The Heart Sodium Channel Phenotype for Inactivation and Lidocaine Block

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SUMMARY

The heart Na channel, although resembling other voltage-gated Na channels, has important functional and structural differences. For heart channels expressed in oocytes, the midpoint of the inactivation relationship was 13 mV negative to that of rat skeletal muscle Na channels, and sensitivity to tonic lidocaine block was approximately 5 times more sensitive for heart. Co-expression with the β subunit increased the difference in inactivation midpoint to 24 mV, largely by changing the midpoint of the rat skeletal muscle channel by 10 mV in the positive direction. Co-expression with $\beta 1$ decreased lidocaine sensitivity for heart but not for skeletal muscle Na channels, and decreased but did not eliminate the greater heart sensitivity to lidocaine block. The differences in inactivation are likely to account for some, but not all, of the differences in lidocaine sensitivity. This cardiac phenotype is important for the role the channel plays in cardiac physiology and pathophysiology, and also may lead to elucidation of structure-function relationships (*Jpn Heart* 1996; 37: 733-739)

Key words: Cardiac Local anesthetic Ion channels Na current
Kinetics Recombinant DNA Tonic block Structure-function

LIDOCAINE, administered systemically, acts as an antiarrhythmic drug. When it is administered locally in higher concentrations, lidocaine acts as a local anesthetic. Both of these actions are caused by block of Na channels and lidocaine is often used as a typical representative of both local anesthetics and antiarrhythmic drugs. Although neurotoxicity occurs with lidocaine when it is used to treat arrhythmias, this side effect is concentration dependent and occurs at higher concentrations (for review see 1). What explains the therapeutic ratio? Is the cardiac Na channel intrinsically more sensitive to lidocaine?

Lidocaine exhibits the property of use-dependent block, that is, extra block accumulates with repetitive depolarizations as a result of higher affinity binding to the inactivated state.²⁾ The relative sensitivity of lidocaine for Na channels in heart may result from an intrinsic difference in lidocaine affinity for the different Na⁺ channel isoforms, but also could result from increased binding to the inacti-

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vated state in heart during the longer action potential without any differences in affinity. In the first comprehensive voltage clamp study of lidocaine block on cardiac Na current, Bean and colleagues³⁾ compared their results with studies published previously for non-heart channels. They concluded that lidocaine showed similar interactions with the channel itself and attributed at least most of the difference to greater block in heart tissue during the greater dwell time in the inactivated state. One of the difficulties of using previously published data for comparison of heart with non-heart lidocaine binding, however, is that lidocaine block is sensitive to experimental conditions including divalent ions, monovalent ions, pH, temperature, and the specific voltage protocols used to elicit the block. The experiments must be done, as much as possible, under identical experimental conditions.

A preliminary report using native cells under nearly identical conditions⁴⁾ suggested that the cardiac channel in rat ventricle had an intrinsically higher affinity for lidocaine than did the brain channel in cultured neuroblastoma cells. More recently, cloned cardiac channels expressed in oocytes^{5,6)} have also suggested an intrinsically higher affinity for the cardiac isoform. This report reviews the previous literature and provides further data to support this contention. In addition, because inactivation is also critical for lidocaine binding, the isoform specific differences in steady-state inactivation are also reviewed.

METHODS

The human heart Na channel α -subunit (hH1) was kindly provided by Drs. H. Hartmann (Baylor University, Houston, TX) and A.M. Brown (Ramelkamp Research Center, Cleveland, OH), the rat skeletal muscle Na channel α -subunit ($r\mu 1$) by Gail Mandel (SUNY Stonybrook, NY), and the Na channel $\beta 1$ subunit by John W. Kyle (University of Chicago, Chicago, IL). The methods for preparation of cRNA, *Xenopus* oocytes, and two microelectrode voltage clamps are standard and have been previously published.⁷⁾ Briefly, *Xenopus* oocytes were injected with 50–150 ng of cRNA for the α -subunit (hH1 or $r\mu 1$) alone, or in some cases a mixture of cRNA for the α and cRNA for the $\beta 1$ subunits in 3–9 fold excess of that for the α subunit (total 50–150 ng). One to seven days after injection I_{Na} was recorded from cRNA-injected oocytes with a two electrode voltage bath clamp. A Dagan CA-1 (bath clamp) with a series resistance compensation circuit (TEV-208, Dagan) was used to make recordings. All recordings were made at 20–22°C in a flowing bath solution consisting of (in mM): 90 NaCl, 2.5 KCl, 1 CaCl₂, 1 MgCl₂, and 5 Hepes, pH 7.2. Lidocaine in appropriate amounts was added from a 100 mM stock solution in distilled water. Electrodes contained 3 M KCl and had resistances that ranged from 0.2 to 1.5 M Ω . Data were ac-

quired using pClamp6 software (Axon Instruments) onto an Intel-486 based computer. Data were digitized at a 42 kHz and were low pass filtered at 10 kHz (-3dB).

Data were fit to model equations using non-linear regression (Procedure NLIN) using SAS (Cary, NC) statistical software running on a SUN ELC workstation. Parameter estimates are given $\pm$ the standard error for the parameter estimate. Steady-state inactivation relationships were fit to Fractional $I_{Na} = I_{Na-max} [1 + \exp (V_c - V_{1/2})/k]^{-1}$ where I_{Na-max} is the predicted maximum peak I_{Na} , $V_{1/2}$ and k are fitted parameters representing the mid-point and slope respectively of the Boltzmann relationship. Tonic lidocaine block data were fit to a single site binding relationship as Fractional $I_{Na} = 1/(1 + [\text{lidocaine}]/K_d)$ where K_d is the dissociation constant (assuming block as an index of binding) and is equivalent to the half blocking dose. Mean data are reported with their standard deviation. All determinations of statistical significance of mean data were performed by using a student t-test for comparisons of two means.

RESULTS

For cloned α -subunit isoforms expressed in oocytes and studied under identical conditions (see example in Figure 1), the midpoint of the steady-state inactivation relationship was significantly more negative in heart by 17 mV in the example and by 13 mV on average (Table). Coexpression with the $\beta 1$ subunit increased this difference to 24 mV (Table) because the $\beta 1$ subunit had a greater effect to shift the relationship in the positive direction for the skeletal muscle channel than for the heart channel. Brain channels ($rB2a + \beta$ in the Table) studied under identical conditions⁵⁾ had the same midpoint as skeletal muscle channels ($r\mu 1 + \beta$ in the Table) suggesting that this relatively negative midpoint is a characteristic phenotype of the cardiac Na channel. These results with co-expression of $\beta 1$ are consistent with those of Nuss et al⁶⁾ summarized in the Table. They are also consistent with results obtained in native rat ventricular myocytes compared with brain channels in neuroblastoma cells⁴⁾ and summarized in the Table. These data taken together strongly suggest that this negative midpoint, although modified by the $\beta 1$ subunit, is an essential feature of the cardiac isoform of the α -subunit.

Lidocaine block of I_{Na} can be classified as phasic and non-phasic block. Non-phasic block, also called rest block, is block occurring either in the absence of prior depolarizations (first pulse block) or at very low pulsing frequencies (tonic block). First pulse block, defined as the relative decrease in I_{Na} for the first depolarization in lidocaine compared with the current measured without lidocaine, was used as a measure of non-phasic or tonic block. The holding potential was

Figure 1. The steady-state inactivation relationship for the heart Na channel is more negative than that for skeletal muscle. Steady-state availability for inactivation was assessed by a standard “h_∞-type” protocol (see protocol inset). Previous results suggested a 10s conditioning duration was required to reach “steady-state”.⁷⁾ The figure shows a typical example from an oocyte injected with hH1 (○) and a second oocyte with rμ1 (□). Data were normalized to the peak current present at -120 mV and plotted against the conditioning potential V_c. Midpoints of fits to a Boltzmann relationship (see methods) are shown on the figure, and summary data are given in Table.

Table. Tonic Lidocaine Block for Cardiac and Non-cardiac Channels

Heart isoform	K _d mM (n)	V _{1/2} (mV)	Non-heart	K _d mM (n)	V _{1/2} (mV)	HP (mV)	Sens. ratio	V _{1/2} diff	Ref
hH1 (Oocytes)	0.41 ± .06 (21)	-78 ± 3	rμ1	2.00 ± 3.7 (10)	-61 ± 4	-120	4.9	13	Present study
hH1 + β (Oocytes)	0.86 ± .15 (16)	-75 ± 5	rμ1 + β	1.72 ± .23 (14)	-51 ± 3	-120	2.0	24	Present study
rh1 (Oocytes)	0.43 ± 0.5 (37)		rB2a + β	2.11 ± .28 (7)	-52 ± 2	-120	4.0		Satin et al 1994
hH1 + β (Oocytes)	.40 ± .04 (22)	-80 ± 1	rμ1 + β	1.17 ± .03 (19)	-60 ± 1	-100	2.9	20	Nuss et al 1995
rat vent. (native)	1.80 ± .22 (16)	-87 ± 6	Mouse Nb2a	4.20 ± .16 (14)	-78 ± 8	-150	2.3	9	Makielski et al 1994

Isoforms of α-subunit of the Na channel are: hH1 human heart Na channel, rh1 rat heart Na channel, rμ1 rat skeletal muscle, rB2a rat brain 2a, + β indicates co-expression with the β1 subunit of the Na channel, all cloned channel studies done in oocytes. For native channels acutely isolated cells from rat heart ventricle were compared to cultured mouse neuroblastoma cells (Nb2a). K_d is the dissociation constant (half block) for lidocaine from a fit to a single site binding relationship ± the standard error for the parameter estimate for the (n) individual experiments at different lidocaine concentrations. V_{1/2} is the midpoint of the steady state inactivation relationship (see Figure 1). The sensitivity ratio for lidocaine was calculated by dividing the K_d in non heart by the K_d in heart. V_{1/2} diff is the difference in inactivation midpoints. All differences in K_d and V_{1/2} between heart and non-heart isoforms are significant by paired t-test at *p* < .05.

Figure 2. The heart channel is more sensitive to lidocaine than the skeletal muscle Na channel. Fractional I_{Na} was measured by taking the peak current for the first depolarization from -120 mV to -10 mV after exposure to lidocaine and dividing by the peak current present for the same step in control solutions. Data points for hH1 ($\circ$) and r μ 1 ($\square$) represent means and the bars represent standard deviations for between 2 and 5 experiments (21 total for hH1, 10 total for r μ 1). The solid lines represent fits to a single-site binding curve (see methods) with the dissociation constant K_d 's as shown on the figure.

-120 mV and the test potential was 10 mV. Figure 2 shows the dose-response relationships for tonic lidocaine block of hH1 and r μ 1 expressed without the β 1 subunit. The heart channel was approximately five times more sensitive (see sensitivity ratio column in the Table). Co-expression of the β 1 subunit decreased this difference but did not eliminate it (hH1 + β and r μ 1 + β in the Table). This result is similar to the result of Nuss et al⁶) also summarized in the Table. The decreased difference with β 1 is caused by a reduced decreased lidocaine sensitivity for hH1 (Table and 7), but β 1 does not have a significant effect on lidocaine sensitivity for r μ 1. Brain channels (rB2a + β in the Table) studied under identical conditions (5) had the same K_d as skeletal muscle channels (r μ 1 + β in the Table) suggesting that the greater tonic block sensitivity is a characteristic phenotype of cardiac channels. A greater sensitivity was also found for Na channels in native rat ventricular myocytes compared with brain channels in neuroblastoma cells⁴) and is summarized in the Table. These data taken together strongly suggest that the greater sensitivity is an essential feature of the cardiac isoform of the α -subunit itself and not a result of different study conditions.

DISCUSSION

The properties of the voltage gated I_{Na} from different tissues bear a remarkable similarity to each other.⁶⁾ Heart, skeletal muscle, and nervous tissue I_{Na} , however, differ in their sensitivity to toxins such as tetrodotoxin, sea anemone toxins, and to divalent cations such as Cd^{2+} (see 9 for review), and subtle kinetic differences have been described. The characterization of various properties of I_{Na} depends greatly upon the experimental conditions under which they are measured including: ionic conditions, pH, temperature, technical quality of the voltage clamp, voltage clamp techniques (ie, patch clamp mode, cell preparation), and the particular voltage clamp protocols used to elicit the properties. For these and other reasons, detecting and describing differences such as gating kinetics and sensitivity to local anesthetics is difficult when comparing various reports in the literature.

More than a dozen isoforms of the α -subunit of voltage-dependent Na channel have been cloned and many of these have been expressed and studied by voltage clamp (see 10 for review). Although similar in structure and overall function, expression and study of different isoforms under identical conditions and with identical protocols have elicited and characterized important specific functional differences. Differences in kinetics and lidocaine sensitivity had been suspected by comparison of results from diverse studies in heart and non-heart preparations under various experimental conditions. The present study reviews previous studies and offers new data to show that under identical study conditions the typical cardiac phenotype for steady-state inactivation is more negative than brain and skeletal muscle Na channels, and that the cardiac phenotype for tonic block by lidocaine is one of greater sensitivity.

An important consideration in measuring tonic block is the holding potential. Lidocaine has higher affinity for the inactivated state, and as the membrane holding potential becomes more depolarized, then tonic block increasingly reflects binding to the inactivated state and becomes greater. This likely explains the greater sensitivities observed by Nuss et al⁶⁾ who measured tonic block at -100 mV compared with our results at -120 mV (Table), and also explains the lower affinities observed at -150 mV in native cells.⁴⁾ Could the greater sensitivity of heart channels to tonic block be simply a reflection of the more negative inactivation kinetics? This certainly accounts for some of the difference and would also be of physiological and pathophysiological significance. That the difference persists (although diminished somewhat) at the more negative holding potentials of -120 mV and -150 mV (Table) suggests that an intrinsic difference in affinity beyond inactivation kinetics may exist.

Isoform specific sensitivities to the tetrodotoxin and saxitoxin have been

exploited to define the structures responsible for tonic toxin block¹¹⁾ and phasic toxin block¹²⁾ by site mutagenesis. Mutagenesis work on the Na channel has suggested residues on domain 4 S6 as forming a putative local anesthetic binding site,¹³⁾ but the possible contributions of the other domains are not known. Recent work with Na channels made chimeric between heart and skeletal muscle channels has suggested that isoform differences in block must be attributed to all four domains.¹⁴⁾ Thus, in addition to helping explain the therapeutic effectiveness and therapeutic ratio of lidocaine for the antiarrhythmic effect, the cardiac phenotype for inactivation and lidocaine block will likely lead to characterization of the structure-function relationship for these important Na channel functions.

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