State-Dependent Blocking Actions of Azimilide Dihydrochloride (NE-10064) on Human Cardiac Na⁺ Channels

Junichiro Miake, MD; Yasutaka Kurata, MD*; Kazuhiko Iizuka, MD; Hitomi Furuichi, MD**; Kasumi Manabe, MD***; Norihito Sasaki, MD***; Yasutaka Yamamoto, MD***; Yoshiko Hoshikawa, MD***; Shin-ichi Taniguchi, MD; Akio Yoshida, MD; Osamu Igawa, MD; Naomasa Makita, MD†; Goshi Shiotani, MD‡‡; Eiji Nanba, MD‡‡; Shigetsugu Ohgi, MD††; Toshio Narahashi, MD¶; Ichiro Hisatome, MD**

Background Azimilide reportedly blocks Na⁺ channels, although its mechanism remains unclear.

Methods and Results The kinetic properties of the azimilide block of the wild-type human Na⁺ channels (WT: hH1) and mutant ΔKPQ Na⁺ channels (ΔKPQ) expressed in COS7 cells were investigated using the whole-cell patch clamp technique and a Markovian state model. Azimilide induced tonic block of WT currents by shifting the h-versus curve in the hyperpolarizing direction and caused phasic block of WT currents with intermediate recovery time constant. The peak and steady-state ΔKPQ currents were blocked by azimilide, although with only a slight shift in the h-versus curve. The phasic block of peak and steady-state ΔKPQ currents by azimilide was significantly larger than the blocking of the peak WT current. The affinity of azimilide predicted by a Markovian state model was higher for both the activated state (KdA=1.4±1.4 μmol/L), and the inactivated state (Kdi=1.4±1.4 μmol/L), of WT Na⁺ channels than that for the resting state (Kds=102.6±10.6 μmol/L).

Conclusions These experimental and simulation studies suggest that azimilide blocks the human cardiac Na⁺ channel in both the activated and inactivated states. (Circ J 2004; 68: 703–711)

Key Words: Activated state; Azimilide; ΔKPQ mutant; hH1; Inactivated state

Azimilide dihydrochloride (NE-10064), a chlorphenylfranyl compound, prolongs cardiac refractoriness by blocking the fast (Ic) and slow (Is) components of the delayed-rectifier potassium currents. These in vitro electrophysiologic properties of azimilide could account for its antiarrhythmic and antifibrillatory actions in a number of animal models, which could be classified as class III antiarrhythmics.1–3 It has been reported that azimilide interacts with other cardiac ion channels, blocking the L-type Ca²⁺ channel, the inward rectifier K⁺ channel, and the Na⁺ channel,11–13 but so far there have not been any systematic studies of the effects of azimilide on the cardiac Na⁺ channels and little is known about the state-dependent azimilide block of the Na⁺ channels, although a few reports indicated that azimilide blocked cardiac peak and late Na⁺ currents.12–15 Therefore, we assessed the effects of azimilide on the Na⁺ current using the wild-type h-subunit of the human cardiac sodium channel (hH1: WT) as well as long QT syndrome type III mutant (ΔKPQ mutant) expressed in COS7 cells.

Methods

Plasmid and Expression The vector pRC/CMV (Invitrogen, San Diego, CA, USA) was used as the expression vector for human WT or ΔKPQ mutant cDNAs. COS7 cells were maintained in Dulbecco’s modified Eagle medium (DMEM; Gibco BRL, Gaithersburg, MD, USA)/10% fetal bovine serum, 1% penicillin and 1% streptomycin at 37°C in a 5% CO₂ incubator. Cells grown on glass coverslips were co-transfected with both pRC/WT or pRC/ΔKPQ mutant and pEGFPC1 (Clontech, Palo Alto, CA, USA) by using Lipofectamine (Gibco BRL) according to the manufacturer’s instructions. Forty-eight h after transfection, cells were visualized by EGFP fluorescence and subjected to the whole-cell patch clamp experiments.

Electrophysiological Recordings Whole-cell patch clamp experiments were performed at 22°C. The external solution had the following composition (μmol/L): NaCl 140, CsCl 5.0, MgCl₂ 1.0, CaCl₂ 1.8, HEPES 10 and glucose 10, pH 7.4 with NaOH. Patch clamp electrodes were filled with a solution of the following composition (μmol/L): CsF 90, CsCl 10, EGTA 10, NaF 10, MgCl₂ 2 and HEPES 10, pH 7.4 with CsOH.

Currents were recorded with a patch clamp amplifier (Axopatch). The membrane current was filtered at 10kHz.
with a two-pore active filter, digitized at a sampling rate of 40 kHz, and recorded on videotape (video recorder, Mitsubishi HV-F73, Mitsubishi, Tokyo, Japan) through a PCM converter (Shoshin EM, PCM-DP16, Shoshin, Tokyo, Japan) for later computer analysis (NEC PC98XL, NEC, Tokyo, Japan). Several criteria described by Colatsky and Tsien that permit indirect determination of the adequacy of space-clamp control in the cardiac preparation were used. Under our experimental conditions, current recordings of the Na+ channels satisfied these criteria.

**Determination of Azimilide Block of Na+ Currents**

Block of the Na+ currents by azimilide was evaluated in two different ways; that is, estimating its tonic and phasic block. The drug-induced decrease in current at a low pulse frequency (0.1 Hz), which was sufficient to ensure full recovery from use-dependent block by drug, was regarded as tonic block. The amount of tonic block was calculated as the % decrease in current after perfusion with the drug with respect to the control. The zero current level was obtained after 100 μmol/L tetrodotoxin application. To determine the values of IC50 and the Hill coefficient, the concentration-dependence data was fitted with the following empirical equation:

\[
\text{% of tonic block} = 1/[1 + (\text{IC}_50/|D|)^n]
\]  

where \( |D| \) is the drug concentration, \( \text{IC}_50 \) is the apparent dissociation constant at various holding potentials (HP) and \( n \) is the Hill coefficient.

To study the shift in the inactivation gating kinetics of the Na+ currents by the drug, the steady-state inactivation curves (\( h_\infty \)) under control conditions and during exposure to the agent were assessed at selected membrane (prepulse) potentials using the following standard two-pulse protocol: A 1,000 ms prepulse to the designated level of membrane potential was followed by a 0.1 ms interpulse interval, then a 30 ms test pulse was applied every 10 s. The curve drawn through the points is described by the following equation:

\[
h_\infty = \frac{1}{1 + \exp[(V_m-V_h)/k]} \]  

where \( V_m \) is the prepulse potential, \( V_h \) is the prepulse potential at \( h=0.5 \) and \( k \) is a slope factor.

To study the phasic block of the Na+ current, the amplitudes of the currents elicited by a train of depolarizing 50 pulses of 30 ms at various frequencies from a holding potential of −100 mV to −20 mV were recorded. The amplitude decreased during a pulse train and reached a new steady state. The amount of phasic block was calculated as the % decrease of current in the new steady state with respect to that of the first pulse. The time constant (\( \tau \)) of current decay during the pulses was determined by fitting with a single exponential function. To quantitatively determine the recovery process of the Na+ channels from the phasic block, a double pulse protocol was employed. A 1,000 ms prepulse was followed by various recovery periods and test pulses of 30 ms to −20 mV to assess the amount of current recovered at a holding potential of −100 mV. Onset of phasic block by the drug after prepulses of various duration was estimated as follows: 50 pulse trains of depolarization to −20 mV with various pulse durations (1, 2, 5, 10, 20, 30, 100, and 200 ms) were applied from a holding potential of –100 mV.
potential of –100 mV and the recovery interval was kept constant at 300 ms.

Azimilide, soluble in water, was kindly supplied by Procter & Gamble Pharmaceuticals. It was applied via a solution chamber from which the solutions around the cell could be changed within 10 s.

**Dynamic Simulation of Azimilide Block of Na⁺ Channel**

To elucidate the kinetic mechanisms of the Na⁺ channel block by azimilide, we determined the $K_d$ and rate constants of the azimilide binding/unbinding for each state of the Na⁺ channel using a Markovian state model. We employed a 3-state model consisting of resting (closed), activated (open), and inactivated states to simulate the Na⁺ channel gating behavior. The dynamics of the Na⁺ channel gating and channel-drug interactions were described by the discrete energy barrier model based on the Eyring absolute reaction rate theory. Rate constants of state transitions were calculated from the rate theory formulas expressed as a function of total free energies at energy peaks and wells. A state diagram, energy profile, and mathematical expressions for the present model are provided in the Appendix. Expressions for the gating kinetics of the drug-free Na⁺ channel were derived from the data of Mitsuyie and Noma for guinea-pig ventricular Na⁺ channels.

The first step to determine the $K_d$ and rate constants of azimilide binding/unbinding was to compute the steady-state inactivation (availability) curve, the concentration dependence curve for azimilide block, and the recovery time course from the model, thereby searching a set of the $K_d$ and rate constant values for the resting and inactivated states to give satisfactory fit for the experimental observations. The $K_d$ and rate constant values for the activated state were then determined by simulating the onset and phasic block of the Na⁺ channel currents. Detailed descriptions of the procedure for parameter adjustments and the parameter values used in the simulations are given in the Appendix.

Programming for mathematical analysis and the numeric calculations with matrix equations were performed on a Power Macintosh G4 computer (Apple Computer, Inc, Cupertino, CA, USA) using MATLAB 5.2 (MathWorks, Inc, Natick, MA, USA).

**Data Analysis**

Analysis of the data was performed on a Macintosh LC630 computer using a custom software. All curve fittings were done with a non-linear least squares algorithm using a Marquardt routine. The results were expressed as means ± SEM. Statistical analysis was done using one-way repeated measures (ANOVA) test or Student’s t-test, and the results were considered to be significant when the p-value was less than 0.05.

**Results**

**Tonic Block of WT and ΔKPQ Mutant Currents Induced by Azimilide**

Azimilide (10 μmol/L) produced tonic block of the WT...
Fig 3. Azimilide-induced phasic block of the wild-type (WT) and mutant ∆KPQ currents. (A) Phasic block of the WT currents induced by 10μmol/L azimilide with a holding potential of –100 mV. Train pulses of 30 ms duration from a holding potential of –100 mV were applied at 2 Hz (a), 3 Hz (b) and 5 Hz (c). The open circles indicate the INa elicited by the first depolarizing pulse to –20 mV of 30 ms duration, and the closed circles are the INa elicited by the 50th depolarizing pulse. The dotted line indicates the zero current level. The % phasic block of INa in the absence (lower open column) and presence (upper closed column) of 10μmol/L azimilide were determined with 2, 3 and 5 Hz train pulses (n=6). (B) Use phasic block of the ∆KPQ currents induced by 10μmol/L azimilide with a holding potential of –100 mV. Train pulses of 30 ms duration from a holding potential of –100 mV were applied at 2 Hz. The open circles indicate the peak (a) and steady state (b) of INa elicited by the first depolarizing pulse to –20 mV of 30 ms duration, and the closed circles are the peak (a) and steady state (b) of INa elicited by the 50th depolarizing pulse. The % phasic block of INa in the absence (open column) and presence of 10μmol/L azimilide (closed column) determined with 2 Hz train pulses of 30 ms duration from a holding potential of –100 mV (n=7). *p<0.05.

Fig 4. Effect of pulse duration on onset of block of wild-type (WT) and mutant ∆KPQ currents by 10μmol/L azimilide. Inset: peak ∆KPQ current elicited by the first (open circle) and prepulse following 50 pulses (closed circles) of either 5 ms test pulse (Left) or 200 ms test pulse (Right). The kinetics of onset block by azimilide was assessed by the double-pulse protocol described in the Methods section. The percentages of phasic block of WT (closed circles: n=7) or ∆KPQ currents (open circles: n=5) in the presence of 10μmol/L azimilide following the prepulse duration (1, 2, 5, 10, 30, 100, and 200) at a holding potential of –100 mV are shown.
currents in a concentration- and voltage-dependent manner (Fig 1A) and the block was reversible after washout (A, Panel a). The current–voltage relationship of the WT currents in the absence and presence of azimilide (10 μmol/L) indicated that azimilide blocked the WT currents without changing the threshold potential (~60 mV), the peak potential (~25 mV), or the reversal potential (~55 mV) (data not shown). The IC50 values for azimilide-induced tonic block of the WT currents at a holding potential of ~100 and ~140 mV, respectively, were calculated to be 9.8±1.4 μmol/L with a Hill coefficient of 0.9±0.1 and 102.6±3.6 μmol/L with a Hill coefficient of 0.9±0.2. The IC50 values for azimilide-induced block of the human Na+ channel was smaller than that of canine ventricular myocytes12 but larger than its plasma concentration in the clinical setting? These results indicate that azimilide produces a greater tonic block in WT currents at more depolarized membrane potentials. The voltage-dependent properties of azimilide block indicated higher affinity of this agent for the inactivated state of the WT currents than for the resting state. It was indeed found that azimilide at 10 μmol/L, significantly shifted h toward a more negative potential by −7.5±2.1 mV (control: Vh=−83.3±6.4 mV vs azimilide 10 μmol/L; Vh=−90.8±6.6 mV; p<0.05; control: slope factor=6.4±0.5 vs azimilide 10 μmol/L; slope factor=6.6±0.7; NS) (Fig 1B). After washout of azimilide, Vh shifted back to −88.1±1.3 mV (p<0.05), suggesting that the natural negative shift of the h curve was at least in part caused by the azimilide effect. Studying the interaction of local anesthetics with the AKPQ channel is clarified their state-dependent block of Na+ channels22–26 As shown in Fig 2A, azimilide (10 μmol/L) also produced tonic block of the peak (Panel a) and steady-state (Panel b) currents of the ΔKPQ currents. The IC50 of the peak and the steady-state currents at a holding potential of ~100 mV were 10.1±1.3 and 11.2±0.8 μmol/L, respectively. However, as shown in Fig 2B, azimilide (10 μmol/L) shifted the h curve of the ΔKPQ channels by only 1.8±0.2 mV (control: Vh=−87.2±2.2 mV, azimilide, Vh=−89.0±2.3 mV, washout: −87.5±2.5 mV) without changes in the slope factor (control: 6.5±0.5, azimilide, 6.6±0.4, washout: 6.5±0.6). This suggests that azimilide blocked the peak ΔKPQ currents with approximately the same affinity for the inactivated and resting states.

**Phasic Azimilide Block of WT and ΔKPQ Currents**

Azimilide (10 μmol/L) produced significant phasic block of the WT currents by 17.0±3.1% at 2 Hz, 27.2±7.0% at 3 Hz and 48.3±3.0 % at 5 Hz (Fig 3A), indicating that stimulation at higher frequencies produced a greater phasic block. The time constant of the decay of the peak WT currents in the presence of azimilide (10 μmol/L) was determined to be t=4.8±0.5 s at 3 Hz (Table 2). Although azimilide (10 μmol/L) produced phasic block of the ΔKPQ mutant at 2 Hz (Fig 3B), the extent of the phasic block of the peak ΔKPQ currents (Panel a: 25.3±2.1%) was significantly larger than that of the WT currents (17.0±3.1%). Moreover, the extent of the phasic block of its steady-state current (Panel b: 40.0±5.9%) was significantly greater than that of its peak current. If azimilide had a high affinity for the inactivated state, prolonging the depolarization would increase the level of phasic block. On the other hand, the extent of phasic block is expected to saturate within the short pulse, if azimilide had a high affinity for the activated state. As shown in Fig 4, the block of the peak WT currents was greater with the long (200 ms) pulse (29.0±4.0%) than with the short (5 ms) pulse (4.3±2.0%), indicating that...
azimilide blocked WT currents as an inactivated state blocker rather than an activated state blocker. Whether the pulse is short (5 ms) or long (200 ms), the azimilide-induced fractional block of the peak ∆KPQ currents (18±1% with the short pulse and 64±5% with the long pulse) was greater than that of the WT currents, indicating that azimilide produced a phasic block of the ∆KPQ currents as both an activated state blocker and an inactivated state blocker. State-dependent block by 10μM azimilide at a holding potential of −100 mV showed that azimilide generated 2 components of the recovery process (WT currents; fast recovery with t=25±2 ms and slow recovery with t=1.8±0.4 s, ∆KPQ currents: fast recovery with t=13±3 ms and slow recovery with t=1.5±0.5 s), indicating that azimilide dissociated from both the WT and ∆KPQ channels with the intermediate kinetics, because the time constant of recovery kinetic with 2–12 s has been classified as intermediate by Campbell et al.27

Model Prediction of the Affinity of Azimilide for Each State of the Na+ Channel

To study the kinetic properties of the state-dependent block of the Na+ channels by azimilide, we estimated the Kd values and rate constants of azimilide binding/unbinding for each conformational state of the WT Na+ channels using a Markovian state model and the rate theory formula (for details, see Methods and Appendix). The Kd and other parameters determined by fitting the experimental data are shown in Table 1. The Kd values for the resting, activated, and inactivated states were 102.6, 1.4, and 1.4μmol/L, respectively. Using the model with the parameters shown in Table 1, we simulated azimilide block of the WT currents and determined the IC50 value for the tonic block, shift in the availability curve (with holding potentials of −100 and −140 mV), recovery time constant with a holding potential of −100 mV, time constant and % block at 200 ms of the onset block, as well as the degree and time constants of the phasic block at various frequencies.

The kinetic properties of the azimilide block of Na+ currents simulated by the model are shown in Table 2, together with the experimental data for comparison. The predicted values were close to the experimental values. These results suggest that azimilide has a relatively high affinity for both the activated state (KdA=1.4μmol/L), and the inactivated state (KdI=1.4μmol/L) of the the WT Na+ channels, whereas its affinity for the resting state (KdR=102.6μmol/L) was relatively low.

### Discussion

Previous studies have reported that azimilide blocks the Na+ channels in a dose-dependent manner by a 1:1 binding stoichiometry and in a use-dependent manner by binding to the inactivated state and suppressing the slowly inactivating component of the Na+ channel to shorten the action potential duration,12 suggesting that azimilide may interact with both the inactivated state as well as the activated state of the Na+ channels. Ours is the first report to show that azimilide can block the human Na+ current by binding to both the activated and inactivated states of the channels, by using a computer model and the ∆KPQ mutant channel to reopen under steady-state conditions.

In the present study, the inhibitory effects of azimilide on the WT Na+ channels were characterized by concentration- and voltage-dependent tonic block (Fig 1A), hyperpolarizing shift in the h curve (Fig 1B), use-dependent block (Fig 3A), increasing block by prolonging depolarization (Fig 4) and intermediate recovery kinetic from the phasic block. Our results are consistent with previous reports11–13,15 suggesting a higher affinity of azimilide for the inactivated and activated states of WT Na+ channels. Because of the absence of the β-subunit, the IC50 value at a holding potential of −100 mV in our study of recombinant human Na+ channels (9.8μmol/L) is almost half of that determined for canine Na+ channels (19μmol/L).12 The IC50 values for azimilide block of ferret ventricular I_K and I_Ks were 0.4μmol/L and 3μmol/L, respectively.12 The IC50 value for block of Na+ channel by azimilide was much larger than that for the delayed rectifier K+ channels, as described elsewhere.12

To test the hypothesis that azimilide may exert dual inhibitory actions (ie, both activated and inactivated state blocks) on WT Na+ channels, we performed a computer simulation using a Markovian state model and determined the Kd value for each state of the channel (Table 1). The

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**Table 1 Parameter Values Used in Dynamic Simulations of the Model**

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>zR=3.974</td>
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<td>zA=0.382</td>
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<tr>
<td>zI=0.557</td>
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<tr>
<td>ΔI=0.904</td>
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<td>ΔR=1</td>
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<tr>
<td>ΔA=0.024</td>
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<tr>
<td>G0=0</td>
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<td>G0A=4.0</td>
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</tr>
<tr>
<td>G0R=13.016</td>
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</tr>
<tr>
<td>G0I=20.706</td>
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<tr>
<td>G1A=18.197</td>
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<tr>
<td>G1R=26.936</td>
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</tr>
<tr>
<td>Ka=1.4 (μmol/L)</td>
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</tr>
<tr>
<td>Kα=1.4 (μmol/L)</td>
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</tr>
<tr>
<td>Ka=102.6 (μmol/L)</td>
<td></td>
</tr>
<tr>
<td>Gα=17.1</td>
<td></td>
</tr>
<tr>
<td>GαR=16.0</td>
<td></td>
</tr>
<tr>
<td>GαI=20.94</td>
<td></td>
</tr>
<tr>
<td>zNamax=1.8 (nS/pF)</td>
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<tr>
<td>Eωo=+42.17 (mV)</td>
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**Table 2 Comparison of the Simulated Kinetics of Na-Channel Block With the Experimental Data**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Experimental data</th>
<th>Simulated values</th>
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<tr>
<td>Vh (ms) in availability curve (mV)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>−83.3 (6.4)</td>
<td>−83.2 (6.5)</td>
</tr>
<tr>
<td>+10μM AZM</td>
<td>−90.8 (6.6)</td>
<td>−90.8 (6.9)</td>
</tr>
<tr>
<td>IC50 (h) value (μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vhold=−140 mV</td>
<td>102.6 (0.94)</td>
<td>95.8 (1.0)</td>
</tr>
<tr>
<td>Vhold=−100 mV</td>
<td>9.0 (0.90)</td>
<td>17.0 (1.0)</td>
</tr>
<tr>
<td>Recovery time constants at −100 mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>△t onset (ms)</td>
<td>23.0</td>
<td>23.7</td>
</tr>
<tr>
<td>△t slow (s)</td>
<td>1.8</td>
<td>1.87</td>
</tr>
<tr>
<td>Phasic block (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2Hz</td>
<td>17.0</td>
<td>27.2</td>
</tr>
<tr>
<td>3Hz</td>
<td>27.2</td>
<td>37.2</td>
</tr>
<tr>
<td>5Hz</td>
<td>48.3</td>
<td>51.0</td>
</tr>
<tr>
<td>Time constant at 3 Hz (s)</td>
<td>4.8</td>
<td>1.14</td>
</tr>
</tbody>
</table>

*Value determined by fitting with a single exponential function.*
model with the parameter values shown in Table I could simulate well the experimentally observed kinetics of the azimilide-induced Na⁺ channel block (Table 2). The Kᵦ values predicted by the model for both the activated (1.4μmol/L) and inactivated states (1.4μmol/L) were much smaller than for the resting state (102.6μmol/L). The Kᵦ value of azimilide for the inactivated state was close to that of lidocaine for the inactivated state (4μmol/L)22 whereas its Kᵦ value for the activated state was much smaller than that of lidocaine (1,300μmol/L). The Kᵦ value of azimilide for the activated state was close to that of flecainide for the activated state (0.61μmol/L)23 whereas its Kᵦ value for the inactivated state was much smaller than that of flecainide (15.9μmol/L). Mexiletine, an inactivated state blocker with an activated channel blocking action, blocked Na⁺ currents with Kᵦ values of 3 and 15μmol/L for the activated and inactivated state, respectively.24 The Kᵦ value of azimilide for the activated and inactivated states (1.4μmol/L) was close to that of mexiletine for the activated state, but smaller than that of mexiletine for the inactivated state. These comparisons support that azimilide has a relatively high affinity for both the activated and inactivated states of the WT Na⁺ channels.

For the ∆KPQ channels, azimilide significantly blocked the peak current during short depolarizations in a use-dependent manner, with the degree of phasic block increasing with prolonged depolarizations (Fig 4). These results suggest that as observed with the WT Na⁺ channels, azimilide has a relatively high affinity for both the activated and inactivated state of the ∆KPQ channels.

Azmilide produced tonic block of the ∆KPQ currents with nearly the same potency (IC₅₀) as for the WT currents, although it produced a smaller shift in the hₜ curve (~1.8 mV), which may indicate that the affinity of azimilide for the resting and inactivated states of the ∆KPQ channels does not differ very much from that for the WT channels. Although we could not precisely determine the Kᵦ values for the azimilide block of the ∆KPQ currents because of the lack of experimental data as well as the need for more complex models, the Kᵦ values of 11.5μmol/L for the resting state and 5.3μmol/L for the inactivated state yielded a shift of ~1.8 mV in the hₛ curve and an IC₅₀ of 10.1μmol/L (with a holding potential of ~100 mV) in the 3-state model. The interaction of local anesthetics with the WT and ∆KPQ channels is different in state-dependent kinetics. The affinity of flecainide and mexiletine has been reported to be higher for the resting state of the ∆KPQ channels than for that of the WT channels.24,25 Taken together with the present results that the azimilide-induced phasic block of both the peak and steady-state ∆KPQ currents was greater than that of the peak WT current (Fig 3B), the interactions of azimilide with the WT and ∆KPQ mutant channels may be different in state-dependency, as described elsewhere.24,25

An activated state blocker, flecainide, selectively blocks the channel in the activated state, and entering the inactivated state does not promote the block.26 Flecainide selectively binds to the ∆KPQ channels in the activated state during reopening after the channel experiences the first opening, producing a greater phasic block of ∆KPQ currents than of WT currents.26 An inactivated channel blocker, lidocaine, interacts preferentially with the inactivated channels, with the block not necessarily enhanced by the channel opening.28,29 Lidocaine produces nearly the same phasic block of the peak and steady-state currents of WT and ∆KPQ channels, but causes a greater tonic block of the steady-state current than peak current of the ∆KPQ mutant channels.26

Our results indicate that the interactions of azimilide with the WT and ∆KPQ channels differ from those of either an activated state blocker (flecainide) or inactivated channel blocker (lidocaine).24,26 The azimilide-induced phasic block of both the peak and steady-state ∆KPQ currents was greater than that of the WT (Fig 3B), but the degree of the azimilide-induced tonic block of the peak and steady-state ∆KPQ currents was nearly the same as that of the peak WT current (Fig 2A). The unique mode of azimilide block of the ∆KPQ channels as compared with that of WT channels (ie, enhanced phasic block without changes in tonic block) may be related to its dual action as an activated and an inactivated channel blocker on the Na⁺ channels.

The pharmacological properties of azimilide on the Na⁺ channel appear to be clinically beneficial, although the IC₅₀ value for azimilide block of the Na⁺ channel was larger than its plasma concentration in the clinical setting (1–5μmol/L).4 The higher affinity of azimilide for the inactivated state channel could cause a strong blocking action on the Na⁺ channels in the diseased heart with depolarized membrane potentials. Because the activated state blocker generally blocks cardiac conduction independent of the action potential duration, azimilide can similarly block the conduction of the atrial action potential with a short effective refractory period as pilsicainide.26,27 Taken together with the prolongation of the post-repolarization refractoriness by its intermediate recovery kinetics from phasic block, azimilide is expected to treat tachyarrhythmias via the Na⁺ channel block, as well as its main effect as a class III antiarrhythmic drug (ie, K⁺ channel block).

Acknowledgments

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References


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**Appendix 1**

**Kinetic Modeling of Azimilide Block of the Na-Channel**

We modeled the gating kinetics of the Na-channel by a 3-state scheme according to Mitsuyie and Noma as shown in Fig 5. Although they proposed an irreversible transition model for the guinea-pig ventricular Na-channel, we developed a reversible model for the human heart Na-channel (H1) in order not to violate the principle of microscopic reversibility. Rate constants of state transitions (Na-channel gating and azimilide binding/unbinding) are formulated in terms of the energy barrier model. General Gibbs free energy profiles for the channel gating are depicted in Fig 6.

The Gibbs free energy level G of a stable state (energy well) or a transition state (energy peak) at the membrane potential VM can be expressed as a sum of the chemical and electrostatic terms; that is, a function of the chemical component (G0) and membrane potential VM. In Fig 6, free energy levels (at the energy peaks and wells) for individual states of the azimilide (AZM)-free Na-channel are given by:

\[ G_{\text{R}} = G_{\text{R0}} + z_I V, \]
\[ G_{\text{A}} = G_{\text{A0}} + z_A V, \]
\[ G_{\text{I}} = G_{\text{I0}}, \]
\[ G_{\text{RA}} = G_{\text{RA0}} + (z_A + (1 - d_I) z_I) V, \]
\[ G_{\text{AI}} = G_{\text{AI0}} + (1 - d_A) z_A V, \]
\[ G_{\text{IR}} = G_{\text{IR0}} + d_I z_I V, \]
\[ G_{\text{AR}} = G_{\text{AR0}} - d_R z_R V \]
\[ G_{\text{IA}} = G_{\text{IA0}} - (1 - d_A) z_A V, \]
\[ G_{\text{RI}} = G_{\text{RI0}} - d_I z_I V, \]
\[ G_{\text{AI}} = G_{\text{AI0}} - (1 - d_A) z_A V. \]

For those of the AZM-bound Na-channel are given by:

\[ G_{\text{RA}} = G_{\text{RA0}} + (1 - d_R) z_R V, \]
\[ G_{\text{AR}} = G_{\text{AR0}} + (1 - d_R) z_R V, \]
\[ G_{\text{IR}} = G_{\text{IR0}} + (1 - d_I) z_I V, \]
\[ G_{\text{AR}} = G_{\text{AR0}} + (1 - d_R) z_R V, \]
\[ G_{\text{IA}} = G_{\text{IA0}} + (1 - d_A) z_A V, \]
\[ G_{\text{RI}} = G_{\text{RI0}} + (1 - d_I) z_I V, \]
\[ G_{\text{AI}} = G_{\text{AI0}} + (1 - d_A) z_A V. \]

The rate constants for AZM binding/unbinding were also formulated as exponential functions of the activation energy DG:

\[ k_{\text{RA}} = k_{\text{RA0}} \cdot \exp(-DG_{\text{RA}}), \]
\[ k_{\text{AR}} = k_{\text{AR0}} \cdot \exp(-DG_{\text{AR}}), \]
\[ k_{\text{IR}} = k_{\text{IR0}} \cdot \exp(-DG_{\text{IR}}), \]
\[ k_{\text{RI}} = k_{\text{RI0}} \cdot \exp(-DG_{\text{RI}}), \]
\[ k_{\text{AI}} = k_{\text{AI0}} \cdot \exp(-DG_{\text{AI}}). \]

**Calculation of Ionic Currents**

The rate equations for the scheme shown in Fig 5 can be written in a matrix form:

\[ \frac{dP}{dt} = K \cdot P. \]
Here, \( P \) and \( K \) are the matrices for probabilities of individual states at time \( t \) and for transition rate constants, respectively, being given by:

\[
P = [P_{R}, P_{A}, P_{B}, P_{R}, P_{B}, P_{R}].
\]

where the probability of a channel (or a channel-AZM complex) to be in state \( X \) is denoted \( P_{X} \). Then, the Na-channel current \( I_{Na} \) in the presence of AZM at a given concentration and \( V_{m} \) can be computed by:

\[
I_{Na} = g_{N_{max}} \cdot (V_{m} - E_{m}),
\]

where \( g_{N_{max}} \) and \( E_{m} \) denote the maximum conductance and reversal potential, respectively.

In a steady-state (at a holding potential), each time derivative term is set equal to zero (ie, \( K \cdot P = 0 \)). Thus, with the conservation equation \( S_{P} = 1 \), we have:

\[
D \cdot P = R.
\]

Once all the rate constants for the transition matrix \( D \) are determined, Eq. A36 can be solved for the matrix \( P \) by deriving the inverse matrix \( D^{-1} \) (ie, \( P = D^{-1} \cdot R \)); and the steady-state probabilities of a channel to be in the allowable states are given by the last column of the matrix \( D^{-1} \).

**Determination of Model Parameter Values**

**Gating Parameters**

The free energy level \( G_{0} \) (at \( V_{m} = 0 \)) for the inactivated state and valency \( z \) (ie, the number of gating charges to move during the transition from the resting to inactivated state) were determined from the half-maximum potential (\( V_{b} = -83.3 \text{ mV} \)) and slope factor (\( s_{b} = 6.4 \text{ mV} \)) of the availability curve in our experiments using the following equations:

\[
G_{0} = V_{b}/s_{b} (= -13.016),
\]

\[
z(t) = (RT/F)/s_{b} (= 3.974).
\]

The free energy level \( G_{i} \) and effective valency \( z_{i} \) (electrical distance \( d_{i} \)) for the resting, activated, and transition states were determined from the rate constant data of Mitsuyie and Noma.19–21 The value of \( G_{R0} \) was calculated from the recovery time constant at \(-100 \text{ mV} \) (\( \tau_{recR} = 25 \text{ ms} \)) using the following equation:

\[
G_{R0} = -\ln((1/\tau_{recR})/(k_{T}/h))/\{\exp(G_{0}) \cdot \exp(-d_{R} \cdot z_{R} \cdot V_{S}) + \exp(G_{R0}) \cdot \exp((1-d_{R}) \cdot z_{R} \cdot V_{S})\}.
\]

**AZM Binding/Unbinding**

The value of \( K_{dR} \) was assumed to be equal to the IC_{50} measured with the holding potential of \(-140 \text{ mV} \), being set to 102.6 \( \mu \text{mol/L} \). The \( G_{RT} \) value was calculated by

\[
G_{RT} = -\ln(1/(\tau_{recS} \cdot ([AZM] + K_{dR})/(k_{T}/h))),
\]

where \( \tau_{recS} \) is the slow time constant of the current recovery at \(-100 \text{ mV} \) (\( \tau_{recS} = 1.8 \text{ s} \)). The values of \( K_{d} \) and rate constant values for the activated state to reproduce the experimental data of the onset and phasic block were then determined.