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*Topical Review*

# Interactions of Local Anesthetics with Voltage-gated $Na^+$ Channels

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**Abstract.** Voltage-gated $Na^+$ channels are dynamic transmembrane proteins responsible for the rising phase of the action potential in excitable membranes. Local anesthetics (LAs) and structurally related antiarrhythmic and anticonvulsant compounds target specific sites in voltage-gated $Na^+$ channels to block $Na^+$ currents, thus reducing excitability in neuronal, cardiac, or central nervous tissue. A high-affinity LA block is produced by binding to open and inactivated states of $Na^+$ channels rather than to resting states and suggests a binding site that converts from a low- to a high-affinity conformation during gating. Recent findings using site-directed mutagenesis suggest that multiple S6 segments together form an LA binding site within the $Na^+$ channel. While the selectivity filter may form the more extracellular-located part of this binding site, the role of the fast inactivation gate in LA binding has not yet been resolved. The receptor of the neurotoxin batrachotoxin (BTX) is adjacent to or even overlaps with the LA binding site. The close proximity of the LA and BTX binding sites to residues critical for inactivation, together with gating transitions through S6 segments, might explain the strong impact of LAs and BTX on inactivation of voltage-gated $Na^+$ channels and might help elucidate the mechanisms underlying voltage- and frequency-dependent LA block.

## Introduction

In the clinical setting, local anesthetics (LAs) are employed in various techniques to produce local or regional anesthesia and analgesia. When applied intrathecally, into the epidural space, or close to nerve trunks or endings, LAs reversibly block the generation and propagation of action potentials in the spinal cord, in spinal nerve roots or peripheral nerves and thus inhibit the sensation of pain without loss of consciousness. Some LAs such as lidocaine can also be administered systemically to alleviate certain types of neuropathic pain. LAs have considerable side effects on the central nervous system and the cardiovascular system when accidentally injected intravascularly or administered in excessive doses.

Various drugs and toxins with different chemical structures exhibit LA properties. However, common structural features of clinically useful LAs include a hydrophobic aromatic tail, an intermediate ester- or amide-containing linker, and a hydrophilic tertiary amine. Other compounds that satisfy similar structural requirements are used as antiarrhythmics and anticonvulsants. The plant alkaloid cocaine (an ester-type agent) was the first LA, introduced clinically in 1884 (Koller, 1884). Soon after its structure was resolved, various synthetic ester-type LAs were developed, the most important of which are procaine, chloroprocaine, and tetracaine. Lidocaine, synthesized in 1944, was the first amide-type LA to be put into clinical use (Lofgren, 1948). Other amide-type LAs include prilocaine, etidocaine, and the piperidine derivatives mepivacaine, ropivacaine, and bupivacaine.

LAs differ in terms of their intrinsic LA potency, onset, duration of action, and differential inhibition of sensory and motor activity. The pharmacological profile of an individual agent is determined by physicochemical characteristics like lipid solubility, pKa, and degree of protein binding.

It is generally agreed that LAs exhibit their clinical effects (and some of their side effects) by interaction with specific sites in voltage-gated $Na^+$ channels. However, LAs also bind to other transmembrane and intracellular proteins at clinically relevant concentrations, such as potassium channels (Valenzuela et al., 1995), nicotinic acetylcholine receptor channels (Neher & Steinbach, 1978), and G

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proteins (Hollmann et al., 2002), and, as amphipathic molecules, are able to cause general membrane perturbation.

Here we review the complex molecular interactions of LAs and voltage-gated $Na^+$ channels from both a biophysical and a structural point of view. Each approach has helped elucidate not only the mechanisms of $Na^+$ channel block by LAs but also the structure and function of voltage-gated $Na^+$ channels themselves.

## Structure and Function of Voltage-gated $Na^+$ Channels

$Na^+$ channels change their configuration in response to membrane potential. They exist in ion-conducting (open) and non-conducting (resting and inactivated) states. The gating characteristics of voltage-gated $Na^+$ channels are highly isoform-specific. However, some generalizations can be made. Voltage-gated $Na^+$ channels exhibit voltage-dependent activation. The dwell time of open $Na^+$ channels is short, less than a millisecond for most isoforms. Unlike the activation process, inactivation from the open state is not voltage-dependent (Aldrich, Corey & Stevens, 1983). $Na^+$ channels may also inactivate without ever opening. This closed-state inactivation is voltage-dependent. The availability of resting channels at various prepulse potentials, at which $Na^+$ channels generally do not open, is termed steady-state inactivation (Hodgkin & Huxley, 1952). Inactivated channels are unavailable for activation for several milliseconds. Again, recovery from inactivation is a voltage-dependent process. After prolonged depolarizations, $Na^+$ channels enter the slow inactivated state, from which they need seconds to minutes of repolarization to recover (for review *see* Hille, 2001).

Voltage-dependent activation of $Na^+$ channels underlies the rising phase of the action potential in excitable membranes (Catterall, 2000). Fast inactivation helps terminate the action potential in non-cardiac cells and regulates the refractory period; slow inactivation is believed to regulate overall membrane excitability, adapt spike frequency, and preserve previous activity (McCollum et al., 2003).

$Na^+$ channels consist of a large α-subunit and auxiliary β subunits. Nine different α-subunit isoforms have been cloned and functionally expressed thus far, and four different β-subunits have been identified (Catterall, Goldin & Waxman, 2003; Yu et al., 2003). α-Subunits are associated with one or more of the four β-subunits. The primary functional properties of $Na^+$ channels reside in the α-subunit. Associated β-subunits modify kinetics and voltage-dependence of channel gating. The α-subunit comprises four homologous domains (D1–D4), each containing six α-helical transmembrane segments (S1–S6; Fig. 1*A*). The linkers between segments S5-S6 form P-loops as a hairpin from the extracellular side and line the narrow outer part of the channel's pore to form the selectivity filter. The S5 and S6 segments of each domain line the inner part of the channel's pore (Catterall, 2000). The highly positively charged S4 segments in each domain serve as voltage sensors (Stuhmer et al., 1989; Kontis, Rounaghi & Goldin, 1997). Their outward movements upon depolarization induce conformational changes in the pore resulting in channel activation (Yang & Horn, 1995; Yang, George & Horn, 1996). During the activation process, S6 segments are likely to exhibit rotational/lateral movements, as suggested by the structures of $K^+$ channels (Perozo, Cortes & Cuello, 1999; Jiang et al., 2002). Segments D3-S4 and D4-S4 are important for coupling channel activation to fast inactivation (Cha et al., 1999; Sheets et al., 1999). The structural determinants of $Na^+$ channel fast inactivation are hydrophobic residues (IFM) in the intracellular D3-D4 loop that may form an inactivation gate (Vassilev, Scheuer & Catterall, 1988; West et al., 1992; Eaholtz, Scheuer & Catterall, 1994). Several residues at the intracellular end of D4-S6 (McPhee et al., 1994, 1995) and within intracellular S4-S5 loops of D3 (McPhee et al., 1994, 1995; Smith & Goldin, 1997) and D4 (McPhee et al., 1998) may function as receptors for the inactivation gate.

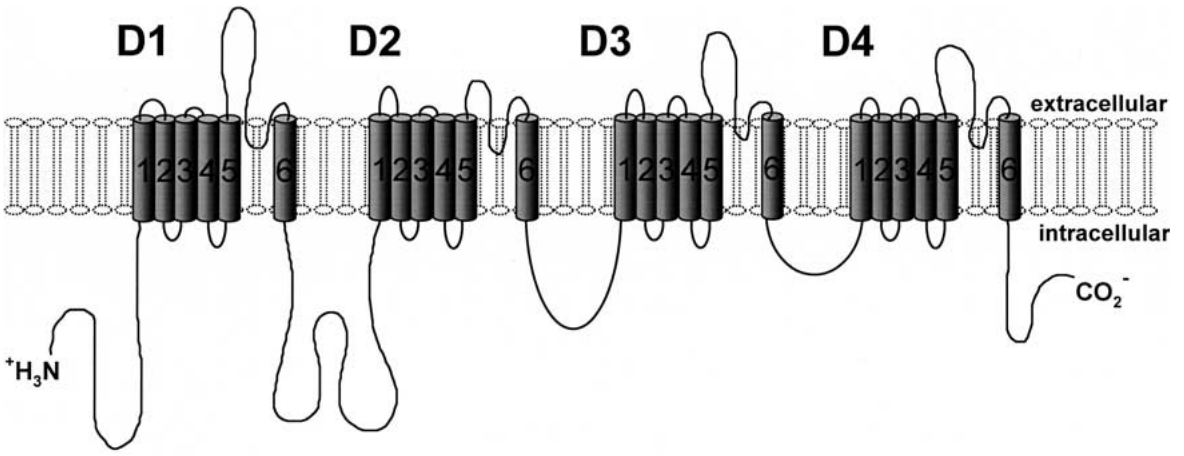
The molecular entity for slow inactivation is distinct from that responsible for fast inactivation and is less well-understood. Slow inactivation apparently involves rearrangements within the P-region (Benitah et al., 1999; Vilin, Fujimoto & Ruben, 2001) and inner parts of the S6 segments (O'Reilly, Wang & Wang, 2000, 2001; Vedantham & Cannon, 2000), along with the contributions of S4 voltage sensors (Mitrovic, George & Horn, 2000) and the D3–D4 linker (McCollum et al., 2003).

**Fig. 1.** (*A*) Putative transmembrane topology of the voltage-gated $Na^+$ channel α subunit. The cylinders represent the putative 6 transmembrane α-helical segments of domains D1, D2, D3, and D4. The interdomain linkers are drawn by eye and do not represent the exact number of amino-acid residues present. (*B*) Amino-acid sequences within S6 segments D1-S6, D2-S6, D3-S6, and D4-S6 of the rat skeletal muscle $Na^+$ channel $Na_v1.4$. Residues in gray are critical for LA binding; residues in a box are critical for BTX binding. The putative gating hinge at position 12 containing a glycine/serine residue (Jiang et al., 2002; Yarov-Yarovoy et al., 2002) is hatched.

## State-dependent Interaction of LAs and Voltage-gated $Na^+$ Channels

Biophysical studies have shown that LAs have access to a binding site within the $Na^+$ channels via a hy-

A




B

| Pos. | D1-S6 | D2-S6 | D3-S6 | D4-S6 |
|---|---|---|---|---|
| 1 | Y417 | C772 | M1264 | G1567 |
| 2 | M418 | L773 | Y1265 | I1568 |
| 3 | I419 | T774 | L1266 | C1569 |
| 4 | F420 | V775 | Y1267 | F1570 |
| 5 | F421 | F776 | F1268 | F1571 |
| 6 | V422 | L777 | V1269 | C1572 |
| 7 | V423 | M778 | I1270 | S1573 |
| 8 | I424 | V779 | F1271 | Y1574 |
| 9 | I425 | M780 | I1272 | I1575 |
| 10 | F426 | V781 | I1273 | I1576 |
| 11 | L427 | I782 | F1274 | I1577 |
| 12 | G428 | G783 | G1275 | S1578 |
| 13 | S429 | N784 | S1276 | F1579 |
| 14 | F430 | L785 | F1277 | L1580 |
| 15 | Y431 | V786 | F1278 | I1581 |
| 16 | L432 | V787 | T1279 | V1582 |
| 17 | I433 | L788 | L1280 | V1583 |
| 18 | N434 | N789 | N1281 | N1584 |
| 19 | L435 | L790 | L1282 | M1585 |
| 20 | I436 | F791 | F1283 | Y1586 |
| 21 | L437 | L792 | I1284 | I1587 |
| 22 | A438 | A793 | G1285 | A1588 |
| 23 | V439 | L794 | V1286 | I1589 |
| 24 | V440 | L795 | I1287 | I1590 |
| 25 | A441 | L796 | I1288 | L1591 |
| 26 | M442 | S797 | D1289 | E1592 |
| 27 | A443 | S798 | N1290 | N1593 |
| 28 | Y444 | F799 | F1291 | F1594 |

drophilic pathway from the intracellular compartment or via a hydrophobic membrane-delimited compartment (Hille, 1977). The binding site may be near the channel's pore, as permeant ions interfere with LA binding. The most obvious effect of LAs is a reversible and concentration-dependent reduction of peak $Na^+$ current. This "tonic block" mirrors a reduction of $Na^+$ channel open-probability at an equilibrium of conformational states. Additionally, LAs (like other structurally related antiarrhythmic and anticonvulsant compounds) produce a voltage- and frequency-dependent block. The voltage-dependent block elicited by some LAs shows a shift of the $Na^+$ channel steady-state inactivation to more negative potentials. Moreover, LAs bind more avidly to open and inactivated channels than to resting channels, suggesting the involvement of a binding site that converts from a low- to a high-affinity conformation during state transitions of the channel. This is known as the modulated-receptor hypothesis and was suggested by Hille (1977) and Hondeghem and Katzung (1977). A competing model, the guarded-receptor hypothesis, suggests that voltage- and frequency-dependent effects are caused by a binding site with access modulated by channel gating (Starmer, Grant & Strauss, 1984). Block of $Na^+$ current by LAs increases during brief and repetitive pulses at high frequency. According to the modulated-receptor hypothesis, this "use-dependent" or "frequency-dependent" block arises from binding of LAs to inactivated channels populated during repetitive pulses and from dissociation of LAs from inactivated states with a time constant slower than the frequency of the pulses.

The central role of $Na^+$-channel inactivation in voltage- and frequency-dependent LA block suggests involvement of the fast inactivation gate in LA binding. Indeed, prevention of fast inactivation by mutating three critical amino acids within the inactivation gate (IFM $\rightarrow$ QQQ) eliminated the high-affinity block of lidocaine (Bennett et al., 1995). The high-affinity block of lidocaine therefore seems to involve either direct binding with one of several structures responsible for fast inactivation or conformational changes initiated by an intact fast inactivation gate.

However, the central role of the fast inactivation gate for voltage- and frequency-dependent LA block is not unequivocal and has been challenged by several observations: First, with a point mutation within the D3-D4 interdomain that exhibited impaired fast inactivation, the antiarrhythmic drug disopyramide exhibited persistent open-channel block (Grant et al., 1996), and flecainide and the lidocaine derivative RAD-243 retained their use-dependent blocking action (Grant et al., 2000), suggesting that the role of the inactivation gate is dependent on the kinetics of drug binding. Second, prevention of fast inactivation by the noncleaving oxidizing reagent chloramine-T did not impair "tonic" or "use-dependent" LA block by either the quarternary lidocaine derivative QX-314 or by etidocaine (Wang et al., 1987). Third, the two antiarrhythmic agents mexiletine and flecainide show pronounced open and use-dependent block in inactivation-deficient $Na^+$ channels with two or three residues in D1-S6 mutated to tryptophan or cysteine (Wang, Russell & Wang, 2003, 2004). Fourth, a study investigating the position of the fast-inactivation gate during lidocaine block demonstrated that lidocaine indeed favored closure of the fast-inactivation gate in a voltage-dependent manner. However, the slowing of $Na^+$-channel recovery caused by lidocaine was not the result of slowing of recovery of the fast-inactivation gate, which was, in fact, rapid (Vedantham & Cannon, 1999).

These findings suggest that the activation pathway may be equally or more important in voltage- and frequency-dependent block than the inactivation pathway.

Yet another hypothesis suggests that use-dependent block results from slow recovery of drug-bound channels between depolarizing stimuli due to an interaction between LAs and slow-inactivated states (Ong, Tomaselli & Balser, 2000).

Whatever model of LA action is favored, it is clear that LA binding and $Na^+$-channel gating influence each other so profoundly that they complicate the interpretation of their interactions.

## Structural Elements of the $Na^+$-Channel Protein Involved in LA Binding

Site-directed mutagenesis studies have provided a more detailed picture of the structural components within the $Na^+$ channel contributing to LA binding. Alanine-scanning mutagenesis of the rat brain $Na_v1.2$ channel and electrophysiological experiments in the *Xenopus* oocyte expression system first revealed specific amino-acid residues in segments D4-S6 that are involved in binding of the LA etidocaine, as several alanine-substitutions drastically reduced voltage- and frequency-dependent block. It has been proposed that the two hydrophobic aromatic residues phenylalanine (F1764, position 13 in Fig. 1*B*) and tyrosine (Y1771, position 20) in D4-S6 (Ragsdale et al., 1994) face the channel pore and interact with the tertiary amine group and the aromatic group of LAs, respectively. The possible modes of interaction (hydrophobic, cation-$\pi$ electron, or aromatic-aromatic) were later demonstrated to depend on the channel's state (Li et al., 1999), implying the existence of a dynamic site that, indeed, changes conformation during state transitions of the channel. In addition to aromatic residues in D4-S6, the amino-acid residues L1465, N1466, and I1469 in D3-S6 (positions 17, 18, and 21, respectively) (Yarov-Yarovoy et al., 2001)

and to some extent residue I409 (position 9) in D1-S6 were also proposed to contribute to the LA binding site (Yarov-Yarovoy et al., 2002), whereas none of the residues in D2-S6 were found to do so (Wang, Barile & Wang, 2001; Yarov-Yarovoy et al., 2002).

Lysine point mutations in the rat skeletal muscle $Na_v1.4$ channel expressed in HEK293t cells confirmed the contribution of phenylalanine F1579 in D4-S6 (Fig. 1*B*; position 13, homologous to F1764 in $Na_v1.2$) to the binding of various LAs (Wright, Wang & Wang, 1998) and revealed that S1276 and L1280 in D3-S6 (positions 13 and 17, homologous to S1461 and L1465 in $Na_v1.2$, respectively) are involved in binding of the enantiomers of the LA bupivacaine (Wang, Nau & Wang, 2000). In addition, N434 and L437 in D1-S6 (positions 18 and 21, homologous to N418 and L421 in $Na_v1.2$, respectively) have also been proposed as critical for LA binding (Wang, Quan & Wang, 1998). The mutation N434R in D1-S6 exhibited significant stereoselectivity for bupivacaine block of inactivated channels, resulting from a selective decrease in block by S(-)-bupivacaine (Nau et al., 1999); and mutation L1280R in D3-S6 exhibited significant stereoselectivity for bupivacaine block of inactivated channels, resulting from a selective decrease in block by R(+)-bupivacaine (Nau, Wang & Wang, 2003). Together, these findings seem to support the idea that these two residues in D1-S6 and D3-S6 face each other while interacting with LAs in the inactivated state.

A cysteine-scanning mutagenesis study of the rat skeletal muscle $Na_v1.4$ channel found several more amino acids that might determine binding of lidocaine (I424, I425, and G428 in D1-S6, and I782 and V786 in D2-S6; positions 8, 9, 12 in D1-S6 and positions 11 and 15 in D2-S6, respectively), based on the observation that cysteine mutations of these residues altered sensitivity independently of mutation-induced changes in gating (Kondratiev & Tomaselli, 2003). Judged by the magnitude of changes in voltage- and frequency-dependent block caused by various mutations, amino-acid residues in segments D3-S6 and D4-S6 are the most prominent determinants of LA binding. Apparent dissimilarities between various studies regarding the contribution of amino acids to LA binding might be due to the use of different substituting amino acids or different LAs as model drugs. It seems plausible that different LAs have slightly different contact points when binding within the $Na^+$ channel. However, major inconsistencies exist in the literature regarding the orientation of segment D1-S6 during interaction with LAs (Nau et al., 1999, 2003; Yarov-Yarovoy et al., 2002; Kondratiev & Tomaselli, 2003).

It is noteworthy that most mutations at sites identified as contributing to LA block also significantly affect $Na^+$ channel gating in the absence of LAs (McPhee et al., 1994, 1995; Nau et al., 1999; Wang & Wang, 1997). This fact has led to concerns in assigning these sites any role in LA binding. Indeed, allosteric changes or unspecific effects at the binding site caused by distant mutations are difficult to control. On the other hand, a contribution by S6 residues to both LA binding and inactivation gating might be more than coincidental and in fact might explain the state-dependent binding of LAs. If S6 segments do exhibit rotational/lateral movements during the activation process, as suggested by the structures of $K^+$ channels (Perozo et al., 1999; Jiang et al., 2002), the orientation of the side chains is likely to change during gating to allow LAs to interact differently with relevant residues depending on channel state. The channel inactivation process might lead to S6 side chain orientations that facilitate LA binding. In this respect, it is not surprising that S6 segments in $Ca^{2+}$ and $K^+$ channels appear to be critical for state-dependent drug binding as well (Hockerman et al., 1997; Chen, Seebohm & Sanguinetti, 2002).

Overall, there is strong evidence that multiple S6 segments together form a single LA binding site within the $Na^+$ channel and that amino acid residues in segments D3-S6 and D4-S6 are the most prominent determinants of LA binding. This implies that the S6 segments must align in close proximity, so that all contributing amino acids have contact with the rather small LA molecule. The contact points are not likely to be static and might depend on both the state of the channel and the type of LA tested.

There is also evidence that the LA binding site may not be limited to S6 segments. Residue $Na_v1.4$-K1237 in the putative domain D3 selectivity filter (DEKA locus) was shown to be involved in interactions with the hydrophilic part of lidocaine. Corresponding residues in D1, D2, and D4 limited extracellular access and escape from the LA binding site (Sunami, Dudley & Fozzard, 1997). The selectivity filter may well be structurally adjacent to the LA binding site and may represent the more extracellular-located part of the binding site.

If LA binding happens within the pore of the $Na^+$ channel, what is the specific role of the inactivation gate in LA binding? As mentioned earlier, some LAs apparently are able to bind and exhibit high-affinity block even if fast inactivation is impaired. For other LAs, the inactivation gate might trap them within the pore (Bennett et al, 1995; Grant et al., 1996; Grant et al., 2000). Yet another concept is derived from long-QT mutations of the cardiac $Na^+$ channel isoform (An et al., 1996; Wang et al., 1997) and partly disrupted inactivation mutations (Balser et al., 1996) that restore the fast inactivation phenotype in the presence of LAs. Some suggest that LAs (to some extent) might mediate or even repair inactivation gating of the $Na^+$ channel or alternatively augment closed-state inactivation through an

allosteric effector mechanism (Kambouris et al., 2000).

## Clues about LA binding from BTX

Voltage-gated $Na^+$ channels are natural targets of various bioactive ligands and neurotoxins. A total of nine distinct receptors have been classified within the voltage-gated $Na^+$ channel (Catterall et al., 2003; Wang & Wang, 2003). Only the receptor for batrachotoxin (BTX) and veratridine (VTD) is adjacent to or even overlaps with the LA receptor.

BTX is abundant in the skin of the South American frog *Phyllobates terribilis* (Daly et al., 1980), whereas VTD is found in plants of the *Liliaceae* family. Like LAs, BTX interacts with the $Na^+$ channel in a state-dependent manner. When BTX preferentially binds to the open $Na^+$ channel, it eliminates both fast and slow inactivation of the channel. BTX also shifts the voltage dependence of activation to the hyperpolarizing direction and may alter the ion selectivity (Hille, 2001). The BTX binding domain has been mapped to positions 17, 18, and 21 within D1-S6 (Wang & Wang, 1998). Further BTX receptor mapping has identified additional residues at D2-S6, D3-S6, and D4-S6 critical for BTX binding (Fig. 1) (for review, *see* Wang & Wang, 2003). Interestingly, several of these residues have been mapped within the LA receptor. Could the BTX binding site also be within the permeation pathway analogous to that proposed for the LA binding site? If so, the LA receptor and BTX receptor are in close proximity within the $Na^+$ permeation pathway and perhaps not located within a hydrophobic pocket as has commonly been assumed (Hille, 2001). Indeed, both BTX and VTD appear to reduce the single-channel conductance significantly, as if they physically impede the ion flow. It is also well-known that LAs strongly antagonize BTX binding and vice versa in native $Na^+$ channels (Catterall, 1980; Catterall & Beneski, 1980). Furthermore, BTX alters the stereoselectivity of +/− cocaine binding by more than 20-fold (Wang & Wang, 1992). Steric hindrance of bound BTX on the adjacent/overlapping LA site on D4-S6 (Linford et al., 1998; Wang & Wang, 1999) could account for such a change in stereoselectivity.

To explain the complex physiological actions of LAs, BTX, and VTD, we hypothesized that S6 receptors and gating transitions are coupled (Wang & Wang, 2003). Several recent studies strongly support this hypothesis. First, the S6 segments apparently contain the docking site for the inactivation gate in homotetrameric $K^+$ channels (Zhou et al., 2001). This finding is consistent with an earlier report that positions 23–25 at D4-S6 are important for normal fast inactivation in $Na^+$ channels (McPhee et al., 1994). If true, receptor mapping for BTX and LAs at multiple S6 segments provides valuable clues as to how BTX and LAs could affect the fast inactivation so strongly. For example, bound BTX could physically extend to this docking site and therefore completely prevent the docking of the inactivation gate. In contrast, a smaller LA molecule could stabilize the binding of the nearby inactivation gate at its docking site. The BTX/LA binding residues include position 21 at D1-S6 and position 20 at D4-S6, both within one helical turn (or 5.4 Å) of position 23–25. Accordingly, the S6 docking site-inactivation gate complex may be critical for binding of LAs.

Second, the $Na^+$ channel S6 segments from D1 to D4 contain a putative activation gating hinge, glycine/serine, at position 12. Recently, Jiang et al. (2002) proposed that, in a "bent" conformation at this flexible hinge, the inner S6 helices may splay open and create a wide entryway for both ions and ligands during $K^+$-channel opening. Indeed, tetra-alkylammonium cations as large as 15 Å could enter and exit this $Na^+$-channel entryway (Huang, Favre & Moczydlowski, 2000). We notice that D2-S6, D3-S6, and D4-S6 contain a BTX-binding residue at position 13, one residue away from the putative gating hinge at position 12. It is feasible that BTX binds to its receptor during the open state while the gating hinge is in the bent configuration. Binding of BTX may stabilize the bent glycine residues and therefore keep the channel open. How LAs interact with this gating-sensitive region will be an interesting subject for further investigation.

## Conclusion

Site-directed mutagenesis of $Na^+$ channel S6 segments has provided invaluable clues on how LAs block $Na^+$ currents. Mounting evidence now suggests that multiple S6 segments jointly form a single LA receptor. Interactions between LAs and their S6 receptor are highly state-dependent. Both the open and the inactivated states of $Na^+$ channels appear to have higher affinities to LAs than the resting states. The open state may have the highest on-rate kinetic for LAs due to the additional hydrophilic access pathway (Starmer et al., 1984). Since small aberrant late $Na^+$ currents can cause cardiac arrhythmia, epilepsy, and neuropathic pain, a rapid high-affinity block of open $Na^+$ channels by antiarrhythmics, anticonvulsants and LAs will likely be beneficial for these diverse syndromes (e.g., Wang et al., 1997). The dynamic nature of $Na^+$ channel S6 segments during gating transitions is consistent with the modulated receptor hypothesis, which requires that the LA receptor changes its configuration upon depolarization. The final proof of the S6 involvement in LA binding must wait for the crystal structure of the LA-S6 complex within $Na^+$ channels.

Such a task was achieved in the homotetrameric $K^+$ channel with an analogous ligand, tetrabutylammonium ions (Zhou et al., 2001)

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