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The Cloned Cardiac Na Channel α-Subunit Expressed in *Xenopus* Oocytes Show Gating and Blocking Properties of Native Channels

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Summary. The neonatal rat cardiac Na channel α-subunit directed currents in oocytes show characteristic cardiac relative resistance to tetrodotoxin (TTX) block. TTX-sensitive currents obtained by expression in *Xenopus* oocytes of the α-subunits of the rat brain (BrnIIa) and adult skeletal muscle (μI) Na channels show abnormally slow decay kinetics. In order to determine if currents directed by the cardiac α-subunit (RHI) exhibit kinetics in oocytes like native currents, we compared RHI-directed currents in oocytes to Na currents in freshly isolated neonatal rat myocytes. The decay rate of RHI currents approached that of neonatal myocytes and was faster than BrnIIa and μI currents in oocytes. The voltage dependence of availability and activation was the same as that in the rat myocytes except for a 12–19 mV shift in the depolarizing direction. The RHI Na currents were sensitive to Cd^{2+} block, and they showed use dependence of TTX and lidocaine block similar to native currents. The current expressed in oocytes following injection of the cRNA encoding for the α-subunit of the cardiac Na channel possesses most of the characteristic kinetic and pharmacological properties of the native cardiac Na current.

Key Words sodium channels · neonatal rat cardiac myocytes · tetrodotoxin · lidocaine · patch clamp

Introduction

Native cardiac Na channels can be distinguished from those in nerve and in adult skeletal muscle on the basis of their drug interactions and their kinetic properties. In contrast to nerve and adult skeletal muscle, cardiac Na current is 100- to 1000-fold less sensitive to block by tetrodotoxin (TTX) and saxitoxin (STX) (Hille, 1968; Baer, Best & Reuter, 1976), while being 100-fold more sensitive to divalent ions of the transition metal type such as Cd^{2+} and Zn^{2+} (Frelin et al., 1986; Sheets et al., 1987; Visentin et al., 1990; Ravindran, Schild & Moczydlowski, 1991). Direct comparisons of cardiac and brain single channel records demonstrate slower kinetics of the cardiac Na channels (Kirsch & Brown, 1989).

Six different sodium channel α-subunits have been sequenced and expressed in *Xenopus* oocytes. Four rat brain channel isoforms (BrnI, II, IIa, and III), a skeletal muscle isoform (μI) and a rat heart isoform (RHI) are all composed of about 2000 amino acids, are 70–90% identical, and share the common motif of six membrane-spanning segments repeated four times (Noda et al., 1986; Trimmer et al., 1989). BrnI-III and μI-directed currents in oocytes are all relatively sensitive to TTX (Noda et al., 1986; Stühmer et al., 1987; Trimmer et al., 1989; for review, *see* Trimmer & Agnew, 1989). Recently we reported that RHI current expressed in *Xenopus* oocytes is less sensitive to TTX (Cribbs et al., 1990), consistent with the native cardiac Na current TTX sensitivity. A second clone from denervated skeletal muscle Skm2 (Kallen et al., 1990) has a structure that is identical to RHI, and it is also less sensitive to TTX (White et al., 1991).

In this study we ask if the cardiac cRNA α-subunit is sufficient for directing in oocytes the expression of Na current with native cardiac Na channel pharmacological and kinetic properties. We compared the RHI current in oocytes to Na currents in neonatal rat myocytes and to the Na currents expressed by μI (Trimmer et al., 1989) and BrnIIa as representative TTX-sensitive Na channels. Those sodium channel α-subunits with high TTX affinity show slow current decay when expressed in the *Xenopus* oocyte system, compared to various native neural (Krafte et al., 1990) and adult skeletal muscle Na currents (Trimmer et al., 1989), or to channels expressed by poly(A)$^+$ mRNA.

RHI current expressed in *Xenopus* oocytes and native cardiac Na current have similar kinetics,

although the voltage dependence of the kinetics and the steady-state availability are shifted in the depolarizing direction. The RHI current decays faster than the TTX-sensitive Na currents μI and BrnIIa and it recovers faster from inactivation. The cardiac Na current expressed in oocytes demonstrates use-dependent block by TTX and lidocaine. In addition, RHI current is sensitive to Cd^{2+} ($K_{1/2}$ = 48 μM). All of these pharmacological properties are typical of the native rat cardiac Na current. We conclude that the α-subunit expressed from the RHI cRNA in *Xenopus* oocytes possesses characteristic pharmacological and kinetic properties of native cardiac Na channels. Preliminary results have been previously reported in an abstract (Satin et al., 1991).

Materials and Methods

Preparation of cRNA

The cDNA for the rat heart I sodium channel (RHI) was initially subcloned into the pSP64t expression vector as previously described (Cribbs et al., 1990). This construct contains the entire RHI cDNA (nucleotides −4 to 6072) cloned into the BgIII site of pSP64t following addition of BamHI adapters to the RHI cDNA. The cDNA in pSP64t is bracketed by *Xenopus* globin untranslated sequences (Krieg & Melton, 1984). The RHI cDNA and *Xenopus* globin sequences were then isolated from pSP64t-RHI by digestion with HindIII and XbaI and ligated into the corresponding sites of the Bluescript II SK plasmid (Stratagene, La Jolla, CA). This construct, named pBSG-RHI, utilizes the T7 promoter for transcription of RHI cDNA. Transcripts prepared from BSG-RHI and injected into oocytes resulted in substantially higher levels of channel expression than previously reported for pSP64t-RHI (*compare* Fig. 1 to Cribbs et al., 1990). Reagents from the mCAP kit (Stratagene) were used for the transcription reaction.

Neonatal Ventricle Isolation and Whole-Cell Recordings

Hearts were dissected from 9–14 rat pups within the first day after birth and placed in cold Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (Sigma, St. Louis, MO). Following washing to remove blood cells, the ventricles were dissected and transferred to 3–4 ml 0.125% pancreatin solution (GIBCO, Grand Island, NY). The tissue was minced, transferred to 10 ml of fresh pancreatin solution, and digested for 15 min at 37° C. The digestion cycle was repeated 8–10 times. After each 15 min digestion cycle, the supernatant containing isolated cells was collected and 1 ml of fetal bovine serum (Hyclone, Logan, UT) per 15 ml of buffer was added. After digestion, cells were transferred to 60-mm tissue culture dishes and plated for 60–90 min to allow fibroblasts to adhere. Suspended cells transferred to fresh culture dishes were incubated overnight (37° C, 5% CO_2) in minimal essential media with Earle's salts (Sigma) supplemented with 10% fetal bovine serum.

A List EPC-7 was used to control voltage and R_{series} compensation was used to minimize the capacity transient. The bath was a modified oocyte Ringers (OR-2) which consisted of (in mM): 90 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 100 sucrose, 10 HEPES, pH 7.4, about 280 mOsm, and the pipette contained (in mM): 120 Cs-glutamate, 20 CsCl, 5 NaCl, 5 EGTA, 2 Mg-ATP, 10 HEPES, pH 7.4. Current was digitized at 33 kHz. Electrode resistance ranged from 1–4 MΩ. All recordings were made at room temperature (18–22° C). Data are displayed as the mean ± SE.

Oocyte Preparation and Electrophysiological Recordings

Stage 5–6 *Xenopus laevis* oocytes (NASCO, Ft. Atkinson, WI) were prepared using procedures previously described (Cribbs et al., 1990). Oocytes were injected with 50 nl of cRNA. Typically RHI cRNA was injected at a concentration of 1 μg/μl whereas BrnIIa and μI cRNA concentrations were 0.1 to 0.5 μg/μl. Oocytes were incubated 2–7 days at 20° C before whole-oocyte recording using a two-electrode voltage clamp (TEV-200, Dagan Instruments, Minneapolis, MN) with a series resistance compensation circuit (TEV-208, Dagan Instruments). Electrodes containing 3 M KCl had resistances ranging from 0.2 to 1 MΩ. A second A/D channel was used to record V_m through electrode #2. Data were discarded if there was a change in the time course of rise of V_m following a $V_{command}$ step during the course of the experiment. The experiments were performed in the presence of a flowing oocyte Ringers solution (OR-2) at 20–22° C. OR-2 consists of (in mM): 90 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4. Cd^{2+} and TTX (Calbiochem, La Jolla, CA) were added to the bath from a concentrated stock solution. Data were digitized at 80 μsec/point and low-pass filtered at 10 kHz (−3dB). Axobasic 1.0 software (Axon Instruments, Foster City, CA) controlled voltage pulse protocols and data storage. All oocyte recordings were made at 20–22° C.

Oocytes were prepared for macropatch recordings as described by Stühmer et al. (1987). Briefly, the vitelline membrane was separated from the plasma membrane by exposing oocytes to hypertonic OR-2 (about 480 mOsm by the addition of 85 mg sucrose per ml OR-2). The vitelline envelope was then completely removed with fine forceps. Oocytes were transferred to isotonic OR-2 for 10 min prior to recording. The cell-attached mode was used with patch pipettes containing OR-2 bath solution, having electrode resistances ranging from 0.5 to 4 MΩ. Oocyte resting potential was recorded after each experiment by switching the patch-clamp amplifier (List EPC-7) to current clamp and applying gentle suction to rupture the patch. Data are displayed as mean ± SE.

Results

Comparison of RHI-Directed Na Current in *Xenopus* Oocytes to Native Cardiac Na Current

Two to four days after RHI cRNA was injected, the oocytes expressed a fast inward Na current (I_{Na}). Figure 1*A* shows a family of currents in response to steps from a holding potential (V_{hold}) of −100 mV to various voltages. The macroscopic RHI-directed

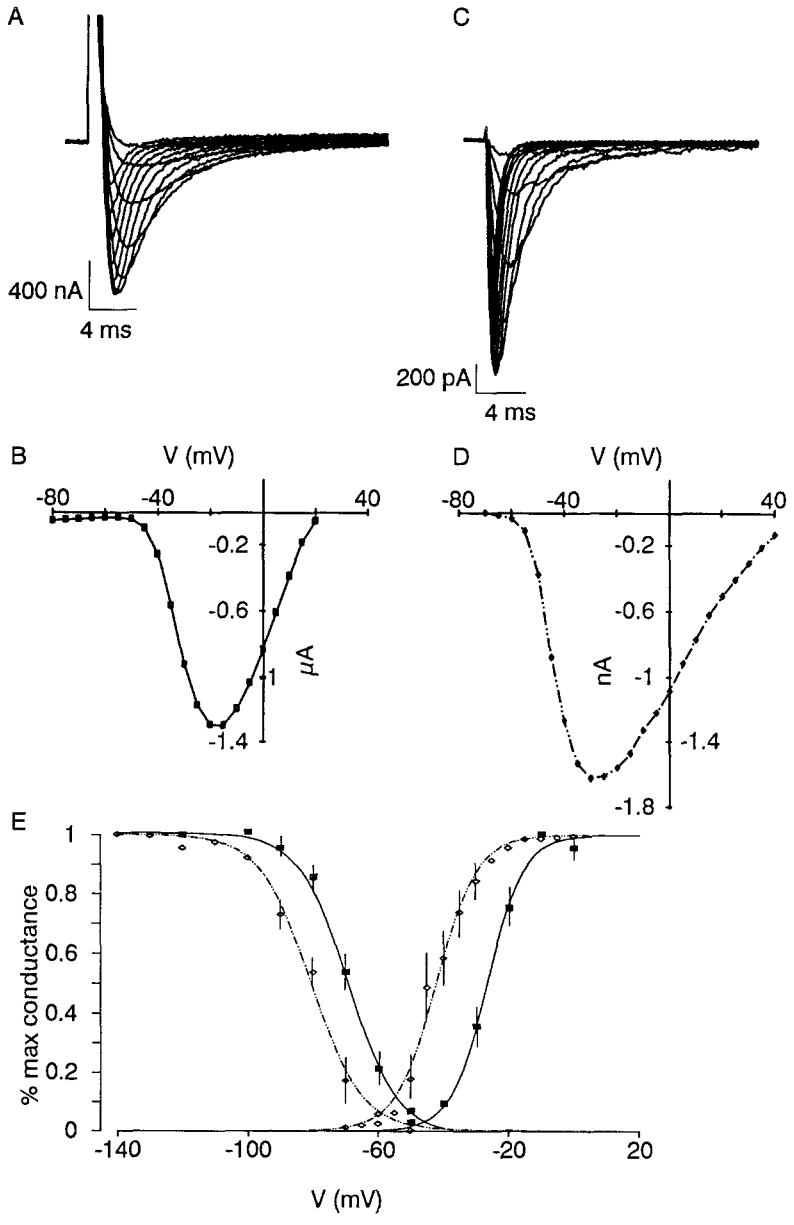

Fig. 1. Comparison of RHI currents in *Xenopus* oocytes to Na currents in neonatal rat myocytes. (*A*) RHI currents in oocytes elicited by step depolarizations to −45 through +10 mV by 5 mV increments. V_{hold} = −100 mV. (*C*) Whole-cell currents from a neonatal ventricular myocyte, V_{test} −55 to −10 by 5 mV increments. V_{hold} = −100. (*B,D*) Corresponding peak *I*(*V*) curves. (*E*) RHI activation and availability curves (solid lines, squares) are shifted to the right of the native neonatal ventricular myocyte whole-cell current (broken lines, diamonds). Error bars ± SD are shown where they exceed the size of the symbol.

current is observed for potentials positive to −50 mV and has its peak value at −20 to −15 mV. Figure 1*C* illustrates a family of whole cell currents from a neonatal rat myocyte, which were obtained within 2 min of establishment of the gigaohm seal. The same protocols were used for myocytes as for oocytes. The neonatal myocyte currents appear to decay somewhat faster. I_{Na} activated at more negative potentials (−60 mV) than I_{Na} in oocytes, and the maximal peak current is at about −30 mV (Fig. 1*D*). The activation curves in Fig. 1*E* were drawn from the normalized chord conductances of the *I*(*V*) curves (Fig. 1*B* and *D*). Activation curves were fitted with a Boltzmann distribution of the form:

$$G_{Na} = 1/[1 + \exp(V_{1/2} - V_{test})/k] \quad (1)$$

where $V_{1/2}$ = −26.0 ± 3.7 mV, and k = 4.83 ± 0.64 mV (n = 8) for the RHI-directed current in oocytes, and $V_{1/2}$ = −44.7 ± 6.7 mV, and k = 6.32 ± 1.32 mV (n = 4) for I_{Na} in neonatal myocytes.

The steady-state availability curves were generated by test pulses to −10 mV following 5 sec at various holding potentials. Steady-state inactivation was completely removed at −100 mV. The availabil-

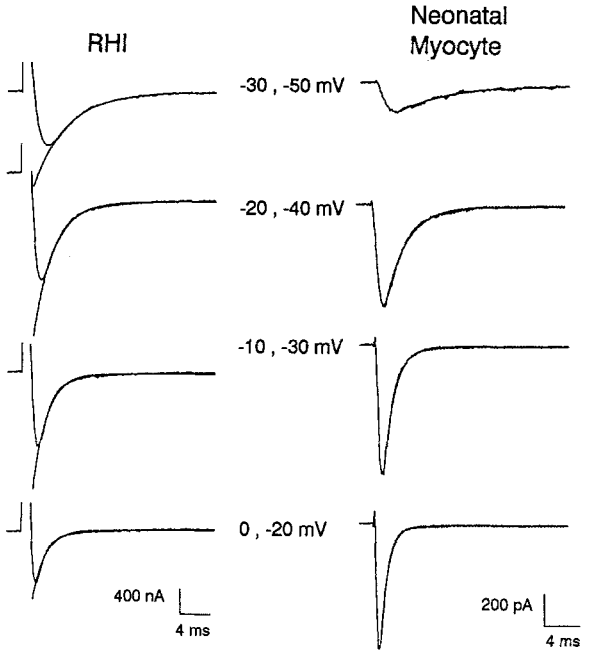

Fig. 2. Decay of RHI and neonatal myocyte Na currents fitted by single exponential functions. Currents shown are for V_{test} displaced by 20 mV to show comparable rates of decay. The decay phases of whole-oocyte current (RHI, *left*) and whole-cell neonatal rat myocyte (*right*) current are superimposed with a single exponential function of the form:

$$I(t) = a^* \exp(-t/\tau) + I_{offset}. \quad (3)$$

ity curves were fitted with a Boltzmann distribution of the form

$$G_{Na} = 1/[1 + \exp(V_{hold} - V_{1/2})/k) \quad (2)$$

where $V_{1/2} = -68.5 \pm 4.1$ mV and the slope factor $k = 6.23 \pm 0.55$ mV ($n = 13$) for the RHI current in oocytes, and $V_{1/2} = -80 \pm 3.7$ mV and $k = 7.24 \pm 0.56$ mV ($n = 3$) for the I_{Na} of neonatal myocytes. The small differences in slope values were not statistically significant.

The decaying phase of RHI current was fit well with a single exponential function. Figure 2 presents representative currents from a RHI-injected oocyte and a whole-cell recording from an isolated neonatal myocyte. For comparison the currents in Fig. 2 are displaced by 20 mV to compensate for the shift seen in the availability and activation curves. Although most of the difference in decay rates is accounted for by the shift, it is apparent that the RHI current decayed somewhat slower than the native current, and a shift of about

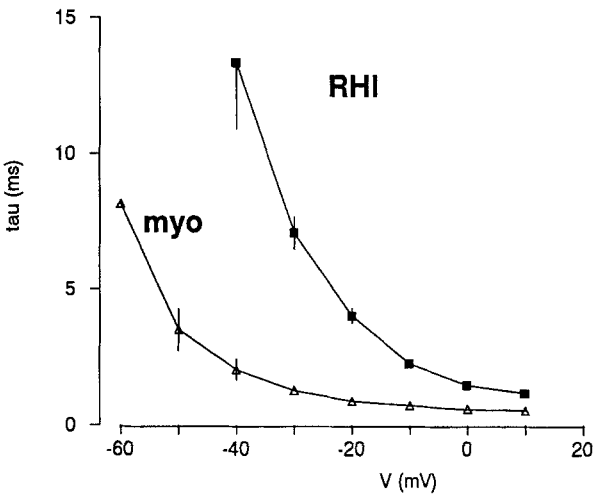

Fig. 3. RHI current decay as a function of voltage is shifted relative to the native current. $\tau(V)$ from Eq. (3) is pooled for five myocytes and eight RHI injected oocytes. Error bars ± SEM are shown where they exceed the size of the symbol.

30 mV would be necessary for the curves to coincide (Fig. 3). Because of the large uncompensated capacitive/ionic current ratio at potentials more positive than +10 mV, we were not able to measure decay rates accurately for RHI currents at these voltages.

RHI Current Decays and Recovers Faster than Currents through TTX-Sensitive Na Channel α-Subunits

The brain and skeletal muscle Na channel α-subunits expressed in oocytes typically exhibit currents with abnormal decay kinetics (Krafte et al., 1990). In order to examine the difference between RHI current decay and the abnormally slow decaying TTX-sensitive currents, we compared their respective time courses for decay and for recovery from inactivation. At near-threshold potentials BrnIIa and RHI current decay τ's are not significantly different. However, the decay rates of BrnIIa or μI currents do not increase at depolarized potentials as much as those for RHI (Fig. 4). The slight dip in the $\tau(V)$ curve between −30 and −20 mV for BrnIIa current corresponds to the steep part of the activation curve and may represent some loss of voltage control. This dip was not seen for the expressed RHI and μI currents where loss of voltage control was less than 2 mV. At more depolarized potentials, where a reduced Na driving force results in smaller amplitude currents allowing for improved voltage control, BrnIIa current decay is significantly slower than RHI current decay. The $\tau(V)$ for μI currents were

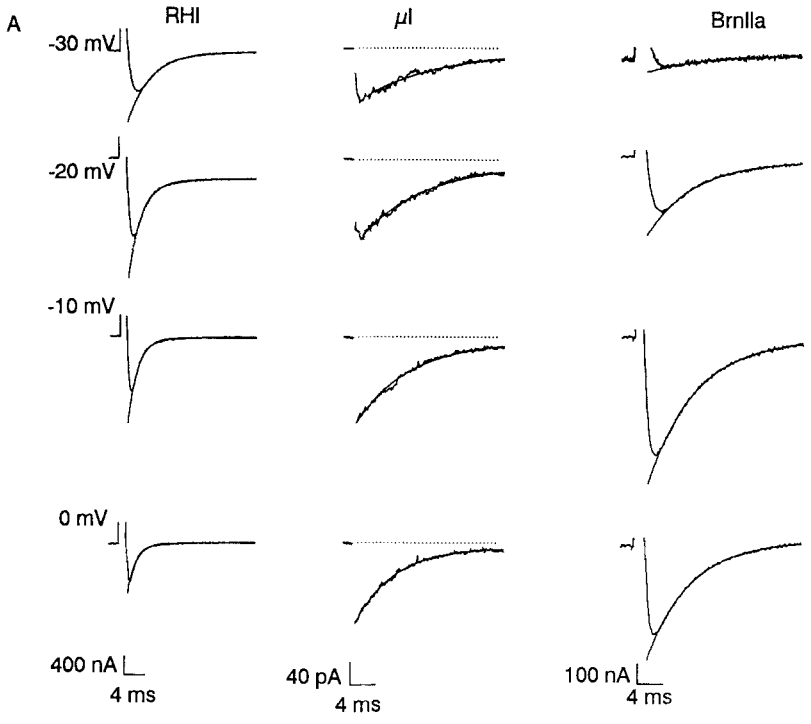

Fig. 4. RHI current decays faster than currents from the two TTX-sensitive isoforms. Interpulse interval was 20 sec to allow complete recovery from slow inactivation. (*A*) Representative currents elicited by depolarizations to −30 through 0 mV in 10 mV increments from V_{hold} = −100 mV. BrnIIa and RHI are whole-oocyte currents; μI is from a cell-attached macropatch. Currents were superimposed with a single exponential function as in Fig. 2. (*B*) Representative BrnIIa whole-oocyte and μI macropatch current shown in (*A*) was used to determine $\tau(V)$. The smooth lines were drawn by eye. $\tau(V)$ for RHI is identical to Fig. 3. τ for BrnIIa and μI currents were extracted from Eq. (3).

obtained with a macro-patch allowing us to examine the current decay at more depolarized potentials. At all potentials tested μI current decayed more slowly than RHI current. Whole-oocyte BrnIIa and RHI currents with similar peak amplitudes were selected for this comparison. Under the conditions of these experiments, we could not detect a second, slower component of current decay.

The kinetics of recovery from inactivation of RHI current were dramatically different from the currents expressed by the TTX-sensitive α-subunits, BrnIIa and μI. We compared the recovery from inactivation with a two-step protocol. Oocytes were voltage-clamped to −10 mV for a 1 sec pre-pulse to inactivate the Na channels, returned to the holding potential of −100 mV for a variable amount of time, and then stepped to a test potential of −10 mV to measure the amount of current that had recovered from inactivation during that test recovery time. Figure 5 illustrates the peak current during the test pulse, normalized to that of the preceding pre-pulse. For RHI current recovery was fit with a two-exponential function (τ_f = 25.0 ± 16 msec, τ_s = 537 ± 184 msec, n = 5). Recovery from inactivation was

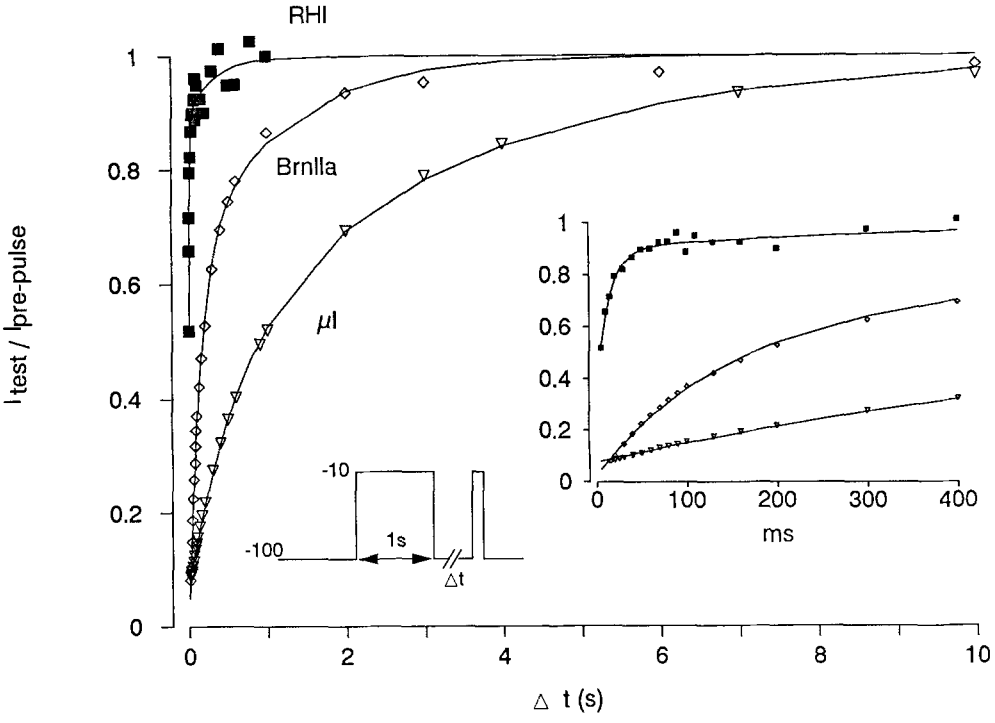

Fig. 5. RHI recovery from inactivation was faster than that for TTX-sensitive α-subunits. Two exponentials were required to fit recovery data for whole-oocyte currents from RHI, BrnIIa, and μI. Both phases of recovery are faster for the cardiac isoform. (*Inset*, right) Expanded time scale illustrates that τ_f is faster for RHI than either TTX-sensitive isoform tested. (*Inset*, left) Two-step protocol used to measure recovery, 20 or 40 sec elapsed between pulse pairs. τ_f for RHI, BrnIIa, and μI is 13.7, 142, and 618 msec, respectively. τ_s for RHI, BrnIIa, and μI was 0.33, 1.10, and 3.24 sec, respectively.

much slower for TTX-sensitive channels. Recovery was measured for BrnIIa and fit to a two-exponential function (τ_f = 143.8 ± 14 msec, τ_s = 1151.8 ± 45 msec, n = 4). Recovery from inactivation is even slower for μI channels. Five of six oocytes were fit with two exponentials (τ_f = 589.0 ± 198 msec, τ_s = 3.549 ± 0.8 sec). One μI-injected oocyte expressed current which required an additional fast time constant to fit the recovery data.

Use-Dependent Block of RHI Current by TTX

Use dependence is a characteristic feature of block by TTX of cardiac Na current (Cohen et al., 1981; Eickhorn et al., 1990). We have shown previously that RHI current is blocked tonically by a relatively high concentration of TTX (K_d = 1.5 μM; Cribbs et al., 1990). We define tonic block as the TTX block that is observed after holding the membrane potential at −100 mV for more than 20 sec. Depolarizations into the voltage range of I_{Na} activation result in further block current which we describe as use-dependent block. To investigate use-dependent block of RHI current by TTX, we applied 2 Hz trains of 7 msec duration voltage pulses from −100 to −10 mV. In the absence of TTX the current was reduced only slightly by this protocol, presumably because of entry of the RHI Na channel into a slow inactivated state (Fig. 6). Addition of 1 μM TTX led to approximately 50% tonic block of RHI current. The train of 7 msec depolarizations stepped at 2 Hz caused an additional 25% block. The rate constant for development of extra block of RHI current by 1 μM TTX during 2 Hz trains was 2.63 ± 0.21 depolarizations (n = 4).

In native cardiac cells TTX slows recovery of Na current from inactivation (Cohen et al., 1981). The fraction of current that recovered quickly was reduced by 1 μM TTX, without a change in time constant of the fast component (Fig. 7). In addition to increasing the fraction of Na current that recovered slowly, 1 μM TTX further slowed its time constant (mean difference $\tau_{slow} - \tau_{slow,toxin}$ = 4.75 ± 1.2 sec, n = 4). Addition of 1 μM TTX did not change the midpoint of the steady-state inactivation relationship under the conditions of these experiments. The mean difference for the $V_{1/2}$'s, measured by a 5 or 10 sec V_{hold} followed by a V_{test} to −10 mV, was

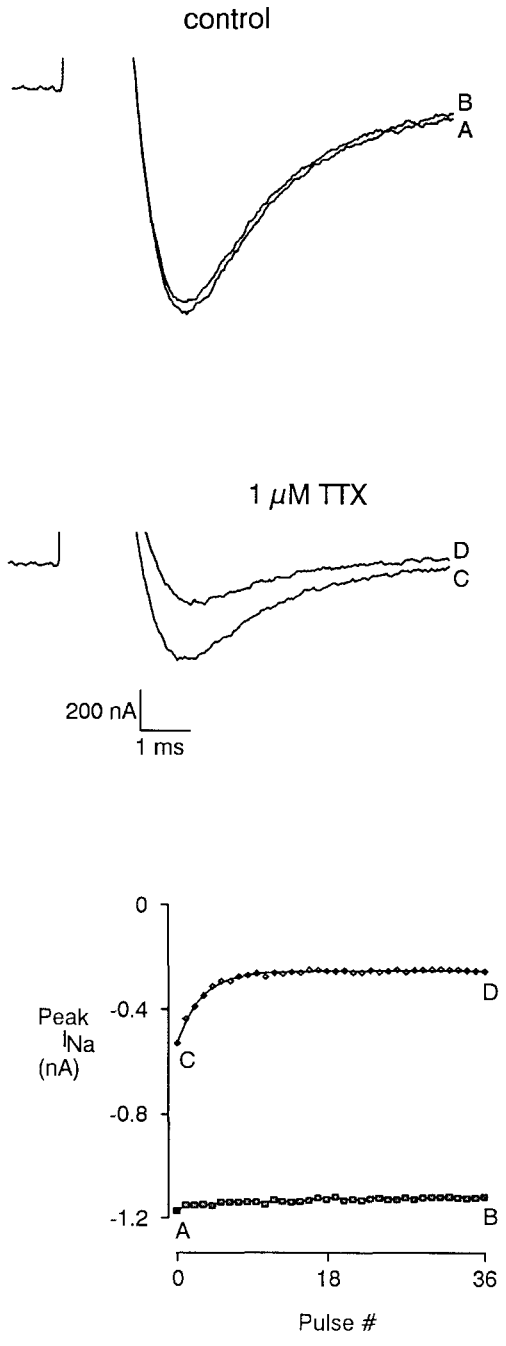

Fig. 6. TTX block of RHI current is use dependent. Trains of 7 msec depolarization were elicited at a frequency of 2 Hz in the absence (*A,B*) and the presence (*C,D*) of 1 μM TTX. Use-dependent block at 2 Hz in this experiment developed with a single exponential time course with a time constant of 2.74 sec.

not significantly different between control measurements and those after toxin was added (mean difference = 1.0 ± 1.8 mV, $n = 4$). The effects of TTX on the recovery of μI and BrnIIa currents were not studied in detail because of their relatively slow recovery.

Divalent Cation Block of RHI

Native cardiac Na current is relatively sensitive to Cd^{2+} in the micromolar range (Visentin et al., 1990; Ravindran et al., 1991; Hanck & Sheets, 1992). To evaluate Cd^{2+} block of the RHI current, we measured peak Na current in RHI-injected oocytes (Fig. 8). Cd^{2+} in the concentrations used on RHI currents had a negligible effect on the potential at which the peak of the $I(V)$ curve occurred. We fitted a single-site curve with a K_d of 48 μM. In contrast, native TTX-sensitive channels are reported to be at least 50-fold less sensitive to Cd^{2+} (Frelin et al., 1986; Ravindran et al., 1991). Consistent with a lower Cd^{2+}-sensitivity in the native tissues, BrnIIa and μI channels expressed in oocytes are 20-fold less sensitive to Cd^{2+} than RHI (Fig. 9). We pooled the μI and BrnIIa current data because their Cd^{2+}-sensitivity was indistinguishable. The Cd^{2+} dose response is drawn from the maximal conductance at each concentration normalized to the control. Because of the very slow recovery from inactivation of μI and BrnIIa Na currents, at least 10 sec were allowed between depolarizations to permit complete recovery. In spite of this long recovery period, these measurements may somewhat overestimate the sensitivity to Cd^{2+} block.

Lidocaine Block of RHI

Native cardiac Na current is blocked in use-dependent fashion by lidocaine. A 2 Hz train of 43 msec long depolarizations results in use-dependent block of RHI current by 100 μM lidocaine. In the absence of drug the peak current decreased less than 10% by the 36th pulse. 100 μM lidocaine resulted in 9.8 ± 3.9% tonic block and a 30.3 ± 3.6% decrease after the 36th pulse of a 2-Hz train ($n = 5$; Fig. 10).

Discussion

The RHI clone, isolated from a neonatal rat heart library (Rogart et al., 1989), directs the expression in *Xenopus* oocytes of a transient voltage-dependent Na current that possesses many of the characteristics of the Na current of isolated neonatal rat myocytes, including relative resistance to the blocking action of STX and TTX (Cribbs et al., 1990). Skm2, a clone derived from rat denervated skeletal muscle, is identical to RHI (Kallen et al., 1990), and in *Xenopus* oocytes it also codes for a Na channel that is relatively resistant to TTX (White et al., 1991). The deduced primary sequence of RHI contains over 2000 amino acids, and codes for the α-subunit of the

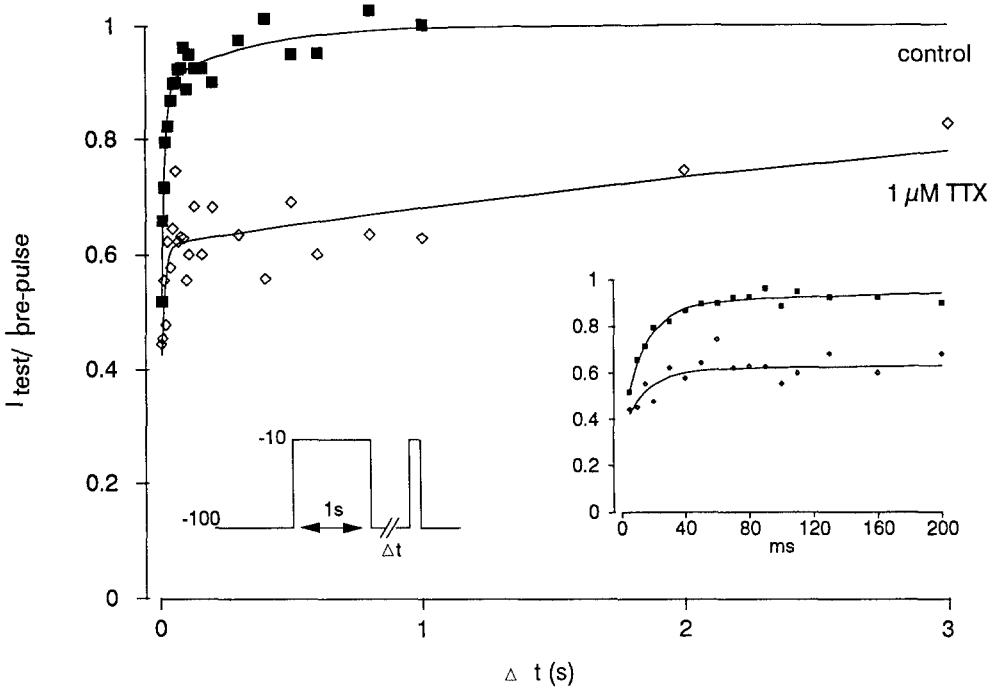

Fig. 7. TTX slows RHI recovery from inactivation. Two-pulse protocol (*inset*, left) used to assess recovery from inactivation. Two exponentials are required to fit recovery data for both control and 1 μM TTX. (*Inset*, right) Expanded time scale shows that τ_f is unaffected by addition of 1 μM TTX. For control τ_f = 13.7 msec, τ_s = 331 msec, and in the presence of 1 μM TTX $\tau_{f,\text{toxin}}$ = 14.4 msec, $\tau_{s,\text{toxin}}$ = 5.511 sec.

Na channel. Other Na channel subunits of unknown function have been identified for skeletal muscle (Tanaka et al., 1984) and brain (Gordon et al., 1988), and perhaps even for heart (Gordon et al., 1988). The presence of subunits and the possibility of tissue-specific post-translational modifications in the Na channel proteins make it important to determine if the expressed α-subunits alone are sufficient to demonstrate the kinetic and pharmacological properties of naturally occurring Na channels in the tissue from which the clones were derived.

The most thoroughly investigated α-subunit is BrnII and its almost identical isoform BrnIIa. Stühmer et al. (1987) demonstrated that BrnII has kinetic properties similar to those of peripheral nerve and muscle, except that the voltage dependence of availability and activation was shifted about 10–15 mV in the positive direction. However, compared to Na currents obtained by co-injection into oocytes of total mRNA isolated from brain tissue, the α-subunit of BrnIIa shows slow current decay rates, which can be restored to normal by coinjection of a low molecular weight fraction of brain mRNA (Krafte et al., 1990). The presumed explanation for this phenomenon is that the RBrn2a α-subunit is kinetically abnormal, but it can be restored to more native-like values by coexpression of a subunit or a protein that modifies the Na channel α-subunit. μI, the channel derived from mature rat skeletal muscle, also shows very slow current decay in oocytes relative to currents expressed from native total mRNA (Trimmer et al., 1989). A single Na channel expressed from a clone of RBrn3 (Moorman et al., 1990) or μI (Zhou, Agnew & Sigworth, 1991) can sequentially show both fast and slow inactivation behavior. The abnormality of current decay found in *Xenopus*-expressed TTX-sensitive α-subunits is not apparent in the cardiac α-subunit.

Comparison of RHI with Native Cardiac Na Current

Native cardiac I_{Na} has been reported to have one or more slowly inactivating components (Carmeliet, 1987; Fozzard et al., 1987). Single Na channels show only transient opening behavior, and ensembles resemble the normal transient I_{Na} (Kunze et al., 1985; Berman et al., 1989; Scanley et al., 1990). However, occasionally a channel will show persistent burst behavior or prolonged opening (Patlak and Ortiz, 1986; Scanley et al., 1990; Kohlhardt, Fröbe & Herzig, 1987), which occurs less than 0.1% of depolarizations (Kiyosue & Arita, 1989). This burst

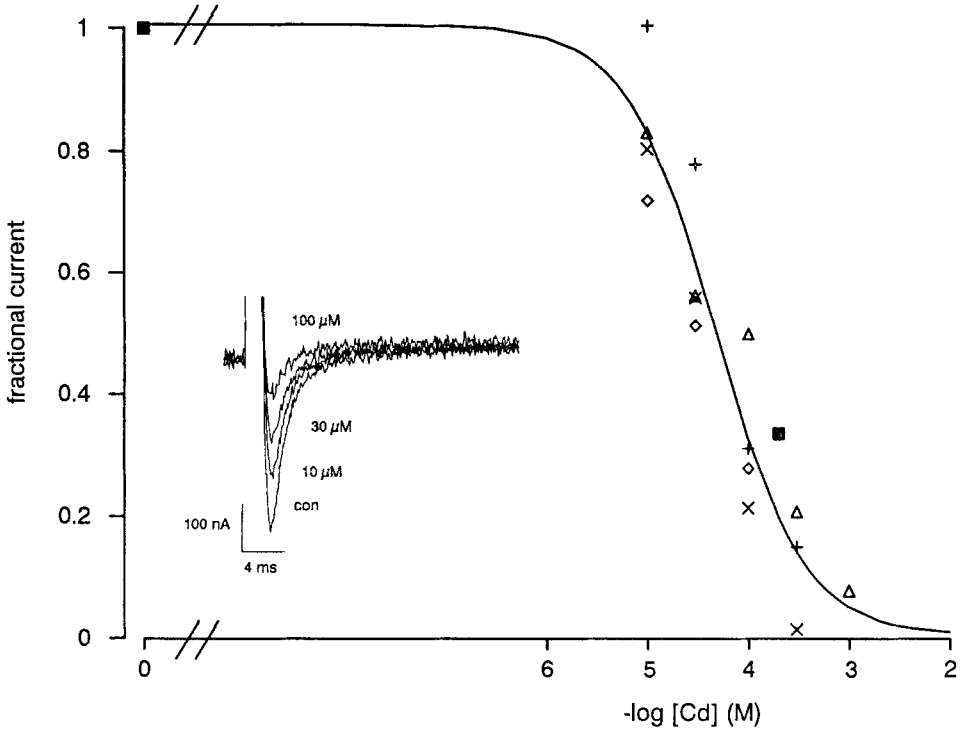

Fig. 8. RH1 current is Cd^{2+}-sensitive. Symbols represent Cd^{2+} dose response from four different RHI-injected oocytes. (*Inset*) current elicited by a voltage step from −100 to −10 mV in control, 10 μM, 30 μM and 100 μM Cd^{2+}. Smooth line is single-site dose-response curve of the form:

$$G_{Na,Cd}/G_{Na,control} = 1/\{1 + ([Cd^{2+}]/K_d)\}, \quad (4)$$

where K_d = 48 μM. G_{Na} was calculated from the chord conductance of the $I(V)$ curve. V_h was −100 mV and at least 10 sec elapsed between depolarizations.

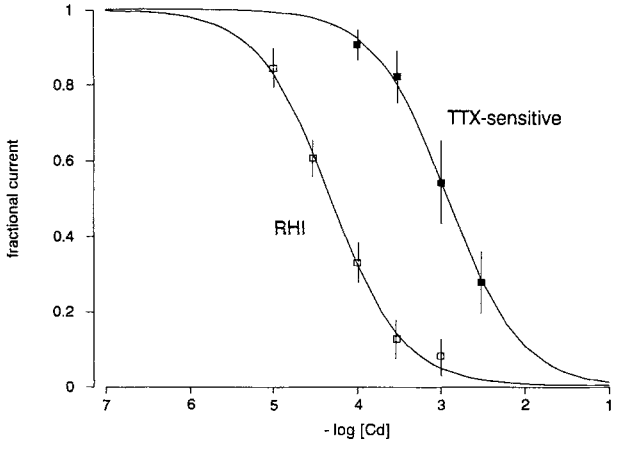

Fig. 9. RHI current is more sensitive to external Cd^{2+} than TTX-sensitive currents. μI (filled) and rat brain IIa (open symbols) data are combined. Single-site curve is fit with a K_d = 964 μM. Error bars are ± SEM (RHI, n = 5; RBrn2a, n = 4; μI, n = 4).

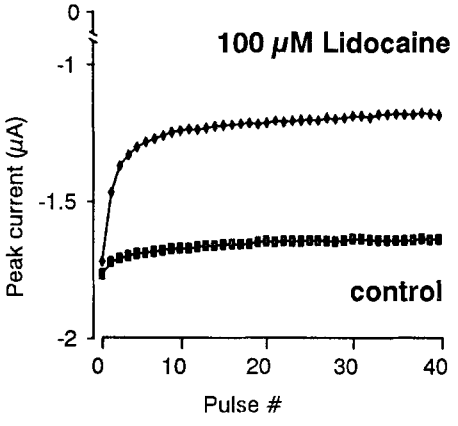

Fig. 10. Lidocaine block of RHI current is use dependent. Forty consecutive peak RHI currents from a representative oocyte are plotted for a 2-Hz train of depolarizations from V_h − 100 to V_{test} − 10 mV. Control current decreased less than 10%. In the presence of 100 μM externally applied lidocaine a negligible tonic block was followed by a 33% decrease of the current at the end of the 2-Hz train.

behavior of cardiac Na channels resembles the burst behavior described by Moorman et al. (1990) for RBrn III and Zhou et al. (1991) for μI. Such a slowly inactivating RHI current could not be seen in these experiments, and if present it must have been a very small component of the current. Certainly, it was not accentuated in the *Xenopus* expression system.

The voltage dependence of availability and activation for the RHI current is the same as that for the Na currents of neonatal rat myocytes, except for a shift in the depolarizing direction. Stühmer et al. (1987) observed a similar shift for RBrnII currents expressed in oocytes, and Trimmer et al. (1989) found a voltage shift in the μI current in oocytes. In addition, Na currents generated by expression in oocytes of brain poly(A)$^+$ mRNA and BrnIIa show the same voltage dependence of availability (Krafte et al., 1990) as that of RHI current. Na currents expressed in oocytes by total heart mRNA (Krafte et al., 1991; J. Satin, *unpublished observation*) also have the same decay kinetics and the same voltage dependence as RHI current in oocytes. In each case, a depolarizing shift is seen when *Xenopus* oocytes are used for the expression of either cloned α-subunit cRNAs or isolated mRNA.

The change in field sensed by the RHI Na channels expressed in oocytes is also apparent in the voltage dependence of the time constants of current decay. Although a 20-mV depolarizing shift does not fully account for this difference in decay between RHI and neonatal myocyte Na currents, the differences in rates after allowing for a 20-mV shift are small compared to the slow decay of the TTX-sensitive Na channels in oocytes. Preliminary results from our lab with currents generated from rat heart poly(A)$^+$ mRNA Na channels show identical decay kinetics to RHI currents.

An additional possible source of difference in the voltage dependence of the Na currents between those expressed by the α-subunit cRNA and those of the neonatal myocyte is a shift caused by myocyte dialysis. Whole-cell Na currents recorded by the patch-clamp method have a slow time-dependent shift in their voltage-dependent kinetics (Kimitsuki, Mitsuiye & Noma, 1990; Ayer, Fujii & DeHaan, 1985). Since the Boltzmann fits of activation and availability resulted in similar slopes, the differences represent simply a translation along the voltage axis. We sought to minimize such shifts in those myocyte experiments by recording I_{Na} quickly after establishment of the whole-cell mode. However, some shift could have occurred; thus, we hesitate to draw any conclusions from the differences seen here.

Comparison to TTX-Sensitive α-Subunits

The largest difference demonstrated in these studies for RHI is the relatively fast current decay rate and current recovery rate after inactivation compared to the two TTX-sensitive Na currents studied. The decay rate for the TTX-sensitive channels is clearly different from that of the Na currents obtained with total native mRNA or to those seen in intact cells. A parallel difference in the TTX-sensitive α-subunits is their very slow recovery from inactivation, although the BrnIIa channel recovers faster than μI. Multiple slow components of recovery from inactivation are consistent with the complex gating of μI single channels reported in oocytes (Zhou et al., 1991). Stühmer et al. (1989) reported that BrnIIa single channel currents showed longer open times than native neuronal single channels. Moorman et al. (1990), as already noted, have also reported fast and slow openings of RBrn3 in cell-attached patches. A common feature of TTX-sensitive Na channel α-subunits expressed in oocytes is gating that is slower than that of the TTX-resistant Na channels expressed by RHI.

Use-Dependent Block by TTX/STX

TTX and STX show two types of block of cardiac Na currents: tonic and use dependent. Use-dependent block is usually considered characteristic of cardiac Na channels, but some nerve preparations show similar properties (Salgado, Yeh & Narahashi, 1986; Patton & Goldin, 1992). RHI Na current shows use-dependent block similar to that shown by Cohen et al. (1981) in short cardiac Purkinje strands, including the slow recovery from inactivation that probably represents slow dissociation of TTX from the channel.

Voltage-dependent block by TTX of native cardiac Na channels is controversial. The lack of a shift of steady-state inactivation of RHI in the presence of TTX is consistent with the results of Cohen et al. (1981). However, several studies demonstrate a shift of the steady-state inactivation curve in the presence of TTX (Vassilev et al., 1986; Carmeliet, 1987; Eickhorn et al., 1990), and the results appear to be protocol-dependent.

RHI Block by Cd^{2+} and Lidocaine

RHI current in oocytes retains distinguishing cardiac pharmacological properties with respect to block by the transition metal Cd^{2+} and the local anesthetic lidocaine. Sub-millimolar concentrations of Cd^{2+}

are sufficient for half-maximal block of cardiac I_{Na} in calf Purkinje fibers (DiFrancesco et al., 1985; Hanck & Sheets, 1992) and canine ventricle (Sheets et al., 1987). Direct comparison of Cd^{2+} block of batrachotoxin-modified Na channels in bilayers from heart and skeletal muscle shows a 46-fold higher affinity for the cardiac channel (Ravindran et al., 1991). Use-dependent block of RHI by 100 μM lidocaine, accompanied by negligible tonic block, is similar to lidocaine block of other native cardiac preparations (Bean, Cohen & Tsien, 1983; Alpert et al., 1989). The retention of native cardiac kinetics and pharmacology by RHI current in oocytes makes this system amenable to the study of structure/function relations of the channel protein using site-directed mutagenesis.

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