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Independent and Joint Modulation of Rat Na_v1.6 Voltage-Gated Sodium Channels by Coexpression with the Auxiliary β 1 and β 2 Subunits

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Abstract

The Na_v1.6 voltage-gated sodium channel α subunit isoform is the most abundant isoform in the brain and is implicated in the transmission of high frequency action potentials. Purification and immunocytochemical studies imply that Na_v1.6 exist predominantly as Na_v1.6+ β 1+ β 2 heterotrimeric complexes. We assessed the independent and joint effects of the rat β 1 and β 2 subunits on the gating and kinetic properties of rat Na_v1.6 channels by recording whole-cell currents in the two-electrode voltage clamp configuration following transient expression in *Xenopus* oocytes. The β 1 subunit accelerated fast inactivation of sodium currents but had no effect on the voltage dependence of their activation and steady-state inactivation and also prevented the decline of currents following trains of high-frequency depolarizing prepulses. The β 2 subunit selectively retarded the fast phase of fast inactivation and shifted the voltage dependence of activation towards depolarization without affecting other gating properties and had no effect on the decline of currents following repeated depolarization. The β 1 and β 2 subunits expressed together accelerated both kinetic phases of fast inactivation, shifted the voltage dependence of activation towards hyperpolarization, and gave currents with a persistent component typical of those recorded from neurons expressing Na_v1.6 sodium channels. These results identify unique effects of the β 1 and β 2 subunits and demonstrate that joint modulation by both auxiliary subunits gives channel properties that are not predicted by the effects of individual subunits.

Keywords

voltage-gated sodium channels; Na_v1.6; β subunits; voltage clamp; kinetics; steady-state properties

Introduction

Voltage-gated sodium channels mediate the transient increase in sodium ion permeability that underlies the rising phase of the electrical action potential in most types of excitable cells [1]. Native sodium channels in the brain are heteromultimeric complexes comprised of one large (~260 kDa) α subunit and two smaller (33–36 kDa) auxiliary β subunits[2; 3].

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Sodium channel α subunits form the ion pore and confer the fundamental functional and pharmacological properties of the channel [2]. Sodium channel β subunits modulate channel function, regulate channel expression at the level of the plasma membrane, and contribute to cell adhesion and cell-cell communication [3]. The α and β subunits of voltage-gated sodium channels are encoded by multi-gene families. Mammalian genomes contain nine genes encoding sodium channel α subunit isoforms, designated $\text{Na}_v1.1$ - $\text{Na}_v1.9$ [4; 5], and four genes encoding sodium channel β subunits, designated $\beta1$ - $\beta4$ [3].

Three sodium channel α subunit isoforms ($\text{Na}_v1.1$, $\text{Na}_v1.2$ and $\text{Na}_v1.6$) are abundantly expressed in the adult brain [4]. The unique expression patterns for these three isoforms suggest that they serve specialized functions in particular cell types and anatomical regions that are subunit determined by their biophysical properties. The $\text{Na}_v1.6$ isoform, the most abundant α isoform in the adult brain [6], is the predominant isoform at nodes of Ranvier and is also expressed in segments of brain axons associated with action potential initiation and in presynaptic and postsynaptic membranes of the neocortex and cerebellum [7; 8]. This pattern of expression implies an important role for $\text{Na}_v1.6$ sodium channels in both electrical and chemical signaling in the brain. A null mutation of the $\text{Na}_v1.6$ (=Scn8a) gene in mice, termed "motor endplate disease" (*med*) causes defective synaptic transmission at neuromuscular junctions, leading to severe paralysis, muscle atrophy and juvenile death [9]. The coincident expression of the $\text{Na}_v1.6$, $\beta1$ and $\beta2$ sodium channel subunits in many brain regions [10; 11; 12] suggests that $\text{Na}_v1.6$ sodium channels exist in the brain predominantly as ternary complexes with the $\beta1$ and $\beta2$ subunits.

Despite the apparent functional importance of $\text{Na}_v1.6$ sodium channels, only limited information exists on the properties of the rat $\text{Na}_v1.6$ isoform [13]. Moreover, there has been no detailed analysis of the independent and joint effects of the $\beta1$ and $\beta2$ subunits on the functional properties of $\text{Na}_v1.6$ sodium channels from any species. Here we describe the expression of rat oocytes, either alone or in binary or ternary complexes with $\text{Na}_v1.6$ sodium channels in *Xenopus* the rat $\beta1$ and $\beta2$ subunits, and the characterization of expressed sodium currents by two-electrode voltage clamp. Our results identify distinctive modulatory effects of each β subunit on the properties of the $\text{Na}_v1.6$ sodium channel.

Materials and Methods

The rat $\text{Na}_v1.6$ α subunit cDNA was provided by L. Sangameswaran (Roche Bioscience, Palo Alto, CA) and the rat $\beta1$ and $\beta2$ subunit cDNAs were provided by W.A. Catterall (University of Washington, Seattle, WA). Plasmid cDNAs were digested with restriction enzymes to provide linear templates for cRNA synthesis *in vitro* using a commercial kit (mMessage mMachine, Ambion, Austin, TX). The integrity of synthesized cRNA was determined by electrophoresis in 1% (w/v) agarose – formaldehyde gels.

Stage V-VI oocytes were removed from female *X. laevis* frogs (Nasco, Ft. Atkinson, WI) as described elsewhere [14]. This procedure was performed in accordance with National Institutes of Health guidelines and followed a protocol that was approved by the Cornell University Institutional Animal Care and Use Committee. Oocytes were injected with 1:1 or 1:1:1 (mass ratio) mixtures of α subunit, $\beta1$ subunit and $\beta2$ subunit cRNAs (0.5 – 5 ng/oocyte); this mixture provided a ~9-fold molar excess of $\beta1$ and $\beta2$ cRNAs to ensure the preferential expression of desired binary or ternary $\alpha+\beta$ complexes. Injected oocytes were incubated in ND-96 medium (in mM: 96 NaCl, 2 KCl, 1.8 CaCl_2 , 1 MgCl_2 , and 5 HEPES; adjusted to pH 7.6 at room temperature with NaOH) supplemented with 5% horse serum (Sigma-Aldrich), 1% streptomycin/penicillin, and 1% sodium pyruvate [15] at 19°C for 3–5 days until electrophysiological analysis of sodium currents.

Sodium currents were recorded from oocytes perfused with ND-96 at room temperature (22–23°C) in the two-electrode voltage clamp configuration using an Axon Geneclamp 500B amplifier (Molecular Devices, Foster City, CA) as described previously [16]. To determine the voltage dependence of activation, oocytes were clamped at a membrane potential of -100 mV and currents were measured during a 40-ms depolarizing test pulse to potentials from -60 mV to 40 mV in 5-mV increments. Maximal peak transient currents were obtained upon depolarization to potentials near 0 mV. To determine the voltage dependence of steady-state inactivation, oocytes were clamped at a membrane potential of -140 mV followed by a 100-ms conditioning prepulse to potentials from -130 mV to 20 mV in 5-mV increments and then a 40-ms test pulse to 0 mV. For determinations of use dependence, oocytes were given trains of 1 to 100 5-ms conditioning prepulses to 10 mV at 66.7 Hz followed by a 40-ms test pulse to 0 mV. Capacitive transients and leak currents were subtracted using the P/4 method [17]. In some experiments, tetrodotoxin (TTX, Sigma Chemical Co., St. Louis, MO; 300 nM final concentration) was used to visualize capacitive transients and voltage clamp artifacts in the absence of sodium currents.

Statistical analyses were performed using the Prism software package (GraphPad Software, La Jolla, CA). Comparisons of two or more mean values to a common control data set employed one-way analysis of variance (ANOVA) followed by an appropriate *post hoc* test for statistical significance. Comparisons of two means employed either a paired or unpaired Student's *t*-test depending on the design of the experiment. Details of statistical analyses and levels of significance are given in footnotes to the Table and in the Figure legends.

Results

Figure 1A shows typical sodium currents recorded from oocytes expressing the $\text{Na}_v1.6$ sodium channel α alone (hereafter designated $\text{Na}_v1.6$ channels), in combination with either the $\beta 1$ or $\beta 2$ subunits ($\text{Na}_v1.6+\beta 1$ and $\text{Na}_v1.6+\beta 2$ channels, respectively), and in combination with both β subunits ($\text{Na}_v1.6+\beta 1 \beta 2$ channels). In the absence of β subunits, $\text{Na}_v1.6$ channels gave sodium currents that activated rapidly but decayed relatively slowly during a 40-ms depolarizing pulse. Coexpression with the $\beta 1$ subunit accelerated fast inactivation, whereas coexpression with the $\beta 2$ subunit retarded it slightly. The initial decay of the peak transient current for $\text{Na}_v1.6+\beta 1 \beta 2$ channels closely matched that of $\text{Na}_v1.6+\beta 1$ channels.

The decay of the peak transient current was fitted best by a two-component, single exponential decay model that yielded first-order decay constants for the initial fast component (τ_{fast}) and the slower secondary component (τ_{slow}). Figure 1B summarizes the impact of coexpression with either or both β subunits on kinetics of peak transient current decay. The shape of the peak transient currents in Fig. 1A was determined principally by the fast component. The $\beta 1$ subunit significantly accelerated τ_{fast} whereas the $\beta 2$ subunit significantly retarded the fast decay component. In the presence of both β subunits τ_{fast} was not significantly different from that obtained with the $\beta 1$ subunit alone. The effects of coexpression with β subunits on τ_{slow} differed from those on τ_{fast} . Each β subunit alone did not significantly alter τ_{slow} when compared to $\text{Na}_v1.6$ channels, but the slow phase for $\text{Na}_v1.6+\beta 2$ channels was accelerated compared to that for $\text{Na}_v1.6+\beta 1$ channels. Coexpression with both β subunits significantly accelerated τ_{slow} in comparison to the three other channel variants.

In Purkinje neurons, a “persistent” (noninactivating) component of sodium current is thought to be carried by sodium channels containing the $\text{Na}_v1.6$ α subunit [18]. We therefore assessed the effects of the $\beta 1$ and $\beta 2$ subunits on the persistent component of currents carried by $\text{Na}_v1.6$ channels. In this study, the persistent current was operationally defined as

the component of current remaining at the end of a standard 40-ms depolarizing pulse. Following depolarization to 0 mV, which yielded maximal or near-maximal peak transient currents with all four channel variants, the persistent component of current varied depending on the subunit composition of the channel (Fig. 1A). With Na_v1.6 channels the persistent component of current was reversed with respect to the peak transient current. The complete block of both the transient and persistent elements of Na_v1.6 currents by 300 nM TTX indicates that the atypical persistent component is mediated by the Na_v1.6 α subunit. Coexpression with the β 1 subunit gave currents that lacked a persistent component, whereas coexpression with the β 2 subunit produced a small inward persistent current. Coexpression with both β subunits a persistent inward current typical of those observed in neurons expressing Na_v1.6 channels.

The amplitude and direction of the persistent currents varied linearly with test potential for potentials between -10 and 10 mV (Fig. 1C). All four channels gave a significant inward persistent current (~6–8% of peak transient current) upon depolarization to -10 mV. The steepness of the voltage dependence upon depolarization to test potentials from -5 mV to 10 mV varied with the subunit composition of the channel. For Na_v1.6 channels, the persistent component of current was outward at -10 mV, near zero at -5 mV, and increasingly positive at potentials above -5 mV. Both β subunits, when expressed alone, attenuated the voltage dependence of the persistent current with the β 2 subunit producing the larger effect, but neither prevented current reversal in this range of test potentials. The voltage dependence of the persistent current carried by Na_v1.6+ β 1 β 2 channels appeared to reflect the additive effects of the β subunits expressed individually, so that persistent currents were inward at all test potentials shown in Fig. 1C.

The independent and joint effects of the β 1 and β 2 subunits on the voltage-dependent gating of Na_v1.6 sodium channels are illustrated in Fig. 2, and the statistical analyses of these data are summarized in Table 1. The β 1 subunit had no detectable effect on the voltage dependence of activation of Na_v1.6 sodium channels but the β 2 subunit caused a small (~2-mV) but significant depolarizing shift in the midpoint potential for activation (Fig. 2A, Table 1). In contrast, the midpoint potential for the activation of Na_v1.6+ β 1 β 2 channels was shifted by ~3.4 mV in the direction of hyperpolarization when compared to channels expressed in the absence of β subunits (Fig. 2A, Table 1). The β subunits, either alone or in combination, had no effect on midpoint potentials for steady-state inactivation of Na_v1.6 channels (Fig. 2B, Table 1). However the β subunits, either alone or in combination, increased the steepness of the voltage response.

High-frequency firing in Purkinje neurons has been associated with the expression of Na_v1.6 channels in these cells [19]. To assess the effects of high frequency stimulation on the stability of the peak transient sodium current we applied trains of up to 100 brief (5-ms) depolarizing pulses to 10 mV at a frequency of 66.7 Hz prior to a standard 40-ms test pulse to 0 mV (Fig. 3). Currents carried by Na_v1.6 channels declined rapidly to ~60% of the control current after 20 pulses but did not decline further. Coexpression with the β 1 subunit completely abolished the stimulus-dependent decline observed with Na_v1.6 channels, whereas coexpression with the β 2 subunit had no effect on current stability. The responses of Na_v1.6+ β 1 β 2 to repeated depolarization were indistinguishable from those of Na_v1.6+ β 1 channels.

Discussion

Sodium channels purified from rat brain occur as heterotrimeric complexes of a pore-forming α subunit and the β 1 and β 2 auxiliary subunits that differ in structure and their mode of association (noncovalent for β 1; covalent for β 2) with the α subunit [2]. Sodium channel

β subunits are multifunctional, acting to modify channel gating, regulate channel expression in the plasma membrane, and serve as cell adhesion molecules in interactions with the extracellular matrix and cytoskeleton [3]. The $\beta 1$ and $\beta 2$ subunits may be replaced in some developmental stages and brain regions by the $\beta 3$ or $\beta 4$ subunit, respectively, but the ubiquitous expression of the $\beta 1$ and $\beta 2$ subunits in adult brain implies that the majority of brain channels are complexes of an α subunit with these two subunits [3]. Moreover, the direct reciprocal interaction between the $\text{Na}_v 1.6$ and $\beta 1$ subunits in promoting neurite outgrowth and determining sodium channel localization [20] demonstrates the functional association of these two subunits.

We employed the *Xenopus* oocyte expression system to assess the effects of the rat $\beta 1$ and $\beta 2$ subunits on the kinetic and gating properties of the rat $\text{Na}_v 1.6$ sodium channel isoform, for which only limited published information exists [13]. Rat $\text{Na}_v 1.6$ channels expressed in the absence of β subunits, like the orthologous mouse channels but unlike rat $\text{Na}_v 1.2$ sodium channels [21], inactivated relatively rapidly during a depolarizing pulse. These channels also gave an atypical “late” or persistent current that was reversed in direction at potentials producing maximal peak transient inward currents. We conclude that this unusual persistent current is intrinsic to the expressed rat $\text{Na}_v 1.6$ channel and not a voltage clamp artifact because it was: (1) completely inhibited by the selective sodium channel blocker TTX; (2) reproducibly observed in all cells expressing the $\text{Na}_v 1.6$ α subunit alone and reproducibly modulated by coexpression with the $\beta 1$ and $\beta 2$ subunits; and (3) never observed in uninjected oocytes (J. T. Tan and D. M. Soderlund, unpublished results) or in our studies of other rat sodium channel isoforms expressed under identical conditions in this system [16; 22].

Coexpression of $\text{Na}_v 1.6$ with either $\beta 1$ or $\beta 2$ produced different effects on channel gating and kinetics. The $\beta 1$ subunit accelerated the decay of the peak transient current by selectively increasing the rate of the fast phase of inactivation by approximately two-fold without altering the slow phase of inactivation. Acceleration of fast inactivation is the most commonly-observed effect of the $\beta 1$ subunit when expressed in combination with most sodium channel α subunits in the oocyte system [4]. Our results with $\text{Na}_v 1.6+\beta 1$ channels are also consistent with the only previous study of rat $\text{Na}_v 1.6$ channels [13]. In contrast to $\beta 1$, $\beta 2$ selectively retarded the fast phase of inactivation. Coexpression of the $\beta 1$ subunit with other sodium channel α subunit isoforms in this system often shifts the voltage dependence of activation in the direction of hyperpolarization [4], but the $\beta 1$ subunit had no significant effect on the voltage dependence of activation for $\text{Na}_v 1.6$. However, the $\beta 2$ subunit caused a small but significant depolarizing shift in the midpoint potential for activation. Coexpression with either the $\beta 1$ or $\beta 2$ subunit attenuated the atypical persistent current observed with $\text{Na}_v 1.6$ channels.

$\text{Na}_v 1.6+\beta 1+\beta 2$ channels, presumed to reflect the heterotrimeric structure of native channels, exhibited kinetic and gating properties distinct from both $\text{Na}_v 1.6+\beta 1$ and $\text{Na}_v 1.6+\beta 2$ channels. Moreover, the combined effects of the $\beta 1$ and $\beta 2$ subunits did not reflect the summation of the effects of these subunits expressed individually. The $\beta 1$ and $\beta 2$ subunits expressed together accelerated both kinetic phases of fast inactivation and modulated the voltage dependence and reversal of the persistent current. The combination of these effects gave a rapidly inactivating peak current and an inward persistent resembling those carried by native $\text{Na}_v 1.6$ channel complexes in Purkinje neurons [19]. $\text{Na}_v 1.6+\beta 1+\beta 2$ channels also exhibited a hyperpolarizing shift in the voltage dependence of activation, an effect not seen with either β subunit alone. In agreement with studies of mouse $\text{Na}_v 1.6$ channels in heterologous expression systems [4], our experiments did not reconstitute the resurgent current observed in Purkinje neurons that is thought to depend in part on $\text{Na}_v 1.6$ channels.

The presumed role of Na_v1.6 sodium channels in the generation of high-frequency action potentials [19] led us to assess the impact of coexpression with the β 1 and β 2 subunits on the fidelity of sodium currents measured following trains of rapid depolarizing pulses. In the absence of β subunits Na_v1.6 channels gave currents that declined rapidly in amplitude following repeated depolarization. This effect, which is also observed with Na_v1.2 and Na_v1.4 channels expressed in oocytes [23; 24], has been attributed to rapid entry of these channels into slow-inactivated states. Coexpression of Na_v1.6 with the β 1 subunit or the β 1 and β 2 in combination, but not the β 2 subunit alone, preserved the stability of peak transient currents following trains of high-frequency depolarizations, a finding consistent with the effects of the β 1 subunit on currents 1 and carried by rat Na_v1.4 channels in intact oocytes [23] and the β 1 and β 2 subunits on mouse Na_v1.6 channels in cut-open oocyte preparations [24]. The latter study also found a significant potentiation of peak transient currents carried by mouse Na_v1.6+ β 1+ β 2 channels following repeated depolarization which was not evident in our assays with the corresponding rat channels. This discrepancy may reflect a species difference in channel properties or may be due to differences in the cellular preparation (intact *vs.* cut-open oocytes) or pulse train protocols employed in these two studies.

To summarize, we have identified unique effects of the rat β 1 and β 2 sodium channel auxiliary subunits on the kinetics, gating and stability following repeated depolarization of currents carried by the rat Na_v1.6 sodium channel α subunit in oocytes. The properties of channels obtained upon expression of Na_v1.6, β 1 and β together as a heterotrimeric complex reflect in part the sum of the effects of the β 1 and β 2 subunits determined separately but also exhibit unique features that appear to result from the joint modulation of Na_v1.6 channels by these subunits. The expression of Na_v1.6+ β 1+ β 2 channels gives currents similar in many respects to those recorded from Purkinje cells and other neurons known to express Na_v1.6 sodium channels.

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References

1. Hille, B. Ion Channels of Excitable Membranes. Sinauer; Sunderland, MA: 2001.
2. Catterall WA. From ionic currents to molecular mechanisms: structure and function of voltage-gated sodium channels. *Neuron*. 2000; 26:13–25. [PubMed: 10798388]
3. Patino GA, Isom LL. Electrophysiology and beyond: multiple roles of Na⁺ channel β subunits in development and disease. *Neurosci Lett*. 2010; 486:53–59. [PubMed: 20600605]
4. Goldin AL. Resurgence of sodium channel research. *Annu Rev Physiol*. 2001; 63:871–894. [PubMed: 11181979]
5. Yu FH, Catterall WA. Overview of the voltage-gated sodium channel family. *Genome Biol*. 2003; 4:207.1–207.7. [PubMed: 12620097]
6. Auld VJ, Goldin AL, Krafte DS, Marshall J, Dunn JM, Catterall WA, Lester HA, Davidson N, Dunn RJ. A rat brain Na⁺ channel α subunit with novel gating properties. *Neuron*. 1988; 1:449–461. [PubMed: 2856097]
7. Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR. Sodium channel Na_v1.6 is localized nodes of Ranvier, dendrites, and synapses. *Proc Natl Acad Sci USA*. 2000; 97:5616–5620. [PubMed: 10779552]

8. Hu W, Tian C, Yang M, Hou H, Shu Y. Distinct contributions of Na_v1.6 and Na_v1.2 in action potential initiation and backpropagation. *Nature Neurosci.* 2009; 12:996–1002. [PubMed: 19633666]
9. Burgess DL, Kohrman DC, Galt J, Plummer NW, Jones JM, Spear B, Meisler MH. Mutation of a new sodium channel gene, *Scn8a*, in the mouse mutant 'motor endplate disease'. *Nature Genet.* 1995; 10:461–465. [PubMed: 7670495]
10. Schaller KL, Caldwell JH. Expression and distribution of voltage-gated sodium channels in the cerebellum. *Cerebellum.* 2003; 2:2–9. [PubMed: 12882229]
11. Shah BS, Stevens EB, Pinnock RD, Dixon AK, Lee K. Developmental expression of the novel voltage-gated sodium channel auxiliary subunit β 3, in rat CNS. *J Physiol.* 2001; 534:763–776. [PubMed: 11483707]
12. Whitaker WRJ, Clare JJ, Powell AJ, Chen YH, Faull RLM, Emson PC. Distribution of voltage-gated sodium channel -subunit and -subunit mRNAs in human hippocampal formation, cortex, and cerebellum. *J Comp Neurol.* 2000; 422:123–139. [PubMed: 10842222]
13. Dietrich PS, McGivern JG, Delgado SG, Koch BD, Eglen RM, Hunter JC, Sangameswaran L. Functional analysis of a voltage-gated sodium channel and its splice variant from rat dorsal root ganglia. *J Neurochem.* 1998; 70:2262–2272. [PubMed: 9603190]
14. Smith TJ, Soderlund DM. Potent actions of the pyrethroid insecticides cismethrin and cypermethrin on rat tetrodotoxin-resistant peripheral nerve (SNS/PN3) sodium channels expressed in *Xenopus* oocytes. *Pestic Biochem Physiol.* 2001; 70:52–61.
15. Goldin AL. Maintenance of *Xenopus laevis* and oocyte injection. *Meth Enzymol.* 1992; 207:266–297. [PubMed: 1528120]
16. Tan J, Soderlund DM. Divergent actions of the pyrethroid insecticides *S*-bioallethrin, tefluthrin, and deltamethrin on rat Na_v1.6 sodium channels. *Toxicol Appl Pharmacol.* 2010; 247:229–237. [PubMed: 20624410]
17. Bezanilla F, Armstrong CM. Inactivation of the sodium channel. *J Gen Physiol.* 1977; 70:549–566. [PubMed: 591911]
18. Vega-Saenz de Miera E, Rudy B, SM, Llinàs R. Molecular characterization of the sodium channel subunits expressed in mammalian cerebellar Purkinje cells. *Proc Natl Acad Sci USA.* 1997; 94:7059–7064. [PubMed: 9192691]
19. Khaliq ZM, Gouwens N, Raman IM. The contribution of resurgent sodium current to high-frequency firing in Purkinje neurons: an experimental and modeling study. *J Neurosci.* 2003; 23:4899–4912. [PubMed: 12832512]
20. Brackenbury WJ, Calhoun JD, Chen C, Miyazaki H, Nukina N, Oyama F, Rascht B, Isom LL. Functional reciprocity between Na⁺ channel Na_v1.6 and β 1 subunits in the coordinated regulation of excitability and neurite outgrowth. *Proc Natl Acad Sci USA.* 2010; 107:2283–2288. [PubMed: 20133873]
21. Smith MR, Smith RD, Plummer NW, Meisler MH, Goldin AL. Functional analysis of the mouse *Scn8a* sodium channel. *J Neurosci.* 1998; 18:6093–6102. [PubMed: 9698304]
22. Tan J, Soderlund DM. Human and rat Na_v1.3 voltage-gated sodium channels differ in inactivation properties and sensitivity to the pyrethroid insecticide tefluthrin. *Neurotoxicology.* 2009; 30:81–89. [PubMed: 19026681]
23. Balser JR, Nuss B, Romashko DN, Marban E, Tomaselli GF. Functional consequences of lidocaine binding to slow-inactivated sodium channels. *J Gen Physiol.* 1996; 107:643–658. [PubMed: 8740377]
24. Zhou W, Goldin AL. Use-dependent potentiation of the Na_v1.6 sodium channel. *Biophys J.* 2004; 87:3862–3872. [PubMed: 15465873]

Figure 1.

Sodium currents carried by Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 sodium channels. (A) Four representative sodium current traces recorded from oocytes expressing Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, or Na_v1.6+β1+β2 sodium channels and a single trace from the oocyte expressing Na_v1.6 sodium channels after the bath application of 300 nM TTX. Peak transient currents in the absence of TTX (~2 – 2.5 μA) were scaled to facilitate visual comparison of decay kinetics. (B) Comparison of the fast and slow kinetic constants for peak current decay obtained from experiments such as those shown in Panel A. Values (means ± SE of 16–44 separate experiments with different oocytes) marked with different letters were significantly different by one-way ANOVA with Tukey's multiple comparison test ($P < 0.05$). (C) Voltage dependence of persistent currents measured at the end of 40-ms test depolarizations from –100 mV to the indicated potentials in oocytes expressing Na_v1.6, Na_v1.6+β1, Na_v1.6+β2 or Na_v1.6+β1+β2 sodium channels. Values are means of 16–44 separate experiments with different oocytes; bars show SE values larger than the data point symbols. Positive values represent inward currents. The arrow indicates the test potential employed in the experiments shown in Panel A.

Figure 2.

Voltage-dependent activation and steady-state inactivation of Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 sodium channels. (A) Conductance-voltage plots for channel activation. Peak sodium currents were obtained using the indicated pulse protocol were transformed to conductances (G) using the equation $G = I/(V_t - V_{rev})$, where I is the peak current, V_{rev} is the reversal potential, and V_t is the voltage of the test potential; conductances were then normalized to the maximum conductance (G_{max}) for that oocyte. Values are means of 15–48 separate experiments with different oocytes; bars show SE values larger than the data point symbols. Curves were fitted to the mean values using the Boltzmann equation. (B) Voltage dependence of steady-state inactivation. Amplitudes of peak transient currents obtained using the indicated pulse protocol are plotted as a function of prepulse potential (V_p). Values are means of 11–34 separate experiments with different oocytes; bars show SE values larger than the data point symbols. Curves were fitted to the mean values using the Boltzmann equation.

Figure 3.

Effect of repeated depolarization on the stability of sodium currents recorded from oocytes expressing Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, or Na_v1.6+β1+β2 sodium channels. Sodium currents were recorded during a 40-ms step depolarization from −100 mV to 0 mV following 0–100 conditioning prepulses (5-ms pulses from −100 mV to 10 mV at 66.7 Hz). Values are means of 11–31 separate experiments with different oocytes; bars show SE values larger than the data point symbols.

Effects of coexpression with the $\beta 1$ and $\beta 2$ subunits on the voltage dependence of activation and steady-state inactivation of $\text{Na}_v 1.6$ sodium channels.^a

Table 1

Channel	Activation			Inactivation		
	$V_{0.5}$	K	n	$V_{0.5}$	K	n
$\text{Na}_v 1.6$	$-13.4 \pm 0.8^{*b}$	$8.77 \pm 0.16^{*}$	15	-51.5 ± 0.4	$8.84 \pm 0.28^{*}$	11
$\text{Na}_v 1.6+\beta 1$	$-14.2 \pm 0.3^{*}$	$9.04 \pm 0.14^{*†}$	48	-50.8 ± 0.3	$7.09 \pm 0.15^{†}$	26
$\text{Na}_v 1.6+\beta 2$	$-11.3 \pm 0.4^{†}$	$8.57 \pm 0.21^{*†}$	20	-50.9 ± 0.4	$8.05 \pm 0.08^{†}$	26
$\text{Na}_v 1.6+\beta 1+\beta 2$	$-16.8 \pm 0.3^{†}$	$7.93 \pm 0.15^{†}$	32	-51.1 ± 0.3	$7.19 \pm 0.08^{†}$	34

^a Values calculated from fits of the data from the indicated number of individual experiments to the Boltzmann equation; $V_{0.5}$, midpoint potential (mV) for voltage-dependent activation or inactivation; K, slope factor.

^b Values in each column that are marked with different symbols were significantly different by one-way ANOVA with Tukey's multiple comparison test ($P < 0.01$).