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Analysis of the action of lidocaine on insect sodium channels

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ABSTRACT

A new class of sodium channel blocker insecticides (SCBIs), which include indoxacarb, its active metabolite, DCJW, and metaflumizone, preferably block inactivated states of both insect and mammalian sodium channels in a manner similar to that by which local anesthetic (LA) drugs block mammalian sodium channels. A recent study showed that two residues in the cockroach sodium channel, F1817 and Y1824, corresponding to two key LA-interacting residues identified in mammalian sodium channels are not important for the action of SCBIs on insect sodium channels, suggesting unique interactions of SCBIs with insect sodium channels. However, the mechanism of action of LAs on insect sodium channels has not been investigated. In this study, we examined the effects of lidocaine on a cockroach sodium channel variant, $BgNa_v1$-1a, and determined whether F1817 and Y1824 are also critical for the action of LAs on insect sodium channels. Lidocaine blocked $BgNa_v1$-1a channels in the resting state with potency similar to that observed in mammalian sodium channels. Lidocaine also stabilized both fast-inactivated and slow-inactivated states of $BgNa_v1$-1a channels, and caused a limited degree of use- and frequency-dependent block, major characteristics of LA action on mammalian sodium channels. Alanine substitutions of F1817 and Y1824 reduced the sensitivity of the $BgNa_v1$-1a channel to the use-dependent block by lidocaine, but not to tonic blocking and inactivation stabilizing effects of lidocaine. Thus, similar to those on mammalian sodium channels, F1817 and Y1824 are important for the action of lidocaine on cockroach sodium channels. Our results suggest that the receptor sites for lidocaine and SCBIs are different on insect sodium channels.

1. Introduction

Local anesthetics (LAs), such as lidocaine, interrupt the initiation and propagation of nerve impulses (i.e., action potentials) by blocking sodium channels, thereby relieving or preventing pain (Catterall, 1987). These drugs preferentially block open and inactivated states of the sodium channel and have a lower affinity to channels in the resting state. This is the basis of use- and frequency-dependent block by LAs, which are of clinical importance because the drugs are more effective in neurons undergoing repetitive firing. Use- and frequency-dependent block of sodium channels by LAs during rapid trains of stimulus pulses result from binding of the drug to open and inactivated channels during depolarization and slowed recovery of drug-bound channels from inactivation during repolarization.

Sodium channels consist of four homologous domains (I–IV), each containing six transmembrane segments (S1–S6) connected by extra- and intracellular loops (Catterall, 2000; Goldin, 2001). Site-directed mutagenesis studies of mammalian sodium channels identified a number of residues in the S6 transmembrane segments of domains I, III, and IV that are critical for the action of a variety of therapeutic sodium channel blockers including LAs (Liu et al., 2003; Nau et al., 1999; Nau et al., 2003; Ragsdale et al., 1994; Ragsdale et al., 1996; Wang et al., 2004; Wang et al., 2000; Wang and Wang, 1998; Yarov-Yarovoy et al., 2001; Yarov-Yarovoy et al., 2002). In particular, two residues in IVS6, F1579 and Y1586 (numbered according to their location in the rat $Na_v1.4$ sodium channel) are key residues in the action of these therapeutic drugs, including LAs (Liu et al., 2003; Ragsdale et al., 1994).

A new class of insecticides, which includes indoxacarb and metaflumizone, exert their toxic effects by blocking sodium channels in a manner similar to that of LAs (Salgado, 1992; Salgado and Hayashi, 2007; Wing et al., 2005; Wing et al., 2000; Wing et al., 1998). Salgado (1992) first documented the similarity between the mode of action of LAs and the pyrazoline RH3421, a progenitor of the indoxacarb/DCJW insecticide family, in crayfish giant axons. Subsequent studies demonstrated that DCJW and

Abbreviations: LA, local anesthetic; IVS6, domain IV transmembrane segment 6 of voltage-sensitive sodium channels; SCBI, sodium channel blocker insecticides.

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metaflumizone cause voltage-dependent block of both mammalian and insect sodium channels (Salgado and Hayashi, 2007; Silver et al., 2009a; Silver and Soderlund, 2005; Song et al., 2006; Zhao et al., 2005). Like LAs, these insecticides, known as sodium channel blocker insecticides (SCBIs), preferably bind to the inactivated state of the sodium channel, blocking them nearly irreversibly. Using dorsal unpaired median (DUM) neurons isolated from the CNS of *Periplaneta americana*, Lapied et al. (2001) showed that lidocaine antagonized the action of DCJW, suggesting that LAs and DCJW possibly share a common or overlapping binding site on insect sodium channels. Furthermore, Silver and Soderlund (2007) showed that one of the LA-interacting residues, F1579, is critical for the binding and action of DCJW and RH3421 on $Na_v1.4$ channels. Though conserved in insect sodium channels, our recent analysis of these residues that interact with LAs in mammals showed that these residues are not molecular determinants of the action of DCJW/metaflumizone on insect sodium channels (Silver et al., 2009b). This raises the question of whether the two key LA-interacting residues identified in mammalian sodium channels are also critical for the binding and action of LAs on insect sodium channels. Therefore, in this study we examined the effects of lidocaine on insect sodium channels and evaluated the involvement of the corresponding residues in the binding and action of lidocaine on insect sodium channels.

2. Materials and methods

2.1. Site-directed mutagenesis

Site-directed mutagenesis was performed by PCR using mutant primers and Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA). All mutagenesis results were verified by DNA sequencing.

2.2. Expression of $BgNa_v$ sodium channels in Xenopus laevis oocytes

The procedures for oocyte preparation and cRNA injection are identical to those described previously (Tan et al., 2002). For robust expression of the $BgNa_v$ sodium channel, cRNA was co-injected into oocytes with *Drosophila melanogaster* tipE cRNA (2:1 ratio), which enhances the expression of insect sodium channels in oocytes (Feng et al., 1995; Warmke et al., 1997).

2.3. Electrophysiological recording and analysis

Sodium currents were recorded using the two-electrode voltage clamp technique. Electrodes were pulled from borosilicate glass and filled with 3 M KCl and 5% agarose. Resistances ranged between 0.5 and 1.5 MΩ. Currents were measured with an oocyte clamp amplifier OC725C (Warner Instrument Corp., Hamden, CT), Digidata 1200A (Axon Instruments, Foster City, CA), and pClamp 8.2 software (Axon Instruments). Capacitive transient leak currents were subtracted using the P/N ($N = 2$) subtraction method. Specific voltage protocols are detailed in the figure legends.

All experiments were performed at room temperature. Peak current was measured with a 20 ms test pulse to −10 mV from the holding potential of −120 mV. Tonic block of sodium channels by lidocaine was then measured by perfusing the drug onto oocytes and allowing them to incubate for 10 min. The ratio of peak sodium current after lidocaine treatment was then compared to peak sodium current recorded prior to drug application. Lidocaine was perfused onto oocytes in a manner similar to that previously described (Tatebayashi and Narahashi, 1994).

Fast and slow inactivation were both measured using two pulse protocols. Oocytes were held at −120 mV, from which conditioning pulses of 200 ms to voltages between −80 and 20 mV were given to induce fast inactivation. A 20 ms pulse to −10 mV immediately followed the conditioning pulse to assess sodium current. Slow inactivation was measured with 60 s conditioning pulses to voltages between −100 and 0 mV, followed by a 10 ms pulse to −120 mV to remove fast inactivation and a 20 ms pulse to −10 mV.

Use- and frequency dependence of block were measured by delivering a train of 50 pulses at a frequency of 20 Hz or at a range of frequencies between 1 and 20 Hz, respectively. The amplitude of sodium current elicited by each pulse was then normalized to the amplitude of the peak sodium current generated by the initial pulse.

Statistical analysis was performed using a one-way ANOVA with a *post hoc* least significant difference test to determine significance ($p \leq 0.05$) with Statistix 7 software (Analytical Software, Tallahassee, FL).

3. Results

3.1. Modification of channel gating properties by the F1817A and Y1824A mutations

Alanine substitutions of F1817 and Y1824 resulted in significant changes in the voltage dependence of inactivation in $BgNa_v1\text{-}1a$ sodium channels (Fig. 1; Table 1). Compared to the wild-type, the Y1824A mutation caused a significant 6.7 mV depolarizing shift in the voltage dependence of fast inactivation (Table 1, $p < 0.05$, $n = 7$). Although neither mutation affected voltage dependence of slow inactivation, both mutations decreased the slope of the slow inactivation curves (Table 1, $p < 0.05$, $n = 7$).

3.2. Tonic block of wild-type and mutant $BgNa_v1\text{-}1a$ channels by lidocaine

We examined the block of $BgNa_v1\text{-}1a$ by various concentrations of lidocaine at a holding potential of −120 mV, which is known as tonic block and reflects lidocaine binding to resting channels. Fig. 2A shows the concentration–effect curve for tonic block of wild-type sodium channels by lidocaine. Lidocaine blocked resting $BgNa_v1\text{-}1a$ sodium channels with an IC_{50} of 2.2 mM. Therefore, a concentration of 2 mM was chosen to evaluate the ability of lidocaine to cause tonic block of mutant and wild-type sodium channels and throughout the rest of this study. Lidocaine caused $45.2 \pm 4.05\%$, $34.8 \pm 4.06\%$, and $42.4 \pm 2.54\%$ tonic block for $BgNa_v1\text{-}1a$, $BgNa_v1\text{-}1a^{F1817A}$, and $BgNa_v1\text{-}1a^{Y1824A}$ sodium channels, respectively; the differences were not statistically significant (Fig. 2B; $p > 0.05$; $n = 7$).

3.3. Modification of channel gating properties by lidocaine

Lidocaine (2 mM) significantly ($p < 0.05$, $n = 7$) shifted the voltage dependences of fast inactivation of all three channel variants to a similar degree in the hyperpolarizing direction by 3–5 mV (Fig. 3, Table 1). Lidocaine treatment also resulted in a steeper fast inactivation curve in $BgNa_v1\text{-}1a^{F1817A}$ sodium channels (Table 1, $p < 0.05$, $n = 7$). Lidocaine (2 mM) also caused large hyperpolarizing shifts in the voltage dependences of slow inactivation in all three channel variants (Fig. 4, Table 1, $p < 0.05$, $n = 7$). The lidocaine-induced shifts in the voltage dependences of slow inactivation were similar (−7 and −8 mV, respectively) for wild-type and $BgNa_v1\text{-}1a^{F1817A}$ channels. However, the shift was slightly greater for $BgNa_v1\text{-}1a^{Y1824A}$ channels (−11 mV, Fig. 4 and Table 1).

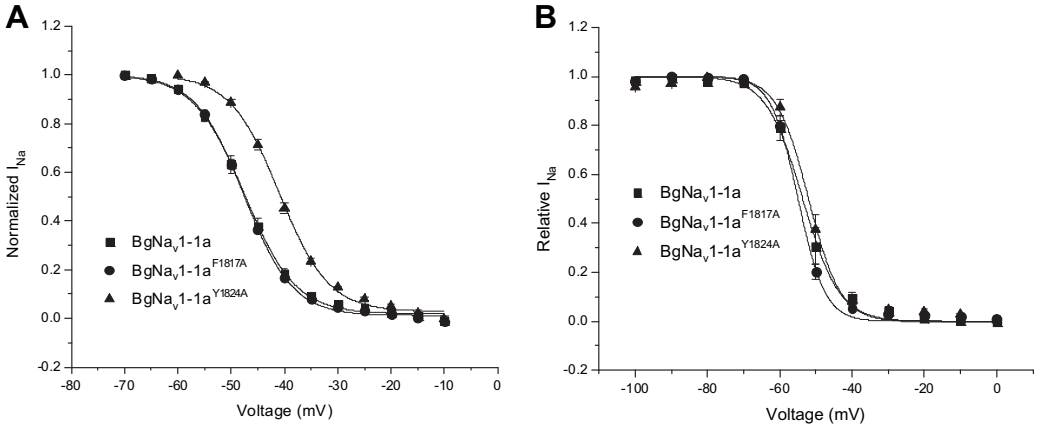

Fig. 1. Effects of mutations on fast inactivation (A) and slow inactivation (B) in BgNa$_v$1-1a sodium channels. Fast inactivation was measured with 200-ms conditioning pulses to potentials ranging from −80 to 20 mV from a holding potential of −120 mV followed by 20-ms test pulses to −10 mV. Current was normalized to the amplitude of the first current recorded. Slow inactivation was measured using 60-s conditioning pulses to potentials ranging from −100 to 0 mV from a holding potential of −120 mV. Conditioning pulses were followed by 10-ms pulses back to the holding potential to remove any fast inactivation and then 20-ms test pulses to −10 mV. Current was normalized to the amplitude of the first current recorded.

3.4. Use-dependent and frequency-dependent inhibition of wild-type and mutant sodium channels by lidocaine

Neither wild-type nor mutant sodium channels showed significant frequency-dependent run-down of sodium current in the absence of lidocaine. In contrast, lidocaine (2.0 mM) caused modest use-dependent or frequency-dependent inhibition of both wild-type and mutant sodium channels (Fig. 5). At a stimulation frequency of 20 Hz, BgNa$_v$1-1a^{Y1824A} and BgNa$_v$1-1a^{F1817A} sodium channels exhibited significantly less use-dependent inhibition than BgNa$_v$1-1a sodium channels after only a single conditioning pulse or as many as 50 ($p < 0.05$, $n = 7$; Fig. 5A). Similarly, when pulse frequency was varied from 1 to 20 Hz, BgNa$_v$1-1a^{F1817A} and BgNa$_v$1-1a^{Y1824A} sodium channels consistently showed significantly less inhibition than wild-type channels after 20 test pulses over the entire range of frequencies tested (Fig. 5B; $p < 0.05$, $n = 6$).

4. Discussion

This study represents the first effort to characterize the action of LAs on insect sodium channels. Though not used on insects for any purpose, the similarity of the effects and site of action of LAs on mammalian sodium channels to that described for sodium channel blocker insecticides (SCBIs) makes this data important in understanding the different receptors on sodium channels and their interaction with each other. Furthermore, these results permit comparison of effects of common chemicals, including molecular determinants of action, on sodium channels of different species. These data will either show a conservation of receptor sites on sodium channels between mammals and insects, or reveal areas where the biding sites of specific toxicants diverge significantly, as was demonstrated in the case of SCBIs (Silver et al., 2009a). To this end, we established the effects of LAs on cockroach BgNa$_v$1-1a sodium channels and examined whether the corresponding residues to those in IVS6 of mammalian sodium channels also constitute a portion of the LA binding site in insect sodium channels.

Lidocaine blocked resting, open, and inactivated BgNa$_v$1-1a sodium channels in a manner similar to that seen in mammalian sodium channels with LAs (Catterall, 1987; Wang and Wang, 2003). Lidocaine caused tonic inhibition of BgNa$_v$1-1 channels with an EC_{50} value of 2.2 mM. This value is similar to, though about twofold higher than the EC_{50} measured on rat Na$_v$1.2 or Na$_v$1.4 sodium channels expressed in oocytes (Pugsley and Goldin, 1998). Furthermore, lidocaine also caused state-dependent inhibition of BgNa$_v$1-1a sodium channels and characteristic hyperpolarizing shifts in the voltage dependences of fast and slow inactivation. Interestingly, however, lidocaine caused only modest use-dependent and frequency-dependent inhibition of BgNa$_v$1-1a sodium channels. Use- or frequency-dependent inhibition of wild-type BgNa$_v$1-1a channels by lidocaine (2 mM) accounted for only about a 30% reduction in current in our experiments. In contrast, use-dependent inhibition of mammalian Na$_v$1.2 and Na$_v$1.4 channels by one-tenth the concentration of lidocaine used in these experiments reduces sodium current by more than 60% (Ahern et al., 2008; Ragsdale et al., 1996). Use-dependent and frequency-dependent block of mammalian sodium channels are key features of block by LAs and are vital for the clinical use of LA drugs (Butterworth and Strichartz, 1990; Catterall, 1987). Use-dependent and frequency-dependent block by LAs can be explained by a modulated drug receptor that has a low affinity when the channel is in resting states and a higher affinity when the channel is in open or inactivated states (Hille, 1977; Hondeghem and Katzung, 1984). The lack of strong use-dependence of block in insect sodium channels could be due to the fast recovery from fast inactivation of insect sodium channels (Song et al., 2006) which limits the availability of inactivated channels for LA binding.

Table 1
Parameters of voltage dependences of fast and slow inactivation for BgNa$_v$1-1a, BgNa$_v$1-1a^{F1817A}, and BgNa$_v$1-1a^{Y1824A} sodium channels in the presence and absence of lidocaine.

	Control		Lidocaine (2 mM)	
	$V_{0.5}$ (mV)	k	$V_{0.5}$ (mV)	k
Fast inactivation				
BgNa$_v$1-1a	−47.2 ± 0.7	4.90 ± 0.05	−52.2 ± 0.5[b]	4.84 ± 0.05
BgNa$_v$1-1a^{F1817A}	−47.4 ± 0.4	4.67 ± 0.03[a]	−50.5 ± 0.6[b]	5.01 ± 0.08[b]
BgNa$_v$1-1a^{Y1824A}	−40.5 ± 0.4[a]	4.92 ± 0.03	−44.4 ± 0.4[b]	5.04 ± 0.07
Slow inactivation				
BgNa$_v$1-1a	−53.7 ± 1.6	4.6 ± 0.2	−60.7 ± 1.2[b]	4.8 ± 0.2
BgNa$_v$1-1a^{F1817A}	−54.9 ± 0.6	3.7 ± 0.1[a]	−63.1 ± 1.0[b]	3.8 ± 0.2
BgNa$_v$1-1a^{Y1824A}	−52.0 ± 1.1	3.8 ± 0.1[a]	−63.0 ± 1.4[b]	6.2 ± 0.6[b]

[a] Significantly different from wild-type (BgNa$_v$1-1a, $p < 0.05$, $n \geq 3$).
[b] Significantly different from control ($p < 0.05$, $n \geq 3$).

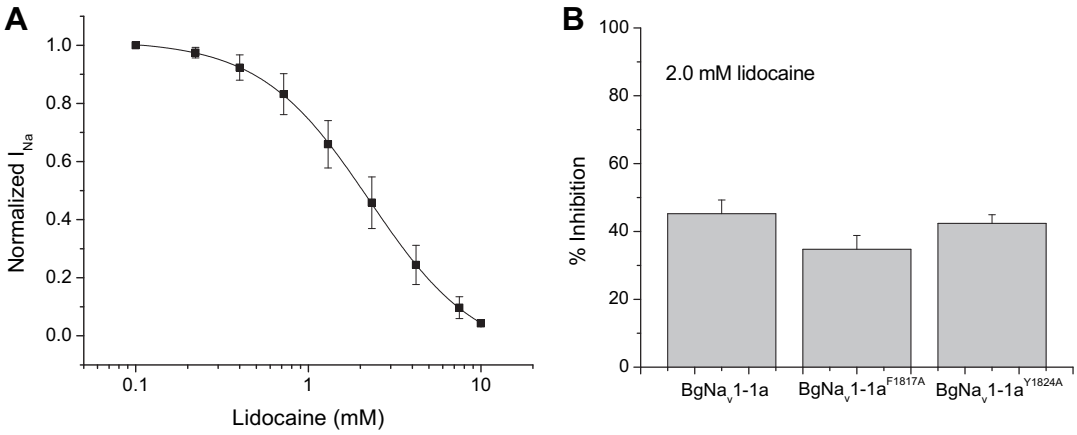

Fig. 2. Tonic block of $BgNa_v1\text{-}1a$, $BgNa_v1\text{-}1a^{F1817A}$, and $BgNa_v1\text{-}1a^{Y1824A}$ sodium channels by lidocaine. (A) Concentration–response relationship of lidocaine block of $BgNa_v1\text{-}1a$ sodium channels. Sodium current was evoked by a 20 ms depolarization to −10 mV from a holding potential of −120 mV. The amplitude of the first peak current recorded after 10 min of incubation in lidocaine was normalized to the amplitude of the peak current recorded before treatment with lidocaine. (B) The percentage of inhibition of wild-type and mutant sodium current by 2.0 mM lidocaine.

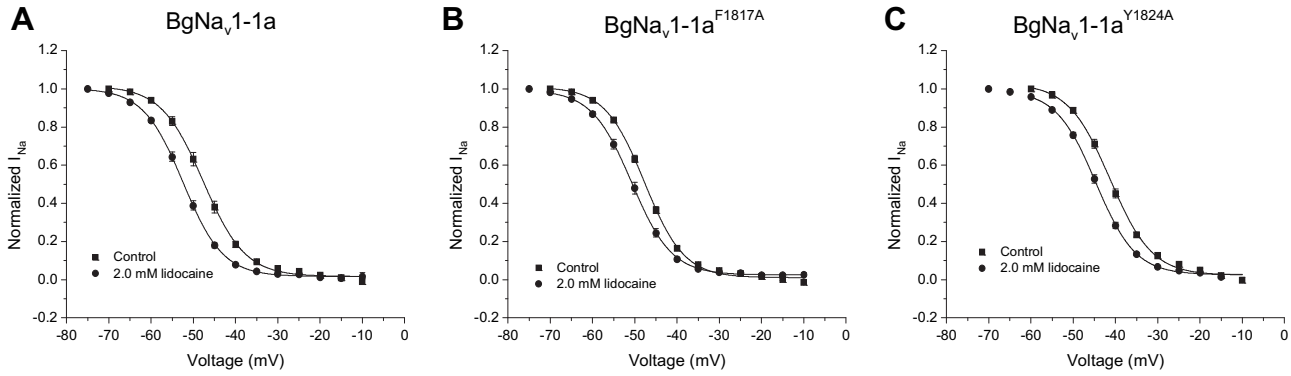

Fig. 3. Effects of lidocaine (2.0 mM) on fast inactivation in $BgNa_v1\text{-}1a$ (A), $BgNa_v1\text{-}1a^{F1817A}$ (B), and $BgNa_v1\text{-}1a^{Y1824A}$ (C) sodium channels. Fast inactivation was measured as in Fig. 1A.

Two specific residues in IVS6 of mammalian sodium channels, F1764 and Y1771 in rat $Na_v1.2$ sodium channels and F1579 and Y1586 in $Na_v1.4$ sodium channels, are required for the action of LAs on mammalian sodium channels (Gauthereau et al., 2005; Li et al., 1999; Liu et al., 2003; Ragsdale et al., 1994, 1996; Wang et al., 2004; Weiser et al., 1999; Wright et al., 1998). We show here that the corresponding residues in the cockroach sodium channel also contribute to the action of lidocaine on insect sodium channels. BgNav1-$1a^{F1817A}$ and BgNav1-$1a^{Y1824A}$ channels were significantly less sensitive to use- and/or frequency-dependent block by lidocaine than wild-type channels, indicating an impaired interaction between LAs and open or fast-inactivated sodium channels. Additionally, shifts in the voltage dependences of fast inactivation by lidocaine were slightly less in mutant channels than in wild-type channels, though this was only a very small effect. However, the F1817A mutation had no effect on the sensitivity of $BgNa_v1\text{-}1$

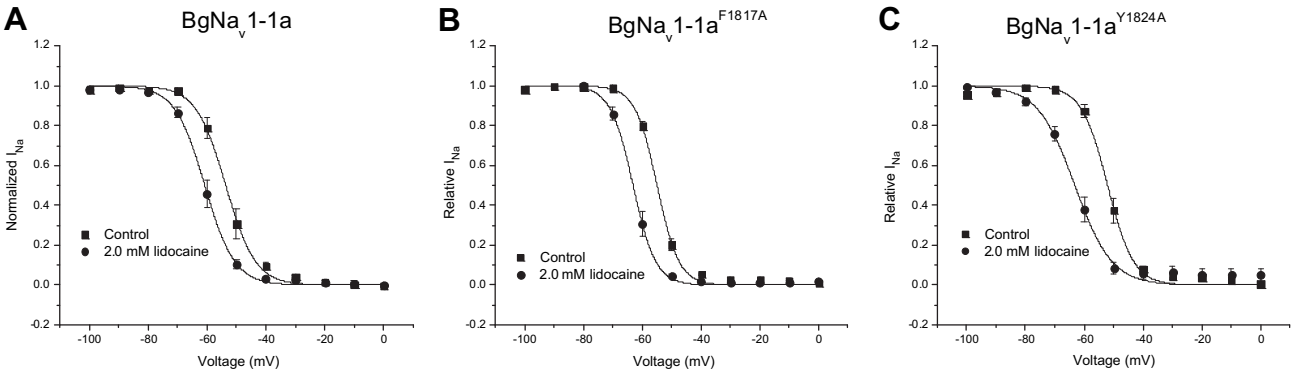

Fig. 4. Effects of lidocaine (2.0 mM) on slow inactivation in $BgNa_v1\text{-}1a$ (A), $BgNa_v1\text{-}1a^{F1817A}$ (B), and $BgNa_v1\text{-}1a^{Y1824A}$ (C) sodium channels. Slow inactivation was measured as in Fig 1B.

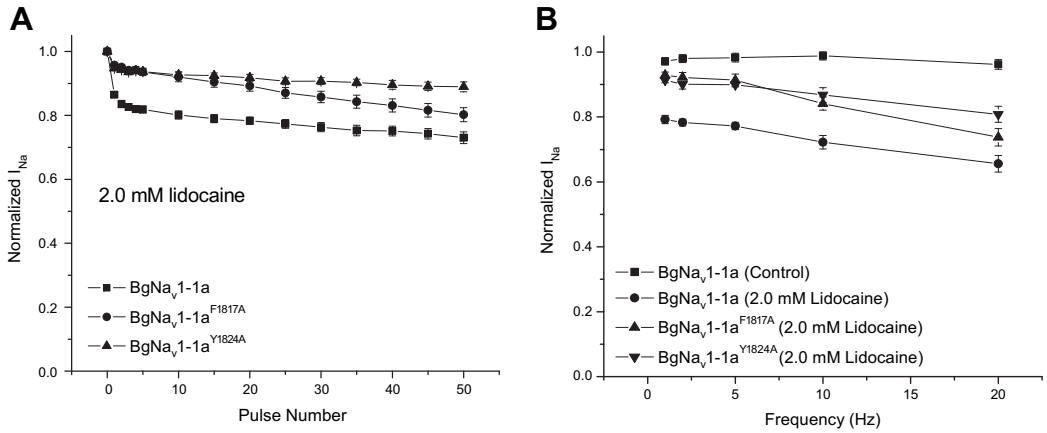

Fig. 5. Use- and frequency-dependent block of wild-type and mutant channels by lidocaine. (A) Use-dependent block. Channels were stimulated with trains of test pulses of differing number (0–50) at a rate of 20 Hz. Currents were normalized to the first pulse in the train. (B) Frequency-dependent block. Trains of 20 pulses were given to wild-type and mutant channels at varying frequencies of stimulation (1–20 Hz). The amplitude of the 20th pulse was normalized to that of the initial test pulse.

channels to stabilization of the slow-inactivated state by lidocaine. Unexpectedly, a negative shift in the voltage dependence of slow inactivation by lidocaine was greater in the BgNav1-1a^{Y1824A} channel than in wild-type channels. Nevertheless, the contribution of these residues to the use- and frequency-dependent activities of lidocaine indicates that these residues participate in the action of lidocaine on BgNa$_v$1-1 channels.

Results reported here are similar to previous studies where mutation of the corresponding residues in rat Na$_v$1.3 channels resulted in decreases in inhibition of open and inactivated channels by lidocaine (Ragsdale et al., 1996). Other studies with other mammalian sodium channels and other LA drugs showed reductions in open and inactivated channel block, with slight variations occurring according to which LA was used (Gauthereau et al., 2005; Li et al., 1999; Liu et al., 2003; Ragsdale et al., 1994, 1996; Wang et al., 2004; Weiser et al., 1999; Wright et al., 1998). Thus, our results indicate that the residues F1817 and Y1824 of IVS6 in cockroach sodium channels are required for mediating the interaction between lidocaine and open or inactivated, but not resting, channel states in a manner similar to mammalian sodium channels.

Previous studies established that the F1579 residue, corresponding to F1817 in the BgNa$_v$1-1a channel, in mammalian sodium channels is an important determinant of both LA and SCBI activity (Silver and Soderlund, 2007), indicating that the receptor sites for these toxins overlap. In contrast, neither F1817 nor the Y1824 of BgNa$_v$1-1 sodium channels, residues orthologous to those in Na$_v$1.4 above, mediate the activity of SCBIs on insect sodium channels (Silver et al., 2009b), yet both residues contribute to LA activity. Thus, these data suggest that unlike on mammalian sodium channels, LAs and SCBIs do not share overlapping receptor sites at F1817 or Y1824 in IVS6 of cockroach sodium channels. These findings also highlight differences between mammalian and insect sodium channels and their interactions with different classes of neurotoxins. These differences may be exploitable to reduce non-target effects of insecticides on mammals.

Changes in gating as a result of mutagenesis of specific residues in BgNa$_v$1-1a channels could account for changes in the sensitivity of mutant channels to lidocaine independent of their effects on the interaction between lidocaine and the LA receptor. However, experiments detailing the effects of these mutations on gating kinetics demonstrated that the F1817A substitution had no effect on the gating of BgNa$_v$1-1a channels. The Y1824A mutation caused a significant depolarizing shift in the voltage dependence of fast inactivation and increased the slope of the slow inactivation curve. However, since little to no effect was observed on the depolarizing shift caused by lidocaine treatment, it is unlikely that the gating changes caused by this residue are affecting lidocaine activity. Therefore, it is unlikely that the changes in channel sensitivity to lidocaine are a result of the alterations in channel function caused by the Y1824A mutation.

In conclusion, our results demonstrate that the residues F1817 and Y1824 of IVS6 of the cockroach voltage-gated sodium channel, BgNav1-1a, are important molecular determinants of the binding of lidocaine to open and inactivated, but not resting, channel states. Although these residues appear to play the same role as the corresponding residues in mammalian sodium channels, these results do not preclude the idea that the LA receptors on mammalian and insect sodium channels may diverge significantly at other important amino acid residues as was shown with SCBIs (Silver et al., 2009b). Additionally, our results indicate that the SCBI receptor site does not overlap with the LA receptor site in insect sodium channels at position F1817 as expected from studies in mammalian sodium channels. Thus, further characterization of the LA and SCBI receptor sites is necessary to fully understand the complex interactions of these two groups of chemicals and their receptors on insect and mammalian sodium channels.

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