Two Components of Use-Dependent Block of Na\(^+\) Current by Disopyramide and Lidocaine in Guinea Pig Ventricular Myocytes

Akihiko Sunami, Zheng Fan, Jun-ichi Nitta, and Masayasu Hiraoka

We studied the kinetics of the use-dependent block of the Na\(^+\) current (I\(_{\text{Na}}\)) by disopyramide and lidocaine. I\(_{\text{Na}}\) was recorded from isolated guinea pig ventricular myocytes by using the whole-cell patch-clamp technique. The use-dependent block of I\(_{\text{Na}}\) by disopyramide with 20- and 200-msec depolarizing pulses developed in two exponential functions. The degree of the use-dependent block and the amplitude of the fast (Af) and slow (A\(_s\)) components with the short (20-msec) pulse protocol were comparable to those with the long (200-msec) pulse protocol. When pH was raised from 7.3 to 8.0, disopyramide increased A\(_s\) without a change in A\(_f\). At pH 6.5, I\(_{\text{Na}}\) block developed with a single exponential function revealing only the slow component. The fast and slow components of I\(_{\text{Na}}\) block by disopyramide could be explained by binding of the uncharged and charged forms, respectively, to the activated state of the channel. Development of I\(_{\text{Na}}\) block by lidocaine also was expressed by two exponentials at all pulse durations (5–200 msec). As pulse durations were prolonged or holding potentials were depolarized, the degree of the use-dependent block and A\(_f\) increased. When pH was lowered to 6.5, the short pulse produced only the slow component, whereas the long pulse caused two exponentials with decreased A\(_f\) and increased A\(_s\). Internal application of QX-314, a permanently charged lidocaine analogue, produced a single exponential block of I\(_{\text{Na}}\) with a very slow onset rate. Therefore, binding of the charged form of lidocaine to the activated state seemed to be responsible for the slow component and binding of the uncharged form to the inactivated state for the fast component. These results suggest that the charged and uncharged forms of disopyramide and lidocaine are responsible for two components of I\(_{\text{Na}}\) block in pulse trains, but their forms are not a sole determinant of state-dependent binding to the Na\(^+\) channel. (Circulation Research 1991;68:653–661)

The action of class I antiarrhythmic agents is assumed to suppress mainly the fast Na\(^+\) current (I\(_{\text{Na}}\)), leading to depression of excitability and conduction of cardiac impulses.\(^1–4\) The mechanism of I\(_{\text{Na}}\) depression by class I agents generally is explained by either the modulated receptor hypothesis\(^4,5\) or the guarded receptor hypothesis.\(^6,7\) According to the modulated receptor hypothesis, the actions of Na\(^+\) channel blocking drugs are modulated by different binding affinities to various states of the Na\(^+\) channel. Transition to drug-bound (blocked) or drug-free (unblocked) states can be expressed by the rate constants of association and dissociation, which are assumed to have first-order kinetics. The guarded receptor hypothesis assumes that the drug–receptor affinity is constant but access to the binding site is limited.

Various attempts have been made to clarify the drug action on the Na\(^+\) channel based on these hypotheses. Among these attempts, the kinetic analysis of use-dependent block by class I agents was used as criteria to characterize their mode of action and to subdivide the drugs into different subgroups.\(^8\) Most previous data on the block kinetics of the maximum rate of rise (V\(_{\text{max}}\)) by class I agents were fitted by a single exponential function.\(^8–11\) However, in a previous study,\(^12\) we found the development of the V\(_{\text{max}}\) block by disopyramide and lidocaine was best expressed by two exponentials. Actually, Clarkson et al\(^13\) have shown two components of I\(_{\text{Na}}\) block by lidocaine. Recently, Sheets et al\(^14\) pointed out that there was a nonlinear relation between V\(_{\text{max}}\) and I\(_{\text{Na}}\). Therefore, the present study was done to clarify the precise mechanisms of the two exponential blocks of the Na\(^+\) channel by disopyramide and lidocaine by measuring I\(_{\text{Na}}\).

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**Materials and Methods**

**Myocyte Preparation**

Single ventricular myocytes from guinea pig hearts were prepared by an enzymatic dissociation procedure as described previously. Isolated single cells were transferred to a recording chamber placed on the stage of an inverted phase-contrast microscope (Diaphot TMD, Nikon Co., Tokyo). The composition of the Tyrode's solution was (mM) NaCl 144.0, KCl 4.0, CaCl₂ 1.8, MgCl₂ 0.53, glucose 5.5, NaH₂PO₄ 0.33, and HEPES 5.0; pH was adjusted to 7.3-7.4 by addition of NaOH. The high-K⁺, low-Cl⁻ solution (KB solution) contained (mM) glutamic acid 70.0, taurine 15.0, KCl 30.0, KH₂PO₄ 10.0, MgCl₂ 0.5, glucose 11.0, EGTA 0.5, and HEPES 10.0; pH was adjusted to 7.3 by adding KOH. For measuring I_{Na}, the external solution contained (mM) NaCl 30.0, tetramethylammonium chloride 110.0, CaCl₂ 1.8, CoCl₂ 1.0, CsCl 5.0, MgCl₂ 1.2, glucose 11.0, and HEPES 20.0 (pH 7.3, adjusted by tetramethylammonium hydroxide). The pipette solution was composed of (mM) NaF 5.0, CsF 125.0, K₇ATP 5.0, K₇ creatine phosphate 5.0, EGTA 5.0, and HEPES 5.0 (pH 7.2, adjusted with CsOH).

**Recording Techniques**

Membrane currents were recorded by a whole-cell patch-clamp technique using an amplifier (AXOPATCH-1C, Axon Instruments, Foster City, Calif.). Details of the recording technique have been described in our previous reports. These experiments were performed at room temperature. At the start of each experiment, the junction potential of each electrode was nulled to zero through adjustment of the compensation circuit in the external bath solution. It was checked again after the end of each experiment. If the junction potential of the second determination exceeded ±2 mV, the value of the membrane potential was corrected accordingly. Current signals were monitored by a storage oscilloscope (model 3091, Nicolet Instrument Corp., Madison, Wis.) and were recorded simultaneously by a video cassette recorder (HR-S 7000, Victor Co., Tokyo) using a PCM converter system (RP-880, NF Instruments, Yokohama, Japan).
Japan). The recorded currents were filtered by an active eight-pole Bessel filter (FV-665, NF Instruments) using a −3-dB cutoff point at 10 kHz. The analog signals were converted into digital signals with an AD converter (CED-1401, Cambridge Electronic Design Ltd., Cambridge, UK) at a sampling frequency of 45 kHz and were stored in an IBM-AT personal computer.

Experimental Protocols

To test the use-dependent block of INa by disopyramide and lidocaine, trains of 30 pulses of 5–200 msec were applied to −30 mV from a holding potential ranging from −90 to −120 mV at various frequencies. Pulse trains were repeated in control solutions and then in test solutions. At least a 4-minute interval in disopyramide and a 1-minute interval in lidocaine was allowed between pulse trains to permit full recovery from block. To study the INa block by QX-314 (Astra Pharmaceuticals Inc., Westborough, Mass.), a permanently charged analogue of lidocaine, trains of 200 pulses with a 10-msec pulse duration were applied to −30 mV from a holding potential of −100 mV at the interpulse interval of 300 msec. QX-314 was dissolved

<table>
<thead>
<tr>
<th>Pulse duration (msec)</th>
<th>Diastolic interval (msec)</th>
<th>UDB (%)</th>
<th>Onset rate (pulse⁻¹)</th>
<th>Amplitude at zero beat (nA)</th>
<th>Aᵣ/Aₛ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fast component</td>
<td>Slow component</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>300</td>
<td>8</td>
<td>30.9±5.9</td>
<td>0.374±0.056</td>
<td>1.581±0.328</td>
</tr>
<tr>
<td>200</td>
<td>300</td>
<td>8</td>
<td>31.0±5.3</td>
<td>0.389±0.049</td>
<td>1.676±0.189</td>
</tr>
</tbody>
</table>

Values are mean±SEM. UDB, use-dependent block of INa; Aᵣ, amplitude of the fast component; Aₛ, amplitude of the slow component.
least-squares algorithm on a personal computer (PC9801, NEC, Tokyo). The formula for the curve fitting used in this study is

\[ A_1 \exp(-bC_1) + A_2 \exp(-bC_2) + A_3 \exp(-bC_3) + A \]

where \( b \) is pulse number and \( C_1, C_2, \) and \( C_3 \) are onset rates. Because we used the data of 30 pulses in the case of disopyramide and lidocaine and 200 pulses of QX-314 for the curve fitting, the certainty of the slower onset rate was limited. Applicability of either one, two, or three exponential functions always was done using the \( F \) test, and \( p<0.01 \) was considered significant. The \( F \) test determines if adding an additional exponential to the fit results in a better fit. All the values were expressed as mean±SEM. Statistical analysis except curve fitting was done using the paired or unpaired \( t \) test, and \( p<0.05 \) was considered significant.

Results

Characteristics of the \( Na^+ \) Current

The properties of \( I_{Na} \) measured in our preparations are shown in Figure 1. Activation of \( I_{Na} \) occurred at the earlier time of pulses, and inactivation of current proceeded more quickly with increasing depolarizations. The current–voltage curve showed an \( I_{Na} \) activation threshold at about −70 mV. The current increased progressively and smoothly with further depolarizations to reach a maximum at −31 mV. The current reversed its polarity at 42.5 mV, which was almost equal to that calculated from \( Na^+ \) concentrations in the external and internal solutions using the Nernst equation. On the average, activation of \( I_{Na} \) was observed at potentials positive to approximately −66.7±3.2 mV, and a maximum value of peak \( I_{Na} \) was observed at −26.2±3.1 mV and reversed at 41.8±3.7 mV (\( n=10 \)). The relation between the steady-state inactivation (\( h_s \)) and the membrane potential (\( V_m \)) was fitted to the equation

\[ h_s = 1/(1+\exp[(V_m-V_h)/s]) \]

where \( V_h \) is the membrane voltage at which \( I_{Na} \) is half maximal, and \( s \) is a slope factor. The values for \( V_h \) and \( s \) determined by a least-squares fit were −85.7±1.6 and 6.0±0.5 mV (\( n=10 \)), respectively. These values are in good agreement with those of other reports.13,18

Use-Dependent Block by Disopyramide

The use-dependent block of \( I_{Na} \) during exposure to 100 \( \mu M \) disopyramide is illustrated in Figure 2. When the 200-msec depolarizing pulses were applied at 2 Hz, the peak \( I_{Na} \) decreased with successive pulses and reached to 80.0% of the first pulse at the 30th depolarization, whereas the peak \( I_{Na} \) showed nearly no change in the absence of the drug. The time course of the \( I_{Na} \) block by disopyramide was best expressed by two exponentials, which were confirmed in all eight cells with the 200-msec pulse protocol at 2 Hz (Table 1). The amplitude of the fast component (\( A_f \)) was larger than that of the slow component (\( A_s \)), resulting in an \( A_f/A_s \) ratio of 2.3. When the 20-msec

in the pipette solution and applied internally. These trains were separated by quiescent periods of more than 15 minutes.

Data Analysis and Curve Fitting

To obtain kinetics of the \( I_{Na} \) block by the drugs, the time course of the development was assessed by a curve-fitting procedure using a simplex nonlinear

Figure 3. Effect of pulse duration on use-dependent block of \( I_{Na} \) by lidocaine. In the presence of 100 \( \mu M \) lidocaine, developments of \( I_{Na} \) block are plotted against pulse number during 30 depolarizing pulses of various durations at the interpulse interval of 500 msec. Pulse durations are 5 (panel A), 20 (panel B), and 200 (panel C) msec. The equation of fit is \( I_{Na}=Af \exp(-bAf) + As \exp(-bAs) + A \), where \( Af \) and \( As \) represent the amplitudes of the fast and slow components, respectively. As pulse durations were prolonged, the fast component of onset rate (\( Af \)) became larger and the \( Af/As \) values increased from 17.1 to 209.4, whereas the slow component of onset rate (\( As \)) did not change. Voltage protocols are indicated at the top right corner.
TABLE 2. Effect of 100 μM Lidocaine on Use-Dependent Block of INa

<table>
<thead>
<tr>
<th>Pulse duration (msec)</th>
<th>Holding potential (mV)</th>
<th>n</th>
<th>UDB (%)</th>
<th>Onset rate (pulse⁻¹)</th>
<th>Amplitude at zero beat (nA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fast component</td>
<td>A₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Slow component</td>
<td>Aₛ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A₁/Aₛ</td>
</tr>
<tr>
<td>A. At diastolic interval of 500 msec</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>−100</td>
<td>4</td>
<td>15.1±2.5</td>
<td>1.185±0.106</td>
<td>0.062±0.006</td>
</tr>
<tr>
<td>20</td>
<td>−100</td>
<td>4</td>
<td>16.7±0.4</td>
<td>1.430±0.073</td>
<td>0.061±0.006</td>
</tr>
<tr>
<td>200</td>
<td>−100</td>
<td>4</td>
<td>28.2±0.2</td>
<td>2.844±0.169</td>
<td>0.061±0.005</td>
</tr>
<tr>
<td>B. At stimulus frequency of 2 Hz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>−90</td>
<td>4</td>
<td>22.6±0.5</td>
<td>1.441±0.029</td>
<td>0.067±0.032</td>
</tr>
<tr>
<td>200</td>
<td>−90</td>
<td>4</td>
<td>55.7±1.1</td>
<td>2.353±0.054</td>
<td>0.089±0.010</td>
</tr>
</tbody>
</table>

Values are mean±SEM. UDB, use-dependent block of INa; A₁, amplitude of the fast component; Aₛ, amplitude of the slow component.

Use-Dependent Block by Lidocaine

When various pulse durations ranging from 5 to 200 msec were applied in the presence of 100 μM lidocaine, two exponential developments of the INa block were observed with all the pulse protocols (Figure 3). Two components of INa block also were confirmed by the studies performed under reduced external Na⁺ concentration (10 mM; n=3) (not shown).

pH Dependence of Two Exponentials of the Na⁺ Current Block by Disopyramide and Lidocaine

Effect of the pH of the external solution on the kinetics of use-dependent block of INa produced by 100 μM disopyramide is shown in Figure 5. When pH was raised from 7.3 to 8.0 to increase the fraction of the uncharged form of disopyramide (i.e., 28.5% in the uncharged form), the degree of use-dependent block and Aₛ were increased significantly (p<0.01) at both the 20- and 200-msec protocols, whereas A₁ was not changed. In two cases of 12 at pH 8.0, the time course of INa block followed a single exponential, of which amplitude was nearly the same as that of A₁ in the cases showing two exponentials at pH 8.0. When pH was lowered to 6.5, reducing the proportion of the uncharged molecules (i.e., 1.2% in the uncharged form and 98.8% in the charged form), the degree of use-dependent block was decreased significantly (p<0.001). At the same time, INa block developed with a single exponential function, of which amplitude was comparable to those of the slow components at both pH 7.3 and 8.0. Furthermore, the degree of use-dependent block and the amplitude of the fast and slow components were nearly the same for 20- and 200-msec pulse protocols at all pH conditions.

When pH was lowered to 6.5 in the presence of 100 μM lidocaine (i.e., 96.2% in the charged form), a train of 200-msec pulses produced two exponential developments of INa block, whereas 20-msec pulse protocols produced a single exponential, of which amplitude was comparable to that of A₁ with 200-msec pulse protocols (Figure 6). The A₁ value with 200-msec pulses at pH 6.5 decreased significantly (p<0.001) compared to that with 200-msec pulses at pH 7.3. The Aₛ values were nearly the same for 20- and 200-msec pulse protocols at both pH conditions; A₁ became larger at pH 6.5, but not significantly so.
Furthermore, application of 1.5 \( \mu \)M QX-314 to the internal solution with 10-msec pulse protocols produced a single exponential development of the \( I_{Na} \) block with a very slow onset rate of 0.0030±0.0013 per pulse (\( n=4 \)) (Figure 7).

**Discussion**

The present study demonstrated that the development of \( I_{Na} \) block produced by disopyramide and lidocaine was best expressed by two exponential functions, confirming our previous report that the \( V_{max} \) block development by disopyramide and lidocaine followed two exponentials.\(^{12} \) Therefore, it is reasonable to assume that two different processes represent the reaction between the drug and the receptor site of the Na\(^+\) channel and cannot be attributed to the technical reasons related to the \( V_{max} \) measurement as an indirect index of the Na\(^+\) current.

Various studies with \( V_{max} \) examining the kinetics of the Na\(^+\) channel block produced by class I agents adopted the process of use-dependent block as a single exponential function.\(^{8−11} \) One of the possible reasons for the discrepancy between these and our results may be attributed to differences in analytical methods. In these reports,\(^{8−11} \) the normalized deviations from the \( V_{max} \) value assumed as the steady state were fitted. However, the assumption of the steady-state value did not represent an exact level, because the component of slowly developing block was present, as shown by the present study.

In fact, two components of development of \( I_{Na} \) block by lidocaine have been reported in ventricular myocytes using a two-pulse protocol.\(^{13} \) Our results...
demonstrate that these two components exist in the form of use-dependent development of \( I_{\text{Na}} \) block in response to pulse trains. Two components of block by lidocaine in pulse trains also have been reported in studies in canine cardiac Purkinje cells.\(^{19} \)

In our experiments, we observed that the use-dependent block of \( I_{\text{Na}} \) by lidocaine developed with two exponential functions. The degree of the use-dependent block increased with membrane depolarization and with prolongation of depolarizing durations. These results agree with the interpretation that lidocaine caused the use-dependent block, preferentially binding to the inactivated state of the channel.\(^{20,21} \)

Lidocaine still had the use-dependent block even with the 5-msec depolarization protocol from the holding potential at \(-120\) mV, where no or little inactivation was assumed to develop. This may indicate that lidocaine binds to the activated state to cause the use-dependent block. Actually, it has been reported that lidocaine interacts with the \( \text{Na}^+ \) channel in the activated as well as the inactivated state.\(^{4,13,22,23} \)

When pH was lowered to 6.5, the short pulse caused a single slow exponential of \( I_{\text{Na}} \) block, of which amplitude \( (A_f) \) was comparable to that of the long pulse. Furthermore, with the short pulse, QX-314 produced a single slow exponential of \( I_{\text{Na}} \) block. On the other hand, \( A_s \) with the long pulse at pH 6.5 decreased compared to that at pH 7.3. \( A_s \) also increased with depolarization of membrane potentials and with prolongation of pulse durations. This can be interpreted to mean that at less negative potentials, there is less recovery of block from the inactivated state between successive pulses. These results indicate that lidocaine in its uncharged and charged forms binds to the inactivated and activated states, producing the fast and slow components, respectively.

In this study, for the first time, we showed that the use-dependent block of \( I_{\text{Na}} \) by disopyramide also developed with two exponential functions, irrespective of pulse duration. The degree of the use-depen-

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**FIGURE 6.** Effect of external pH on use-dependent block of \( I_{\text{Na}} \) by 100 \( \mu \text{M} \) lidocaine. UDB, \( A_f \), and \( A_s \) represent the degree of use-dependent block, and the amplitude of the fast and slow components, respectively. Lidocaine increased UDB without changing \( A_s \) when pulse durations were prolonged at both pH 6.5 and 7.3. At pH 6.5, 20-msec pulses produced only the slow component of \( I_{\text{Na}} \) block, whereas 200-msec pulses caused two exponentials. With 200-msec pulses, \( A_f \) became smaller at pH 6.5 than at pH 7.3. Numbers in parentheses (panel A) indicate number of experiments. **p<0.01; ***p<0.001.

**FIGURE 7.** Use-dependent block of \( I_{\text{Na}} \) by QX-314. Application of 1.5 \( \mu \text{M} \) QX-314, a permanently charged lidocaine analogue, to the internal solution produced a single exponential function of \( I_{\text{Na}} \) block with a very slow onset rate of 0.0055 per pulse. Depolarizations of 10-msec pulse durations were applied to \(-30\) mV from a holding potential of \(-100\) mV at 3.2 Hz.
dent block with the short pulse was comparable to that with the long pulse. The results indicate that disopyramide mainly binds to the activated states, confirming previous reports. When pH was raised from 7.3 to 8.0, $A_1$ increased. When pH was lowered to 6.5, disopyramide always produced only the slow component of $I_{Na}$ block. Furthermore, $A_1$ and $A_2$ values with the short pulse were comparable to those values obtained with the long pulse at all pH conditions. Therefore, these findings indicate that the fast and slow components of $I_{Na}$ block by disopyramide may be related to binding of the uncharged and charged forms, respectively, to the activated states.

It then can be suggested that different drugs can have different modes of binding to their receptors. The charged form of both disopyramide and lidocaine appears to bind to the activated state through the hydrophilic pathway. Uncharged binding for lidocaine seems to occur to the inactivated state through the hydrophobic route. On the other hand, disopyramide in its uncharged form seems to bind primarily to the activated state and unbind readily through the hydrophilic pathway to produce the fast component of block. Because the hydrophobic pathways are available at all times to lipid-soluble molecules irrespective of gating, these observations do not contradict the studies in nerve by Hille.

Two components of block have been explained either by the modulated receptor model in terms of specific binding of drug to different states of the Na"+ channel or by the guarded receptor model in terms of block by the uncharged and charged forms of drug. We not only demonstrate that these two components of block are due to the drug’s uncharged and charged species, but also show that the uncharged and charged forms are not sole determinants to state-dependent binding.

The class I agents have been subdivided into the fast, intermediate, or slow drugs using the kinetic analysis of the use-dependent block of $V_{max}$, which was based on a single exponential development of $V_{max}$ block. Basically, the fast drugs like lidocaine have been thought to have advantages in preventing premature beats with short coupling intervals; but in practice, the actual advantage of such usage of the fast drugs over the slow drugs has not yet been established. Existence of the drugs that have two exponential developments of $I_{Na}$ block might fill a gap between basic study and clinical work. In addition to the onset rates, the ratio of the amplitudes of the fast and slow components of $I_{Na}$ block might give important information for the use of drugs showing two exponentials of block development.

Acknowledgments

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References

8. Campbell TJ: Kinetics of onset of rate-dependent effects of class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. Cardiovasc Res 1983;17:344–352.

**KEY WORDS**
- sodium current
- use-dependent block
- disopyramide
- lidocaine
- ventricular myocytes
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