Cardiac Sodium Channels (hH1) Are Intrinsically More Sensitive to Block by Lidocaine Than Are Skeletal Muscle (μ1) Channels

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ABSTRACT When lidocaine is given systemically, cardiac Na channels are blocked preferentially over those in skeletal muscle and nerve. This apparent increased affinity is commonly assumed to arise solely from the fact that cardiac Na channels spend a large fraction of their time in the inactivated state, which exhibits a high affinity for local anesthetics. The oocyte expression system was used to compare systematically the sensitivities of skeletal (μ1-β1) and cardiac (hH1-β1) Na channels to block by lidocaine, under conditions in which the only difference was the choice of α subunit. To check for differences in tonic block, Na currents were elicited after 3 min of exposure to various lidocaine concentrations at -100 mV, a potential at which both hH1-β1 and μ1-β1 channels were fully reprimed. Surprisingly, hH1-β1 Na channels were threefold more sensitive to rested-state block by lidocaine (402 ± 36 μM, n = 4–22) than were μ1-β1 Na channels (1,168 ± 94 μM, n = 7–19). In contrast, the inactivated state binding affinities determined at partially depolarized holding potentials (hV = 0.2) were similar (Kd = 16 ± 1 μM, n = 3–9 for hH1-β1 and 12 ± 2 μM, n = 4–11 for μ1-β1). Lidocaine produced more use-dependent block of peak hH1-β1 Na current elicited by trains of short-(10 ms) or long- (1 s) duration step depolarizations (0.5 Hz, -20 mV) than of μ1-β1 Na current. During exposure to lidocaine, hH1-β1 channels recover from inactivation at -100 mV after a prolonged delay (20 ms), while μ1-β1 channels begin repriming immediately. The overall time course of recovery from inactivation in the presence of lidocaine is much slower in hH1-β1 than in μ1-β1 channels. These unexpected findings suggest that structural differences in the α subunits impart intrinsically different lidocaine sensitivities to the two isoforms. The differences in steady state affinities and in repriming kinetics are both in the correct direction to help explain the increased potency of cardiac Na channel block by local anesthetics.

INTRODUCTION

Lidocaine is used clinically as both an antiarrhythmic and a local anesthetic. Its sites of action are Na channels in heart, skeletal muscle and nerve. While microm
lar concentrations of lidocaine are clinically efficacious in the treatment of arrhythmias, much higher doses are required to induce local anesthesia by block of sodium channels in nerve and skeletal muscle. Lidocaine's therapeutic value in the treatment of arrhythmias is derived from its ability to block Na channels in depolarized cells before affecting the excitability of healthy polarized cells (Hondeghem, Grant, and Jensen, 1974; Hondeghem and Katzung, 1977; Lazzara, Hope, El-Sherif, and Scherlag, 1978). Thus, lidocaine helps to prevent abnormal impulse propagation (Abete, Ferrara, Rengo, and Vassalle, 1991), although such an action sometimes paradoxically enhances arrhythmias (Podrid, 1989).

Preferential block of cardiac Na channels by lidocaine has been proposed to arise solely because lidocaine binds with particularly high affinity to the inactivated state (Hille, 1977a; Bean, Cohen, and Tsien, 1983) and cardiac Na channels spend a large fraction of their time in the inactivated state; the duration of the ventricular action potential is long, on the order of hundreds of milliseconds, while action potentials in nerve and skeletal muscle last only a few milliseconds. The notion of high-affinity inactivated state binding is supported both by theory (Hille, 1977a; Hondeghem and Katzung, 1977) and by experimental findings that block of Na current by lidocaine increases with faster stimulus rates, longer depolarization durations, and less negative holding potentials (Schwarz, Palade, and Hille, 1977; Hondeghem and Katzung, 1977; Bean et al., 1983).

The lidocaine sensitivity of Na channels in the heart, skeletal muscle and nerve has historically been determined only for native channels either in tissue preparations or in isolated cells. Heterologous expression of Na channels enables study of the lidocaine binding properties without potentially confounding differences in the environment of the host cell or tissue. Tetrodotoxin (TTX) and lidocaine sensitivity are preserved in Na channels expressed in oocytes from cardiac mRNA (Krafte, Volberg, Dillon, and Ezrin, 1991; Gellens, George, Chen, Chahine, Horn, Barchi, and Kallen, 1992; Satin, Kyle, Chen, Rogart, and Fozzard, 1992) and skeletal muscle mRNA (Trimmer, Cooperman, Tomiko, Zhou, Crean, Boyle, Kallen, Sheng, Barchi, Sigworth, Goodman, Agnew, and Mandel, 1989). Cardiac Na channels are relatively resistant to blockade by TTX, requiring micromolar concentrations, whereas skeletal muscle Na channels are quite sensitive to block by TTX and $\mu$-conotoxin GIIIA at nanomolar concentrations (Moczydowski, Uehara, Guo, and Heiny, 1986). The relative sensitivities of the two isoforms are reversed when describing block by cadmium (Satin et al., 1992; Backx, Yue, Lawrence, Marban, and Tomaselli, 1992). These examples indicate that structural differences within the Na channel itself can impart tissue-specific drug sensitivity to cardiac and skeletal muscle Na channels.

We used the oocyte expression system to compare systematically the intrinsic sensitivities of cardiac (hH1) and skeletal muscle (\(\mu.1\)) Na channels to block by lidocaine under conditions designed so that the only difference between channel types is the identity of the $\alpha$ subunit. Because the $\beta_1$ subunit associates with the hH1 and $\mu.1$ Na channel $\alpha$ subunits and modifies Na channel function (Nuss, Chiamvimonvat, Pérez-García, Tomaselli, and Marban, 1995), we have coexpressed the $\beta_1$ subunit in the present study. Coexpression increases the size of the current, speeds the rate of current decay, shifts the voltage dependence of inactivation to
more negative potentials, and reduces use-dependent decay (Nuss et al., 1995). For both cardiac and skeletal muscle Na channels, coexpression with a β1 subunit more closely reproduces in vivo Na channel function. It is not yet clear whether structural differences exist within the lidocaine receptor in cardiac vs skeletal muscle Na channels which confer differential lidocaine sensitivity to these two tissues; a direct comparison of lidocaine sensitivity of heterologously expressed cardiac and skeletal muscle channels may provide functional evidence for different receptor conformations.

MATERIALS AND METHODS

Detailed descriptions of the methods employed in this study can be found in the preceding paper (Nuss et al., 1995). Cardiac and skeletal muscle Na channels were expressed in Xenopus laevis oocytes by coinjection of human heart (hH1) or rat skeletal muscle (μ1) α subunit RNA with saturating amounts of rat brain β1 subunit RNA. Macroscopic hH1-β1 and μ1-β1 Na currents were recorded in ND-96 solution using the two-electrode voltage clamp technique as described (Nuss et al., 1995). Lidocaine HCl (USP 2%; Abbott Laboratories, North Chicago, IL) was added to the recording solution to achieve final concentrations ranging from 3 μM to 3 mM. Control data plots which characterize the voltage dependence of inactivation (Figs. 3 and 4), use dependence (Fig. 5) and recovery from inactivation (Fig. 6) of μ1-β1 and hH1-β1 Na channels are reproduced from the companion paper (Nuss et al., 1995). With these exceptions, all of the data shown here are original to this paper.

Pooled data are presented as means and standard deviations of the mean unless noted otherwise. Significance of differences between two populations (hH1-β1 and μ1-β1) over a range of lidocaine concentrations was assessed using multivariate analysis of variance (MANOVA; Systat Inc., Evanston, IL).

RESULTS

Voltage Dependence of Lidocaine Block

Skeletal muscle Na channels expressed in oocytes by coinjection of the rat skeletal muscle (μ1) α subunit with excess rat brain β1 subunit mRNA demonstrated sensi-
tivity to block by lidocaine. Families of \( \mu_1-\beta_1 \) currents were recorded by depolarization to potentials between \(-60\) and \(30\) mV from a holding potential of \(-100\) mV in the absence and presence of lidocaine (Fig. 1). Lidocaine (700 \( \mu \)M) caused a reduction by \( \approx 35\% \) in peak \( \mu_1-\beta_1 \) current at all voltages tested, without a shift in the current-voltage relationship or an obvious acceleration of current decay.

Sodium currents recorded in oocytes expressing cardiac Na channels after coinjection of the human heart \( \alpha \) subunit (hH1) with excess rat brain \( \beta_1 \) subunit RNA were also sensitive to block by lidocaine. Peak hH1-\( \beta_1 \) currents are reduced by more than 60\% during exposure to 700 \( \mu \)M lidocaine (Fig. 2). In this example, the peak of the current-voltage relationship was shifted by 5 mV in the depolarizing direction. Such a depolarizing shift in the \( I-V \) relationship may reflect improved voltage control after reduction of peak \( I_{\text{Na}} \) or a genuine lidocaine effect. The kinetics of macroscopic current decay did not appear to be affected by lidocaine (see Discussion).

Comparison of the effect of lidocaine in Figs. 1 and 2 reveals that cardiac Na channels appear to be more sensitive to block by lidocaine than are skeletal muscle channels: the same concentration of drug (700 \( \mu \)M) inhibits a larger fraction of the hH1-\( \beta_1 \) Na current (62\%) than in \( \mu_1-\beta_1 \) channels (33\%). To elucidate the basis of this difference in sensitivity to lidocaine, we used a variety of venerable voltage protocols designed to determine how avidly drugs bind to the various states of the Na channel (Hille, 1977a; Hondeghem and Katzung, 1977; Bean et al., 1983).

**Lidocaine Binding to the Inactivated State**

Lidocaine is thought to bind particularly tightly to the inactivated state of Na channels. As one manifestation of this phenomenon, the steady state availability curve typically shifts to the left when Na channels bind lidocaine, reflecting a shift in equilibrium from resting to inactivated states (Hille, 1977a; Bean et al., 1983). The voltage dependence of inactivation (\( h_a \) curve; Hodgkin and Huxley, 1952) was ex-
amined in the absence and presence of lidocaine to determine whether exposure to drug shifts the availability of \( \mu_1-\beta_1 \) Na channels. Prepulses (1 s) were applied to potentials between \(-140\) and \(-20\) mV to alter the distribution of channels between inactivated and resting states, and current was assayed during test pulses to \(-20\) mV. While one second prepulses were sufficiently long to recruit slow inactivation (Fig. 6), long periods of rest at the holding potential of \(-100\) mV (15 s in the absence of lidocaine and 45 s in its presence) were used to ensure that recovery from slow inactivation was complete before the next prepulse. Exposure to 700 \( \mu \)M lidocaine induced a 20 mV hyperpolarizing shift in the availability curve of \( \mu_1-\beta_1 \) channels (Fig. 3). In the drug-free condition, 50% of the channels were available at \(-60 \pm 1\) mV \((n = 8)\). Lidocaine shifted the midpoint of the \( \mu_1-\beta_1 \) availability curve by \(-20\) mV to \(-80 \pm 1\) mV \((n = 6)\) and increased its slope factor from 5.2 to 7.2.

Lidocaine also induced a hyperpolarizing shift of the \( h_{\mu} \) curve in hH1-\( \beta_1 \) Na channels; Fig. 4 shows the effect of 300 \( \mu \)M lidocaine. Exposure to lidocaine shifted the midpoint of the hH1-\( \beta_1 \) availability curve by \(-10\) mV to \(-90 \pm 1\) mV \((n = 5)\) from \(-80 \pm 1\) mV \((n = 6)\) while increasing the slope factor from 4.9 to 7.7.

**Use-dependent Block**

Lidocaine produces both "tonic" blockade, which is the reduction of the current evoked by the first pulse after a long rest, and use-dependent block, defined as a further reduction of current which accumulates with repetitive pulsing in the presence of drug. The amount of use-dependent block produced by a given concentration of lidocaine is influenced by the magnitude and duration of the test pulse and the duration of the recovery interval (Schwarz et al., 1977; Wendt, Starmer, and Grant, 1993).

We assessed use dependence by measuring peak \( I_{Na} \) during 20-step trains of depo-
Figure 4. Voltage dependence of inactivation of hH1-\(\beta_1\) Na channels in the absence (open circles) and presence of lidocaine (300 \(\mu\)M, solid circles). The experiments were performed as described in the legend to Fig. 3. Lidocaine shifted the voltage dependence of inactivation relationship by \(-10\) mV, from \(-80\pm1\) mV \((n=9)\) to \(-90\pm1\) mV \((n=5)\) and increased the slope factor from 4.9 to 7.7.

Figure 5. Use-dependent decay of peak \(\mu1-\beta_1\) and hH1-\(\beta_1\) Na currents in the absence (open symbols) and presence of lidocaine (700 \(\mu\)M or 300 \(\mu\)M, solid symbols). The voltage pulse protocols shown illustrate that a train of short (10 ms, upper panels) or long (1 s, lower panels) step depolarizations (0.5 Hz) were initiated after a long rest to mimic the action potential durations of skeletal muscle and heart, respectively. Measurements of peak currents were normalized to the first pulse current and plotted as a function of pulse number. The lidocaine data were normalized to first pulse current in presence of lidocaine (=1) even though these currents measured \(\sim60\%\) of the drug-free currents. This was done to compare the amount of use-dependent block which developed in the absence and presence of lidocaine. Use-dependent decay of current induced by lidocaine is in addition to the drug-free use-dependent current decay and is illustrated as the difference between the open and solid symbols. Lidocaine induced use-dependent decay of peak hH1-\(\beta_1\) Na currents by trains of short and long pulses equals 12\% (10-ms pulses) and 51\% (1-s pulses) and is greater than that of \(\mu1-\beta_1\) channels (6 and 16\%).
larizations to \(-20\) mV, a potential which is positive enough to activate and then inactivate most channels (Fig. 1). Currents were elicited by short (10 ms) or long (1 s) pulses applied every 2 s. A long rest (1 min) at \(-100\) mV between pulse trains sufficed to allow reversal of the effects of previous trains.

In the absence of drug, peak \(\mu_1\beta_1\) Na current elicited by the second pulse in a train of short or long pulses equaled \(98 \pm 1\%\) and \(92 \pm 3\%\) of the first pulse current (Fig. 5). No additional decay of peak current occurred during trains of short pulses. However, trains of long pulses did produce additional drug-free use dependence of peak \(\mu_1\beta_1\) current which decayed to \(83 \pm 4\%\) of the post-rest current by pulse 20. Exposure to lidocaine (700 \(\mu\)M) produced tonic block evident by reduction of the first pulse current to \(71\%\) (short pulses) and \(69\%\) (long pulses) of control (not shown). Additional use-dependent block developed during the pulse trains, reducing peak \(\mu_1\beta_1\) current to \(93 \pm 3\%\) (short pulses) and \(67 \pm 4\%\) (long pulses) of the partially blocked first pulse current by the 20th pulse in the train. We define the use-dependent decay of peak \(\mu_1\beta_1\) current attributed to block by lidocaine as equal to the amount of decay in peak current measured in the presence of drug minus that in the absence of drug. Accordingly, use-dependent block of \(\mu_1\beta_1\) Na channels by lidocaine equaled \(5\%\) (0.98-0.93) during a train of short pulses and \(16\%\) (0.83-0.67) during a train of long pulses.

Prolonging the depolarization duration to 1 s increased the amount of block, which again is consistent with the idea that lidocaine binds with high affinity to the inactivated state. Although the interval between short pulses and long pulses was not the same (1,990 ms vs 1,000 ms), the increased block occurred from increased binding during depolarization and not because of differences in the intervals between pulses when channels reprimed. Drug-free \(\mu_1\beta_1\) channels reprimed very rapidly after a 1-s prepulse to \(-20\) mV (see Fig. 6).

The same protocols were used to measure use-dependent block of \(hH1\beta_1\) current by lidocaine (Fig. 5). In the absence of drug, trains of short- and long-duration pulses reduced peak \(hH1\beta_1\) current to \(99 \pm 1\%\) and \(84 \pm 2\%\) of the post-rest current. In the presence of lidocaine (300 \(\mu\)M), repeated stimulation by short and long pulses reduced peak \(hH1\beta_1\) current to \(87 \pm 5\%\) and \(53 \pm 9\%\) of the first pulse partially blocked current. Use-dependent block of \(hH1\beta_1\) current by lidocaine equaled \(12\%\) (0.99-0.87) by the end of a train of short pulses and \(31\%\) (0.84-0.53) during a train of long pulses. It is notable that 300 \(\mu\)M lidocaine produced a greater amount of use-dependent block of \(hH1\beta_1\) Na channels than did 700 \(\mu\)M lidocaine on \(\mu_1\beta_1\) Na channels (see Discussion).

Lidocaine Prolongs Repriming, Particularly in \(hH1\beta_1\) Channels

In addition to quantifying use dependence at a single frequency, we measured directly the effects of lidocaine on recovery from inactivation. The time course of repriming at \(-100\) mV, after 1-s depolarizations to \(-20\) mV, was first tracked in the absence of drug and then repeated in the presence of sufficient lidocaine to block approximately half of the channels (300 \(\mu\)M for \(hH1\beta_1\), 700 \(\mu\)M for \(\mu_1\beta_1\)). Fig. 6 shows that, even in the absence of drug, the repriming of \(hH1\beta_1\) Na channels is slower than that of \(\mu_1\beta_1\) (see also Nuss et al., 1995). The differences are particularly apparent after short coupling intervals: \(hH1\beta_1\) exhibits an initial delay in re-

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Figure 6. Recovery from inactivation of \( \mu_1\)-fl and hH1-B\( 1 \) Na currents in the absence and presence of lidocaine (700 or 300 \( \mu \)M). Repriming of \( \mu_1\)-fl and hH1-B\( 1 \) Na channels at \(-100\) mV was assessed using a standard two-pulse protocol (see voltage pulse). Channels were inactivated by a 1 s prepulse to \(-20\) mV and after a variable recovery interval tested at \(-20\) mV (40 ms). The repriming data for drug-free channels were best fit by three exponentials. The time constants were obtained by repetitive fitting sessions and then fixed while the amplitudes of each exponential were allowed to float for the final fitting. Drug-free \( \mu_1\)-fl channels recovered from inactivation with a very rapid time-course and without any delay (see inset at faster time base). The fitting parameters are: \( t_1 = 1\) ms, \( A_1 = -0.60\), \( t_2 = 100\) ms, \( A_2 = -0.23\), \( t_3 = 900\) ms, \( A_3 = -0.17\). Lidocaine (700 \( \mu \)M) acted on \( \mu_1\)-fl channels by slowing the rate of repriming such that the majority of repriming occurred with a time constant of 175 ms (\( t_1 = 6.6\) ms, \( A_1 = -0.23\), \( t_2 = 175\) ms, \( A_2 = -0.58\), \( t_3 = 900\) ms, \( A_3 = -0.19\)). In the absence of lidocaine, hH1-B\( 1 \) channels also reprimed rapidly but after a slight delay (1-2 ms). Drug-free repriming of hH1-B\( 1 \) channels was also best fit with three exponentials: \( t_1 = 4\) ms, \( A_1 = -0.45\), \( t_2 = 16\) ms, \( A_2 = -0.38\), \( t_3 = 900\) ms, \( A_3 = -0.17\). In contrast to results in \( \mu_1\)-fl channels, lidocaine (300 \( \mu \)M) greatly prolonged the initial delay (deactivation) before any repriming occurred up to 20 ms (see inset at faster time base) and then proceeded at a very slowed rate. Repriming of hH1-B\( 1 \) channels in the presence of lidocaine could be described by two exponentials with slow time constants (\( t_1 = 427\) ms, \( A_1 = -0.86\), \( t_2 = 6443\) ms, \( A_2 = -0.14\)).

Repriming, while no delay can be resolved for the beginning of recovery of \( \mu_1\)-fl (Fig. 6, see insets at fast time base). Although real, such differences in the absence of drug are dwarfed by those that emerge when lidocaine is added. Cardiac channels recover very slowly in the presence of lidocaine: 400 ms must transpire before a majority of the channels are again available to open. In contrast, \( \mu_1\)-fl channels exposed to lidocaine reprimed with a half time of only 100 ms.

It should be noted that the hH1-B\( 1 \) channels recovered much more slowly despite being exposed to a lower concentration of lidocaine than the \( \mu_1\)-fl channels. Thus,
these kinetic differences do not merely reflect an increase in the number of drug-bound hH1-β1 channels. The cardiac isoform-specific accentuation of lidocaine block revealed by Fig. 6 may have important implications for antiarrhythmic efficacy in vivo. At the short coupling intervals typical of ventricular arrhythmias that complicate cardiac ischemia and reperfusion (Wit and Janse, 1993), extrasystoles would be much more effectively suppressed in cardiac Na channels than in those from skeletal muscle.

**Lidocaine Sensitivity of Rested vs Inactivated States**

The sensitivity of the rested state to block by lidocaine was estimated by applying lidocaine at a negative holding potential and measuring tonic block (Fig. 7). A holding potential of −100 mV was selected so that most channels would start off in their resting state (see Figs. 3 and 4). A lidocaine dose-response relationship (Fig. 7) was constructed by exposing oocytes expressing hH1-β1 and μ1-β1 Na channels to increasing concentrations of lidocaine from 3 μM to 3 mM and using infrequent pulses to elicit Na currents. Oocytes were exposed to each concentration of lidocaine for at least 3 min, during which intermittent pulses were used to assay its effect on peak \( I_{\text{Na}} \). Each concentration of lidocaine was applied long enough for peak \( I_{\text{Na}} \) to reach a steady state. Peak currents were measured after 45 s of rest to

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**Figure 7.** Rested-state lidocaine dose-response relationships for cardiac (hH1-β1) and skeletal muscle (μ1-β1) Na channels are compared. All channels were fully reprimed at the −100 mV holding potential. Oocytes were exposed to concentrations of lidocaine ranging from 3 μM to 3 mM while infrequent test pulses to −20 mV indicated when a steady state blocking condition was achieved. Low concentrations of lidocaine (3–30 μM) did little to reduce both hH1-β1 and μ1-β1 Na current. Higher concentrations of lidocaine were more effective at blocking hH1-β1 Na channels (EC\(_{50} = 402 ± 36 \mu M\), \( n = 4–22 \)) than μ1-β1 channels (1,168 ± 34 μM, \( n = 7–19 \)). A power logistic function fit the mean data (Origin, MicroCal Software Inc., Northampton, MA). Representative currents shown in the upper panels were recorded at equivalent lidocaine concentrations (0, 30, 100, 300, 700, 1,000, and 3,000 μM). In a few instances in which a small amount of run-up of \( I_{\text{Na}} \) (<3%) occurred during exposure to a low concentration of lidocaine, the lidocaine recording solution was washed out and another control measurement of peak \( I_{\text{Na}} \) was made. The amount of block by lidocaine was determined by normalizing peak \( I_{\text{Na}} \) recorded during exposure to a given concentration of lidocaine to the average of the control and washout measurements which sandwiched that particular measurement.
avoid extra use-dependent block. Low concentrations of lidocaine (3–30 μM) had little blocking effect on either hH1-β1 or μ1-β1 Na channels: lidocaine binding to the resting state is weak. At higher concentrations, lidocaine was more effective at reducing hH1-β1 Na currents than μ1-β1 currents. The apparent EC₅₀ for rested state block of hH1-β1 Na channels equaled 402 ± 36 μM (n = 4–22), while the EC₅₀ for lidocaine block of μ1-β1 Na channels in the rested state was 1,168 ± 34 μM (n = 7–19). Thus, approximately a twofold higher concentration of lidocaine is required to block μ1-β1 Na channels compared to hH1-β1 Na channels (p < 0.002).

The sensitivity of the inactivated state to block by lidocaine was also compared in hH1-β1 and μ1-β1 Na channels. The strategy followed that of Bean et al. (1983): partially depolarized holding potentials were selected so that ~80% of all channels would be steady state inactivated (see Figs. 3 and 4). Oocytes expressing μ1-β1 Na channels were held at −55 mV and those expressing hH1-β1 Na channels were held at −70 mV (hₐ = 0.2 in either case). As described above, oocytes were exposed to concentrations of lidocaine ranging from 3 to 700 μM while infrequent test pulses to −20 mV indicated when a steady state blocking condition had been achieved. Under these conditions, block arises mostly from lidocaine binding to inactivated channels. The enhanced affinity for lidocaine binding to the inactivated state is demonstrated by EC₅₀ values much lower than those obtained at negative holding potentials (Fig. 8). In contrast to the isoform-specific differences in rested-state block, the EC₅₀'s for lidocaine binding to the inactivated state of hH1-β1 (16 ± 1 μM, n = 3–9) and μ1-β1 Na channels (12 ± 2 μM, n = 4–11) are similar (P = NS).

**Influence of Holding Potential on Tonic Block**

Given that lidocaine binds more tightly to the inactivated state, we compared the amount of block produced by 300 μM lidocaine using a holding potential of −120
mV, where inactivation was completely removed, to block at the −100 mV holding potential where a small fraction of channels may have been inactivated (Fig. 4). The reduction of first pulse hH1-β1 Na current was slightly greater when the holding potential was set at −100 mV. Lidocaine (300 μM) reduced peak hH1-β1 Na current to 60 ± 5% and 67 ± 6% (n = 3) of the drug-free current elicited from −100 and −120 mV, respectively. The observation that tonic block was reduced by 7% at the more negative holding potential confirmed suspicions that a small fraction of hH1-β1 channels were inactivated at −100 mV.

We used the results of this experiment to estimate empirically the actual dissociation constant for lidocaine binding to the rested state of hH1-β1 channels. The dose-response curve generated by a logistic fit to the mean data obtained using −100 mV as the holding potential (Fig. 7, dotted line) was shifted to the right until it intersected the new data indicating the amount of block produced by applying 300 μM lidocaine at −120 mV (not shown). Via this manipulation, the dissociation constant for lidocaine block of hH1-β1 channels when inactivation is completely removed equals 486 μM. Despite the underestimation of lidocaine sensitivity obtained at −100 mV, hH1-β1 and μL-β1 Na channels still differ substantially in their rested-state lidocaine affinities. Support for this interpretation is bolstered by the observation that the differences in sensitivity to lidocaine between hH1-β1 and μL-β1 channels persist even after 1-s prepulses to −140 mV (Figs. 3 and 4).

**DISCUSSION**

*Lidocaine Preferentially Blocks Cardiac Na Channels via Differences in Intrinsic Sensitivity or Duty Cycle?*

Given that lidocaine's main action is the blockade of Na channels, the question remains: how does lidocaine act as an effective antiarrhythmic without producing muscle and nerve paralysis? Two mechanisms have been postulated. In the first scenario, Na channels in skeletal muscle or nerve are intrinsically less sensitive to block by lidocaine compared to those in cardiac muscle. If true, one would expect that structural differences in the lidocaine binding site exist in cardiac and skeletal muscle Na channels and that such differences may help to localize the receptor. In the second hypothesis, the combination of an action potential that is dramatically longer in cardiac muscle than in skeletal muscle with high-affinity binding of lidocaine to the inactivated state produces preferential block of cardiac Na channels. If the plateau phase of the action potential represents a time during which most Na channels are in the inactivated state, lidocaine has greater opportunity to bind avidly to Na channels in cardiac muscle than in tissues with brief, sporadic action potentials. Support for the latter hypothesis is founded on comparisons of lidocaine dissociation constants from studies in different native preparations. On the basis of such comparisons, cardiac Na channels have been said to resemble those in skeletal muscle and nerve with respect to lidocaine binding (Bean et al., 1983). One should be careful when comparing dissociation constants between different studies because variable experimental conditions, including voltage protocols, temperature and ionic conditions, influence lidocaine binding (Hille, 1977b; Schwarz et al., 1977; Makielski and Falleroni, 1991; Wendt et al., 1993). In addition,
differences in the cellular environment among various native preparations may in part determine the sensitivity of native channels to block by lidocaine.

We have explored the possibility that the intrinsic sensitivity of the two Na channel isoforms to lidocaine is different, as is known to be the case for tetrodotoxin binding. Until this study, there had not been a head-to-head comparison of lidocaine binding affinities in cardiac and skeletal muscle Na channels that are unmodified and independent of a host cell's influences. We used the oocyte expression system to compare the sensitivity to block by lidocaine of cardiac (hH1-β1) and skeletal muscle (μ1-β1) Na channels expressed in oocytes such that the only difference would be the choice of the α subunit. Under identical experimental conditions, hH1-β1 Na channels were threefold more sensitive to rested-state block by lidocaine than were μ1-β1 Na channels, whereas inactivated-state block was similar in hH1-β1 and μ1-β1 Na channels. These results provide the first evidence that the lidocaine binding site in cardiac Na channels may be structurally different from its counterpart in skeletal muscle channels (see Discussion).

Verification of Rested-State Binding Affinity

Using a modulated-receptor model (Hille, 1977a; Hondeghem and Katzung, 1977) to describe 1:1 lidocaine binding and equilibrium between resting and inactivated states, the relationship between the apparent dissociation constant (Kapp) and the actual dissociation constants for rested (Kr) and inactivated states (Kι) is $1/K_{\text{app}} = h/K_r + (1-h)/K_\iota$, where $h$ and $1-h$ represent the fraction of channels populating the resting and inactivated states, respectively (Bean et al., 1983). The actual dissociation constant for lidocaine binding to the inactivated state (Kι) equals 14 μM when $K_r$ is set to 486 μM (−120 mV) and $K_{\text{app}}$ is 16 μM ($h = 0.16$ at −70 mV). Using the true state-specific dissociation constants ($K_r = 486$ μM, $K_\iota = 14$ μM) and setting $K_{\text{app}}$ equal to 402 μM (the experimentally measured half-blocking concentration at $V_h = −100$ mV), the fraction of channels in the rested state when holding at −100 mV was calculated to be 99.4% ($h = 0.994$). By comparison, the drug-free availability data (Fig. 4) indicate that the fraction of channels residing in the resting state at −100 mV equals 98.6% ($h = 0.986$). The discrepancy is small (0.8%) and certainly within the bounds of experimental error considering that the availability experiments and the dose-response experiments were performed on different populations of oocytes. Even if 1% of channels had been inactivated, the rested state affinity ($K_r$) for lidocaine binding to hH1-β1 calculated with the above equation would have equaled 532 μM: hH1-β1 channels would still be significantly (220%) more sensitive to tonic block by lidocaine than are μ1-β1 channels.

Verification of Inactivated State Binding Affinity

The observation that lidocaine induces a negative shift in the voltage dependence of inactivation is evidence for lidocaine binding with high affinity to the inactivated state (Hondeghem and Katzung, 1977; Bean et al., 1983). Again using a model based on the modulated-receptor hypothesis with equilibrium binding of lidocaine to resting and inactivated states, the shift in the availability curve induced by a given concentration of lidocaine is $\Delta V_h = k \ln \left( \frac{(1 + [L]/K_r)/(1 + [L]/K_\iota)}{1 + [L]/K_\iota} \right)$, where $k$ is the
slope factor of the Boltzmann function which describes the availability data and \([L]\) is the lidocaine concentration (Bean et al., 1983). Instead of using this relationship to predict the amount of shift in the availability curves, we employed it to calculate the dissociation constants for inactivated state binding. Although this method is less direct than determining \(K_a\) from measurements of lidocaine block at a partially depolarized holding potential, we use it here as an internal consistency check on those results. For \(hH1-\beta_1\) Na channels, 300 \(\mu\)M lidocaine shifted the midpoint of availability curve from \(-80\) mV \((k = 4.9)\) to \(-91\) mV \((\Delta V_h = -11\) mV, Fig. 4). With \(K_R\) equal to 486 \(\mu\)M, the dissociation constant for inactivated state binding calculates to 20 \(\mu\)M. For \(\mu1-\beta_1\) channels, 700 \(\mu\)M lidocaine shifted the midpoint of the availability curve from \(-60\) mV \((k = 5.2)\) to \(-80\) mV \((\Delta V_h = -20\) mV, Fig. 3). In this case, \(K_R\) equaled 1,168 \(\mu\)M and the estimated dissociation constant for inactivated state binding equals 10 \(\mu\)M. The values of \(K_a\) calculated from the lidocaine-induced shifts in the availability curve compare favorably to the values of \(K_a\) measured directly (16 \(\mu\)M for \(hH1-\beta_1\) and 12 \(\mu\)M for \(\mu1-\beta_1\) channels, Fig. 8).

**Comparison of \(K_R\) and \(K_i\) to Other Studies**

The salient results of our study are summarized in Fig. 9. The dissociation constant for rested state lidocaine binding to \(hH1-\beta_1\) channels \((K_R)\) equals 486 \(\mu\)M. Similar affinities have been determined for cardiac cells by direct measurements of Na currents in rabbit Purkinje fibers (440 \(\mu\)M) (Bean et al., 1983), guinea-pig ventricular myocytes (540 \(\mu\)M) (Wasserstrom, Liberty, Kelly, Santucci, and Myers, 1993) and *Xenopus* oocytes expressing rat heart (RH1) \(\alpha\) subunits (420 \(\mu\)M) (Satin, Kyle, Rogart, and Makielski, 1994) and by \([3H]batrachotoxinin\ A 20-\alpha\)-benzoate binding in rat cardiac myocytes (455 \(\mu\)M) (Hill, Duff, and Sheldon, 1989). Other studies have
reported rather different lidocaine affinities, for example 1.2 mM in canine Purkinje cells (Alpert, Fozzard, Hanck, and Makielski, 1989) and 226 μM for oocytes injected with cRNA encoding only the hH1 α subunit (Chahine, Chen, Bar-chi, Kallen, and Horn, 1992); it is likely that the variability among determinations of lidocaine sensitivity arise because of differences in experimental conditions. This underscores the value of comparing lidocaine affinities in a heterologous expression system where the only difference between Na channel isoforms is the α subunit.

Voltage Dependence of Inactivation and Membrane Potential in Diastole

The enhanced sensitivity of cardiac Na channels to block by lidocaine may partly be determined by the value of the resting membrane potential. The range of membrane potentials at which cardiac muscle cells are maintained during diastole is nearly identical to the half-inactivation potential for hH1-β1 channels (−80 mV, Fig. 4). During the diastolic interval, Na channels distribute themselves between resting and inactivated states in accordance to their voltage dependence of inactivation relationship. As such, a small change in resting membrane potential in the depolarizing direction would increase the fraction of channels populating the inactivated state and increase the sensitivity of the entire population of channels to block by lidocaine. In contrast, lidocaine sensitivity of skeletal muscle with its voltage dependence of inactivation centered at −60 mV (Fig. 3) would not be dramatically influenced by a small depolarization of the resting membrane potential.

Increased Use-dependent Block in Cardiac Channels

Lidocaine produced more use-dependent block of peak hH1-β1 Na current (12%) during trains of short depolarizations (10 ms, 0.5 Hz, −20 mV) than of μ1-β1 Na current (6%). Prolonging the depolarization (1 s) increased the amount of use-dependent block of both hH1-β1 (31%) and μ1-β1 (16%) channels, consistent with lidocaine binding with high affinity to the inactivated state. If “tonic block occludes use-dependent block” (Bean et al., 1983), then one would predict less use-dependent block in hH1-β1 channels because 300 μM lidocaine produces more tonic block of hH1-β1 channels than does 700 μM lidocaine on μ1-β1 channels. Instead, we observed the opposite result (Fig. 5). As with μ1-β1 channels, the increased use-dependent decay of hH1-β1 current primarily reflects increased binding during depolarization, because drug-free hH1-β1 channels rapidly recover from inactivation: recovery from a 1-s prepulse (−20 mV) is 93% complete after a 1-s recovery interval. Even larger differences in the amounts of use-dependent block between hH1-β1 and μ1-β1 channels would be expected under conditions where the contribution of tonic block is reduced (at lower concentrations of lidocaine) and at faster stimulation rates.

Slow Recovery from Inactivation in hH1-β1, Channels Exposed to Lidocaine

We have observed striking isoform-specific differences in the ability of hH1-β1 and μ1-β1 channels to recover from inactivation, particularly in the presence of lidocaine (Fig. 6). Relatively minor differences in the initial rates of repriming in the
absence of drug are magnified in its presence. Recent experimental and theoretical work by Kuo and Bean (1994) may help to rationalize the basis for such marked isoform-specific differences in drug sensitivity. These workers postulated that recovery from inactivation is tightly linked to deactivation: the binding of an inactivation particle to Na channels becomes progressively weaker as more of the m gates return to their resting position. In this framework, local anesthetics (diphenylhydantoin in the case of Kuo and Bean, 1994) might act simply as surrogate inactivation gates; in so doing, the rate of recovery from drug block would become very voltage-dependent. Thus, the differences in repriming that we observe between the two isoforms may reflect relatively small shifts in the voltage dependence of deactivation, apparent but subtle in the absence of drug, which are magnified in the presence of lidocaine. New lines of experimentation will be required to test this interpretation. To the degree that it is correct, the Kuo and Bean (1994) model points out one important limitation of the present analysis: not all rested states exhibit equal affinities to drug, calling into question the practice of lumping all R states together (as in classical modulated receptor theory).

**Structural Implications**

While our results indicate that structural differences in the α subunit underlie the differences in lidocaine sensitivity and repriming in the presence of lidocaine, they only indirectly suggest which specific regions of the molecule might be responsible. The affinities for binding to the inactivated state are nearly identical. Perhaps not coincidentally, the region that mediates inactivation (the III-IV linker) is amongst the most conserved in the various Na channel isoforms (Patton, West, Catterall, and Goldin, 1992). The structural determinants of available but nonconducting (i.e., resting) states are less clear. By site-directed mutagenesis of rat brain type IIA Na channels, Ragsdale, McPhee, Scheuer, and Catterall (1994) have localized various residues that may delineate the local anesthetic receptor site. Single point mutations of naturally occurring residues for alanine in transmembrane segment S6 of domain IV increased tonic block by etidocaine without altering the affinity for the inactivated state at three sites (I1761, V1766, N1769) and decreased use-dependent block at three other locations (I1760, F1764, Y1771) (Ragsdale et al., 1994). Given that rested state binding and the repriming rate of drug-bound channels represent the two most prominent differences in lidocaine sensitivity between hH1-β1 and μ1-β1, we compared aligned sequences of rat brain IIA, μ1 and hH1 Na channels and found that the six residues implicated by Ragsdale et al. (1994) are conserved amongst all three Na channel isoforms. While two amino acids within the S6 segment of domain IV do differ between μ1 and hH1, mutation of the equivalent residues in the rat brain IIA channel (V1757 and S1758) to alanine had only small effects on the sensitivity to etidocaine (Ragsdale et al., 1994). These results suggest that the relevant differences between cardiac and skeletal muscle channels reside in as-yet indeterminate structures. Thus, examination of tonic block and repriming in various chimeric hH1-μ1 constructs, including IV-S6 and III-IV linker chimeras, may further delineate the lidocaine binding site and the nature of its interactions with the gating machinery.
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REFERENCES

Abete, P., N. Ferrara, F. Rengo, and M. Vassalle. 1991. Mechanisms of lidocaine actions on normal and abnormal rhythms in canine cardiac tissues in vivo and in vitro. Clinical and Experimental Pharmacology and Physiology. 18:179–191.

Alpert, L. A., H. A. Fozzard, D. A. Hanck, and J. C. Makielaki. 1989. Is there a second external lidocaine binding site on mammalian cardiac cells? American Journal of Physiology. 257:H79–H84.

Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. The Journal of General Physiology. 58:413–437.

Backx, P. H., D. T. Yue, J. H. Lawrence, E. Marban, and G. F. Tomaselli. 1992. Molecular localization of an ion-binding site within the pore of mammalian sodium channels. Science. 257:248–251.

Bean, B. P., C. J. Cohen, and R. W. Tsien. 1983. Lidocaine block of cardiac sodium channels. The Journal of General Physiology. 81:615–642.

Chahine, M., L. Q. Chen, R. L. Barchi, R. G. Kallen, and R. Horn. 1992. Lidocaine block of human heart sodium channels expressed in Xenopus oocytes. Journal of Molecular and Cellular Cardiology. 24:1231–1236.

Clarkson, C. W., R. E. Follmer, R. E. Ten Eick, L. M. Hondeghem, and J. Z. Yeh. 1988. Evidence for two components of sodium channel block by lidocaine in isolated cardiac myocytes. Circulation Research. 63:615–628.

Gellens, M. E., A. L. George, L. Chen, M. Chahine, R. Horn, R. L. Barchi, and R. G. Kallen. 1992. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proceedings of the National Academy of Sciences, USA. 89:554–558.

Gingrich, K. J., D. Beardsley, and D. T. Yue. 1993. Ultra-deep blockade of Na+ channels by quaternary ammonium ion: catalysis by a transition-intermediate state? Journal of Physiology. 471:319–341.

Hill, R. J., H. J. Duff, and R. S. Sheldon. 1989. Class I antiarrhythmic drug receptor: biochemical evidence for state-dependent interaction with quinidine and lidocaine. Molecular Pharmacology. 36:150–159.

Hille, B. 1977a. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. The Journal of General Physiology. 69:497–515.

Hille, B. 1977b. The pH-dependent rate of action of local anesthetics on the node of Ranvier. The Journal of General Physiology. 69:475–496.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. Journal of Physiology. 117:500–544.

Hondeghem, L. M., A. O. Grant, and R. A. Jensen. 1974. Antiarrhythmic drug action: selective depression of hypoxic cardiac cells. American Heart Journal. 87:602–605.

Hondeghem, L. M., and B. G. Katzung. 1977. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochimica et Biophysica Acta. 472:373–398.

Kuo, C.-C., and B. P. Bean. 1994. Na+ channels must deactivate to recover from inactivation. Neuron. 12:819–829.

Krafte, D. S., W. A. Volberg, K. Dillon, and A. M. Ezrin. 1991. Expression of cardiac Na channels with appropriate physiological and pharmacological properties in Xenopus oocytes. Proceedings of the National Academy of Sciences, USA. 88:4071–4074.
Lazzara, R., R. R. Hope, N. El-Sherif, and B. J. Scherlag. 1978. Effects of lidocaine on hypoxic and ischemic cardiac cells. *The American Journal of Cardiology*. 41:872–879.

Makielski, J. C., and M. J. Falleroni. 1991. Temperature dependence of sodium current block by lidocaine in cardiac Purkinje cells. *American Journal of Physiology*. 260:H681–H689.

Moczydlowski, E., A. Uehara, X. Guo, and J. Heiny. 1986. Isochannels and blocking modes of voltage-dependent sodium channels. *Annuals New York Academy of Sciences*. 479:269–292.

Nuss, H. B., N. Chiamvimonvat, M. T. Pérez-García, G. F. Tomaselli, and E. Marban. 1995. Functional Association of the β Subunit with Human Cardiac (hH1) and Rat Skeletal Muscle (µ1) Sodium Channel α subunits Expressed in *Xenopus* Oocytes. *The Journal of General Physiology*. 106:1171–1191.

Patton, D. E., J. W. West, W. A. Catterall, and A. L. Goldin. 1992. Amino acid residues required for fast Na⁺ channel inactivation: charge neutralizations and deletions in the III–IV linker. *Proceedings of the National Academy of Sciences, USA*. 89:10905–10909.

Podrid, P. J. 1989. Aggravation of arrhythmia: a complication of antiarrhythmic drug therapy. *European Heart Journal*. 10:66–72.

Ragsdale, D. S., J. C. McPhee, T. Scheuer, and W. A. Catterall. 1994. Molecular determinants of state-dependent block of Na⁺ channels by local anesthetics. *Science*. 265:1724–1728.

Satin, J., J. W. Kyle, M. Chen, R. B. Rogart, and H. A. Fozzard. 1992. The cloned cardiac Na channel α-subunit expressed in *Xenopus* oocytes show gating and blocking properties of native channels. *The Journal of Membrane Biology*. 130:11–22.

Satin, J. C., J. W. Kyle, R. B. Rogart, and J. C. Makielski. 1994. Lidocaine block of heart and brain Na channel isoforms. *The Journal of General Physiology*. 104:18a. (Abstr.)

Schwarz, W., P. T. Palade, and B. Hille. 1977. Local anesthetics: effect of pH on use-dependent block of sodium channels in frog muscle. *Biophysical Journal*. 20:343–368.

Trimmer, J. S., S. S. Cooperman, S. A. Tomiko, J. Zhou, S. M. Crean, M. B. Boyle, R. G. Kallen, A. Sheng, R. L. Barchi, F. J. Sigworth, R. H. Goodman, W. S. Agnew, and G. Mandel. 1989. Primary structure and functional expression of a mammalian skeletal muscle sodium channel. *Neuron*. 3:33–49.

Wasserstrom, J. A., K. Liberty, J. Kelly, P. Santucci, and M. Myers. 1993. Modification of cardiac Na⁺ channels by batrachotoxin: effects on gating, kinetics, and local anesthetic binding. *Biophysical Journal*. 65:386–395.

Wendt, D. J., C. F. Starmer, and A. O. Grant. 1993. pH dependence of kinetics and steady-state block of cardiac sodium channels by lidocaine. *American Journal of Physiology*. 264:H1588–H1598.

Wit, A. L., and M. J. Janse. 1993. Ventricular arrhythmias in the acute phase of myocardial ischemia and infarction. In *The Ventricular Arrhythmias of Ischemia and Infarction*. Futura Publishing Co., Inc., Mount Kisco, NY. 161–260.