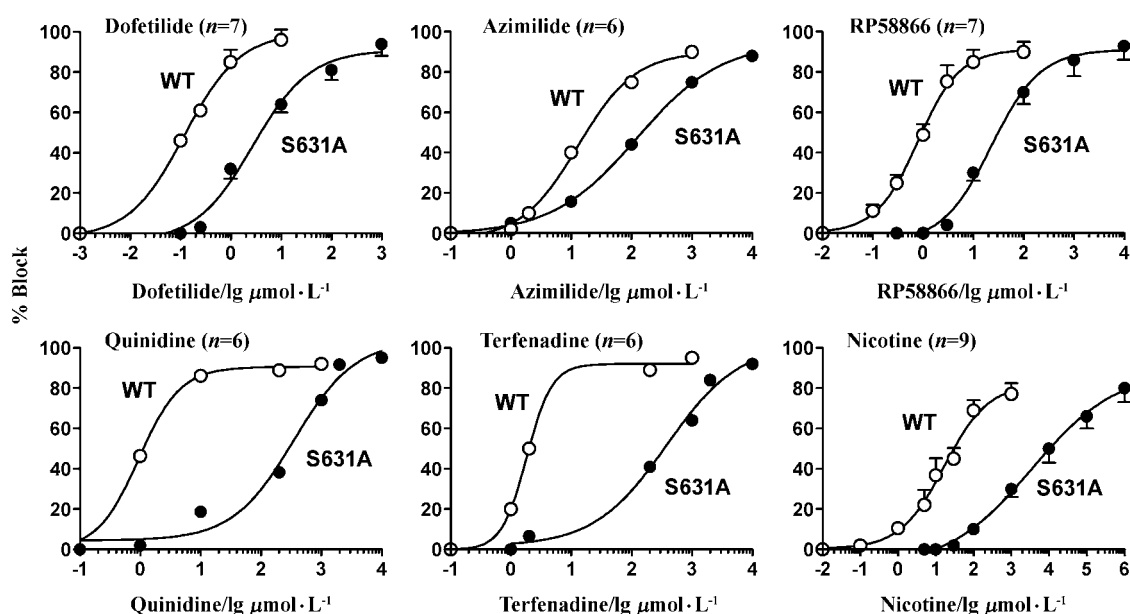


Fig 2. Concentration-dependent blockade of wild type and S631A mutant HERG channels by various drugs. Mean±SEM.

Tab 1. Effects of various drugs on wild-type and S631A mutant of HERG channels.

Drug	WT		S631A	
	$IC_{50}/\mu\text{mol}\cdot\text{L}^{-1}$	n	$IC_{50}/\mu\text{mol}\cdot\text{L}^{-1}$	n
Dofetilide	0.1±0	0.8±0.1	2.7±0.3	0.8±0.2
Alzimilide	13.1±2.1	0.9±0.2	123±10	0.6±0.1
RP58866	0.8±0.1	1.0±0.2	22.7±1.9	0.9±0.1
Quinidine	1.0±0.2	1.3±0.2	350±42	0.9±0.2
Terfenadine	1.8±0.2	2.1±0.2	381±36	0.7±0.1
Nicotine	16.8±2.0	0.7±0.1	3510±257	0.4±0.1

IC_{50} -drug concentration for 50 % channel block; n : Hill coefficient.

Voltage-dependence of HERG blockade by various compounds The above results indicated that the drugs blocked HERG more in the inactivated state. If this is true, then voltage protocols favoring channel inactivation should enhance the inhibition. To test this notion, effects of drugs on HERG channels with varying membrane (holding) potentials were assessed.

As illustrated in Fig 1C, approximately 10 % of the channels were inactivated at an HP of -100 mV compared to the value at a strongly hyperpolarized potential of -140 mV. The channel availability was further decreased with stronger depolarization (more positive HPs). For instance, at -60 mV, around 50 % of the channels entered into inactivated state. There was no

significant channel activation at potentials between -100 mV and -60 mV, or in the other words, there was no overlapping of channel activation and inactivation within this voltage range. This property provides an opportunity for assessing inactivation-dependence (set by holding potential) of drug effects without concomitant channel activation. The effects of drugs at potentials between -100 mV and -60 mV were therefore compared. Currents were elicited by 2-s depolarizing pulses to -20 mV from an HP of -100 mV or -60 mV, and the current amplitude was measured at 400 ms after the beginning of the pulses. The test potential and the time-point for current measurement were chosen because no apparent time-dependent inactivation of the channels was seen under such conditions and changes observed should more likely represent voltage-dependent effects.

The blockade of HERG channels by various compound was substantially accentuated at more depolarized potentials (or more positive holding potentials) (Fig 3).

In order to explore whether the HERG block by the drugs also depended on open channels, effects of the drugs at different test potentials were determined. Comparison was made between test potentials of -50 mV and 0 mV. These two test potentials were chosen because they covered the voltage range with the steepest change in the activation conductance curve (Fig 1C). The percentage of channels in the open state was increased from about 20 % to about 90 % when the test

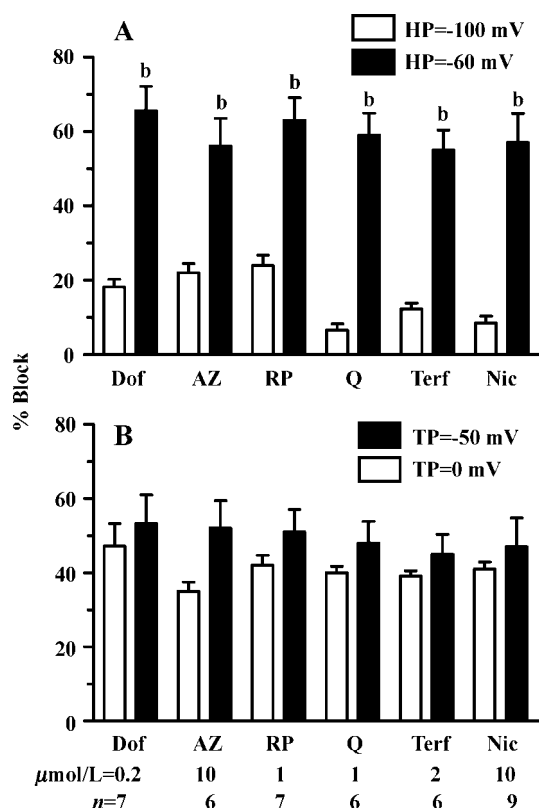


Fig 3. Voltage-dependent effects of various drugs on wild type HERG channels at different holding potentials (A) and different test potentials (B). ^b $P < 0.05$ vs -100 mV. Dof-dofetilide; AZ-azimilide; RP-RP58866; Q-quinidine; Terf-terfenadine; Nic-nicotine. "n" indicates the number of oocytes studied. Mean \pm SEM.

potential was increased from -50 mV to 0 mV (Fig 3B). In sharp contrast to the changes in the holding potential, no statistically significant differences were found between the two different test potentials for any of the drugs examined, indicating that the effects of these drugs required channel inactivation more than opening.

[K⁺]_o-dependence of HERG blockade by various compounds Rapid C-type inactivation of the HERG channels is highly sensitive to external K⁺ concentrations^[25]. Elevating [K⁺]_o markedly slowed the kinetics of the HERG channel inactivation and decreased the magnitude of the inactivation. Obviously, the voltage-dependent inactivation parameters were markedly shifted toward more positive potentials. For example, the channel availability at -60 mV remained as high as about 85 % when [K⁺]_o was raised to 20 mmol/L (vs only 50 % with 5 mmol/L [K⁺]_o) (Fig 4A). Comparison of drug effects under different [K⁺]_o (5 vs 20 mmol/L) was made and the current amplitude was measured at 0 mV because the channels reached nearly full activation at this potential. The degree of HERG inhibition induced by all

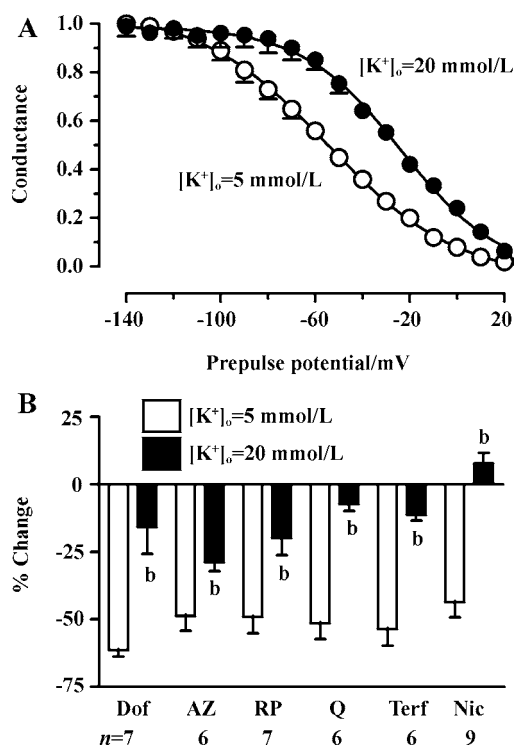


Fig 4. Effects of various drugs on wild type HERG channels under different external K⁺ concentrations. (A) Inactivation curves constructed under normal [K⁺]_o (5 mmol/L) and under high [K⁺]_o conditions (20 mmol/L), respectively. (B) Comparison of the HERG channel blockade induced by various drugs under [K⁺]_o=5 mmol/L and [K⁺]_o=20 mmol/L, respectively. Mean \pm SEM. ^b $P < 0.05$ vs 5 mmol/L [K⁺]_o. Dof-dofetilide; AZ-azimilide; RP-RP58866; Q-quinidine; Terf-terfenadine; Nic-nicotine. "n" indicates the number of oocytes studied.

compounds tested was significantly diminished without exception. To achieve the same extents of block, drug concentrations had to be raised to about 100-times higher (Fig 4B).

DISCUSSION

In the present study, we characterized the effects of various compounds (including dofetilide, quinidine, RP58866, azimilide, terfenadine and nicotine) on the HERG channels. Our experiments demonstrated that all the drugs tested caused a concentration-dependent inhibition and the drugs preferentially acted on the inactivated channels to produce the effects. The results suggest that agents of different categories interact with the HERG channels with a common mechanism: inactivation gating determines the potency of drug blockade. Except for RP58866 and nicotine which were studied previously by our laboratory^[18,21], inactivation blocking properties of other drugs have not been reported at all

or characterized in detail.

The data from our experiments revealed that maneuvers that favored (or prevented) channel inactivation promoted (or relieved) drug blockade. For example, the drugs produced more pronounced inhibition of the HERG channels with the voltage protocols which rendered the channels more in the inactivated state. This was clearly demonstrated by the enhanced effects with depolarized membrane potentials or less negative holding potentials. On the contrary, changes in the test potential with a fixed holding potential, which altered the fraction of channels in the open state without altering the inactivation, caused a little changes in the drug effects. Moreover, elevating the external potassium concentration, which led to markedly decreased degree of channel inactivation, substantially relieved the channels from blocking by the drugs. These observations are in agreement with the studies by other laboratories, in which the drug action on I_{Kr} ^[26] and HERG^[2] was found to be considerably weakened with increase in $[K^+]_o$. Furthermore, the drug effects were remarkably blunted on the S631A mutant, in which the voltage- and time-dependent inactivation was virtually absent within the voltage range and pulse duration employed in our experiments. Our data therefore suggested that blockade of the HERG channels was mainly due to the binding of the drugs to the inactivated channels.

Inactivation block of HERG channels has also been previously documented in several studies using antiarrhythmic and non-antiarrhythmic agents. For example, Suessbrich *et al*^[8] characterized the blockade of HERG by haloperidol (an antipsychotic drug). The mechanism of haloperidol block was found to involve binding to inactivated channels as inactivation enhanced and removal of inactivation weakened the inhibition. A study utilizing inactivation-deficient mutant (S620T) by Brown's group^[24] also elegantly demonstrated that the inhibitory potency of dofetilide on HERG was considerably reduced in the absence of channel inactivation gating, as in our study with S631A mutant. The authors concluded that a C-type inactivation process was crucial for high-affinity binding of dofetilide^[24]. Similarly, Herzberg *et al*^[27] also clearly demonstrated that the blockade of HERG by E-4031 was largely diminished with the mutations disabling the rapid HERG channel inactivation.

In the study reported by Snyders *et al*^[4], open-state block of HERG channels by dofetilide was postulated to account for their observations such as use-dependent block, acceleration of inactivation, etc.

However, these data could also be readily explained by inactivated channel blocking paradigm. Particularly, the prepulse voltage-dependence (depolarized holding potentials) of the effects as clearly stated in their article was difficult to understand on the basis of open channel blocking property, because open channel block should show the opposite prepulse voltage-dependence, ie, effects diminished with stronger depolarization which renders stronger channel inactivation. Together with the observation by Ficker *et al*^[24] and us^[18,21], hence, their results on dofetilide could also be interpreted as the drug binding preferentially to the inactivated channels.

Effects of azimilide on HERG channels have also been characterized by Busch *et al*^[2]. They found that azimilide blockade was reverse use-dependent, ie, the relative block and apparent affinity of azimilide decreased with an increase in channel activation frequency. Consistent with our data, they also observed a reduced HERG inhibition by azimilide under conditions of high $[K^+]_o$. In an envelope of tail test, HERG channel blockade was found to increase with an increasing channel activation. Based on this latter observation, they postulated binding of azimilide to open channels. However, the first two observations are better explained by inactivation block because as an open channel blocker, the effects are expected to be greater with higher channel activation frequencies and with more channels trapped in the open state when $[K^+]_o$ is raised. It is possible that inactivation block and open channel block are both contributing to azimilide's action, and our data from the present study seem to be in favor of this notion. Among the drugs tested in this study, azimilide stands a compound with the least selectivity toward inactivated channels, as indicated by only 10-times difference in the IC_{50} for the wild type and the S631A HERG channels. By comparison, other drugs had at least 50-fold difference. Together all these studies would suggest that azimilide could act on both inactivated and activated channels.

Based on the present and previous studies, we postulated that preferential inactivation block might be a common mechanism for drug action on HERG channels, or in the other words, the inactivation gating determined to a large extent the potency of drug block of HERG channels.

Although our data indeed provide some insights into the mechanisms of the interactions between various drugs and HERG channels, definite conclusions cannot be drawn from our study. Moreover, our experiments do not exclude the open channel and/or closed

state-dependent block by the drugs. There are several other limitations in our study. First, we are unable to provide information regarding the detailed structural determinants of drug binding and blocking. Extensive site-directed mutagenesis is required to address this issue. Second, our data also fail to answer why a variety of structurally unrelated compounds have rather similar mode of actions on HERG channels. Although these topics are beyond the scope of the present work, it is of great interest and importance to make further investigation to have better understanding of mechanisms of drug actions.

REFERENCES

- Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* 1995; 81: 299-307.
- Busch AE, Eigenberger B, Jurkiewicz NK, Salata JJ, Pica A, Suessbrich H, *et al*. Blockade of HERG channels by the class III antiarrhythmic azimilide: mode of action. *Br J Pharmacol* 1998; 123: 23-30.
- Kiehn J, Laceda AE, Wible B, Brown AM. Molecular physiology and pharmacology of HERG. Single-channel currents and block by dofetilide. *Circulation* 1996; 4: 2572-9.
- Snyders DJ, Chaudhary A. High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol Pharmacol* 1996; 49: 949-55.
- Spector PS, Curran ME, Keating MT, Sanguinetti MC. Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K^+ channel. Open-channel block by methanesulfonanilides. *Circ Res* 1996; 78: 499-503.
- Roy M, Dumaine R, Brown AM. HERG, a primary human ventricular target of the nonsedating antihistamine terfenadine. *Circulation* 1996; 94: 817-23.
- Suessbrich H, Waldegger S, Lang F, Bush AE. Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists. *FEBS Lett* 1997; 385: 77-80.
- Suessbrich H, Schönherr R, Heinemann SH, Attali B, Lang F, Bush AE. The inhibitory effect of the antipsychotic drug haloperidol on HERG potassium channels expressed in *Xenopus* oocytes. *Br J Pharmacol* 1997; 120: 968-74.
- Yang BF, Li GR, Xu CX, Nattel S. Effects of RP58866 on transmembrane K^+ currents in mammalian ventricular myocytes. *Acta Pharmacol Sin* 1999; 20: 961-9.
- Rampe D, Roy ML, Dennis A, Brown AM. A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett* 1997; 417: 28-32.
- Schönherr R, Heinemann SH. Molecular determinants for activation and inactivation of HERG, a human inward rectifier potassium channel. *J Physiol* 1996; 493 (Pt 3): 635-42.
- Wang Z, Kiehn J, Yang Q, Brown A, Wible B. Comparison of K^+ channel binding and block produced by alternatively spliced Kv 3.1 subunits. *J Biol Chem* 1996; 271: 28311-7.
- Wang Z, Yue L, White M, Pelletier G, Nattel S. Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. *Circulation* 1998; 98: 2422-8.
- Yang BF, Luo DL, Bao LH, Zhang YC, Wang HZ. Artemisinin blocks activating and slowly activating K^+ current in guinea pig ventricular myocytes. *Acta Pharmacol Sin* 1998; 19: 269-72.
- Yang BF, Nattel S. Effects of RP58866 and 62719 on transmembrane K^+ currents in mammalian ventricular myocytes. *Biophysical* 1997; 72: 405.
- Andersen HR, Wiggers HS, Knudsen L, Simonsen I, Thomsen PEB, Christiansen N. Dofetilide reduces the incidence of ventricular fibrillation during acute myocardial ischemia. A randomized study in pigs. *Cardiovasc Res* 1994; 28: 1635-40.
- Jurkiewicz NK, Wang J, Fermini B, Sanguinetti MC, Salata JJ. Mechanism of action potential prolongation by RP58866 and its active enantiomer, terikalant: block of the rapidly activating delayed rectifier K^+ current, I_{Kr} . *Circulation* 1996; 94: 2938-46.
- Wang H, Shi H, Wang Z. Inactivation block of the HERG human cardiac K^+ channels by RP58866. *Br J Pharmacol* 1999; 127: 1899-907.
- Benowitz NL. Pharmacological aspects of cigarette smoking and nicotine addiction. *New Engl J Med* 1988; 319: 1318-30.
- Benowitz NL, Gourlay SC. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. *J Am Coll Cardiol* 1997; 29: 1422-31.
- Wang H, Shi H, Liao S, Wang Z. Inactivation gating determines nicotine blockade of the human HERG channels. *Am J Physiol* 1999; 277 (3 Pt 2): H1081-8.
- Smith PL, Baukowitz T, Yellen G. The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* 1996; 379: 833-6.
- Wang S, Liu S, Morales MJ, Strauss HC, Rasmusson RL. A quantitative analysis of the activation and inactivation kinetics of HERG expressed in *Xenopus* oocytes. *J Physiol* 1998; 502: 45-60.
- Ficker E, Jarolimek W, Kiehn J, Baumann A, Brown AM. Molecular determinants of dofetilide block of HERG K^+ channels. *Circ Res* 1998; 82: 386-95.
- Wang S, Morales MJ, Liu S, Strauss HC, Rasmusson RL. Time-, voltage- and ionic-concentration dependence of rectification of h-erg expressed in *Xenopus* oocytes. *FEBS Lett* 1996; 389: 167-73.
- Yang T, Roden DM. Extracellular potassium modulation of drug block of I_{Kr} : implications for *Torsade de Pointes* and reverse use-dependence. *Circulation* 1996; 93: 407-41.
- Herzberg IM, Trudeau CM, Robertson GA. Transfer of rapid inactivation and sensitivity to the class III antiarrhythmic drug E-4031 from HERG to M-eag channels. *J Physiol* 1998; 511: 3-14.