Lithium Increases Potency of Lidocaine-Induced Block of Voltage-Gated Na\textsuperscript{+} Currents in Rat Sensory Neurons in Vitro

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Received March 15, 2001; accepted July 30, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

We and others have obtained data both in vivo and in isolated nerve preparations suggesting that Li\textsuperscript{+} increases the potency of local anesthetics in the block of conduction. In the present study we have tested the hypothesis that Li\textsuperscript{+} increases the potency of local anesthetic-induced block of conduction via a shift in the potency of local anesthetic-induced block of voltage-gated Na\textsuperscript{+} channels. To test this hypothesis we have used whole cell patch-clamp electrophysiological techniques on isolated adult rat sensory neurons. The presence of Li\textsuperscript{+} significantly increased the potency of lidocaine-induced block of both tetrodotoxin (TTX)-sensitive and TTX-resistant voltage-gated Na\textsuperscript{+} currents: ED\textsubscript{50} values for lidocaine-induced block of both currents in the presence of Li\textsuperscript{+} were less than 35% of the values obtained in the presence of Na\textsuperscript{+}. Li\textsuperscript{+} effects were dependent on the state of the Na\textsuperscript{+} channel. It increased the potency of lidocaine-induced block of resting or closed channels, without a detectable influence on use-dependent block or block of channels in the inactivated state. Li\textsuperscript{+} alone had no detectable effect on the gating properties of voltage-gated Na\textsuperscript{+} currents present in sensory neurons. The effects of Li\textsuperscript{+} were concentration-dependent. These results support the suggestion that the influence of Li\textsuperscript{+} on lidocaine-induced conduction block reflects an increase in potency of lidocaine-induced block of voltage-gated Na\textsuperscript{+} channels. This increase in potency appears to reflect an increase in the affinity of the low-affinity binding site for local anesthetics. Including Li\textsuperscript{+} in lidocaine preparations may be an effective way to increase the safety factor associated with the use of this anesthetic clinically.

Although local anesthetics, such as lidocaine are used routinely for local and regional anesthesia, these compounds may be toxic. Problems associated with systemic toxicity (ranging from vertigo to respiratory arrest) have long been appreciated. More recently, reports of neurological injury after continuous spinal anesthesia suggest that local anesthetics can cause nerve injury (Rigler et al., 1991; Auroy et al., 1997). Consistent with these clinical observations, we have recently demonstrated that lidocaine is toxic to sensory neurons at concentrations used clinically (Gold et al., 1998). A lidocaine-induced increase in intracellular Ca\textsuperscript{2+} appeared to be an underlying mechanism of this toxicity. Because the mechanisms underlying neuronal toxicity appear to be distinct from those underlying local anesthetics, identification of ways to increase the potency of lidocaine may have important clinical implications.

Evidence suggests that the addition to lithium (Li\textsuperscript{+}) to local anesthetic preparations may be one way to increase the potency of local anesthetics with no loss of efficacy. For example, we have obtained preliminary data both in vivo and in an isolated nerve preparation indicating that substituting only a fraction of the extracellular Na\textsuperscript{+} with Li\textsuperscript{+} increased the potency of local anesthetic-induced anesthesia and conduction block (Turner et al., 1994). More recently, data obtained in an isolated frog sciatic nerve preparation indicated that replacing Na\textsuperscript{+} with Li\textsuperscript{+} increased the potency of lidocaine-induced block of compound actions potentials more than 2-fold, as measured by a change in IC\textsubscript{50} value (Lilley and Robbins, 1998). Whether these results reflect an interaction between local anesthetics and Li\textsuperscript{+} at a Na\textsuperscript{+} channel has yet to be determined. Therefore, we have used whole cell patch-clamp electrophysiological techniques on isolated adult rat sensory neurons to determine whether the effects on conduction reflect a shift in the potency of local anesthetic block of voltage-gated Na\textsuperscript{+} channels. Portions of this study have been published previously in abstract form (Gold et al., 1999).

Materials and Methods

Cell Culture. Primary cultures of dissociated adult rat DRG neurons were prepared as described previously (Gold et al., 1996a). Male Sprague-Dawley rats (150–250 g; Harlan Bioproducts for

ABBREVIATIONS: DRG, dorsal root ganglion; MEM-BS, minimal essential medium-10% heat-inactivated fetal bovine serum; TTX-R \textsubscript{Na}, tetrodotoxin resistant voltage-gated Na\textsuperscript{+} current; TTX-S \textsubscript{Na}, tetrodotoxin-sensitive voltage-gated Na\textsuperscript{+} current; TTX, tetrodotoxin; I-V, current-voltage; ANOVA, analysis of variance.
Science, Indianapolis, IN) were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg); lumbar (L1-L6) DRGs were removed, and rats were subsequently killed by an overdose of sodium pentobarbital. DRGs were desheathed in ice-cold minimal essential medium-10% heat-inactivated fetal bovine serum (MEM-BS) composed of 90% MEM (Invitrogen, Carlsbad, CA), 10% BS, and 1000 units/ml each of penicillin and streptomycin. DRGs were then incubated 120 min at 37°C in 5 ml of MEM-BS, to which collagenase P (Roche Molecular Biochemicals, Indianapolis, IN) had been added to a final concentration of 0.125%. DRGs were then incubated 5 min at 37°C in Ca2+- and Mg2+-free Hank’s balanced salt solution (Invitrogen) containing 0.25% trypsin (Worthington, Bristol, UK) and 0.025% EDTA (Sigma Chemical, St. Louis, MO). Trypsin activity was inhibited by the addition of MEM-BS containing 0.125% MgSO4, and DRGs were dissociated by trituration with a fire-polished Pasteur pipette. DRG cells were plated onto glass coverslips, previously coated by a solution of 5 µg/ml mouse laminin (Invitrogen) and 0.1 mg/ml poly-l-ornithine (Sigma Chemical). The cells were incubated in MEM-BS at 37°C, 3% CO2, and 90% humidity overnight. To minimize neurite outgrowth, DRG neurons were then transferred to a modified L15 medium composed 90% L-15, 10% BS, 5 mM HEPES, 5 mM glucose, and 1000 units/ml each of penicillin and streptomycin. DRG neurons were studied between 14 and 24 h after plating.

Electrophysiology. Voltage-clamp recordings were performed using a HEKA EPC-9 amplifier (HEKA Electronik, Lembrecht/Pfalz, Germany). Data were low-pass filtered at 5 to 10 kHz with a four-pole Bessel filter and digitally sampled at 25 to 100 kHz with Pulse (version 8.31; HEKA Electronik) software running on a Pentium PC. Capacity transients were canceled and series resistance was compensated (>80%); a P/4 protocol was used for leak subtraction. Electrodes (1–2.5 MΩ) were filled with 100 mM CsCl, 40 mM tetraethylammonium chloride, 5 mM NaCl, 1 mM CaCl2, 2 mM MgCl2, 11 mM EGTA, 10 mM HEPES, 2 mM Mg-ATP, 1 mM Li-GTP; pH was adjusted to 7.2 with Tris base, osmolality was adjusted to 310 mOsml. Bath solution used to record whole cell Na+ currents in isolation contained 35 mM NaCl, 30 mM tetraethylammonium chloride, 65 mM choline chloride, 0.1 mM CaCl2, 5 mM MgCl2, 10 mM HEPES, 10 mM glucose; pH adjusted to 7.4, osmolality adjusted to 325 mOsml. Tetrodotoxin-resistant voltage-gated Na+ current (TTX-R I Na) was isolated from TTX-sensitive Na+ currents (TTX-S I Na) by adding TTX (250 nM) to the bath solution or with an inactivating prepulse to −50 mV. To determine the influence of Li+ on TTX-R and TTX-S I Na, and the lidocaine-induced block of these currents, LiCl was substituted for NaCl on a mole per mole basis. All salts were obtained from Sigma Chemical. Because TTX-R I Na is preferentially expressed in small-diameter DRG neurons (Gold et al., 1996b), all experiments were performed on neurons less than 35 µm in diameter.

Experimental Protocol. To assess Li+-induced changes in the current-voltage (I-V) relationships data were collected for an I-V curve every 2 min in the presence of either 35 mM Li+ or 35 mM Na+. The membrane potential was held at −60 mV. Current was evoked after a 500-ms prepulse to either −100 or −50 mV to potentials between −50 and +50 mV in 5-mV increments. At least three complete I-V curves were collected before the application of Li+. These I-V curves were used to establish the baseline response from which Li+-induced changes were compared. To assess the influence of Li+ on lidocaine-induced block of I Na, lidocaine, concentrations between 0.3 and 3 mM were applied sequentially in either ascending or descending order. Lidocaine-free solution was applied between each lidocaine application so that the lidocaine-induced inhibition could be assessed relative to the average of the peak current evoked before and after the application of lidocaine.

Data Analysis. Conductance-voltage (G-V) curves were constructed from I-V curves by dividing the evoked current by the driving force on the current, such that G = I(V m − V rev), where V m is the potential at which current was evoked and V rev is the reversal potential for the current determined by extrapolating the linear portion of the positive slope of the I-V curve through 0 current. Activation and steady-state inactivation data were fitted with a Boltzmann equation of the following form: G = Gmax/[1 + exp(V 0.5 − V m/k)], where G is the observed conductance, Gmax is the calculated maximal conductance, V 0.5 is the potential for half activation or inactivation, V m is command potential, and k is the slope factor. Once Gmax was calculated, data were normalized with respect to Gmax. Activation time constants were estimated from exponential fits to the rising phase of the current traces. Inactivation time constants were estimated from exponential fits to the falling phase of the current traces. Recovery from inactivation data were fitted with a two-exponential function of the following form: fractional recovery (I Na rec = α·(1 − exp(−x/τ1)) + (1 − α)·(1 − exp(−x/τ2))), where I is the peak inward current evoked after a hyperpolarizing voltage step to enable recovery from inactivation, I Na rec is the peak inward current evoked during a conditioning voltage step used to induce inactivation, and x is the fraction of recovery that was accounted for by the first time constant, τ1, τ2 is the second time constant. Dose-response data were fitted with a Michaelis-Menten equation: fractional inhibition (1 − I Na/lido/cont) = [(lidocaine) n/1/2]/(lidocaine) n/1/2 + ED50 n/1/2), where I Na/lido is the peak current evoked in the presence of lidocaine, I Na/cont is the peak current evoked in the absence of lidocaine, lidocaine concentration is the concentration of lidocaine used, n is the exponential term, ED50 is the maximal inhibition of I Na, and ED50 is the effective dose of lidocaine that produces a change in I Na that is 50% of the maximum. Data were fitted using a nonlinear least-squares method (Sigma Plot; SPSS, Inc., Chicago, IL).

Statistics. Data are expressed as mean ± S.E.M. Student’s t test and one-way ANOVA and two-way repeated measures ANOVA (both with Tukey’s post hoc tests) were used to assess for the presence of statistically significant differences; p < 0.05 was considered statistically significant.

Results

Although several TTX-S voltage-gated I Na have been described in sensory neurons through the use of whole cell voltage-clamp experiments, TTX-S I Na described in the present study had a low threshold for activation and inactivation and was rapidly activating and inactivating (Fig. 1; Table 1). Similarly, at least five TTX-R I Na have been described in sensory neurons. The current referred to as TTX-R I Na in the present study was a current with biophysical properties similar to that originally described by Kostyuk et al. (1981), with relatively high thresholds for activation and steady-state inactivation and relatively slow rates of activation and inactivation (Fig. 1; Table 1). The rapidly activating and inactivating currents illustrated in Fig. 1 were completely blocked by 500 nM TTX (data not shown), and therefore are unlikely to reflect TTX-R currents described by Rush et al. (1998) or Scholz et al. (1998a). The low-threshold TTX-R current recently described by Cummins et al. (1999) activates so slowly it may not have been detected with the voltage-clamp protocols used in the present study.

Lidocaine dose dependently blocked TTX-S I Na evoked in sensory neurons in the presence of Na+ and in the presence of Li+. Data were fitted with Michaelis-Menten equation described under Materials and Methods. The ED50 of lidocaine-induced block of TTX-S I Na in the presence of Na+ was 50.2 ± 8.2 µM (Fig. 2; n = 6). Substituting extracellular Na+ with Li+ increased the potency of lidocaine-induced block of
Fig. 1. Voltage-gated $I_{Na}$ in DRG neurons are distinguished by sensitivity to tetrodotoxin and gating properties. Left, voltage-gated Na$^+$ current (total $I_{Na}$) evoked from a 28-μm-diameter DRG neuron during 15-ms command potentials ranging between −80 and +50 mV from a prepulse potential of −100 mV (prepulse duration of 500 ms). The voltage-clamp protocol used to evoke the current is shown beneath the current traces. Middle, TTX-R $I_{Na}$ is isolated with a 500-ms prepulse to −50 mV. Bath application of 200 nM TTX produced similar results. Right, TTX-S $I_{Na}$ is isolated as the difference between the current evoked before and after a 500-ms prepulse to −50 mV.

TTX-S $I_{Na}$ such that the ED$_{50}$ was decreased to 11.2 ± 6.1 μM (Fig. 2; $n = 6$, $p < 0.01$). There was no significant difference between the Hill coefficients derived from fitted data: $n_H = 0.99 ± 0.26$ and 1.5 ± 0.37 in the presence of Na$^+$ and Li$^+$, respectively ($p > 0.05$).

Lidocaine also dose dependently blocked TTX-R $I_{Na}$ in the presence of Na$^+$ and in the presence of Li$^+$. The ED$_{50}$ of lidocaine-induced block of TTX-R $I_{Na}$ in the presence of Na$^+$ was 119.5 ± 25.4 μM (Fig. 3; $n = 6$). As with TTX-S $I_{Na}$, substituting Na$^+$ with Li$^+$ increased the potency of lidocaine-induced block of TTX-R $I_{Na}$ such that the ED$_{50}$ was decreased to 39.9 ± 10.6 μM (Fig. 3; $n = 6$, $p < 0.05$). Again, there was no significant difference between the Hill coefficients derived for lidocaine-induced block of TTX-R $I_{Na}$: $n_H = 1.13 ± 0.09$ and 1.26 ± 0.17 in the presence of Na$^+$ and Li$^+$, respectively ($p > 0.05$). Similar to our previous observations and those of others, the potency of lidocaine-induced block of TTX-R $I_{Na}$ was less than that of TTX-S $I_{Na}$ (Figs. 2 and 3). This was observed in the presence of either Na$^+$ ($p < 0.01$) or Li$^+$ ($p < 0.05$).

As a preliminary experiment to determine whether the effects of Li$^+$ on the potency of lidocaine-induced block of voltage-gated Na$^+$ channels reflected a nonspecific action of Li$^+$, we determined the influence of Li$^+$ on lidocaine-induced block of high-threshold voltage-gated Ca$^{2+}$ currents. Ba$^{2+}$ was used as the charge carrier by adding 5 mM Ba$^{2+}$ to bath solutions containing either 35 mM Na$^+$ or 35 mM Li$^+$. Ba$^{2+}$ currents were studied in isolation after digital subtraction of current evoked in the absence of Ba$^{2+}$ from those evoked in the presence of Ba$^{2+}$ (data not shown). Ba$^{2+}$ currents were evoked with a 100-ms voltage step to 0 mV from a holding potential of −80 mV. Currents were evoked once every 5 s. Lidocaine (10 mM) blocked 88 ± 2.2% ($n = 8$) of the Ba$^{2+}$ current evoked in the presence of Na$^+$, and it blocked 82 ± 2.5% ($n = 8$) of the current evoked in the presence of Li$^+$ ($p > 0.05$).

Subsequent experiments were performed to identify mechanisms that may underlie the influence of Li$^+$ on the potency of lidocaine-induced block of voltage-gated Na$^+$ currents in sensory neurons. Given that lidocaine-induced block of voltage-gated Na$^+$ channels involves at least two mechanisms (i.e., stabilizing the closed state of the channels and stabilizing the inactivated state of the channels; Li et al., 1999), we investigated the possibility that Li$^+$ influences lidocaine-induced block of TTX-S and TTX-R $I_{Na}$ by altering the gating properties of these currents. To assess the influence of Li$^+$ on the closed state of TTX-S and TTX-R $I_{Na}$, we compared the voltage dependence of activation as well as the rates of activation of these currents in the presence of Na$^+$ to those of

| **TABLE 1** |  |
| --- | --- | --- | --- |
| **Boltzmann values for steady-state properties** | **Inactivation** | **Activation** |  |
|  | $V_{1/2}$ | Slope | $V_{1/2}$ | Slope |
|  | mV |  | mV |  |
| TTX-R $I_{Na}$ |  |  |  |  |
| Na$^+$ ($n = 11$) | −32.2 ± 2.2 | 5.0 ± 0.4 | −6.9 ± 0.2 | 5.5 ± 0.2 |
| Li$^+$ ($n = 11$) | −29.8 ± 1.1 | 5.0 ± 0.32 | −5.8 ± 1.2 | 5.4 ± 0.2 |
| TTX-S $I_{Na}$ |  |  |  |  |
| Na$^+$ ($n = 7$) | −78.5 ± 3.6 | 10.0 ± 0.9 | −17.8 ± 1.6 | 8.1 ± 1.6 |
| Li$^+$ ($n = 5$) | −82.1 ± 4.8 | 9.4 ± 1.1 | −20.1 ± 1.6 | 6.8 ± 0.7 |

Data are presented as a mean ± SEM.
obtained in the presence of Li⁺. Li⁺ had no significant influence on the voltage dependence of activation of either TTX-S or TTX-R (Fig. 4; Table 1). Furthermore, there was no detectable difference between the rates of activation of either current obtained in the presence of Na⁺ and Li⁺ (i.e., the time constant for TTX-R activation in response to a voltage step from −50 to 0 mV in the presence of Na⁺ was 754.8 ± 92 μs, whereas that in the presence of Li⁺ was 800.5 ± 117 μs; p > 0.05).

There are several ways that an influence of Li⁺ on the inactivation of \( I_{Na} \) could be manifest at the whole cell current level. These include changes in steady-state inactivation, recovery from inactivation, and/or rate of inactivation. However, Li⁺ did not significantly influence steady-state inactivation of either TTX-S or TTX-R \( I_{Na} \) (Fig. 4; Table 1). Nor did Li⁺ significantly influence the recovery from inactivation of either current (Fig. 5). Nor did Li⁺ significantly influence the rate of inactivation or either current (Fig. 5).

Scholz et al. (1998b) recently reported that local anesthetic-induced block of \( I_{Na} \) in DRG neurons is facilitated if Na⁺ channels are inactivated. Such a mechanism could explain the observation that lidocaine blocked TTX-S \( I_{Na} \) with a greater potency than TTX-R \( I_{Na} \), given that the magnitude of lidocaine-induced block of TTX-S \( I_{Na} \) was assessed on currents evoked from −100 mV, a potential at which a significant fraction of channels are inactivated (Fig. 4). Therefore, to determine whether Li⁺ influences lidocaine-induced block of inactivated channels, we assessed the influence of Li⁺ on TTX-R \( I_{Na} \) evoked after a 500-ms prepulse to −35 mV, a voltage step that inactivates roughly 50% of TTX-R \( I_{Na} \) channels (Fig. 6). Consistent with the observations of Scholz et al. (1998), lidocaine blocked partially inactivated TTX-R \( I_{Na} \) with a greater potency than it blocked of resting channels: \( ED_{50} \) of lidocaine-induced block of TTX-R \( I_{Na} \) evoked from −35 mV was 65.0 ± 14.6 μM, significantly less than that observed for currents evoked from −50 mV in the absence of Li⁺ (Fig. 3; p < 0.05). However, substituting Li⁺ for Na⁺ had no detectable influence on lidocaine-induced block of TTX-R \( I_{Na} \) evoked from −35 mV: \( ED_{50} = 64.7 ± 14.7 \) μM (n = 6).

In addition to the tonic block of \( I_{Na} \), observed when lidocaine is applied to sensory neurons held at their resting membrane potential, lidocaine is able to induce a use-dependent, or phasic block of \( I_{Na} \). To determine whether Li⁺ influences lidocaine-induced phasic block of \( I_{Na} \), TTX-R \( I_{Na} \) was activated at frequencies ranging between 0.3 and 30 Hz in the presence of Na⁺ or Li⁺-based bath solutions containing various concentrations of lidocaine. There was no detectable influence of Li⁺ on the use-dependent block of TTX-R \( I_{Na} \) observed in the absence of lidocaine (Fig. 7). The presence of lidocaine significantly increased the use-dependent block of TTX-R \( I_{Na} \) both in the presence of Na⁺ and Li⁺ (Fig. 7). When concentrations of lidocaine were such that produced roughly an equivalent degree of tonic block of TTX-R \( I_{Na} \) (100 μM in Na⁺ and 30 μM in Li⁺), there was no statistically significant difference between Li⁺ and Na⁺ with respect to the phasic block induced (two-way repeated measures ANOVA; p > 0.05).

Finally, we wished to determine whether a complete substitution of Li⁺ for Na⁺ is necessary to induce an increase in the potency of lidocaine-induced block of \( I_{Na} \). Lidocaine dose-response curves were generated in the presence of a series of bath solutions in which Li⁺ was incrementally replaced, on a mole per mole basis with Na⁺. \( ED_{50} \) was determined as described under Materials and Methods. Incrementally increasing Li⁺ in the bath solution increased the potency of lidocaine-induced block of TTX-R \( I_{Na} \). The threshold for a Li⁺-induced increase in the potency of lidocaine-induced block of TTX-R \( I_{Na} \) was between 3.5 and 17.5 mM (Fig. 8).

**Discussion**

We have demonstrated that Li⁺ increases the potency of lidocaine-induced block of voltage-gated Na⁺ currents.
present in sensory neurons. A complete substitution of Li\textsuperscript{+} for Na\textsuperscript{+} in the bath solution resulted in a 5-fold decrease in the ED\textsubscript{50} of lidocaine-induced block of TTX-S I\textsubscript{Na} and more than a 2-fold decrease in the ED\textsubscript{50} of lidocaine-induced block of TTX-R I\textsubscript{Na}. That these changes could be achieved with a partial substitution of Li\textsuperscript{+} for Na\textsuperscript{+} was suggested by the observation that a significant decrease in the ED\textsubscript{50} of lidocaine used in the presence of Na\textsuperscript{+} was 100 \mu M, whereas that used in the presence of Li\textsuperscript{+} was 30 \mu M. At these concentrations, the used-dependent block of TTX-R I\textsubscript{Na} in the presence of Na\textsuperscript{+} and Li\textsuperscript{+} were similar. B, example of use-dependent block of TTX-R I\textsubscript{Na} evoked at 3 Hz before the application of lidocaine. C, same neuron shown in B stimulated at 3 Hz after the application of 100 \mu M lidocaine.

That Li\textsuperscript{+} had differential effects on Na\textsuperscript{+} channel block depending on the state of the channel suggests that Li\textsuperscript{+} did not influence lidocaine in a nonspecific manner. That is, if the effects of Li\textsuperscript{+} were on lidocaine, rather than on Na\textsuperscript{+} channels, we would have expected Li\textsuperscript{+} to increase the potency of lidocaine-induced block of resting, open, and inactivated channels. However, this was not what we observed. Additional evidence in support of our suggestion that Li\textsuperscript{+} influences Na\textsuperscript{+} channels rather than lidocaine was our observation that Li\textsuperscript{+} had no significant influence on the potency of lidocaine-induced block of voltage-gated Ca\textsuperscript{2+} channels.

Voltage-gated Na\textsuperscript{+} channels are thought to reside in three distinct states: closed or resting, open, and inactivated. That local anesthetic-induced block of voltage-gated Na\textsuperscript{+} channels
is voltage- and use-dependent lead to the hypothesis that the affinity of drug receptor in the channel protein depends on the state of the channel (Hille, 1977): a high-affinity receptor is accessible when the channel is in the open or inactivated states, whereas a low-affinity site is accessible when the channel is in the closed state (Hille, 1977). Consistent with this hypothesis, studies using scanning mutagenesis of cloned voltage-gated Na⁺ channels lead to the identification of two residues in the transmembrane segment IVS6 of the channel α-subunit that are critical for state dependence of local anesthetic-induced block of these channels (Ragsdale et al., 1994). These residues, a phenylalanine at position 1712 and a tyrosine at position 1719 in NaV1.8, the α-subunit underlying the TTX-R $I_{Na}$ in the present study (Akopian et al., 1999), are thought to face the inner pore of the Na⁺ channel (Li et al., 1999). It has yet to be determined whether these residues constitute a local anesthetic receptor or whether they influence the actions of local anesthetics at other residues. However, it is interesting to note that manipulation of these residues suggested that it is possible to differentially influence local anesthetic binding to closed versus open or inactivated channels (Li et al., 1999).

We suggest that Li⁺ increases the potency of lidocaine-induced block of voltage-gated Na⁺ channels in DRG neurons by increasing the affinity of the low-affinity binding site for local anesthetics. This suggestion is based on the observations that Li⁺ increased the potency of lidocaine-induced block of TTX-R $I_{Na}$ evoked from a resting or closed state, but failed to influence the potency of block of open or inactivated channels and failed to influence properties of channel gating that could have secondarily influenced the actions of the local anesthetic. Li⁺ had access to residues lining the channel pore because these channels are clearly permeable to this ion and because our method of assessing the magnitude of current block involved evoking currents before the application of local anesthetic. Thus, it is possible that Li⁺ increased the potency of lidocaine through an interaction with a residue such as the phenylalanine at position 1712 in NaV 1.8, possibly increasing the apparent hydrophobicity of this residue and thereby stabilizing binding of lidocaine to channels in the resting state (Li et al., 1999). Additional site-directed mutagenesis experiments would be required to further test this suggestion.

The clinical utility of the use of Li⁺ to increase the potency of local anesthetics may be tempered by the potential for local and/or systemic toxicity associated with Li⁺ and the possibility for adverse drug interactions. That said, there are several recent lines of evidence to suggest that Li⁺ may have neuroprotective properties (Chalecka-Franaszek and Chuang, 1999; Chen and Chuang, 1999; Mora et al., 1999). Indeed, we have tested the influence of Li⁺ on lidocaine-induced neurotoxicity and obtained evidence suggesting Li⁺ attenuates lidocaine-induced cell death (unpublished observation). In a preliminary study, we also assessed the impact of systemic administration of Li⁺. Specifically, Li⁺ was administered intravenously at a dose and volume 5 times that used in vivo to produce complete block of reflexive responses to noxious stimulation with the local administration of a local anesthetic-Li⁺ mixture. This systemic administration of Li⁺ resulted in no detectable adverse reactions in the animals tested and a plasma concentration of Li⁺ 2 orders of magnitude lower than the target concentration recommended for the treatment of bipolar disorder (Turner et al., 1994).

Our results suggest that it is possible to alter the functional profile of local anesthetics such as lidocaine. The clinical implications of this possibility are that compounds such as Li⁺ may be administered in combination with local anesthetics to maximize specific anesthetic properties for specific purposes. For example, in surgical settings where any activation of primary afferent nociceptors may have deleterious effects such as the induction of central sensitization, a compound such as Li⁺ that appears to stabilize resting block of Na⁺ channels may enable a more effective anesthesia with a lower risk for side effects. Alternatively, there may be compounds that facilitate block of Na⁺ channels in open or inactivated states. These compounds would be useful for the local anesthetic treatment of disorders such as neuropathic pain, where the block of ongoing or aberrant activity depends on the use-dependent properties of the local anesthetic.

Acknowledgments

We thank Lei Zhang for technical assistance with experiments.

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