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Distinct Local Anesthetic Affinities in Na^+ Channel Subtypes

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ABSTRACT Lidocaine is a widely used local anesthetic and antiarrhythmic drug that is believed to exert its clinically important action by blocking voltage-gated Na^+ channels. Studies of Na^+ channels from different species and tissues and the complexity of the drug–channel interaction create difficulty in understanding whether there are Na^+ channel isoform specific differences in the affinity for lidocaine. Clinical usage suggests that lidocaine selectively targets cardiac Na^+ channels because it is effective for the treatment of arrhythmias with few side effects on muscle or neuronal channels except at higher concentrations. One possibility for this selectivity is an intrinsically higher drug-binding affinity of the cardiac isoform. Alternatively, lidocaine may appear cardioselective because of preferential interactions with the inactivated state of the Na^+ channel, which is occupied much longer in cardiac cells. Recombinant skeletal muscle (hSkM1) and cardiac sodium channels (hH1) were studied under identical conditions, with a whole-cell voltage clamp used to distinguish the mechanisms of lidocaine block. Tonic block at high concentrations of lidocaine (0.1 mM) was greater in hH1 than in hSkM1. This was also true for use-dependent block, for which 25-μM lidocaine produced an inhibition in hH1 equivalent to 0.1 mM in the skeletal muscle isoform. Pulse protocols optimized to explore inactivated-state block revealed that hSkM1 was five to eight times less sensitive to block by lidocaine than was hH1. The results also indicate that relatively more open-state block occurs in hSkM1. Thus, the cardiac sodium channel is intrinsically more sensitive to inhibition by lidocaine.

INTRODUCTION

Lidocaine is a widely used local anesthetic and antiarrhythmic drug that is believed to exert its clinically important action by blocking voltage-gated Na^+ channels (see Hille, 1992). The pattern of block that occurs is both time and voltage dependent with multiple components. These complex dynamics can be summarized in models in which block depends on channel state (Hille, 1977; Hondeghem and Katzung, 1977).

Molecular genetics and cloning have revealed the existence of at least five unique voltage-gated Na^+ channels encoded by distinct genes (Noda et al., 1984, 1986; Kayano et al., 1988; Rogart et al., 1989; Trimmer et al., 1989; Gellens et al., 1992). In adult human skeletal muscle, the dominant form of the voltage-gated Na^+ channels (hSkM1) is encoded by a gene on chromosome 17q23–25 (SCN4A) (George et al., 1992). The cardiac isoform (hH1) is represented on chromosome 3p21 (SCN5A) (George et al., 1995). Earlier studies (Strichartz, 1973; Hille, 1977; Schwarz et al., 1977; Cahalan and Almers, 1979; Bean et al., 1983; Starmer et al., 1984; Sanchez-Chapula et al., 1989; Bennett et al., 1988; Clarkson et al., 1988; Grant et al., 1989; McDonald et al., 1989; Ragsdale et al., 1994; Kuo and Bean, 1994; Bennett et al., 1995a) utilized Na^+ channels from different species and tissues to characterize local anesthetic block. However, the complexity of action and the variety of different experimental preparations lead to a difficulty in understanding whether there are Na^+ channel isoform specific differences in the affinity for lidocaine. Therefore, we have directly compared block by lidocaine in heterologously expressed, recombinant human cardiac (hH1) and human skeletal muscle (hSkM1) Na^+ channels. We find that the cardiac sodium channel is intrinsically more sensitive to inhibition by lidocaine. Furthermore this higher intrinsic affinity is augmented by the dynamics of the cardiac cycle. The high-affinity inactivated state is occupied much longer in cardiac cells, which have very long action potentials relative to skeletal muscle, leading to a further kinetic enhancement of the cardiac selective block in vivo.

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MATERIALS AND METHODS

Voltage clamp methods

The methods used were described previously (Valenzuela and Bennett, 1994; Wang et al., 1995). Briefly, macroscopic sodium currents were recorded by the whole-cell method of the patch clamp technique (Hamill et al.,1981). Electrode resistances ranged from 0.8 to 2 MΩ. Voltage clamp command pulses were generated by pCLAMP software (v6.0; Axon Instruments, Inc., Foster, CA). Currents were filtered at 5 kHz (−3-dB, four-pole Bessel filter). An Axopatch 200 patch clamp amplifier was used with series resistance compensation. The standard holding potential for all pulse protocols was -120 mV. Data are presented as mean ± SE. Experiments were carried out at room temperature (20–22°C).

Solutions

The bath solution contained 145-mM NaCl; 4-mM KCl; 1.8-mM $CaCl_2$; 1.0-mM $MgCl_2$; 10-mM HEPES; and 10-mM glucose, pH 7.35. The pipette solution contained (intracellular solution) 10-mM NaF; 110-mM CsF; 20-mM CsCl; 10-mM EGTA; and 10-mM HEPES, pH 7.2.

Transfection of hH1 and hSkM1 cDNA for expression in tsA-201 cells

A full-length hH1 coding sequence (nt. 151-6214) was constructed in the mammalian expression vector pRc/CMV (Invitrogen Corp., San Diego, CA) by subcloning from pSP64T-hH1 (Gellens et al. 1992) into the *Hind*III and *Xba*I sites of the vector. Construction of full-length pRc/CMV-hSkM1 was described previously (Chahine et al., 1994). Additional details are provided in Wang et al. (1995).

Transient transfection with calcium phosphate was used to express hSkM1 or hH1 in a transformed human kidney cell line (HEK 293; tsA-201) stably expressing the SV40 T-antigen. Cell transfection was carried out with 10 μg of plasmid DNA encoding channel.

Pulse protocols and data analysis

Sodium channels are known to exist in three primary classes of states: 1) preopen closed states, 2) open state(s), and 3) a nonconducting state or states occupied during depolarization (inactivated state).

The measured sodium current, I_{Na}, is given by

$$I_{Na}(t, v) = i \cdot NP_{open}(t, v), \quad (1)$$

where i is the unitary current, N is the number of channels, and P_{open} is the probability that a channel is open. The open probability can be defined as a convolution integral of three probability-density functions:

$$P_{open} = \int PDF_{OT} \times PDF_{L} \times PDF_{RO}\, dt, \quad (2)$$

where PDF_{OT}, PDF_{L},and PDF_{RO} are the probability-density functions for channel open times, latencies for first opening, and latencies for channel reopening. Thus, the time integral of $I_{Na}(t)$ at a fixed membrane potential will reflect this open probability scaled by the number of channels and the unitary current. Block is defined as the reduction of sodium current and could occur by reduction of any of the parameters in Eq. 1. This current–time integral is a sensitive measure of block. A blocking reaction that has a time course similar to channel gating will appear to alter the Na^+ current kinetics. In this case, one can distinguish block that has occurred before the peak I_{Na} from block that occurs later by measuring the change in area under the I_{Na}-time curve. Block that is slow relative to gating and that is fully established before opening of the channel will appear to decrease the number of functional channels. In this case peak I_{Na} and the area under the transient will scale down similarly. We can use this distinction to discriminate between block that affects the open state (disproportionate change in area versus peak) versus block that is established before opening (apparent decrease in N with current and area scaled down equally). We investigated lidocaine interactions with the inactivated state by first causing inactivation with a 500-ms prepulse. This duration is long enough to allow significant drug association but short enough to avoid slow inactivation of the channels. A subsequent test pulse (V_{test}) to −20 mV was used to assess the fraction of unblocked channels.

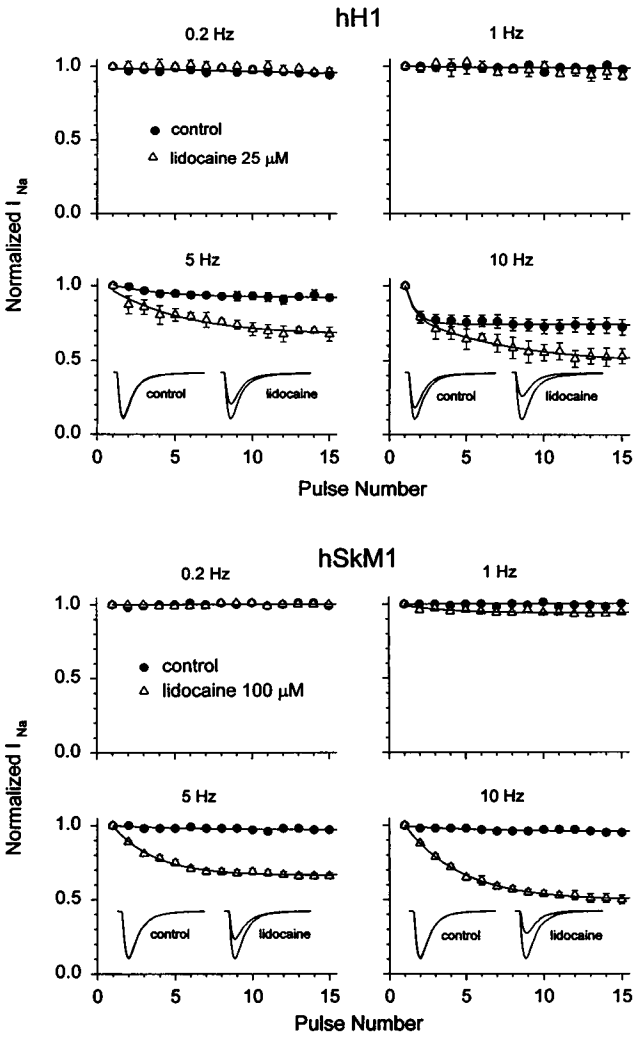

FIGURE 1 Use- and frequency- dependent block by lidocaine of hH1 and hSkM1. Trains of 20-ms pulses to −20 mV from a holding potential of −120 mV were applied at the rates shown. Preliminary studies had shown that significant block could be induced with 25-μM lidocaine in hH1. Therefore this concentration was used. With these brief pulses use-dependent block becomes apparent at 5 Hz for hH1 and hSkM1. The concentration of lidocaine used in the hSkM1 experiments was 100 μM. The symbols represent the means ± SE of 7–10 different cells. In some cases the standard error bars are smaller than the symbol size. The insets show Na^+ current recorded during the 1st and 15th voltage clamp steps for both control and lidocaine.

RESULTS

Frequency and use dependence

Fig. 1 compares use- and frequency-dependent block by lidocaine in each of the two Na^+ channel isoforms: hH1 and hSkM1. In these experiments trains of voltage clamp pulses were used to activate Na^+ channels repetitively in control and in the presence of lidocaine. At rates less than 1/s, there was no discernable block of either isoform. At a frequency of 5 Hz, 25-μM lidocaine produced approximately 30% inhibition of hH1 Na^+ current after 15 pulses (see Fig. 1). In contrast, to achieve approximately the same degree of block for hSkM1 at the same rate required 100-μM lidocaine. In this case 100-μM lidocaine produced approximately 35% block after 15 pulses. Additional block occurred at 10 Hz for hSkM1 and for hH1, although for hH1 measurement of the additional block was complicated by the development of slow inactivation in control. Thus, based on these train protocols under identical conditions, hH1 appears more sensitive to block than does hSkM1. However, as mentioned above, the particular nature of the pulse protocol and the relative amounts of time in each channel state may greatly influence the level of block. Therefore we

have employed pulse protocols to emphasize occupancy of different channel states.

Tonic block

We next compared block by using a high concentration of lidocaine (100 μM) and very infrequent pulsing. This protocol should reveal tonic block, which can be due to lidocaine interactions with rested or open states.

Fig. 2 shows current–voltage relationships for hH1 and hSkM1 in control and in lidocaine. Peak inward HH1 Na^+ current was reduced approximately 45% by 100-μM lidocaine. Less suppression was observed in hSkM1 at the same concentration. We also noted a difference in the shape of the Na^+ current transients after lidocaine exposure. Whereas the cardiac current appears simply scaled down by lidocaine, the modification of the hSkM1 Na^+ current was asymmetric. The rising phase occurred coincident with the control, and the decay appeared modified. Possible mechanisms for this differential effect are discussed below.

Inactivation kinetics

One possible consequence of open-channel block is a change in the rate of decay of the macroscopic Na^+ current (see Discussion). We analyzed the time course of decay of the macroscopic inactivation by fitting an exponential function to the decay of the macroscopic Na^+ current. The cardiac isoform was resolved into the sum of two exponentials, whereas the skeletal muscle isoform was well fitted by a single exponential function. The best-fitted time constants are shown in Fig. 3. Neither the biexponential nature of the cardiac isoform nor the rate of decay was significantly changed in the presence of lidocaine concentrations that

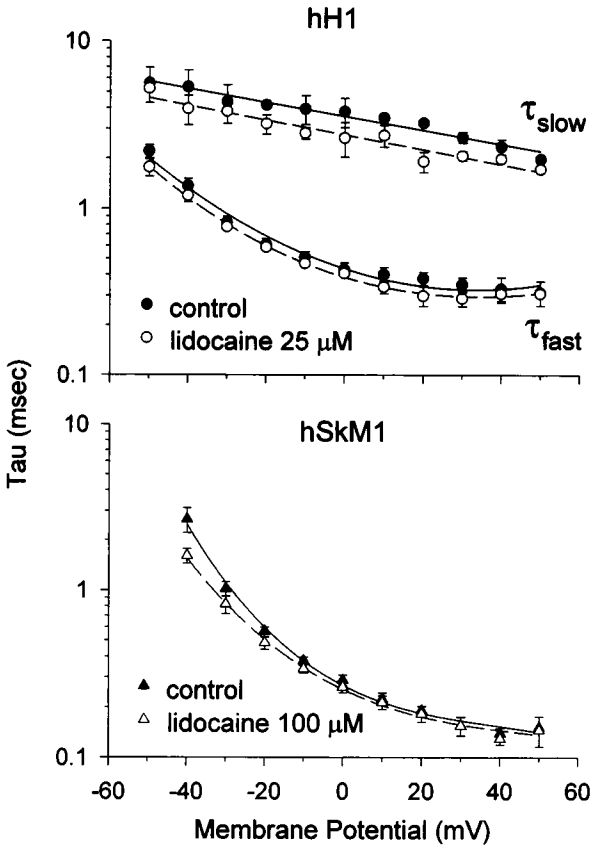

FIGURE 3 Time constants of decay of Na^+ current in control and in the presence of lidocaine. (*Top panel*) hH1 time constants resolved into two components. Symbols represent means, with error bars indicating the standard error of the mean. (*Bottom panel*) The time constant of decay of hSkM1 Na^+ current in control and in the presence of lidocaine. No statistically significant difference was detected in either time constant in the presence of lidocaine for either isoform.

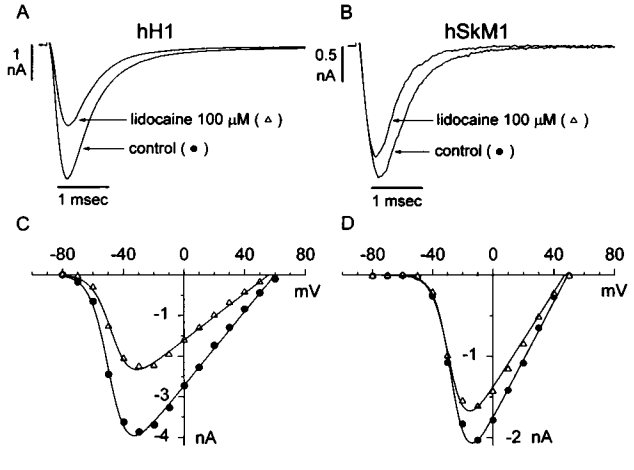

FIGURE 2 Tonic block by lidocaine for hH1 and hSkM1. *Top*: Na^+ current records obtained in control and in the presence of 100-μM lidocaine. The voltage clamp step was to −20 mV. The holding potential was −120 mV. *Bottom*: Cells were clamped to a holding potential of −120 mV and test pulses were applied to the potentials shown on the abscissae both in control and after 5 min of superfusion of 100-μM lidocaine. Pulses were delivered once every 30 s to prevent accumulation of use-dependent block.

clearly block the channel. Such changes are expected if block occurs by an open-channel mechanism (but see the Discussion). Likewise we could not resolve an accelerated or slowed decline in the current for the skeletal muscle isoform in the presence of 100-μM lidocaine. Much higher concentrations did cause an enhanced rate of decay consistent with open-channel block. At 300 and 1000 μM, the mean time constants at −20 mV were reduced to 0.31 ± 0.02 and 0.24 ± 0.03 ms ($p < 0.05$, $N = 5$), respectively, compared with the control time constant of 0.4 ± 0.03 ms. Likewise in hH1, an enhanced rate of decay was observed only with very high concentrations of lidocaine (100 μM). The two time constants were significantly reduced to 0.45 ± 0.06 and 1.5 ± 0.29 (τ_1, τ_2, $p < 0.05$; $N = 5$) from 0.6 ± 0.04 and 4.9 ± 1.25 ms, respectively. Measurements at higher concentrations in hH1 were not feasible because of the large degree of block. The expectation for various models of block and the effects that they would have on the rate of decay of the macroscopic Na^+ current are discussed below.

Development of block

Fig. 4 compares the time course of onset of block for hH1 and hSkM1. For hH1 we used an approximate therapeutic level of lidocaine (25 μM) as well as a much higher concentration (100 μM). To produce sufficient block in hSkM1 we used 100 μM. Fig. 4 shows normalized Na^+ current as a function of prepulsed duration plotted on a logarithmic axis. Under this transformation, an exponential time course appears sigmoidal. The time constant can be judged by the half-point for decline. To separate lidocaine block from inactivation, we interposed a 300-ms step to -120 mV between the prepulse and the test pulse to permit recovery of inactivated drug-free channels. More than 95% of control channels had recovered within 300 ms (data not shown). Therefore, reduction of current during the test pulse is due to lidocaine block. We were able to observe significant inhibition of cardiac current by lidocaine by using this protocol. The major component of the onset of block of hH1 channels had a time constant of 400 ms in 25-μM lidocaine, and this decreased to 65 ms in 100-μM lidocaine (see Fig. 4). Note that in the control the decline for durations longer than 1 s was due to slow inactivation of the channels. To control for this we made a ratio of current in the presence of lidocaine divided by current in the control. This transformation is shown in the bottom panels of Fig. 4 for both hH1 and hSkM1. The fitted time constants derived from the averaged data were 390 and 65 ms for 25- and 100-μM lidocaine, respectively. For the skeletal muscle isoform, inactivated-state block occurred with a time constant of approximately 65 ms in 100-μM lidocaine. Note that, even at the higher concentrations, the time course of block development is too slow for equilibrium to occur with the transient open state.

Inactivated- versus open-state block

Preliminary experiments using single-channel analysis to explore open-channel block revealed only modest changes in open times and no effect on single-channel current. This finding is consistent with the small effect observed on the Na^+ current decay kinetics except at very high concentraions of lidocaine. Therefore, to maximize the possibility of observing open-state interactions, we measured the time integral of the sodium current transients. The Na^+ current transient is a scaled probability waveform, which is a convolution integral of the probability-density functions for all the ways in which a channel can be open at a particular membrane potential and time (Aldrich et al., 1983; Yue

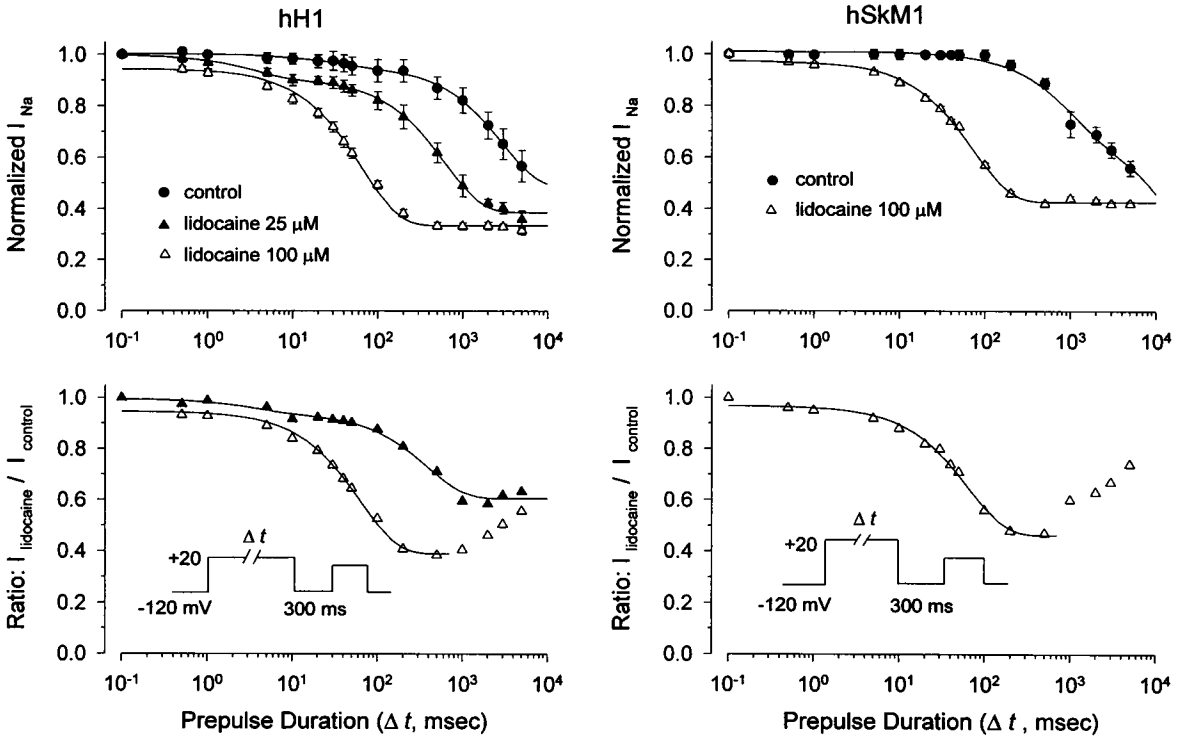

FIGURE 4 Time course of onset of block in hH1 and hSkM1. A double-pulse protocol was used to induce block. The holding potential was -120 mV, and test pulses of varying durations as indicated on the abscissae were applied to allow development of block. This test pulse was followed by a 300-ms interval at -120 mV to permit recovery of unblocked channels. Following this interval a test pulse was applied to -20 mV to permit the fraction of unblocked current to be assessed. This pulse protocol was repeated for progressively longer prepulse durations. The symbols represent means ± SE. Sodium current during the test pulse is normalized to the shortest pulse (100 μs) and plotted as a function of prepulse duration on a logarithmic time axis. The solid curves represent best-fit exponential functions to the averaged data as shown. In the bottom panels the sodium current in lidocaine at each prepulse duration has been normalized by the corresponding control value to control for the onset of slow inactivation as shown in the top panels by the filled circles.

et al., 1989; Hille, 1992). Thus, we can use the area under the Na^+ current waveform as a measure of this function.

To elucidate the contribution of inactivated- versus open-state block in these distinct Na^+ channels, we first compared the peak Na^+ current and the area during single voltage clamp steps and those preceded by a prepulse to allow inactivated-state interactions. Additional block that occurred in the presence of a prepulse could then be attributed to interactions of lidocaine with the inactivated state of the channel. Suppression of peak current can reflect only blocking events that have occurred up to the time of the peak current and will not reveal effects of the drug on channel open times and reopenings after the peak. A disproportionate decrease in the area relative to the peak Na^+ current indicates additional block that occurs during channel opening after the peak has occurred.

Comparison of Fig. 5*A* and *C* shows that 100-μM lidocaine caused more tonic block for the cardiac isoform than for the skeletal muscle isoform. Likewise, when the double-pulse protocol was used to induce inactivated-state block, the cardiac isoform was significantly more inhibited than was the skeletal muscle isoform. Summary data from of a number of experiments are shown in Fig. 5 *E*. For the cardiac isoform there was a small (8.0 $\pm$ 2.2%; $n = 12$) increase in the block detected with the single-pulse area compared with that which resulted from the single-pulse peak measurements. Therefore, evaluating the area revealed a small additional component of block. No additional block was seen when we compared the peak and the area in the double-pulse experiments for hH1 (2.9 $\pm$ 0.3%; NS). This "extra block" was larger for the skeletal muscle isoform. Whether we used single (14.1 $\pm$ 3.4%) or double (15.5 $\pm$ 2.5%) pulses, a significant increase in the amount of block was observed when we used the area versus the peak ($p < 0.05$; $n = 12$, paired t-test). This then demonstrates that, in addition to the inactivated-state block that occurs in both isoforms, a small but significant additional component of block occurs during the test pulse. This additional block was significantly ($p < 0.05$) greater in hSkM1 (14%) than in hH1 (8%). Thus, the area measurements revealed more block than detected by the peak measurement, suggesting that block was continuing to occur during the Na^+ current transient, especially in hSkM1.

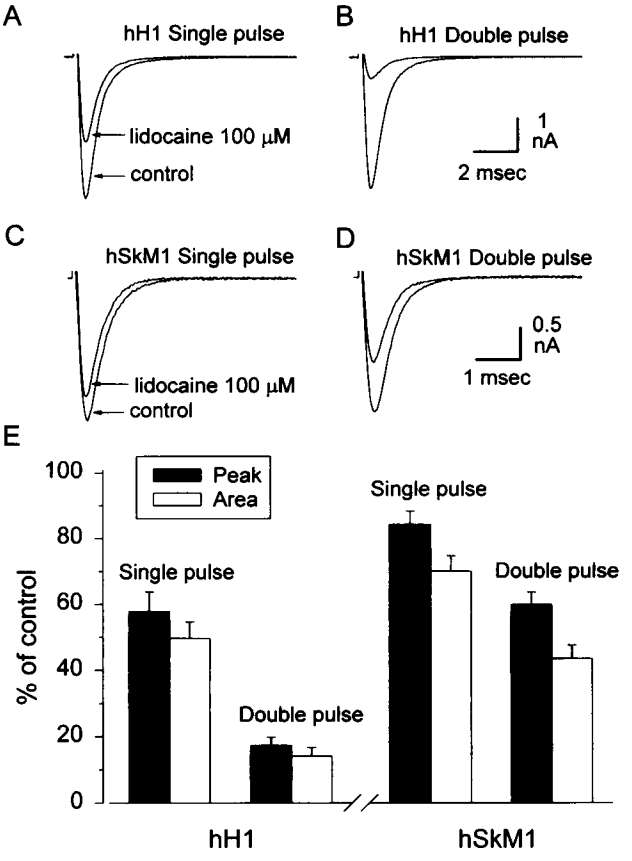

FIGURE 5 Suppression of hH1 and hSkM1 Na^+ currents by 100-μM lidocaine by use of single-pulse or double-pulse protocols. The prepulse was 500 ms. This is long enough to allow significant block to develop in both isoforms but short enough to prevent significant amounts of slow inactivation to occur. The same concentration of lidocaine was used in both cases (100 μM) to ensure that the association rates would be similar. Block by lidocaine was measured during a single pulse to -20 mV after holding at -120 mV for 1 min to ensure full availability of channels. In this case reduction of current should reflect only tonic block, which can be either drug interactions with the rested state or open-channel block. Two methods of assessing block were used. One was to measure the suppression of the peak Na^+ current and the other was to integrate the area under the Na^+ current transient and look for changes in area as an index of block. For the cardiac isoform, there was no difference in the amount of block assessed by the peak measurement or area measurement. Both were decreased proportionally in the presence of lidocaine. This was not the case for the skeletal muscle isoform, for which in all circumstances measurement of the area revealed additional block that was not evident simply by measurement of the peak current suppression. In both isoforms the imposition of a prepulse induced substantial inactivated-state block. However, significantly more block was induced by a prepulse for the cardiac isoform than for the skeletal muscle isoform. For the skeletal muscle isoform, the additional block revealed by the area on both the single-and double-pulse protocols was statistically significant.

Concentration-response relationships

Fig. 6 shows concentration-response curves for the cardiac and skeletal muscle isoforms when the double-pulse block induction protocols were used. When we measured the peak Na^+ current, the fitted IC_{50} for the skeletal muscle isoform was 172 μM, compared with an IC_{50} of 21 μM in the cardiac isoform. The concentration-response derived from the area under the curve measurements is shown in the bottom panel. The observed IC_{50} for the skeletal muscle isoform was decreased to 83 μM, and that for the cardiac isoform was 16 μM. These results show that, when the peak is compared with the area for the cardiac isoform, the area measurement revealed only a small amount of additional block (IC_{50} peak versus area: 21 vs 16 μM). In the skeletal muscle isoform the area measurement revealed a large amount of additional block (IC_{50}: 172 vs 83 μM). We can draw two conclusions from these results. First, the cardiac isoform is significantly more sensitive than the skeletal

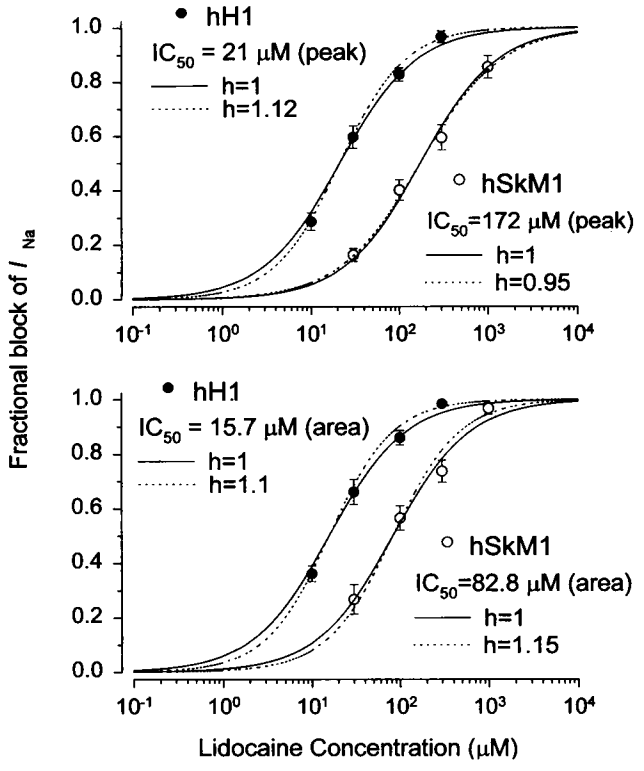

FIGURE 6 Concentration-response relationships for block by lidocaine of the cardiac and skeletal muscle isoforms by use of double-pulse protocols. (*Top panel*) The fractional inhibition of Na^+ current as assessed by measurement of suppression of the peak Na^+ current. (*Bottom panel*) The fractional suppression of Na^+ current assessed from the area under the Na^+ current time transient. Little additional block is revealed when the area rather than the peak measurement is used for the cardiac isoform, whereas substantial additional block is revealed when the area measurement is used in the skeletal muscle isoform. These data suggest that, in addition to the lower affinity of the inactivated state in the skeletal muscle than in the cardiac isoform, the skeletal muscle isoform also had a larger fraction of channels that were blocked by an open-channel pathway. The dashed curves represent the best fits of the Hill equation with the Hill coefficients (*h*) shown. The solid curves represent the best fits with a Hill coefficient of 1.

muscle isoform to block by lidocaine. Second, a significant amount of the block of the skeletal muscle isoform occurs while the Na^+ channels are open, as revealed by the additional block that is uncovered by measuring the area of the Na^+ current. It should be noted that in each case, because of the nature of the pulse protocols that we used and the complications from slow inactivation, we have underestimated the true affinity for lidocaine block for both isoforms.

DISCUSSION

The observed Na^+ current waveform is a scaled probability transient whose form is an integral of all the ways in which the channel can be open at a given time during the pulse at a particular membrane potential. The area under a Na^+ current transient quantifies this relationship. A disproportionate reduction in the area relative to the peak I_{Na} owing to block by lidocaine can result from interaction of lidocaine with the activated or open state of the channel. We can discriminate between inactivated- and activated-state block in the two channel isoforms and ascertain their relative contributions to the total block observed. We define activated-state block as inhibition that occurs at some time following depolarization as the channel proceeds from distal closed states toward and through the open state. Inactivated state or states are the states entered during depolarizations and are in general fully occupied within approximately 10 ms of a depolarization (see Fig. 2). Previous studies showed that the time course of onset of lidocaine block with the inactivated state occurred over several hundreds of milliseconds (Bean et al., 1983; Clarkson et al., 1988; Bennett et al., 1995a). If block is fully established before the test pulse, then peak and area measurements should decrease identically. Only if additional block occurs during the test protocol will the area and peak measurements be in disagreement.

Fig. 7 shows the predicted consequences for various types of channel block. Here we adopted a basic multistate Na^+ channel model taken from the literature (Patlak, 1991). Only states adjacent to the open state are shown. In the control channels, fast inactivation proceeds at a rate of 500/s, leading to rapid macroscopic inactivation. Although the multistate nature of the simulation gives rise to multiple eigenvalues (multiple time constants), the decay phase, which we will refer to as inactivation, can be fitted with a single dominant time constant. In the control case this value was 2.4 ms and is sufficient to characterize adequately the speed of this process. In Fig. 7 *A* we have simulated the effects of a very rapid open-channel blocker. Here all the control features of the channel are left intact. An additional reaction has been added to the simulation where the blocking molecule interacts with the open state. The effective concentration of the drug used is equivalent to the thermodynamic equilibrium dissociation constant. The association rate and the dissociation rate are equal, 10^4/s. Note that in this model block can occur only during the brief time during which the channel is in the open state. However, inasmuch as the drug–channel interaction is much faster than the rate of entering into the inactivated state, the consequence of this interaction is effectively to delay channel inactivation. At the single-channel level this is revealed as a very rapid blocking process with bursts of blocked events that eventually terminate when the channel is finally inactivated. The apparent macroscopic time constant for this slowed rate of decay was 4.3 ms. Note also that the peak Na^+ current is reduced but the area under the Na^+ current transient remains essentially at the control level. Thus three features of this model are an increase in the apparent time constant for macroscopic inactivation, a suppression of peak current, and no change in the area under the Na^+ time transient. Therefore we can reject this model because it is not consistent with our data. A second possible model is shown in Fig. 7 *B*. In this case the drug-association rate is rapid but the dissociation rate is much slower. This sort of blocking mechanism would have the consequence of decreasing the

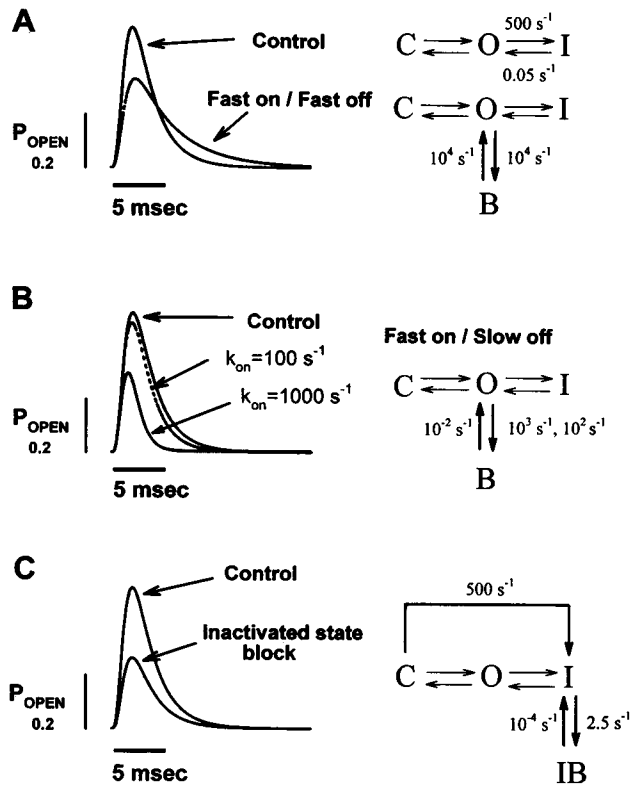

FIGURE 7 Possible mechanisms of block of voltage-gated Na^+ channels. (*A*) Theoretical drug with rapid association and dissociation kinetics. In this case a blocker would interact with the channel only after it had entered the open state. Rapid interactions between the open and blocked states would have the consequence of shortening channel open times and essentially delaying entry into the inactivated state. This results in a crossover of the decay phase of the control and the drug macroscopic Na^+ currents, resulting in a slower apparent time constant of inactivation, and for appropriate kinetics the area under the curve in the presence of drug would be similar to that in control. The peak Na^+ current would be suppressed. (*B*) Example of a fast on-rate slow off-rate open-channel blocker. In this case the kinetics of onset of block (i.e., the rate constant) is similar to or greater than the inactivated-state rate constant, but the off rate of the blocked state is relatively slow. In this model, depending on the kinetics of the drug–receptor interaction, the macroscopic time constant of decay of the Na^+ current could be accelerated or unchanged. The open times of the channel would be reduced, and the area under the Na^+ current transient would be reduced proportionally more than the peak current. (*C*) Inactivated-state block. Channels are placed into the inactivated state by a prepulse where lidocaine association occurs. These drug-associated channels no longer contribute to the sodium conducting pool of channels and appear as a decrease in the number of functional channels during a test pulse. This procedure has the consequence of simple scaling down both the peak and the area measurement of Na^+ current, with no effect on channel kinetics.

time constant for macroscopic decay, reducing the peak current, and reducing the area. In this simulation, we explored the effect of two different drug concentrations, one that gives an effective association rate constant (k_{on}) of 100 s^{-1} and another with a k_{on} of 1000 s^{-1}. For the case of k_{on} = 1000 s^{-1}, the decay time constant was changed from 2.4 ms in the control to 1.1 ms, and the area under the Na^+ time transient decreased significantly more than the suppression of the peak current (56% vs 43%, respectively). The greater reduction in area versus peak current provides an indication for open-channel block. With the smaller association rate (k_{on} = 100 s^{-1}), the peak is suppressed by 8% and the area is decreased by 13%. The characteristics of this type of mechanism are in part similar to our data for the skeletal muscle isoform. Note that, in addition to the small changes in the decay rate, the rising phase occurred coincident with the control, as seen for hSkM1 in Figs. 2, 3, and 5.

A third possible mechanism is shown in Fig. 7 *C*. Here we have explored interactions with the inactivated state of the channel. The drug can interact with the channel in this simplified model only when the channels are in the inactivated state. Drug binding occurs as a consequence of a prepulse, which places channels into the inactivated state. The rate of association with the inactivated state is 2.5/s (τ = 400 ms). Consequences of this form of block are that channels that have accumulated in the inactivated-blocked state (IB) will no longer be available for opening. Thus, both the peak and the area of the Na^+ transient will scale down by an equivalent amount, and no change in the apparent macroscopic decay time constant is observed in this model. This model is largely consistent with our data for the cardiac isoform. Although the skeletal muscle isoform shows characteristics of this form of block, we note that it also shows characteristics for the open-channel block more than does hH1. Thus, we conclude that the models shown in Fig. 7 *B* and *C*, although they are an oversimplification of the actual blocking processes, can account for the observed block, that inactivated-state block is dominant in both isoforms, and that there is significantly more open-state block occurring in hSkM1 than in hH1.

Molecular interpretation

Recent studies have focused on the molecular localization of the local anesthetic binding domain in the Na^+ channel protein (Ragsdale et al., 1994; Bennett et al., 1995b). Ragsdale et al. (1994) demonstrated that amino acids in the S6 region of domain 4 (D4S6) are involved in local anesthetic block of the rat brain IIA sodium channel. Specifically, mutation of phenylalanine 1764 to alanine resulted in nearly complete loss of block, as did mutation of tyrosine 1771. Mutations between these positions resulted in smaller perturbations. These sites cannot, however, account for the differences that we observe in lidocaine affinity between hH1 and hSkM1, as they are completely conserved between these isoforms. We have demonstrated (Bennett et al., 1995a) that removal of fast inactivation by mutations in the 3–4 interdomain (ID34) of hH1 results in loss of high-affinity inactivated-state block of the channel by lidocaine, consistent with the hypothesis that lidocaine stabilizes the inactivated conformation of the channel. This domain is also highly conserved between these channels in regions known to be important for inactivation (West et al., 1992; Bennett et al., 1995b). Another possible explanation for the

differences between isoforms could be the presence of a second lidocaine receptor site (Alpert et al. 1989). Additional experiments will be required, focusing on isoform-specific amino acid differences, to localize further the regions involved in the distinct lidocaine affinities.

Therapeutic implications

Clinical usage has suggested that lidocaine may block cardiac Na^+ channels more potently than neuronal or skeletal muscle channels simply because it is effective for the treatment of arrhythmias and has few side effects related to muscle or neuronal channels except at higher concentrations. Our data demonstrate that one reason for this greater effect on the cardiac isoform is that it has an intrinsically higher binding affinity for the drug. A second mechanisms for channel subtype specificity is suggested by the fact that lidocaine preferentially interacts with the inactivated state of the Na^+ channel. This state is occupied within a few milliseconds of the beginning of an action potential and exists for as long as the cell remains depolarized. Neuronal and skeletal muscle action potentials are only a few milliseconds in duration, and the inactivated state is minimally occupied. In contrast, cardiac action potentials are several hundred milliseconds in duration, and as a consequence the cardiac Na^+ channel spends a large fraction of the cardiac cycle in the inactivated state. Thus, an additional basis for the apparent difference in sensitivity to block by lidocaine is dependent on the accessibility of the inactivated state for channel block. Cardiac channels are preferentially inhibited because they spend a larger fraction of time in the high-affinity inactivated state. Neuronal and skeletal muscle channels are less affected because of their brief action potentials and because the inactivated state is minimally available for binding.

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