Evidence for Two Components of Sodium Channel Block by Lidocaine in Isolated Cardiac Myocytes


The effects of lidocaine on sodium current in cardiac myocytes isolated from cat and guinea pig were investigated using the whole-cell variation of the patch-clamp technique. Lidocaine (43–200 μM) reduced sodium current during repetitive depolarizing pulses in a use-dependent manner. To clarify the nature of the use-dependent block, we characterized the time course of block development using a two-pulse protocol. Two distinct phases of block development were found: a rapid phase (τ = 1–6 msec) having a time course concurrent with the time course of channel activation, and a slower phase (τ = 100–900 msec), which developed after channels inactivated. The amplitude of the block during the rapid phase of development was a steep function of transmembrane voltage over the range of −70 to +20 mV. The voltage-dependence was similar to that for sodium channel activation (sodium conductance) but was too steep to be attributed solely to the passive movement of a singly charged molecule under the influence of the transmembrane voltage gradient. These results suggest that use-dependent block of sodium channels in cardiac tissue may result from an interaction of lidocaine with sodium channels in the activated as well as the inactivated channel states. Possible mechanisms underlying the fast component of block are discussed. (Circulation Research 1988;63:869–878)

Local anesthetic drugs appear to block sodium channels in nerve, skeletal muscle, and cardiac tissue in a similar manner. In addition to producing a resting or “tonic” block, lidocaine-induced block of sodium channels in all three tissue types has also been shown to be use- or rate-dependent.1–5 Measurements of sodium current (INa) in both nerve4 and skeletal muscle3 suggest that lidocaine’s use-dependent block results in part from interaction of the drug with both activated and inactivated channel states. Previous studies based upon measurements of maximum upstroke velocity (Vmax) of cardiac action potentials have also suggested that lidocaine interacts with both activated (i.e., open) and inactivated (i.e., closed) states of the cardiac sodium channel.4,7 However, evidence consistent with open channel block has not been convincingly demonstrated in recent studies in which the effect of lidocaine on cardiac INa was directly measured.5,8

The present study with isolated cardiac myocytes was designed to assess the time course of lidocaine block of the cardiac INa and to determine whether lidocaine block has a component that would be consistent with block of activated sodium channels. Experiments were performed under voltage clamp conditions on single cardiac myocytes using the whole-cell variation of the patch clamp technique.9 We found that the use-dependent block of cardiac sodium channels in cat and guinea pig myocytes can be divided into two components: one closely associated with sodium channel activation in both time course and voltage-dependence, and a second that develops following channel inactivation. We have published some of our results in preliminary form.10

Materials and Methods

Isolation of Cardiac Myocytes

The method for cell isolation used in this study was essentially identical to that described by Silver et al.11 Briefly, hearts from 2–4-kg adult mongrel cats or 200–500-g guinea pigs, of either sex, were removed under pentobarbital anesthesia, rinsed of blood with saline solution (0.9%), and mounted.
immediately on the cannula of a perfusion apparatus for retrograde perfusion through the aorta (Langendorff perfusion). The heart was initially perfused with a modified, nominally Ca\(^{2+}\)-free Krebs-Henseleit solution of the following millimolar composition: NaCl 130, KCl 4.8, MgSO\(_4\) 1.2, and NaH\(_2\)PO\(_4\) 0.33, and warmed to 37° C, equilibrated with 95% O\(_2\)-5% CO\(_2\), and titrated to pH 7.3 with hydrochloric acid. Once the initial perfusate was free of blood, the heart was perfused with recirculating Krebs-Henseleit supplemented with 0.15% collagenase (type II, Cooper Biomedical, Malverne, Pennsylvania). After the heart had become adequately digested (20-30 minutes for guinea pig; 40-50 minutes for cat), the atria and ventricles were cut away from each other, minced with scissors, and incubated at 37° C for 10 minutes in the collagenase solution. Harvesting of atrial and ventricular myocytes was the same as that described by Silver et al.\(^{11}\) Cells were incubated in Eagle’s Minimum Essential Medium with Earle’s Salts containing L-glutamine and supplemented with 0.15 mg/mlgentamicin sulfate. Cells were kept in an incubator maintained at 37° C and equilibrated with 5% CO\(_2\) until they were used (on the day of isolation) in electrophysiological experiments.

At the beginning of each experiment, cells were transferred from the storage solution to a shallow dish using a modified, nominally Ca\(^{2+}\)-free Krebs-Henseleit solution of the following composition: NaCl 130, KCl 4.8, MgSO\(_4\) 1.2, and NaH\(_2\)PO\(_4\) 0.33, and warmed to 37° C, equilibrated with 95% O\(_2\)-5% CO\(_2\), and titrated to pH 7.3. When washout of lidocaine was complete, peak 1\(^{\pm}\)I\(_{\text{Na}}\) amplitudes were measured before and after addition of lidocaine to the external solution, an equilibration period of 5-15 minutes was allowed for the effects of lidocaine on I\(_{\text{Na}}\) to approach steady state. After adding drug, washout of lidocaine was immediate and I\(_{\text{Na}}\) recovered completely.

### Solutions and Drugs

The experimental chamber was perfused with a low-sodium, N-2-hydroxyethyleneepiperoxazine-N’-2-ethanesulfonic acid (HEPES)—buffered salt solution of the following millimolar composition: NaCl 25, tetramethylammonium chloride 115, CaCl\(_2\) 1.8, CsCl 5, MgCl\(_2\) 1.2, CoCl\(_2\) 1.0, p-glucose 11, and HEPES 20. In some experiments, the external Na\(^+\) concentration was raised to 50 mM by equimolar substitution for tetramethylammonium chloride. The solution was titrated to a pH of 7.3 ± 0.01 with 1 Mtetramethylammonium hydroxide. The solution inside the suction pipette had the following millimolar composition: CsF 145, NaF 5.6, and HEPES 5 and was titrated to a pH of 7.2 ± 0.02 with 1 M CsOH. After addition of lidocaine to the external solution, an equilibration period of 5-15 minutes was allowed for the effects of lidocaine on I\(_{\text{Na}}\) to approach steady state. Peak I\(_{\text{Na}}\) amplitudes were measured before and after adding drug. When washout of lidocaine was possible, I\(_{\text{Na}}\) recovered completely.

### Evaluation of Method

To accurately measure the effects of drugs on cardiac I\(_{\text{Na}}\), certain requirements must be met. First, all other ionic currents that could contaminate measurement of I\(_{\text{Na}}\) must be eliminated. Second, errors resulting from current flowing across a residual series resistance (R\(_s\)) must be reduced to acceptable values. Isolation of I\(_{\text{Na}}\) current from other ionic currents was effectively accomplished using Cs\(^+\) to replace K\(^+\), and by placing Co\(^{2+}\) on the outside and F\(^-\) on the inside of the membrane (see “Solutions and Drugs”) to block current through calcium channels. Under these conditions, no evidence for transient potassium or calcium currents was observed.

### Electrophysiological Techniques

The voltage clamp circuit used to record membrane currents from cat myocytes was the same as that designed by M. Yoshii and described elsewhere.\(^{12}\) An Axoclamp voltage clamp amplifier (Axon Instruments, Burlingame, California) was used for experiments using guinea pig myocytes. Suction pipettes were made using a vertical pipette puller (Kopf 700D, Tujunga, California) and were fire polished. When filled with internal solution, pipettes had tip resistances of about 0.3 to 0.5 MΩ. On occasion, electrodes with slightly higher resistances were used to patch-clamp small atrial cells.

Capacitative transients evoked by 10 mV hyperpolarizing pulses were well-described by single exponential functions and had mean time constants of 110 ± 10 μsec in cat atrial myocytes (n = 9) and 85 ± 8 μsec in guinea pig ventricular myocytes (n = 13). Mean cell capacitance was estimated from the equation C = Q/ΔV, where Q is the total charge discharged during a voltage step, ΔV. Q was derived from integration of the capacitative transient. Mean values of cell capacitance derived by this method were 78 ± 10 pF for cat atrial myocytes and 66 ± 4 pF for guinea pig ventricular myocytes.

The total series resistance for the connective pathway between the pipette and cell membrane was calculated from estimates of cell capacitance and the time constant of the capacitative current’s decay (Cy = R\(_s\) × C\(_m\)). The mean series resistance was 1.5 ± 0.1 MΩ for cat atrial myocytes (n = 9), and 1.3 ± 0.1 MΩ for guinea pig ventricular myocytes (n = 13). Compensation for this resistance was done empirically to speed the decay of the capacitative transient by applying a level of electronic series resistance compensation just short of producing oscillation.

Command pulses and data acquisition were controlled by either a DEC PDP-11/23 computer or an IBM PC/AT. The membrane current (i.e., I\(_{\text{Na}}\)) signal was filtered at 10 kHz, fed into an A/D converter (sample interval 30-100 μsec), and the digitized data were stored on floppy disks. Since leak currents were relatively small and can be a nonlinear function of voltage, no electronic compensation for voltage-dependent leak current was used.

### References

1. Silver et al. Cells were incubated in Eagle’s Minimum Essential Medium with Earle’s Salts containing L-glutamine and supplemented with 0.15 mg/ml gentamicin sulfate. Cells were kept in an incubator maintained at 37° C and equilibrated with 5% CO\(_2\) until they were used (on the day of isolation) in electrophysiological experiments.

2. Solutions and Drugs: The experimental chamber was perfused with a low-sodium, N-2-hydroxyethyleneepiperoxazine-N’-2-ethanesulfonic acid (HEPES)—buffered salt solution of the following millimolar composition: NaCl 25, tetramethylammonium chloride 115, CaCl\(_2\) 1.8, CsCl 5, MgCl\(_2\) 1.2, CoCl\(_2\) 1.0, p-glucose 11, and HEPES 20. In some experiments, the external Na\(^+\) concentration was raised to 50 mM by equimolar substitution for tetramethylammonium chloride. The solution was titrated to a pH of 7.3 ± 0.01 with 1 M tetramethylammonium hydroxide. The solution inside the suction pipette had the following millimolar composition: CsF 145, NaF 5.6, and HEPES 5 and was titrated to a pH of 7.2 ± 0.02 with 1 M CsOH. After addition of lidocaine to the external solution, an equilibration period of 5-15 minutes was allowed for the effects of lidocaine on I\(_{\text{Na}}\) to approach steady state. Peak I\(_{\text{Na}}\) amplitudes were measured before and after adding drug. When washout of lidocaine was possible, I\(_{\text{Na}}\) recovered completely.
was observed at any test potential. Also, at the concentration used, Co^2+ did not alter the time course or voltage-dependence of I_{Na} when examined in the presence of 1.8 mM Ca^2+.

Voltage errors related to the flow of I_{Na} across the series resistance were minimized by a combination of using low resistance electrodes (300–500 KΩ), reducing peak current to values less than 15 nA using 25–50 mM external sodium concentration and low temperature (14–17° C), and by electronic series resistance compensation. To test for the presence of uncompensated series resistance, we defined the peak sodium conductance (G_{Na}) versus E_{m} relation at holding potentials of -140 and -90 mV. If a significant uncompensated series resistance error exists, reduction of peak I_{Na} amplitude by partial inactivation should cause both a depolarizing shift of the G_{Na} versus E_{m} relation, as well as a decrease in its slope. We calculated G_{Na} using a Hodgkin-Huxley model (where G_{Na} = I_{Na}/(E_{m} - E_{Na})). The relation between G_{Na} and E_{m} was approximated by a least-squares fit to Equation 1:

\[ G_{Na} = \frac{G_{Na,\text{max}}}{1 + \exp\left(\frac{(E_{m} - E_{\text{mid}})}{S}\right)} \]  

where G_{Na,\text{max}} is the maximal value of G_{Na}, E_{\text{mid}} is the voltage at which G_{Na} is half maximal, and S is a slope factor.

In ventricular myocytes, the peak I_{Na} amplitude during pulses to -20 mV from a holding potential of -140 mV was 10.1 ± 2.1 nA (mean ± SEM; n = 6). Reducing the holding potential to -90 mV reduced maximum G_{Na} by 44 ± 4%, but produced only a very small shift (1.6 ± 0.5 mV) of E_{\text{mid}} (from -36.2 to -34.6 mV), and a reduction of the slope factor from 6.9 ± 0.7 mV to 6.2 ± 0.8 mV, indicating a slight steepening of the slope. These small changes suggest that the measurement error due to uncompensated series resistance was within acceptable limits. Similar results were obtained after inhibiting approximately two thirds of I_{Na} with tetrodotoxin.

To evaluate the adequacy of voltage clamp control we estimated the length constant (\Lambda) at the time of peak sodium conductance. According to Belles et al., the length constant can be approximated by the following equation:

\[ \Lambda = \sqrt{(2 \cdot \frac{\pi}{Q} \cdot R_{m}/R_{s})} \]  

where L is cell length, Q is cross sectional area of the cell, R_{m} is average input resistance, and R_{s} is specific resistivity of the cell interior. R_{m} at peak sodium conductance, calculated from the positive slope of the current voltage relation, was 9.9 ± 1.5 \times 10^{4} \Omega (mean ± SEM; n = 10). L and Q were assumed to be 98 \mu m and 314 \mu m^2,16 and R_{s} = 605 \Omega cm. With these values, the estimated length constant at peak sodium conductance is 317 \mu m, more than five times the distance from the pipette tip to the end of a typical myocyte when the pipette is placed centrally along the cell length.

Thus, we conclude that problems concerning I_{Na} resolution, voltage errors due to series resistance, and nonuniformity are within acceptable limits under our experimental conditions and should not preclude interpretation of the data.

Statistics and Data Analysis

Fits of experimental data to exponential equations were performed with a nonlinear least-squares fitting algorithm. Paired comparisons between data points were done using Student's t test. Grouped or multiple comparisons were made using an analysis of variance with a Scheffe test for critical difference. Differences were considered significant at p<0.05. All results are expressed as mean ± SEM.

Results

Tonic Block

Lidocaine had three distinct effects on the steady-state sodium current availability curve. It reduced the maximum available current, increased the slope factor (k), and shifted the voltage-dependence of I_{Na} to more-negative potentials. Figure 1 shows a representative example of the effects of lidocaine on the steady-state availability curve for I_{Na} in cat atrial cells. In the example shown, the maximum available current was reduced 14% by lidocaine. This block, produced at very negative potentials and at low stimulus rates, was defined as "tonic block." In cat atrial myocytes, 200 \mu M lidocaine reduced I_{Na} by 14 ± 1.3% (mean ± SEM; n = 5). A similar level of tonic block was also observed in guinea pig ventricular myocytes exposed to 200 \mu M lidocaine (23 ± 3.5%, n = 4). As illustrated in Figure 1, typi-
Under control conditions, the $I_{Na}$ availability curve was maximal (i.e., $h_{Na}(t)=1$) at $-120 \text{ mV}$, half maximal at $-86 \text{ mV}$, and 0 at $-50 \text{ mV}$. The solid line representing a simple Boltzmann equation has a slope factor of $5.3 \text{ mV}$ and adequately describes the data. In the presence of $200 \mu M$ lidocaine, the potential at which $I_{Na}$ was half maximal shifted to $-96 \text{ mV}$, that is, $10 \text{ mV}$ more negative than the control. The slope factor was increased to $7.3 \text{ mV}$ in the presence of lidocaine. On average, $200 \mu M$ lidocaine shifted the steady-state availability curve in the negative potential direction $10 \pm 1 \text{ mV}$ and increased the slope factor by $1.7 \pm 0.3 \text{ mV}$ ($5.7 \pm 0.4$ to $7.5 \pm 0.3$; $n=5$) in cat atrial myocytes.

**Use-Dependent Block**

In addition to producing tonic block of $I_{Na}$, lidocaine suppressed $I_{Na}$ in a use-dependent manner when cells were repetitively stimulated at rates faster than 0.5 Hz. Use-dependent block is illustrated in Figure 2. In the absence of drug, repetitive depolarization to $-20 \text{ mV}$ for 20 msec at 2–5 Hz produced very little decrease in $I_{Na}$ (Figure 2B). However, in the presence of 100 $\mu M$ lidocaine, there was a substantial decrease in $I_{Na}$ amplitude following a single 20-msec pulse, and peak $I_{Na}$ amplitude decreased further with each pulse until a steady-state level was approached within 12 pulses (Figures 2B and 2C). This block, which is over and above the tonic block, is referred to as "use-dependent block." An increase in the stimulus rate enhanced use-dependent block (Figure 2C). The amplitude of use-dependent block at 5 Hz produced by 100–200 $\mu M$ lidocaine ranged from 50% to 79% in guinea pig ventricular myocytes ($n=5$).

**Two Phases of Lidocaine Block Development**

Cumulative reduction of peak $I_{Na}$ during repetitive pulsing is usually thought to result from the drug blocking sodium channels in either the activated or the inactivated states, or from blocking channels in both states. To determine in which states sodium channels can be blocked by lidocaine during a depolarizing step, the time course of block development was characterized with the two-pulse protocol shown in the inset of Figure 3A. The block produced by conditioning pulses having selected durations ranging from 1 msec to 10 seconds was determined by a test pulse applied after a short (500 msec) recovery interval. At the holding potential used ($-120 \text{ mV}$), the 500 msec recovery interval was sufficient for the drug-free channels to recover from the inactivation produced by the conditioning pulse. The pulse sequence was applied at 30-second intervals to obviate cumulative block by lidocaine and to allow full recovery from slow inactivation.

In guinea pig ventricular myocytes, under control conditions, test $I_{Na}$ was not appreciably decreased even after a 1-second conditioning prepulse (Figure 3). In contrast, in the presence of 172 $\mu M$ lidocaine, test $I_{Na}$ was decreased markedly even after short conditioning pulses (1–10 msec). Further increase in prepulse duration enhanced the degree of block produced by lidocaine. Two distinct phases of block onset that can be fit by a sum of two exponentials were observed, a rapid phase with a time constant of 3 msec accounting for approximately 37% of the block and a slow phase ($\tau = 351$ msec) accounting for the remainder. The rapid phase of block occurred during the period (0–10 msec at $16^\circ C$) when most sodium channels were, or had been, in the activated state, whereas the slow phase of block did not become appreciable until most channels had become inactivated. Qualitatively similar results were obtained over a wide range of drug concentrations in guinea pig ventricular myocytes (Table 1), as well as in cat atrial myocytes (Figure 3B).
Pulse protocol was 30 seconds. The peak amplitude of the lNa during the test pulse was normalized to the value obtained without a conditioning prepulse. Under control conditions there was little noticeable change in peak current amplitude following conditioning prepulses of up to 1 second duration. In the presence of lidocaine, peak current was inhibited in two distinct phases having constants of 3 and 351 msec. Temperature, 14°C; extracellular sodium concentration, 50 mM. A: Guinea pig ventricular myocyte, plotted with a linear time scale; Preparation 4221. B: Cat atrial myocyte plotted with a linear time scale; Preparation 4221.

Voltage-Dependence for the Rapid Phase of Lidocaine Block

Previous studies have shown that the slow phase of block is not strongly voltage-dependent at voltages in which sodium channels are fully inactivated (e.g., at membrane voltages positive to −50 mV). However, the voltage-dependence of the rapid phase was not defined during these studies. Therefore, the voltage-dependence of the previously unresolved rapid phase of block was determined using the protocol shown in Figure 4A. A train of 2-msec pulses of selected amplitudes (E_c) were applied at 3 Hz to produce a steady-state level of I_{Na} block. The amount of block produced by the train of pulses to voltage E_c was then assessed with a test pulse to −20 mV delivered 300 msec after the conditioning train. As illustrated, block was a steep function of membrane voltage over a range of conditioning potentials from −70 to 0 mV. The block exhibited voltage-dependence at potentials positive to −80 mV, had a midpoint near −44 mV, and saturated at approximately 20 mV. The line drawn through the data points describing the voltage-dependence of lidocaine-induced block represents an equation that has been used previously by Cahalan and co-workers, and in a different form by Strichartz, to describe the open-channel block of Na^+ channels in nerve preparations by local anesthetics:

$$\{(1-B)/[1 + \exp((E_c - E_{mid})/S)]\} + B$$

where B is the steady-state level of I_{Na} remaining after the series of prepulses, E_c is the membrane potential during the conditioning pulse, E_{mid} is the membrane potential at which drug-induced block is half-maximal, and S is the slope of the voltage-dependent block. This model assumes that channel block results from the passive movement of a monovalent cation under the influence of the membrane field to a blocking site within the sodium channel. According to this model, any voltage-dependence derived from the opening and closing of sodium channels is ignored, and the slope factor (S) is equal to RT/ZF, where F, R, and T have their usual meaning, Z is the drug molecule's valency, and δ is the fractional distance (0 to 1) of the binding site across the membrane when measured from the inside. Under our experimental conditions, RT/ F = 24 mV and Z = 1. Therefore, the minimum possible value for the slope factor is 24 mV (when δ = 1). In our experiments, the slope factor S for
greater than that predicted for passive movement of ions during opening, we compared the voltage-dependence of the initial 2 msec of depolarizing pulses (i.e., for the channel block with that of gNa. gNa was calculated from either peak INa, or the integral of INa • dt during the initial 2 msec of depolarizing pulses (i.e., for the same pulse duration used to define channel block) (Figure 4, Table 2). As shown in Table 2, the best agreement between channel block and channel conductance was obtained using the time integral of gNa. However, while the voltage-dependencies of channel block and of the integrated gNa had similar voltage midpoints, the slopes for the two relations were significantly different (p < 0.05). For comparison, the voltage-dependence of gNa was also defined using a 50 msec integral of INa elicited at each of the several different voltages. However, the disparity between the characteristics of gNa as defined using 50 msec integrals (E mid = -51.0 ± 3.5 mV; slope = 2.7 ± 0.2 mV) and those of lidocaine-induced block (E mid = -34.7 ± 5.5 mV; slope = 13.7 ± 0.7 mV) was not lessened (n = 8).

Effect of Lidocaine on Sodium Current Time Course

As illustrated in Figure 5A, lidocaine (100-200 μM) produced a barely perceptible change in the time course of lNa decay in cells with intact inactivation. Under control conditions, the decay of lNa following peak inward current at -20 mV could be well described as the sum of a fast and a slow exponential plus a small constant (Figure 5A, Table 3). Exponential fits to the time-course of lNa decay after a 5-20 minute exposure to 200 μM lidocaine indicated that the rapid and slow time constants were slightly reduced by 9 ± 9% and 18 ± 12%, respectively, but this reduction was not significant (p > 0.1). The relative fractions of current decay attributed to the fast and slow components were also not significantly altered by 200 μM lidocaine (p > 0.1). Similar results were also observed in two additional cells exposed to 100 μM lidocaine (Table 3).

Many putative open-channel blockers (including lidocaine) have been reported to produce relatively small effects on lNa time course in nerve\textsuperscript{1,18-21} and cardiac preparations\textsuperscript{5,8,22} when lNa inactivation is intact. In contrast, open-channel blockers have been demonstrated to exert very marked kinetic effects in nerve cells in which inactivation has been "removed" (i.e., modified) by pretreatment with chemical reagents or enzymes.\textsuperscript{19-20} Therefore, we examined the effects of lidocaine on lNa of myocytes that had their inactivation process modified by exposure to α-chymotrypsin (0.7 to 0.8 mg/ml, applied intracellularly by addition to the pipette solution).\textsuperscript{23} After treatment with α-chymotrypsin for 30-60 minutes, there was little, if any, decay of lNa during 20 msec depolarizing pulses (Figure 5B). Nevertheless, subsequent exposure to lidocaine induced a time-dependent decay in lNa. This finding was obtained in five out of five cells exposed to 100 or 200 μM lidocaine and is consistent with the notion that lidocaine can block sodium channels when they are in an open, or activated, state.
The voltage dependence of rapid channel block and $G_{Na}$

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Discussion

In 1977, Hondeghem and Katzung developed a set of differential equations for a model derived from the modulated receptor hypothesis. When available data on the effects of lidocaine on maximum upstroke velocity were fit to the model, it predicted that lidocaine has a low affinity for sodium channels in the closed, rested state ($K_d>1 \text{mM}$) and a relatively high affinity for channels in both activated (open) and inactivated (closed) states ($K_d=0.3-40 \mu M$). Based upon the association and dissociation rate constants estimated from the model for the three primary channel states, the lidocaine-induced use-dependent block that developed during trains of ventricular action potentials was predicted to consist of two kinetically different components: a rapid component related to binding of lidocaine to activated (open) channels, and a second slower component related to lidocaine binding to inactivated (closed) channels. Direct evidence for the presence of two components was subsequently obtained from measurement of $V_{\text{max}}$ of action potential upstrokes in guinea pig ventricular muscle when action potential plateau duration was controlled by voltage clamp.

In contrast to the studies using $V_{\text{max}}$, recent studies investigating the effect of lidocaine directly on $I_{Na}$ during exposure to cool temperature and low external sodium concentration provided strong evidence that lidocaine can bind to inactivated channels, but little or no evidence that lidocaine can bind to channels in an activated, or open, state. Reasoning that the voltage clamp protocols used in previous studies were not particularly well adapted for detection of open-channel block, in the present study we have reexamined the effects of lidocaine on $I_{Na}$ in voltage clamped cardiac myocytes and found evidence for an interaction between lidocaine and sodium channels while in or going to an open state. During a depolarizing pulse, we observed two components of block development (Figure 3). One component developed rapidly with a time constant of a few milliseconds at potentials positive to threshold for channel opening. This rapid component was then followed by a slower component having a time constant 100-fold larger and which is essentially identical to that previously described by others in cooled Purkinje fibers and isolated rat myocytes.

Role of the Membrane Field Acting on the Cationic Form

Lidocaine (100–200 μM) induced a time-dependent decay of $I_{Na}$ in cells whose inactivation had been slowed or removed by internal application of α-chymotrypsin (Figure 5B). This effect of lidocaine on cardiac $I_{Na}$ is similar to the reported effects of a variety of tertiary and quaternary amine local anesthetics documented to block open channels in...
nerve fibers.\textsuperscript{19–20} Block of open sodium channels in nerve fibers has also been shown to be strongly voltage-dependent.\textsuperscript{1,18–20} This voltage-dependence of open channel block has been proposed to result from the voltage-sensitive movement of the cationic form of the drug between the cytoplasm and a receptor site which is located partway across the transmembrane voltage gradient.\textsuperscript{18,19}

To determine whether the rapid component of lidocaine block in cardiac tissue could be attributed to a similar mechanism, the voltage-dependence of the rapid component of block was defined and found in the present study to be a steep function of membrane voltage between $-70$ and $0$ mV, saturating at $\sim +20$ mV (Figure 4). However, the data could not be fit well to an equation describing passive movement of a cation within a voltage gradient\textsuperscript{19} unless either an ionic charge greater than unity or a ratio of drug-channel binding greater than $1:1$ was assumed. Since lidocaine cannot bear more than one positive charge, and the available evidence supports $1:1$ stoichiometry for lidocaine block of sodium channels,\textsuperscript{5,23} it seems unlikely that the observed steepness in the relation between membrane voltage and channel block can be attributed to simple passive movement of charged lidocaine molecules across a portion of the transmembrane electric field. The observed sharp steepness in voltage-dependent block by lidocaine is in contrast with the more shallow voltage-dependence previously reported for etidocaine and tetracaine in nerve\textsuperscript{19} (however, see Reference 21).

**Voltage-Dependence of Rapid Component May Result From Channel Gating**

Since the voltage-dependence of channel block was too steep to be simply attributed to voltage-dependent movement of the charged form of lidocaine into sodium channels, we explored the possibility that the voltage-dependence of block results indirectly from state-dependent drug-binding to the open state of the channels in a manner similar to that described by Yeh and Ten Eick\textsuperscript{26} for the block of $I_{Na}$ in squid giant axon by derivatives of disopyramide. To evaluate this possibility, we compared the voltage-dependence of sodium channel block with that for channel opening (as defined peak $G_{Na}$ or by integrated $G_{Na}$) and found them to be similar, but not identical. Both channel block and the integral of $G_{Na}$ could be described as sigmoid functions of membrane voltage that develop over the range of $-70$ to $+20$ mV and have similar midpoints (Table 2, Figure 4). This result suggests that the activation gate for the sodium channel plays an important role in the development and modulation of use-dependent block of $I_{Na}$. The voltage dependence of channel block, however, was consistently less steep than that derived for $G_{Na}$ (Table 2). The meaning of this difference is not clear. It is conceivable that other factors, such as a competitive interaction between lidocaine and monovalent cations (e.g., Na$^+$) for a common binding site, may contribute to the observed difference between the voltage-dependence of channel gating and channel block.\textsuperscript{18,21,22,26,27}

**Implications of Rapid Open Channel Block**

The results of this study support the notion that lidocaine can produce a rapidly developing time-dependent block of channels that can reduce the whole cell $I_{Na}$ measurably within a very few milliseconds after the onset of $I_{Na}$ activation. Since the time-to-peak for $I_{Na}$ in cooled preparations is approx-
approximately 1 to 3 msec at -20 mV, a significant fraction of the block occurring during the first pulse following a long rest period (i.e., the block termed "tonic block") appears to result from a rapidly developed block of open channels. Therefore, especially at strongly depolarized potentials and high drug concentrations, tonic block may not be an accurate reflection of drug binding to channels that are in the closed (i.e., rested) state since in part tonic block can result from open channel block.

The time course for the rapidly developing component of block (see Figure 3) falls within the period during which channel transitions between the closed (rested), open and inactivated states are occurring, and the fraction of channels in the open state is not constant. Therefore, the onset rate for block development, characterized by applying conditioning depolarizing pulses, should not be regarded as an accurate reflection of the rate of drug-to-open-channel binding.

Although not necessarily anticipated in every instance, open channel block also may accelerate the decay phase of the $I_{Na}$-time course. Table 3 shows that in the presence of lidocaine the slower inactivation time constant decreased in five out of six instances. The decrease, however, was not statistically significant ($p>0.1$). Figure 5 also suggests that lidocaine may modestly accelerate the inactivation time course even when inactivation is intact. The basis of any acceleration would be quite complex, however. $I_{Na}$ decay in the presence of lidocaine would be influenced by several factors, among which would be the usual normal (i.e., in the absence of lidocaine) time course of $I_{Na}$ inactivation, the rate of development of open-channel block, and any effects with a potential for altering $I_{Na}$ inactivation gating kinetics. Therefore, it is unlikely that the difference between the time courses of $I_{Na}$ decay when in the presence and absence of lidocaine will reflect the true drug-to-channel binding kinetics. Despite the quantitative uncertainties, the finding that lidocaine accelerated the time-dependent decay of $I_{Na}$ in cells whose sodium inactivation had been modified (by exposure intracellularly to α-chymotrypsin), is consistent with the notion that the change in time-course involved open channel block, and represents a second line of evidence supporting this view.

**Use-Dependence Involves Both Activation and Inactivation Gating**

In summary, the present data obtained using voltage clamped isolated guinea pig ventricular and cat atrial myocytes supports earlier findings suggesting that use-dependent block of cardiac $I_{Na}$ produced by lidocaine can involve binding to inactivated channels.5,8 In addition, the data provide evidence suggesting that binding with fairly rapid kinetics of drug to an open channel conformation can also contribute significantly to the production of use-dependent block. The data also suggest that lidocaine-induced tonic block of $I_{Na}$ may not be a reliable measure of drug-affinity for channels in the closed, rested state because at least some portion of the tonic block should result from lidocaine binding to channels in the open state so quickly that peak $I_{Na}$, even during the first pulse following a long rest, is reduced compared with control. The rapidity of drug binding to the sodium channels may play an important role in lidocaine’s ability to depress conduction under conditions that can substantially shorten the duration of cardiac action potentials, such as hypoxia and ischemia.

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References

21. Wang GK, Brodwick MS, Eaton DC, Strichartz GR: Inhibition of sodium current by local anesthetics in chloramine-T-treated squid axons. The role of channel activation. J Gen Physiol 1987;89:645-657

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