Evidence supports a role for the tetrodotoxin-sensitive NaV1.7 and the tetrodotoxin-resistant NaV1.8 in the pathogenesis of pain. Ranolazine, an anti-ischemic drug, has been shown to block cardiac (NaV1.5) late sodium current (INa). In this study, whole-cell patch-clamp techniques were used to determine the effects of ranolazine on human NaV1.7 (hNaV1.7 + β1 subunits) and rat NaV1.8 (rNaV1.8) channels expressed in HEK293 and ND7-23 cells, respectively. Ranolazine reduced hNaV1.7 and rNaV1.8 INa with IC50 values of 10.3 and 21.5 μM (holding potential = -120 or -100 mV, respectively). The potency of INa block by ranolazine increased to 3.2 and 4.3 μM when 5-sec depolarizing prepulses to -70 (hNaV1.7) and -40 (rNaV1.8) mV were applied. Ranolazine caused a preferential hyperpolarizing shift of the steady-state fast, intermediate and slow inactivation of hNaV1.7 and intermediate and slow inactivation of rNaV1.8, suggesting preferential interaction of the drug with the inactivated states of both channels. Ranolazine (30 μM) caused a use-dependent block (10-msec pulses at 1, 2 and 5 Hz) of hNaV1.7 and rNaV1.8 INa, and significantly accelerated the onset of, and slowed the recovery from inactivation, of both channels. An increase of depolarizing pulse duration from 3 to 200 msec did not affect the use-dependent block of INa by 100 μM ranolazine. Taken together, the data suggest that ranolazine blocks the open state and may interact with the inactivated states of NaV1.7 and NaV1.8 channels. The state-and use-dependent modulation of hNaV1.7 and rNaV1.8 Na+ channels by ranolazine could lead to an increased effect of the drug at high firing frequencies, as in injured neurons.

Introduction

Upon membrane depolarization, voltage-gated Na+ channels rapidly undergo conformational changes that lead to channel activation and result in rapid entry of Na+ ions into the cell. During prolonged depolarization, Na+ channels progressively enter slow inactivated states with lifetimes ranging from hundreds of milliseconds to several seconds.3 Predominantly, voltage-gated Na+ channels are heterodimers, composed of an α-subunit (~260 kDa) and β (auxiliary) subunits (33–39 kDa). Ten isoforms of Na+ channel α-subunits (NaV1.1–1.9, NaV) have been identified by cloning and expression.2,3 Despite amino acid sequence similarities within the Na+ channel family, isoforms have different gating kinetics and varying sensitivities to block by tetrodotoxin (TTX). Thus, NaV1.1, NaV1.6 and NaV1.7 are rapidly-inactivating TTX-sensitive (TTX-S) Na+ channels, whereas NaV1.8 and NaV1.9 are slowly-inactivating TTX-resistant (TTX-R) channels.3,6

In nociceptive neurons, varying levels of expression of NaV1.7, NaV1.8, NaV1.9, and the brain sodium channels NaV1.1, NaV1.2, NaV1.3 contribute to electrical excitability.7 Changes in the expression levels of these Na+ channels have been implicated in the alterations of neuronal excitability associated with acute and chronic pain syndromes.8,11 Neuropathic pain has multiple etiologies and develops in many disease states, including diabetic mellitus, alcoholism, entrapment syndromes, herpes and HIV infections, and after lesions of the brain, spinal and peripheral nervous tissues. Loss-of-function as well as gain-of-function congenital mutations of Na+ channel α-subunits have been identified in patients with epilepsy, seizures, ataxia and increased/decreased sensitivity to pain. The alpha polypeptide (pore-forming unit) of the NaV1.7 Na+ channel encoded by the gene SCN9A is a rapidly-inactivating TTX-S Na+ channel that is highly expressed in spinal dorsal root ganglion (DRG) neurons.12,13 Goldberg and colleagues14 showed that humans lacking functional NaV1.7 channels exhibit a complete insensitivity to pain with apparently no other deficits, suggesting that the loss of NaV1.7 activity may selectively reduce pain. Furthermore, Cummins and colleagues15 have shown that gain-of-function mutations in NaV1.7 cause inherited painful neuropathies, indicating a central role for NaV1.7 in nociception. The alpha polypeptide (pore-forming unit) of the NaV1.8 Na+ channel encoded by the gene SCN10A is a slowly-inactivating TTX-R Na+ channel that is found in DRG neurons and small nociceptive C-type pain fibers.16,17 Using a nociceptor-specific NaV1.8 knockout mouse model, Matthews and colleagues18 showed significant reduction in spontaneous firing as...
well as reduced responses of spinal wide dynamic range neurons to mechanical stimulation. Furthermore, following peripheral nerve damage, protein expression of Na\textsubscript{v}1.8 channels is reduced in injured neurons; however, the expression level of Na\textsubscript{v}1.8 is increased in uninjured neurons. Activity in the uninjured neurons has been shown to be critical in neuropathic pain states. Recently, selective block of Na\textsubscript{v}1.8 has also been shown to alleviate both inflammatory and neuropathic pain in animals.

Nonselective inhibitors of Na\textsuperscript{+} channels, such as lidocaine and mexiletine, have been shown to attenuate hyperalgesia in animal models of neuropathic pain and in humans. However, the therapeutic application of these agents is limited due to their side-effects. Consequently, there is a great need for new drugs to treat pain. Ranolazine has been shown to reduce the cardiac Na\textsubscript{v}1.5 persistent (late) I\textsubscript{Na}\textsuperscript{c} The drug has been approved for reduction of chronic angina and shown to be safe. Ranolazine (a structural analog of lidocaine, Fig. 1) block of late I\textsubscript{Na}\textsuperscript{c} was significantly reduced after mutation of an amino acid residue in the putative local anesthetic binding site of the sodium channel, suggesting that its site and mechanism of action may be similar to those of lidocaine, a known blocker of both Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8. Further, ranolazine was reported to block the open state of Na\textsubscript{v}1.7 I\textsubscript{Na}\textsuperscript{c} in a use-dependent manner.

In this study, we investigated the open as well as the inactivated state block of Na\textsubscript{v}1.7 by ranolazine. Because evidence from several studies suggests that Na\textsubscript{v}1.8 also has a critical role in peripheral pain sensing, the effects of ranolazone on Na\textsubscript{v}1.8 were also determined. Thus, we performed voltage-clamp experiments on human embryonic kidney (HEK293) cells transfected with human Na\textsubscript{v}1.7 (hNa\textsubscript{v}1.7 + β\textsubscript{1} subunit) and on the DRG-derived cell line ND7-23 expressing rat Na\textsubscript{v}1.8 (rNa\textsubscript{v}1.8) Na\textsuperscript{+} channels to investigate whether ranolazine blocks these two neuropathic pain Na\textsuperscript{+} channel targets at concentrations of the drug that are achieved therapeutically (i.e., 2–8 μM) during treatment of patients with ischemic heart disease.

**Results**

Characterization of Na\textsuperscript{+} channels in untransfected and transfected ND7-23 and transfected HEK293 cells. Figure 1 shows the effect of 300 nM TTX on HEK293 cells stably expressing hNa\textsubscript{v}1.7 + β\textsubscript{1} subunits (Fig. 2A) and untransfected ND7-23 cells (Fig. 2B) or ND7-23 cells stably expressing rNa\textsubscript{v}1.8 Na\textsuperscript{+} channels (ND7-23/ rNa\textsubscript{v}1.8; Fig. 2C). TTX (300 nM) completely blocked the hNa\textsubscript{v}1.7 I\textsubscript{Na}\textsuperscript{c} in HEK293 and endogenous I\textsubscript{Na}\textsuperscript{c} in ND7-23 cells. In contrast, 300 nM TTX caused a minimal block of rNa\textsubscript{v}1.8 I\textsubscript{Na}\textsuperscript{c}, confirming previous reports of the resistance of rNa\textsubscript{v}1.8 to the toxin.

Ranolazine blocks recombinant human and native rat Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8 currents. The application of 30 μM ranolazine to either HEK293 cells stably expressing hNa\textsubscript{v}1.7 or ND7-23/rNa\textsubscript{v}1.8 Na\textsuperscript{+} channels produced a significant reduction of peak current (Fig. 3A) and suggested a considerable acceleration of the rate of inactivation (transition from open to inactivated states). To quantify the changes in I\textsubscript{Na}\textsuperscript{c} decay rates (in the absence and presence of 30 μM ranolazine) the current traces (hNa\textsubscript{v}1.7 and rNa\textsubscript{v}1.8) were fit with single exponentials. At -20 mV the decay of hNa\textsubscript{v}1.7 currents for control conditions and in the presence of 30 μM ranolazine had time constants of 1.51 ± 0.31 and 0.68 ± 0.15 msec (n = 4 cells, p < 0.05), respectively. Similarly, at +20 mV the decay of rNa\textsubscript{v}1.8 currents for control conditions and in the presence of 30 μM ranolazine had time constants of 3.40 ± 0.13 and 1.60 ± 0.04 msec (n = 4 cells, p < 0.05), respectively.

Ranolazine caused a concentration-dependent block of hNa\textsubscript{v}1.7 and rNa\textsubscript{v}1.8 at holding potentials of -120 or -100 mV, respectively (Fig. 3B). When the holding potential in experiments was set at a voltage close to the midpoint of the voltage-dependent steady-state inactivation relationship (voltage at which 50% of channels are inactivated) for each channel (-70 mV for Na\textsubscript{v}1.7 and -40 mV for Na\textsubscript{v}1.8), the concentration-response relationship for ranolazine block of I\textsubscript{Na}\textsuperscript{c} was shifted to the left (i.e., to lower ranolazine concentrations) (Fig. 3B). Ranolazine also blocked the endogenous TTX-S
Ranolazine blocks NaV1.7 and NaV1.8 Na+ channels

Table 1

| Na\(_V\) isoform | \(V_0\) | IC\(_{50}\) value (\(\mu\)M) | \(V_\text{h}\) |
|-----------------|--------|-----------------------------|--------|
| hNaV1.7 | 10.36 ± 1.25 | 3.25 ± 0.17 |
| rNaV1.8 | 21.53 ± 3.01 | 4.33 ± 0.52 |
| TTX-S (endogenous I\(_{Na}\)) | 9.05 ± 0.56 | N.T. |

Data were recorded using voltage-clamp protocols described in Figure 2. Data were fitted with Hill equation. IC\(_{50}\) values are given in \(\mu\)M and Hill coefficients are listed in parenthesis. \(V_\text{h}\) = -120 and -100 mV for hNaV1.7 and rNaV1.8, respectively. N.T. = not tested.

Voltage dependence of activation in the presence of ranolazine.

Current-voltage (I-V) relationships for hNaV1.7 and rNaV1.8 I\(_{Na}\) were determined in the absence of 10 \(\mu\)M ranolazine using a series of 50-msec depolarizing steps from a holding potential of -120 mV to +100 mV with an interpulse interval of 10 sec. Figure 4A and B shows the voltage clamp protocols and representative current traces recorded from a HEK293 cell stably expressing hNaV1.7 (left) and from ND7-23/rNaV1.8 I\(_{Na}\) (right, recorded in the presence of 300 nM TTX), respectively. From the peak amplitude of I\(_{Na}\) measured, sodium conductance (G\(_{Na}\)) was calculated (see Methods for details) and the voltage-dependence of G\(_{Na}\) was plotted in the absence (○, hNaV1.7; ●, rNaV1.8, Fig. 4C) and presence (□, hNaV1.7; ○, rNaV1.8, Fig. 4C) of 10 \(\mu\)M ranolazine. The values of mean half-maximal voltage (\(V_\text{½}\)) for activation and the slope (\(k\)) factors of the relationships in the absence (control) and presence of ranolazine are shown in Table 2. Ranolazine did not significantly shift the voltage range across which channel activation occurred (Fig. 4, Table 2, Activation). Figure 4D shows the decay of the hNaV1.7 (left) and rNaV1.8 (right) I\(_{Na}\) (current traces described in Fig. 4B) in the absence (●) and presence of 10 \(\mu\)M (○) ranolazine to a single exponential equation. Ranolazine caused a significant effect to decrease on the time constants of current decay at voltages between -40 to +5 mV for hNaV1.7 and -35 to +30 mV for rNaV1.8, respectively.

Voltage dependence of steady-state fast, intermediate and slow inactivation in the presence of ranolazine.

Results of experiments to determine the voltage dependence of steady-state fast, intermediate and slow inactivation of hNaV1.7 (left) and rNaV1.8 (right) I\(_{Na}\) are shown in Figure 5. Figure 5A shows voltage-clamp protocols and summary results of experiments for steady-state fast inactivation of hNaV1.7 and rNaV1.8 (inactivating prepulse of 100 msec) in the absence (○, hNaV1.7; ●, rNaV1.8) and presence of 10 \(\mu\)M ranolazine (□, hNaV1.7; ○, rNaV1.8). Ranolazine caused a significant (p < 0.05) leftward shift in the \(V_\text{½}\) of fast-inactivation without affecting the slope (\(k\)) factor of hNaV1.7, and a minimal (p = 0.15) leftward shift in the \(V_\text{½}\) of fast-inactivation without affecting the slope (\(k\)) factor of rNaV1.8 I\(_{Na}\) (see Fig. legends for values). Figure 5B shows voltage-clamp protocols and summary results of experiments for steady-state intermediate inactivation of hNaV1.7 and rNaV1.8 (inactivating pulse of 1 sec) in the absence (○, hNaV1.7; ●, rNaV1.8) and presence of 10 \(\mu\)M ranolazine (□, hNaV1.7; ○, rNaV1.8). Ranolazine caused a concentration-dependent (1–30 \(\mu\)M) leftward shift in the \(V_\text{½}\) of intermediate inactivation without affecting the slope (\(k\)) factor for hNaV1.7 (n = 4 cells at each concentration) and rNaV1.8 (n = 4–5 cells at each concentration) I\(_{Na}\) (Table 2, Inactivation). The data for midpoints of activation and steady-state inactivation for control conditions (hNaV1.7 and rNaV1.8) in the present study are comparable to values found previously for ND7-23/rNaV1.8 and native TTX-S and TTX-R currents in DRG neurons.\(^9,27\) To test the voltage...
dependence of the steady-state slow inactivation process, the pulse protocol shown in Figure 5C was employed for both hNa_v1.7 and rNa_v1.8. Using this protocol, slow inactivation (physiological) became evident at potential of -80 mV and -75 mV for hNa_v1.7 and rNa_v1.8, respectively. However, slow-inactivation was only 50 and 70% complete at the maximum conditioning test pulse of -10 mV. Figure 5C shows voltage-clamp protocols and summary results of experiments for steady-state slow inactivation of hNa_v1.7 and rNa_v1.8 (inactivating prepulse of 10 sec) in the absence (■, ○) and presence of 10 μM ranolazine (□, △). Ranolazine caused a significant (p < 0.05) leftward shift in the V½ of slow inactivation without affecting the slope (k) factor of hNa_v1.7 and rNa_v1.8 INa (see Fig. legends for values).

The ranolazine-induced shift in the mid-point (V½) of inactivation (Fig. 5) and voltage-dependent block of hNa_v1.7 and rNa_v1.8 (Fig. 3, Table 1, at V½ holding potential IC50 values) suggest that ranolazine might be interacting with the inactivated states of these channels. To estimate the extent of block of inactivated channels by ranolazine, an indirect approach based on the concentration-dependence of the shift of the steady-state inactivation curve28 was used (Kdr and Kdi values, calculated as described in Methods). Estimates of dissociation constants for ranolazine to bind to rested (Kdr) and inactivated (Kdi) states of hNa_v1.7 and rNa_v1.8 channels were found to be 12.12 and 22.84 μM and 0.47 and 0.64 μM, respectively.

Development of inactivation in the presence of ranolazine. Ranolazine caused a hyperpolarizing shift in the voltage dependence of Na_v1.7 and 1.8 INa availability (Fig. 5, Table 2 and the estimated Kdi values using Bean equation), suggesting that the drug interacts with the inactivated state of these Na+ channels. To better understand the interaction of ranolazine with Na_v1.7 and Na_v1.8 channels, the rate of development of slow inactivation was determined by depolarizing the cells to -40 and -20 (hNa_v1.7) or -20 and +20 mV for a variable interval (0.1 to 10 sec) to allow development of block. A 20-msec hyperpolarizing step was inserted to allow recovery of unbound channels from fast inactivation before a standard test pulse to assess channel availability. The time dependence of development of inactivation of hNa_v1.7 (-20 mV, Fig. 6A, n = 4–5 cells) and rNa_v1.8 (+20 mV, Fig 6B, n = 4–5 cells) INa in the absence (■) and presence (□) of 30 μM ranolazine is shown in Figure 6. For control conditions, the progressive decay of currents with increasing conditioning pulse duration reflects entry of channels into inactivated states. The development of slow inactivation of hNa_v1.7 + β1 and rNa_v1.8 channels could be fit with double and triple exponential functions, respectively (see Table 3, control, Development of slow inactivation). As shown previously,29 the onset of slow inactivation of Na_v1.7 channels is rapid when compared to Na_v1.7 channels (~ four-fold, see Table 3, control, τf = 10.78 and 43.97 msec for Na_v1.8 and Na_v1.7 channels, respectively). The rate of development of slow inactivation was 2–5 fold faster in the presence of ranolazine (30 μM) (Table 3, ranolazine, Development of inactivation). The time constants for development of inactivation of hNa_v1.7 (n = 4 cells) and rNa_v1.8 (n = 5 cells) INa with a depolarizing prepulse to -40 mV (hNa_v1.7) or -20 mV (rNa_v1.8) in the absence and presence of 30 μM ranolazine are plotted in Table 3. The rate of development of slow inactivation was 4–10 fold faster in the presence of ranolazine (30 μM) (Table 3, ranolazine, Development of inactivation at -40 (hNa_v1.7) and -20 mV (rNa_v1.8), respectively).

Recovery from ranolazine block. The effects of ranolazine on recovery from inactivation of hNa_v1.7 and rNa_v1.8 were assessed with a standard two-pulse protocol as described in Methods. The time dependence of recovery from inactivation of hNa_v1.7 (n = 5 cells)
Ranolazine blocks NaV1.7 and NaV1.8 Na⁺ channels

Figure 5. Voltage dependence of steady-state inactivation for hNaV1.7 (left) and rNaV1.8 (right) Na⁺ channel currents in the absence (filled symbols) and presence of 10 μM ranolazine (open symbols) are shown. Conditioning prepulses of 100 msec (A), 1 sec (B) and 10 sec (C) were used. Inset: voltage-clamp protocols. (A) Ranolazine 10 μM caused a minimal shift in the mid-point (V½) without affecting the slope factor (k) of steady-state fast inactivation of hNaV1.7 (n = 4 cells) and rNaV1.8 (n = 4 cells). The estimated V½ and k values in the absence (■) and presence of ranolazine (□) for hNaV1.7 are -74.49 ± 2.79; 6.01 ± 0.3 and -86.15 ± 3.62 (p < 0.05); 7.55 ± 0.82 (p = 0.14), and the estimated V½ and k values in the absence (●) and presence of ranolazine (○) for rNaV1.8 are -33.12 ± 1.10; 9.69 ± 1.10 and -40.66 ± 3.23 (p = 0.15); 11.45 ± 1.21 (p < 0.02), respectively. (B) Ranolazine caused a concentration-dependent (1–30 μM) shift in the V½ of steady-state intermediate inactivation without affecting k values for both hNa1.7 and rNaV1.8 (Table 2). (C) Ranolazine (10 μM) caused a significant leftward shift in the V½ of steady-state slow inactivation without affecting the k values of hNaV1.7 (n = 4 cells) and rNaV1.8 (n = 6 cells). The estimated V½ and k values in the absence (■) and presence of ranolazine (○) for hNaV1.7 are -37.22 ± 4.21; 13.52 ± 0.93 and -61.39 ± 3.54 (p < 0.05); 14.22 ± 2.14 (p = 0.80) and the estimated V½ and k values in the absence (●) and presence of ranolazine (○) for rNaV1.8 are -37.13 ± 2.42; 7.31 ± 0.81 and -54.57 ± 3.69 (p < 0.05); 8.38 ± 0.76 (p = 0.23), respectively. Data represent mean ± SEM.

Table 2

|       | hNaV1.7 | rNaV1.8 |
|-------|---------|---------|
|       | V½ (mV) | k (mV/e-fold) | V½ (mV) | K (mV/e-fold) |
| Activation |         |         |         |             |
| Control (■, ●) | -32.65 ± 2.16 | 4.84 ± 0.38 | 8.72 ± 3.51 | 11.23 ± 1.62 |
| Ranolazine 10 μM (□, ○) | -33.96 ± 2.06 | 4.77 ± 0.32 | 4.71 ± 2.58 | 8.55 ± 1.62 |
| Inactivation |         |         |         |             |
| Control (■) | -74.06 ± 2.96 | 4.67 ± 0.16 | -37.43 ± 3.13 | 7.58 ± 1.03 |
| Ranolazine 1 μM | -78.97 ± 3.09 | 4.80 ± 0.21 | -47.25 ± 3.93 | 7.38 ± 0.93 |
| 3 μM | -84.44 ± 3.64* | 4.76 ± 0.11 | -47.61 ± 4.56* | 7.14 ± 0.61 |
| 10 μM | -86.99 ± 2.86* | 4.56 ± 0.1 | -57.88 ± 5.73* | 7.59 ± 0.79 |
| 30 μM | -89.07 ± 5.41* | 5.15 ± 0.31 | -59.52 ± 2.18* | 7.59 ± 1.09 |

Data were recorded using voltage-clamp protocols described in Figure 3A and B and fitted with a Boltzmann equation *p < 0.05.
and rNaV1.8 (n = 5 cells) INa in the absence (□) and presence (■) of 30 μM ranolazine is shown in Figure 6. For control conditions, recovery from inactivation of hNaV1.7 INa (Fig. 6C, repolarizing potential = -100 mV) could be fit with a double exponential equation, with fast (τF) and slow time constants, (τS), respectively. In contrast, recovery from inactivation of rNaV1.8 INa was slow, and could be better fit with three exponentials. The time course of recovery from inactivation of rNaV1.8 INa (Fig. 6D, repolarizing potential = -100 mV) had fast (τF), intermediate (τI) and slow (τS) time constants. As summarized in Table 3 (Recovery from inactivation at -100 mV), the fast component (τF) of hNaV1.7 INa recovery from inactivation was not different in the absence and presence of 30 μM ranolazine, whereas the slow component (τS) was significantly (p < 0.05) slowed in the presence of 30 μM ranolazine (see Table 3, hNaV1.7, Recovery from inactivation). The fast (τF), intermediate (τI) and slow (τS) components of rNaV1.8 INa recovery from inactivation were significantly (p < 0.05) slowed in the presence of 30 μM ranolazine (Table 3, rNaV1.8, Recovery from inactivation). The time dependence of recovery from inactivation of hNaV1.7 (n = 5 cells) and rNaV1.8 (n = 4 cells) INa with a depolarizing prepulse to -40 mV (hNaV1.7) or -20 mV (rNaV1.8) in the absence and presence of 30 μM ranolazine are plotted in Table 3. As summarized in Table 3 (Recovery from inactivation at -80 mV), the fast (τF) and slow (τS) components of hNaV1.7 INa recovery from inactivation were significantly (p < 0.05) slowed in the presence of 30 μM ranolazine. Similarly, ranolazine (30 μM) caused a significant (p < 0.05) slowing of the fast (τF), intermediate (τI) and slow (τS) components of rNaV1.8 INa recovery from inactivation (see Table 3, rNaV1.8, Recovery from inactivation at -80 mV).

Use-dependent block by ranolazine. To study the use-dependent block of hNaV1.7, rNaV1.8 and TTX-S INa by ranolazine, a series of 40 short repetitive impulses (10 msec in duration) to -20 mV (for hNaV1.7 and TTX-S INa) or to +50 mV (for rNaV1.8 INa) from a holding potential of -100 mV were applied at rates of 1, 5 and 10 Hz. The amplitude of current evoked by the 40th impulse was normalized to that of the current evoked by the first impulse. The short depolarizing pulse duration of 10 msec was chosen to approximate the somatic action potential duration of C fibers (0.6–7.4 msec).30 For hNaV1.7 and TTX-S INa, pulsing frequencies up to 10 Hz had small effects on the amplitude of currents (Fig. 7A and C, filled symbols), suggesting that these channels recovered rapidly from inactivation (τS = -50 msec, Table 3) and could cycle quickly through open, closed and inactivated conformations at these tested frequencies.6,29,31,32 In contrast, rNaV1.8 INa in control conditions showed a reduction in amplitude that depended on stimulating frequency (Fig. 7B, filled symbols). This frequency-dependent reduction in INa amplitude suggests that rNaV1.8 channels in ND7-23 cells recover slowly from inactivation (τS = ~847 msec, Table 3). Ranolazine (30 μM) caused a frequency-dependent reduction (p < 0.05, n = 4–5 cells, each) in amplitude of hNaV1.7, rNaV1.8 and TTX-S INa, indicating marked use-dependent block. At the lowest stimulating frequency (1 Hz, □), -20–40% (depending on the channel isoform) of available channels were readily blocked by the drug. Increasing the stimulation frequency from 1 to 5 (∇) or 10 Hz (∆) revealed additional rapidly-equilibrating channel block, although block appeared to saturate at 5 and 10 Hz (Fig. 7). Interestingly, ranolazine caused only little use-dependent block of rNaV1.8 channels (block of INa at 1, 5 and 10 Hz were 60.20 ± 2.04%, 67.96 ± 4.68% and 70.16 ± 2.09% (p < 0.05 when compared to 1 Hz), respectively). One possible explanation could be that dissociation of ranolazine from inactivated rNaV1.8 channels is fast, much faster than its dissociation from inactivated hNaV1.7 channels.

Open channel block by ranolazine. The voltage-dependent block (Fig. 1, Table 1, V½ holding potential experiments) and concentration-dependent shift in the mid-points (V½) of inactivation of hNaV1.7 and rNaV1.8 (Fig. 5, Table 2) caused by ranolazine, and the estimated (using Bean equation) Ki values of hNaV1.7 and rNaV1.8, suggest that ranolazine interacts with the inactivated

Figure 6. (A and B) Plots of development of slow inactivation in the absence and presence of 30 μM ranolazine (inset: voltage-clamp protocol). Data represent mean ± SEM. The smooth curves are fits of the data with two (A; hNaV1.7, n = 3–5 cells, each) or three (B; rNaV1.8, n = 3–5 cells, each) component exponential equations (see Table 3 for values of the individual parameters). (C and D) Plots of recovery from inactivation in the absence and presence of 30 μM ranolazine (inset: voltage-clamp protocol). Data represent mean ± SEM. The smooth curves are fits of the data with two (C; hNaV1.7, n = 5 cells, each) or three (D; rNaV1.8, n = 5 cells, each) component exponential equations (see Table 3 for values of the individual parameters).
Ranolazine blocks NaV1.7 and NaV1.8 Na+ channels

Table 3  Development of slow inactivation and recovery from inactivation parameters of hNaV1.7 and rNaV1.8 in the absence (control) and presence of 30 μM ranolazine

| Channel          | Development of slow inactivation | Recovery from inactivation |
|------------------|----------------------------------|---------------------------|
|                  | Control (at -20 mV) | Ranolazine | Control (at -100 mV) | Ranolazine |
| hNaV1.7          | A_F 0.16 ± 0.08, A_S 0.82 ± 0.03, τ_F 39.97 ± 15.76, τ_S 7372.66 ± 654.66 | 0.24 ± 0.02*, 0.56 ± 0.01*, 9.14 ± 2.49*, 1657.71 ± 180.23* | 0.82 ± 0.04, 0.12 ± 0.02, 1.94 ± 0.31, 54.85 ± 3.53 | 0.73 ± 0.03*, 0.16 ± 0.02, 2.15 ± 0.22, 546.44 ± 171.03* |
|                  | (at -40 mV) | | | |
|                  | A_F 0.11 ± 0.02, A_S 0.89 ± 0.02, τ_F 39.12 ± 4.91, τ_S 7923.74 ± 850.36 | 0.30 ± 0.02*, 0.67 ± 0.02*, 23.99 ± 2.92*, 1845.75 ± 129.03* | 0.88 ± 0.01, 0.12 ± 0.01, 30.31 ± 1.01, 2279.19 ± 609.28 | 0.75 ± 0.02*, 0.19 ± 0.02*, 37.72 ± 3.51*, 4724.79 ± 1301.69* |
|                  | (at +20 mV) | | | |
|                  | A_F 0.59 ± 0.02, A_S 0.89 ± 0.02, τ_F 10.78 ± 1.05, τ_S 2171.4 ± 57.43 | 0.53 ± 0.07, 0.99 ± 0.02*, 5.59 ± 1.08*, 34.11 ± 14.14* | 0.37 ± 0.01, 0.32 ± 0.01, 10.19 ± 0.76, 123.70 ± 17.92 | 0.56 ± 0.02*, 0.19 ± 0.05*, 19.62 ± 1.16*, 371.16 ± 127.40* |
|                  | (at -100 mV) | | | |
| rNaV1.8          | A_F 0.62 ± 0.02, A_S 0.20 ± 0.05, τ_F 29.55 ± 4.72, τ_S 334.23 ± 89.43 | 0.52 ± 0.05*, 0.18 ± 0.03, 22.75 ± 2.31*, 98.08 ± 13.65* | 0.51 ± 0.04, 0.14 ± 0.01, 15.55 ± 3.30, 389.01 ± 83.41 | 0.39 ± 0.07*, 0.25 ± 0.03*, 25.04 ± 5.19*, 701.39 ± 44.00* |
|                  | (at -20 mV) | | | |
|                  | A_I 0.1 ± 0.02, A_S 0.20 ± 0.05, τ_I 334.23 ± 89.43 | 0.30 ± 0.06*, 0.18 ± 0.03 | 0.29 ± 0.04, 0.14 ± 0.01 | 0.36 ± 0.07, 0.25 ± 0.03 * |
|                  | (at -80 mV) | | | |
|                  | A_F 0.59 ± 0.02, A_S 0.89 ± 0.02, τ_F 10.78 ± 1.05, τ_S 2171.4 ± 57.43 | 0.53 ± 0.07, 0.99 ± 0.02*, 5.59 ± 1.08*, 34.11 ± 14.14* | 0.37 ± 0.01, 0.32 ± 0.01, 10.19 ± 0.76, 123.70 ± 17.92 | 0.56 ± 0.02*, 0.19 ± 0.05*, 19.62 ± 1.16*, 371.16 ± 127.40* |
|                  | (at -100 mV) | | | |
|                  | A_I 0.1 ± 0.02, A_S 0.20 ± 0.05, τ_I 334.23 ± 89.43 | 0.30 ± 0.06*, 0.18 ± 0.03 | 0.29 ± 0.04, 0.14 ± 0.01 | 0.36 ± 0.07, 0.25 ± 0.03 * |
|                  | (at +20 mV) | | | |
|                  | A_I 0.23 ± 0.02, A_S 0.18 ± 0.04, τ_I 2171.4 ± 57.43 | 0.25 ± 0.07, 0.09 ± 0.02* | 0.27 ± 0.02, 0.32 ± 0.01 | 0.21 ± 0.06, 0.19 ± 0.05* |
|                  | (at -100 mV) | | | |
|                  | A_I 0.23 ± 0.02, A_S 0.18 ± 0.04, τ_I 2171.4 ± 57.43 | 0.25 ± 0.07, 0.09 ± 0.02* | 0.27 ± 0.02, 0.32 ± 0.01 | 0.21 ± 0.06, 0.19 ± 0.05* |
|                  | (at +20 mV) | | | |

Data were recorded using voltage-clamp protocols described in Figure 4 and fitted with double or triple exponential equations. *p < 0.05.

Discussion

In this study we show that ranolazine blocked NaV1.7 and NaV1.8 I_Na at clinically relevant (2–8 μM) concentrations. These channels are present in peripheral pain-sensing neurons and are reported to play an important role in the etiology of neuropathic pain. Ranolazine inhibited hNaV1.7 and rNaV1.8 Na+ channels in a voltage- and use-dependent manner. Ranolazine did not alter the activation voltage range of either NaV1.7 or NaV1.8 I_Na, or the voltage at which half-maximal activation (V_1/2) of current occurred. However, ranolazine caused a concentration-dependent hyperpolarizing shift of the inactivation voltages of both currents. These data suggest that the binding of ranolazine was state-dependent. This interpretation was further confirmed by the finding that the potency of the drug state of these Na+ channels. However, it is unclear whether block of hNaV1.7 or rNaV1.8 Na+ channels by ranolazine with 10-msec depolarizing pulses at 1, 5 and 10 Hz also involved the transient open state in addition to the inactivated state of the channel. Wang and colleagues25 have demonstrated that both muscle NaV1.4 and neuronal NaV1.7 are equally sensitive to ranolazine block, and they also demonstrated that the drug preferentially blocks the open state of these Na+ channels. To investigate block of the open state of NaV1.7 and NaV1.8 channels, the effect of pulse duration on magnitude of use-dependent block by ranolazine was investigated. Figure 6 shows representative records of rNaV1.8 current elicited by 5 (Fig. 8A) or 200 msec (Fig. 8B) long test pulses to +50 mV at a frequency of 5 Hz in the presence of 100 μM ranolazine. Peak current elicited by each pulse was measured, normalized to the current of the first pulse, and plotted against the pulse number in Figure 8C. The plot shows that the development of use-dependent block of rNaV1.8 I_Na evoked by 3 (▽), 5 (△), 20 (□) or 200 (□□) msec-long test pulses to +50 mV in the presence of 100 μM ranolazine reached a steady-state of 71.69 ± 0.85% (n = 4–5 cells, each) with a time constant of 2.34 ± 0.22 pulses. In the absence of drug, repetitive stimulation caused small or no reductions in the amplitude of I_Na (data not shown). Thus, our data show that ranolazine blocked open states of hNaV1.7 and rNaV1.8 INa.

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Ranolazine blocks NaV1.7 and NaV1.8 Na+ channels

was greater at more positive membrane potentials. Interestingly, the effects of ranolazine to reduce $I_{Na}$ were identical (~80% $I_{Na}$ inhibition) when different conditioning pulse durations (3, 5, 20 and 200 msec) were employed (Fig. 8), suggesting that the drug could rapidly bind to open Na+ channels. Recently, Wang and colleagues have demonstrated that the muscle and neuronal Na+ channels, NaV1.4 and NaV1.7, respectively, are equally sensitive to ranolazine block. These investigators reported that the drug preferentially interacts with the open, but not resting or inactivated states, of Na+ channels. Our results (Fig. 8) also indicate that ranolazine interacted with the open states. But importantly, ranolazine also accelerated the onset of inactivation (Fig. 6) and delayed recovery of hNaV1.7 and rNaV1.8 Na+ channels from slow inactivation (Fig. 6), suggesting that it interacts with inactivated states of these channels.

Block of voltage-gated Na+ channels by local anesthetics is highly state-dependent with a preference for open and inactivated channel states. The effect of ranolazine on the voltage dependence of steady-state inactivation of hNaV1.7 and rNaV1.8 was dependent on the length of the depolarizing prepulse that was used to inactivate the channel (Fig. 5). Using 100-msec prepulses, 10 μM ranolazine caused -11 mV (11.41 ± 4.27 mV; p < 0.05) shift in the voltage dependence of steady-state fast inactivation of hNaV1.7, but caused -7 mV (7.54 ± 4.04 mV; p = 0.15) shift of rNaV1.8. Interestingly, when the depolarizing prepulse was increased to 1 sec (Fig. 5B, Table 2), even 3 μM ranolazine caused -10 and 20 mV shifts in the voltage dependence of steady-state intermediate inactivation of hNaV1.7 and rNaV1.8 channels, respectively. Similarly, with a 10 sec depolarizing prepulse, ranolazine (10 μM) caused -24 and 17 mV shifts of the voltage dependence of steady-state slow inactivation hNaV1.7 and rNaV1.8, respectively. Further, it was demonstrated previously that the binding affinity of drug to inactivated channels could be estimated indirectly using the concentration-dependent shift in the voltage dependence of inactivation curve ($\Delta V_{1/2}$ shift in midpoint of the steady-state inactivation curve). The $K_{di}$ (dissociation constant for block of inactivated channels) values of 0.47 and 0.64 μM calculated for ranolazine inhibitions of hNaV1.7 and rNaV1.8 were lower than the IC$_{50}$ values of 3.25 and 4.33 μM measured using a protocol with conditioning prepulses to -70 and -40 mV, respectively. The differences of these $K_{di}$ and IC$_{50}$ values for block of hNaV1.7 and rNaV1.8 by ranolazine might be due to overestimation of drug affinity by the equation (model). Nevertheless, either set of estimates suggests that ranolazine inhibits NaV1.7 and NaV1.8 at concentrations that are achieved clinically during administration of the drug. Our data suggest that ranolazine could be interacting with fast, intermediate and slow inactivated states of hNaV1.7 and intermediate and slow inactivated states of rNaV1.8. The effects of ranolazine are similar to the effect of lidocaine and lacosamide on hNaV1.7 (stable expression in HEK293 cells), rNaV1.8 (recorded from DRG neurons) and Na+ currents recorded from neocortical neurons and mouse neuroblastoma cells. Furthermore, the state-dependence of block of hNaV1.7 and rNaV1.8 Na+ channels by ranolazine could be desirable and lead to a greater effect of the drug when neuronal firing frequencies are increased, as during prolonged neuronal depolarization.

Slow inactivation in TTX-R Na+ channels has been suggested to play a central role in controlling adaptation of action potential firing in nociceptive sensory neurons. In the absence of drug, repetitive stimulation (at rates up to 10 Hz) of hNaV1.7 and TTX-S $I_{Na}$ endogenously expressed in ND7-23 cells caused minimal reduction in current amplitude (~9%, Fig. 7). In contrast, repetitive stimulation of rNaV1.8 at rates of 1, 5 and 10 Hz caused ~21%, ~35 and 42% reductions in current amplitude, respectively, in the absence of drug (Fig. 7). This reduction could be due to entry of rNaV1.8 Na+ channels into inactivated states during a brief depolarization (10 msec). Vijayaragavan and colleagues reported that the time course of NaV1.8 entry into a slow inactivated state ($\tau_F = 8.4$ msec) was faster than that of NaV1.7 ($\tau_F = 33$ msec). Similarly, our results (Fig. 6 and

![Figure 7. Use-dependent block of hNaV1.7 (A), rNaV1.8 (B) and TTX-S $I_{Na}$ (C) by 30 μM ranolazine. Each protocol included a train of 40 pulses from -120 to -20 mV (hNaV1.7 + β1 or endogenous TTX-S $I_{Na}$) or from -100 to +50 mV (rNaV1.8) at frequencies of 1, 5 and 10 Hz in the absence (control; filled symbols) or presence of 30 μM ranolazine (open symbols). The amplitude of currents evoked by the nth impulse (40th) was normalized to that of the current evoked by the first pulse and plotted versus respective pulse number.](image-url)
Table 3) further indicate that the onset of slow inactivation is fourfold faster for Na\textsubscript{v}1.8 than Na\textsubscript{v}1.7 channels (\(\tau\_f = 10.78\) and 43.97 msec for Na\textsubscript{v}1.8 and Na\textsubscript{v}1.7 channels, respectively). Thus, during the brief 10-msec depolarization used in the repetitive stimulating protocol, a significant fraction of rNa\textsubscript{v}1.8, but not hNa\textsubscript{v}1.7, may undergo slow inactivation. Drug-induced use-dependent block arises from binding of drug to, and stabilization of, inactivated states of channels (which increase as the frequency of stimulation increases) and/or from dissociation of drugs from inactivated states with a time constant slower than the frequency of the pulses (i.e., a slow repriming rate). Ranolazine (30 \(\mu\)M) caused a use-dependent block of hNa\textsubscript{v}1.7, rNa\textsubscript{v}1.8 or TTX-S Na\textsuperscript{+} channels (Fig. 7). Also, ranolazine (30 \(\mu\)M) caused a significant delay in recovery (at both -80 and -100 mV) from slow inactivation (\(\tau\_s\)) of hNa\textsubscript{v}1.7 and rNa\textsubscript{v}1.8 Na\textsuperscript{+} channels. This finding suggests that the use-dependent block by ranolazine of TTX-S and TTX-R \(I\textsubscript{Na}\) reflects the binding of ranolazine to inactivated states of Na\textsuperscript{+} channels. However, further studies using site-directed mutagenesis are needed to better understand the inactivated state interactions of ranolazine to rNa\textsubscript{v}1.8 Na\textsuperscript{+} channels.

Most Na\textsuperscript{+} channels are multimeric proteins in which the pore-forming \(\alpha\)-subunit is associated with accessory \(\beta\) subunits.\textsuperscript{37} In this study, hNa\textsubscript{v}1.7 was co-expressed with a human \(\beta\) subunit. Vijayaragavan and colleagues\textsuperscript{29} showed that co-expression of hNa\textsubscript{v}1.7 with a \(\beta_1\) subunit in Xenopus oocytes caused a hyperpolarizing shift in gating and an increase in rates of inactivation and recovery from inactivation. The ND7-23 cells used in this study endogenously express \(\beta_1\) and \(\beta_3\) subunits.\textsuperscript{27} The overexpression of rNa\textsubscript{v}1.8 in this cell line may lead to formation of membrane Na\textsuperscript{+} channels composed of pore-forming \(\alpha\)-subunits with or without adjacent \(\beta\) subunits. Thus, direct extrapolation of effects of ranolazine on Na\textsuperscript{+} channels expressed in the heterologous cell models used in this study to cells expressing native Na\textsuperscript{+} channels should be done with caution.

Local anesthetics (lidocaine, mexiletine, tocainide) have been reported to be useful in therapy of painful diabetic polyneuropathies, neuralgic pain and traumatic peripheral nerve injuries.\textsuperscript{38} Lidocaine has been shown to inhibit the inactivated state of TTX-R \(I\textsubscript{Na}\), with an IC\textsubscript{50} value of 59.5 \(\mu\)M. In addition, lidocaine has been shown to suppress the number of TTX-R action potentials during repetitive firing and block of TTX-R Na\textsuperscript{+} channels by local anesthetics (lidocaine and bupivacaine) was thought to be the mechanism for their pain suppression.\textsuperscript{39} Furthermore, lidocaine has been shown to relieve neuropathic pain at serum concentrations as low as 10 \(\mu\)M (2.4 \(\mu\)g/mL).\textsuperscript{40} Ranolazine (structurally related to lidocaine), a newly approved anti-ischemic agent, has been shown to inhibit cardiac late \(I\textsubscript{Na}\),\textsuperscript{41} and may also have anti-arrhythmic properties.\textsuperscript{24,42} In the present study, ranolazine blocked hNa\textsubscript{v}1.7 and rNa\textsubscript{v}1.8 Na\textsuperscript{+} channels with IC\textsubscript{50} values of 3.25 and 4.33 \(\mu\)M, concentrations that are achieved therapeutically (2 to 8 \(\mu\)M). Our finding that ranolazine binds to inactivated states of Na\textsubscript{v}1.7 and Na\textsubscript{v}1.8 channels with high affinity suggests that the drug may reduce firing frequencies of neurons that express these channels. Thus, investigation of the effects of ranolazine on neuronal function, and on models of neuropathic pain, is warranted.

**Materials and methods**

**Expression of sodium channels.** HEK293 cells stably expressing the hNa\textsubscript{v}1.7 (\(\alpha\)-subunit) along with a human \(\beta_1\) subunit were purchased from Scottish-Biomedical, Glasgow, UK. Cells were continuously maintained using MEM (Gibco-Invitrogen, Carlsbad, CA) supplemented with 10% heat inactivated fetal bovine serum, 1% penicillin-streptomycin, 600 \(\mu\)g/mL geneticin (Gibco-Invitrogen), 2 \(\mu\)g/mL blastocydin (Calbiochem, NJ, USA), and were incubated at 37°C in an atmosphere of 5% CO\textsubscript{2} in air.

The cell line ND7-23 (rat DRG/mouse neuroblastoma hybrid, untransfected) was purchased from European Collection of Animal Cell Cultures (Porton Down, UK). Recombinant ND7-23/rNa\textsubscript{v}1.8 cells were purchased from Millipore (UK) limited, Cambridge, UK. It has been reported that ND7-23 cells also express a TTX-S \(I\textsubscript{Na}\) that has rapid kinetics, but the molecular identity of these Na\textsuperscript{+} channels is still not clear.\textsuperscript{27} Cells were maintained using DMEM (Gibco-Invitrogen) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% non-essential amino acids, 1% penicillin-streptomycin, 400 \(\mu\)g/mL geneticin (Gibco-Invitrogen), and were incubated at 37°C in an atmosphere of 5% CO\textsubscript{2} in air.

**Solutions and chemicals.** For recording hNa\textsubscript{v}1.7 \(I\textsubscript{Na}\), HEK293 cells were superfused with an extracellular solution containing (in mM): 140 NaCl, 3 KCl, 10 HEPES, 10 glucose, 1 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, pH 7.4 (with NaOH). Patch pipettes were filled with an internal solution containing (in mM): 140 CsF, 10 NaCl, 1 EGTA, 10 HEPES, pH 7.3 (with CsOH). For recording either endogenous \(I\textsubscript{Na}\) in ND7-23
cells or rNaV1.8 INa, cells were superfused with an extracellular solution containing (in mM): 140 NaCl, 5 HEPES-Na, 1.3 MgCl₂, 1 CaCl₂, 11 glucose, 4.7 KCl, pH 7.4. Patch pipettes were filled with an internal solution containing (in mM): 120 CsF, 10 HEPES, 10 EGTA, 15 NaCl, pH 7.25. To determine the use-dependence of drug block of rNaV1.8, experiments were performed using a test potential of +50 mV (at which the Na⁺ current is outward) and an extracellular solution containing (in mM): 65 NaCl, 85 choline Cl, 2 CaCl₂, 10 HEPES, pH 7.4 (with tetramethylammonium hydroxide). Patch pipettes were filled an internal solution containing (in mM): 100 NaF, 30 NaCl, 10 EGTA, 10 HEPES, pH 7.2 (with CsOH). The reversed Na⁺ gradient was employed to minimize series resistance artifacts, which are less serious with outward than with inward I_Na flow. Unless otherwise mentioned, patch-clamp studies using ND7-23 cells were performed in the continuous presence of 300 nM TTX to block the endogenous TTX-S INa.6 Research grade ranolazine was synthesized by the Department of Bio-Organic Chemistry at CV Therapeutics, Inc., (Palo Alto, CA) and TTX was purchased from Sigma (St. Louis, MO). Ranolazine was dissolved in 0.1 N HCl to give a stock solution of 10 mM and further dilutions were freshly made in Tyrode solution on the day of the experiments. TTX was dissolved in distilled water.

**Electrophysiological technique and data acquisition.** Whole-cell I_Na was recorded as described previously43 using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, USA). Signals were filtered at 5 kHz and sampled at 20 kHz. Patch pipettes were formed from borosilicate glass (World Precision Instruments, Sarasota, USA) using a micropipette puller (Dagan Corporation, Minneapolis, USA). The offset potential was zeroed before the pipette was attached to the cell and the voltages were not corrected for the liquid junction potential. In all recordings, 75–80% of the series resistance compensation was achieved, thus yielding a maximum voltage error of ~5 mV and leak currents were cancelled by P/-4 subtraction. pCLAMP 10.0 software (Molecular Devices) was used to generate voltage clamp protocols and acquire data. Cells were held at a membrane potential of -100 or -120 mV and were dialyzed with pipette solution for 5–7 minutes before current was recorded, to avoid time-dependent shifts in Na⁺ channel gating within the first several minutes after patch rupture. In all experiments, the temperature of experimental solutions was maintained at 20 ± 1°C using a CL-100 bipolar temperature controller (Warner Instruments, Hamden, USA).

Data were analyzed using Clampfit and Microcal Origin (MicroCal, Northampton, USA) software. Results are expressed as mean ± S.E.M, and n refers to number of cells. All experiments were repeated on at least two different experimental days. Statistical significance of differences between responses of a cell in the absence and presence of drug was determined using the Student t-test, with p < 0.05 indicating statistical significance. Concentration-response relations were fit using the Hill equation:

\[
\frac{I_{\text{drug}}}{I_{\text{control}}} = 1/[1 + (D/IC_{50})^{nH}]
\]

where \(I_{\text{drug}}/I_{\text{control}}\) is fractional block, D is drug concentration, IC_{50} is the drug concentration that causes 50% block and nH is the Hill coefficient. The voltage dependence of activation was determined using 50-msec depolarizing pulses from a holding potential of -120 or -100 mV to test potentials ranging from -80 to +40 mV, in 5 mV increments. To determine the voltage dependence of channel activation, Na⁺ conductance (\(G_{Na}\)) was calculated from the peak current (\(I_{Na}\)), using the equation:

\[
G_{Na} = \frac{I_{Na}}{(V - V_{rev})}
\]

where \(V\) is the test pulse potential and \(V_{rev}\) is the calculated reversal potential. Normalized Na⁺ conductance was plotted against test pulse potential and fit to a Boltzmann equation:

\[
G/G_{\text{max}} = 1/[1 + \exp((V - V_{1/2})/k)]
\]

where \(G\) is the measured conductance, \(G_{\text{max}}\) is the maximal conductance, \(V_{1/2}\) is the membrane potential at which the half-maximal channel open probability occurs and \(k\) is the slope of the curve. For assessing the voltage dependence of steady-state fast and intermediate inactivation, prepulses ranging from -120 to 0 mV (for hNaV1.7 \(I_{Na}\)) or -100 to +20 mV (for rNaV1.8 \(I_{Na}\)) were applied for a period of 100 msec (to measure fast inactivation), or 1 sec (to measure intermediate inactivation), followed by a 50-msec depolarizing step to 0 mV (for hNaV1.7 \(I_{Na}\)) or to +20 mV (for rNaV1.8 \(I_{Na}\)). The peak current (\(I\)) was normalized relative to the maximal value (\(I_{\text{max}}\)) obtained at a holding potential (\(V_h\)) of -100 or -120 mV and plotted against the conditioning pulse potential. Data were fit to a Boltzmann equation:

\[
III_{\text{max}} = 1/[1 + \exp((V - V_{1/2})/k)]
\]

where \(V\) is the membrane potential during the pre-pulse, \(V_h\) is the potential at which the half-maximal channel inactivation occurs and \(k\) is the slope factor. For assessing the voltage dependence of steady-state slow inactivation, prepulses ranging from -120 to -10 mV (for hNaV1.7 \(I_{Na}\)) or -100 to -10 mV (for rNaV1.8 \(I_{Na}\)) were applied for a period of 10 msec, followed by a 100-msec hyperpolarizing step to -160 (for hNaV1.7) or -140 mV (for rNaV1.8) and then stepped to 0 (for hNaV1.7 \(I_{Na}\)) or +20 mV (rNaV1.8 \(I_{Na}\)) for a period of 50-msec to measure the available current. The brief 100-msec hyperpolarizing step was employed to allow channels (both without and with drug bound) to recovery from fast, but not slow-inactivation. Data from the voltage dependence of steady-state slow inactivation were fit with a modified Boltzmann equation:44,45

\[
III_{\text{max}} = (1 - I_{\text{resid}})/[1 + \exp(-(V - V_{1/2})/k)]
\]

where \(I_{\text{resid}}\) is the residual (noninactivating) fraction of the current.

To estimate the extent of block of inactivated channels by ranolazine, an indirect approach based on the concentration-dependence of the shift of the steady-state inactivation curve was used (reviewed in ref. 28, see equation below).

\[
\Delta V_{1/2} = k \log([1 + [\text{Ranolazine}]/K_{dR}]/(1 + [\text{Ranolazine}]/K_{dR}))
\]

where \(\Delta V_{1/2}\) is the shift in midpoint of the steady-state (intermediate) inactivation curve, \(k\) is the slope factor of the steady-state (intermediate) inactivation curve derived from a Boltzmann fit, \(K_{dR}\) is the affinity constant for the resting state of the channel and \(K_{di}\) is the affinity constant for the inactivated state of the channel.

The time course of entry into the slow inactivated state was measured using a three pulse protocol of incremental time delay (0.1...
to 10 sec) to -40 or -20 mV (hNaV1.7) or -20 or +20 mV (rNaV1.8) to allow development of block ($I_f$). A 20-msec hyperpolarizing step to -100 mV was inserted to allow recovery of unbound channels (from inactivation), followed by a standard test pulse to assess channel availability. The test current was normalized and plotted against the conditioning pulse interval and fit to a double or triple exponential function,

$$I/I_0 = A_F \cdot (1 - \exp(-t/\tau_F)) + A_S \cdot (1 - \exp(-t/\tau_S))$$

where $t$ = conditioning pulse duration, $\tau_F$ and $\tau_S$ = fast and slow time constants, $A_F$ and $A_S$ = relative amplitude of the fast and slow recovery component, or

$$I/I_0 = A_F \cdot (1 - \exp(-t/\tau_F)) + A/I \cdot (1 - \exp(-t/\tau_I)) + A_S \cdot (1 - \exp(-t/\tau_S))$$

where $t$ = conditioning pulse duration, $\tau_F$, $\tau/I$ and $\tau_S$ = fast, intermediate and slow time constants, and $A_F$, $A/I$ and $A_S$ = relative amplitude of the fast and slow recovery component.

Recovery from inactivation was measured with a standard two-pulse protocol of 50-msec duration with an incremental time delay of 1 msec to 8 sec between the two pulses at a repolarizing potential of the fast and slow recovery component.

$$I/I_0 = A_F \cdot (1 - \exp(-t/\tau_F)) + A_S \cdot (1 - \exp(-t/\tau_S))$$

where $t$ = recovery time interval, $\tau_F$ and $\tau_S$ = fast and slow recovery components, and $A_F$ and $A_S$ = relative amplitude of the fast and slow recovery component, or

$$I/I_0 = A_F \cdot (1 - \exp(-t/\tau_F)) + A/I \cdot (1 - \exp(-t/\tau_I)) + A_S \cdot (1 - \exp(-t/\tau_S))$$

where $t$ = recovery time interval, $\tau_F$, $\tau/I$ and $\tau_S$ = fast, intermediate and slow time constants, and $A_F$, $A/I$ and $A_S$ = relative amplitude of the fast and slow recovery component.

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