Molecular Model of Anticonvulsant Drug Binding to the Voltage-Gated Sodium Channel Inner Pore

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ABSTRACT

The tricyclic anticonvulsant drugs phenytoin, carbamazepine, and lamotrigine block neuronal voltage-gated Na^+ channels, and their binding sites to domain IV-S6 in the channel's inner pore overlap with those of local anesthetic drugs. These anticonvulsants are neutral, in contrast to the mostly positively charged local anesthetics, but their open/inactivated-state blocking affinities are similar. Using a model of the open pore of the Na^+ channel that we developed by homology with the crystal structures of potassium channels, we have docked these three anticonvulsants with residues identified by mutagenesis as important for their binding energy. The three drugs show a common pharmacophore, including an aromatic ring that has an aromatic-aromatic interaction with Tyr-1771 of $Na_v1.2$ and a polar amide or imide that interacts with the aromatic ring of Phe-1764 by a low-energy amino-aromatic hydrogen bond. The second aromatic ring is nearly at a right angle to the pharmacophore and fills the pore lumen, probably interacting with the other S6 segments and physically occluding the inner pore to block Na^+ permeation. Hydrophobic interactions with this second aromatic ring may contribute an important component to binding for anticonvulsants, which compensates energetically for the absence of positive charge in their structures. Voltage dependence of block, their important therapeutic property, results from their interaction with Phe-1764, which connects them to the voltage sensors. Their use dependence is modest and this results from being neutral, with a fast drug off-rate after repolarization, allowing a normal action potential rate in the presence of the drugs.

Introduction

Voltage-gated sodium channels are the molecular targets for several important commonly used classes of drugs: local anesthetic and antiarrhythmic drugs, anticonvulsant drugs (antiepileptics), and antidepressants, even though the therapeutic goals for these drugs are quite different. All three types of drugs block sodium currents with noticeable voltage dependence, showing low affinity at resting states and strong block for open-inactivated states. The mechanisms of action of local anesthetic drugs have been studied extensively, and previously we have modeled their interactions (etidocaine, lidocaine, and bupivacaine) with the inner pore of Na^+ channels (Lipkind and Fozzard, 2005). Local anesthetics have a characteristic bipolar structure with an aromatic ring at one end and a tertiary amine at the other. Anticonvulsant drugs typically have a tricyclic structure, with a polar amide in the middle (Fig. 1). These molecules are wider and more rigid than local anesthetics. Although structurally different, anticonvulsant drugs interact with some of the same amino acid residues in the Na^+ channel's inner pore and have similar overall open/inactivated state affinity (Ragsdale et al., 1996; Ragsdale and Avoli, 1998; Clare et al., 2000; Liu et al., 2003; Tarnawa et al., 2007). Most local anesthetic drugs with high affinity for the open/inactivated state are tertiary amines that are easily protonated at physiological pH, and the positive charge is thought to contribute importantly to their binding (Hille, 2001; McNulty et al., 2007; Ahern et al., 2008). In contrast, the anticonvulsant drugs with similar affinity are neutral (Fig. 1). It is unclear how these larger, neutral molecules can bind to the same site in the pore with similar affinity, yet have different therapeutic effects.

Anticonvulsants are characterized by their ability to block seizures in patients with epilepsy, mediated by their interaction and inhibition of brain Na^+ channels (Rogawski and Löscher, 2004: McNamara, 2006). Anticonvulsant drugs have little effect on normal brain activity, and they block Na^+ channels poorly at slower firing rates from normal resting potentials. Seizures, however, are characterized by depolarized resting potentials and high frequency trains of action potentials. The drugs are thought to suppress seizures by inhibiting this high frequency repetitive firing, allowing the

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ABBREVIATIONS: $Na_v1.2$, one of the three brain isoforms of the Na^+ channel; KcsA and MthK, two isoforms of the potassium channel; S6, the sixth transmembrane helix of a domain of the Na channel.

drugs to have selective action on hyperactive rather than normal neurons (MacDonald and Kelly, 1993; McNamara, 2006). The functional factors that could underlie this voltage and use dependence are a higher affinity for depolarized cells and an off-rate slower than the repetition frequency of the action potentials. For local anesthetics and antiarrhythmic drugs interacting with the heart Na^+ channel, this results from a higher affinity to the open/inactivated conformation of the channel. Benzocaine, a neutral local anesthetic, also has a voltage-dependent affinity, but it does not show use dependence, partly because the increase in affinity to the open/inactivated state is modest and the drug off-rate is rapid (Wang et al., 1998). Neutral anticonvulsant drugs also seem to have rapid off-rates because they show little frequency-dependent block at moderate concentrations (Xie et al., 2001; Liu et al., 2003) and allow normal neuronal firing rates in the presence of the drugs. Anticonvulsants have little effect on skeletal muscle or heart, even though excitation in those tissues depends on Na^+ channels with similar structures.

Three typical anticonvulsant drugs, phenytoin, carbamazepine, and lamotrigine, share a common binding site in neuronal Na^+ channels (Kuo, 1998). Despite the differences in their chemical structure, it is likely that their mechanisms of block and pharmacological action are similar (Xie and Garthwaite, 1996). Although much evidence favors overlap between the local anesthetic drug binding site and that for these three anticonvulsants (Ragsdale et al., 1996; Liu et al., 2003), anticonvulsant drug action is from outside the cell, rather than from the intracellular compartment, and this has raised doubts about a common binding site (Kuo, 1998). Here we explore the interactions of these three drugs with our homology model of the Na^+ channel's inner pore to test the idea of a common binding site and to characterize their binding interactions. Although the model constructed by homology with potassium channel structures was reasonably successful in accounting for inner pore residue interactions with local anesthetics, anticonvulsant drug interactions further test the model. Insight into the structural basis of their block of neuronal Na^+ channels may help to explain their target specificity and assist in a rational search for new anticonvulsant drugs.

Materials and Methods

Methods similar to those employed for modeling of local anesthetic drug binding to the Na^+ channel were used (Lipkind and Fozzard, 2005). The model of the open (activated) Na^+ channel pore was based on homology with the Ca^{2+}-gated MthK channel (Jiang et al., 2002). For the closed channel, we used the KcsA structure (Doyle et al., 1998) as a template. Modeling was accomplished in the Insight and Discover graphical environment (MSI, Inc., San Diego, CA). Molecular mechanics energetic calculations used the consistent valence force field approximation. For minimization procedures, the steepest descents and conjugate gradients were used.

Results

Phenytoin. Phenytoin (diphenylhydantoin) contains a hydantoin ring and two phenyl substitutions at C5 of the hydantoin (Fig. 1). However, the second aromatic ring is not obligatory; 5-alkyl-5-phenyl-hydantoins also have a comparable affinity for Na^+ channels (Brown et al., 1997, 1999), with an optimal length of the aliphatic chain corresponding to pentyl, hexyl, and heptyl. Most likely, this aliphatic chain and the corresponding aromatic ring participate in less specific hydrophobic interactions, whereas the second aromatic ring has specific aromatic-aromatic interactions. Methyl (or alkyl) substitutions of the amide nitrogens of the hydantoin ring reduced binding significantly (Brown et al., 1997), which underlines the importance of these hydrogens for binding. It has been proposed that one aromatic ring and one amide group of the hydantoin form the pharmacophore core of phenytoin (Unverferth et al., 1998; Brown et al., 1999).

There are several estimates of binding affinity for phenytoin with the open/inactivated states of the neuronal Na^+ channels ranging from ~9 to 19 μM (Kuo and Bean, 1994; Ragsdale et al., 1996; Kuo, 1998), whereas binding to the resting state was at least 100-fold weaker. Alanine scanning mutagenesis of the transmembrane helix of domain IV (IVS6) has identified two highly conserved amino acid residues crucial for the binding of local anesthetic drugs: Phe-1764 and Tyr-1771 ($rNa_V1.2$ numbering) (Ragsdale et al., 1994). In subsequent studies, Ragsdale et al. (1996) showed that the same two residues are crucial for the action of phenytoin. It is noteworthy that the affinity of binding of both drugs with the inactivated state of the brain Na^+ channel, estimated
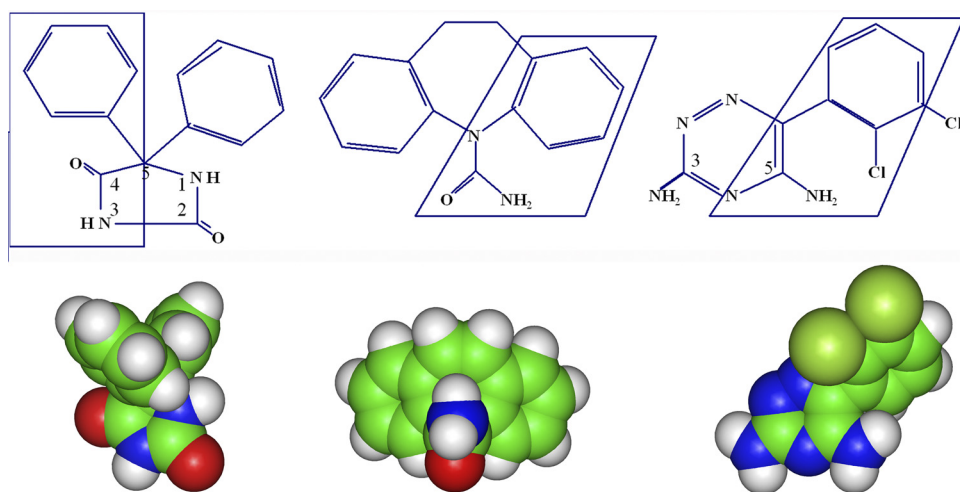
Fig. 1. Structural formulae of anticonvulsants and optimized three-dimensional molecular structures of anticonvulsants. The essential pharmacophore elements are indicated by rectangles.

from concentration-block relationships at depolarized holding potentials (−35 or −40 mV) were similar (11 ± 2.7 μM, lidocaine; 19 ± 4.2 μM for phenytoin) (Ragsdale et al., 1996). Both the F1764A and Y1771A mutations reduced affinities of binding of both lidocaine and phenytoin. However, the IC$_{50}$ for lidocaine increased 25- and 13-fold for the two mutations, respectively, whereas the IC$_{50}$ increases for phenytoin were only 8- and 3-fold, respectively. Although both domain IV S6 side chains were important determinants for binding of phenytoin, the interactions were weaker than for lidocaine, implying that other sites of interaction are more important for phenytoin than for lidocaine. Phenytoin did not show noticeable use-dependent block (Ragsdale et al., 1996; Xie et al., 2001; Lenkowski et al., 2007). Two obvious structural factors that could be responsible for these differences are the geometrical fit of the structures and the presence of a positive charge on lidocaine.

Using the evidence provided by Ragsdale et al. (1996), we examine the docking of phenytoin in the same site as that for local anesthetics in the Na$^+$ channel open state (Lipkind and Fozzard, 2005). The spacial structure of phenytoin was energy-minimized with the Discovery module of Insight II (Fig. 1) (Accelrys, San Diego, CA) for this docking interaction. In the open conformation of the inner pore model, the C-ends of the S6 α-helices of domains I to IV form a wide opening, and the side chains of Phe-1764 and Tyr-1771 face the pore. We recognize that in the complex structure of the pore, the aromatic rings may be oriented differently in different states, but this represents the most logical starting point. Phenytoin was arranged between the two aromatic side chains of Phe-1764 and Tyr-1771, with one of its aromatic rings in the proximity of Tyr-1771 in the position to form a nonpolar aromatic-aromatic interaction, similar to that found for local anesthetics (Lipkind and Fozzard, 2005). This placed the amino groups of the hydantoin ring in position for a polar interaction with the aromatic ring of Phe-1764. The hydrogen atoms of these amino groups within peptide bonds bear partial positive charges (+0.2–0.3; Creighton, 1993). Neutral polar amino groups in proteins (for example, inside amide groups of Asn and Gln side chains) have a high tendency to interact with the partial negative charges of a π-electron system of an aromatic ring, and the hydrogen atoms of these amino groups are located closer to the centers of aromatic rings. Burley and Petsko (1986) call this an amino-aromatic interaction, and Levitt and Perutz (1988) further propose that these interactions are an aromatic-hydrogen bond (-N-H. . . aromatic ring). Therefore, we propose that the hydantoin ring of phenytoin behaves similarly to the tertiary amine of local anesthetics in their interactions with the side chain of domain IV Phe-1764. The complex conformations of both side chains were optimized in the next step.

We can consider two possibilities for binding of phenytoin—either the right or the left aromatic ring in the immediate proximity of the side chain of Tyr-1771. The two possibilities in principle correspond to the binding of two stereoisomers of 5-alkyl-5-phenyl-hydantoin, where the *R*-configuration has an upper location of the 5-phenyl ring and a lower location of the 5-alkyl chain relative to the plane N1-C5-C4 of the hydantoin ring, and the converse is true for the *S*-configuration (Fig. 2). Optimization of location of phenytoin inside the inner pore with the flexible

side chains of Phe-1764 and Tyr-1771 showed that the two modes of binding are energetically indistinguishable; optimal interaction of the hydantoin ring with the side chain of Phe-1764 is achieved in both cases (Figs. 3 and 4). The 5-phenyl ring participates in the perpendicular aromatic-aromatic interaction with Tyr-1771, whereas the N3-H3 amino group at N3 is directed to the center of the aromatic ring of Phe-1764 by formation of an optimal aromatic hydrogen bond. However, this interaction is energetically weak; its experimental value, estimated from the F1764A mutation, is only approximately −1 kcal/mol (Ragsdale et al., 1996). In the modeled interaction, the calculated estimate of weak electrostatic interactions of the hydantoin ring with the aromatic ring of this side chain (bearing the partial charges of −0.1 on its carbons; Levitt and Perutz, 1988) is also −1 kcal/mol.

In contrast to local anesthetic molecules, in which pharmacologically important groups are located on opposite ends of an approximately planar structure, phenytoin contains the polar hydantoin ring in the middle of the molecule, with the two aromatic rings occupying approximately perpendicular locations. Consequently, with binding of one 5-phenyl ring and one amino group of the hydantoin by the DIV-S6 helix, the second 5-phenyl ring (or its 5-alkyl substituent) is directed into the inner pore, closer to DII-S6 and DIII-S6 helices and physically occluding the pore lumen (Figs. 3 and 4). In this configuration, the second 5-phenyl ring is in contact with the side chain of Leu-1465 of DIII-S6, making this a candidate for hydrophobic interaction. This DIII-S6 Leu-1465 also contributes to local anesthetic interaction, and it also interacts with lamotrigine (Yarov-Yarovoy et al., 2001). Because phenytoin has an affinity almost as great as that of charged local anesthetics, but mutation of its close packing with DIV-S6 reduces its affinity less than local anesthetics, there must be additional interactions for neutral phenytoin. Although local anesthetics are located in the interface between DIII-S6 and DIV-S6 and do not contact DII-S6 (Yarov-Yarovoy et al., 2002; Lipkind and Fozzard, 2005; Hanck et al., 2009), the almost perpendicular location of the second 5-phenyl ring of phenytoin approaches DII-S6. Because interactions of phenytoin with DI-S6 and DII-S6 have not been studied, such interactions would be worth investigating. In addition, the perpendicular 5-phenyl ring almost occludes the pore lumen, in contrast to our modeling results with local anesthetics, which are docked eccentrically, leaving the inner pore open but creating a high
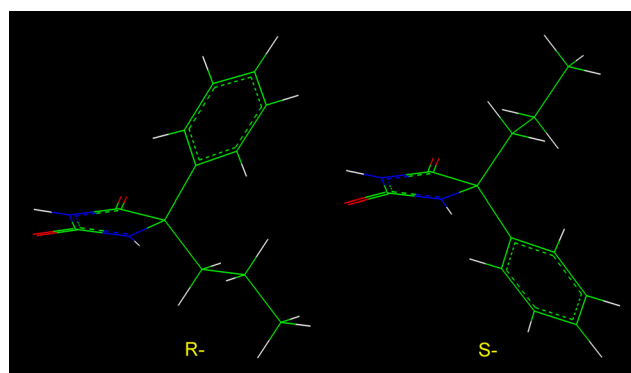

Fig. 2. *R*- and *S*-configurations of 5-alkyl-5-phenyl-hydantoin.

electrostatic barrier for Na^+ permeation (McNulty et al., 2007). Consequently, their mechanism of current reduction may be different.

Substitutions of the second aromatic ring of phenytoin by aliphatic hydrophobic chains produced derivatives with high affinity of binding with the Na^+ channel (Brown et al., 1999). Figure 5 presents the complex of 5-hexyl-5-phenyl-hydantoin with the open pore, showing the interactions of the *n*-hexyl

Fig. 3. Phenytoin inside the inner pore of the Na^+ channel ($Na_v1.2$) (top view). Optimal location of phenytoin (*R*-) shown by space-filled images. The backbones of S6 α-helices of domains I-IV are shown by yellow, red, blue, and green ribbons, correspondingly. The same colors are used for amino acid residues in proximity to phenytoin, also shown by space-filled images. One aromatic ring of phenytoin is closer to the side chain of Tyr-1771, whereas another is located in the central part of the pore, occluding the pore and blocking Na^+ permeation.

Fig. 4. Phenytoin in the inner pore of the Na^+ channel (side view). The amino group N-H at N3 of the hydantoin ring makes immediate van der Waals contacts, interacts with the center of the aromatic ring of Phe-1764, and participates in weak electrostatic interactions with its π-electron system ("the amino-aromatic bond"). The outer vestibule forming P loops of domains I, III, and IV are also shown (pink ribbons), including Asp(I) and Lys(III) (shown by balls and sticks) of the selectivity filter. IIP and IIS6 are omitted.

chain with nonpolar side chains of S6 helices of domains I to III. The energy estimates for nonbonded interactions for both phenytoin and 5-hexyl-5-phenyl-hydantoin are practically equal.

Improvement of binding of the derivatives of phenytoin with increase in size of the 5-alkyl chains (from *n*-propyl to *n*-nonyl; Brown et al., 1999) certainly reinforces the hydrophobic nature of their interactions with the inner pore in the open/inactivated state, where the wide opening fills the pore with bulky water. Lenkowski et al. (2007) determined the affinity of binding of 5-heptyl-5-phenyl-hydantoin to the $Na_v1.2$ channel more precisely with IC_{50} = 2.5 μM for the fast inactivated state. If the IC_{50} of phenytoin is ~20 μM (Ragsdale et al., 1996), then 5-heptyl-5-phenyl-hydantoin has shown an improvement of binding by ~8- to 10-fold or ~1 to 1.2 kcal/mol. This estimate approximately corresponds to the differences in hydrophobicities of the aromatic side chain of phenylalanine (−2.5 kcal/mol) and the hypothetical side chain including *n*-heptyl (-3.5 kcal/mol), if we accept the idea that hydrophobicity of one methylene group is equal to the hydrophobicity of the side chain of alanine (−0.5 kcal/mol) (Tanford, 1980). Therefore, with the inside the open inner pore filled by bulk water, hydrophobic interactions can play an important role in the binding of phenytoin.

In the closed state the four S6 α-helices at their C-ends form the so-called S6-crossing (Doyle et al., 1998), producing an inner cavity of restricted size below the selectivity filter. Voluminous phenytoin practically fills this space in the closed channel, excluding water and making direct contacts with the walls of this cavity. Such low-affinity interactions would likely be only nonbonded van der Waals interactions. In this case the interface between nonpolar surfaces of the drug and bulk water is lacking, and therefore the additional stabilizing effect of hydrophobic interactions is absent. This comparative analysis by binding of phenytoin in the open and closed states could explain why even neutral molecules of anticonvulsants show low affinities of binding in the rested
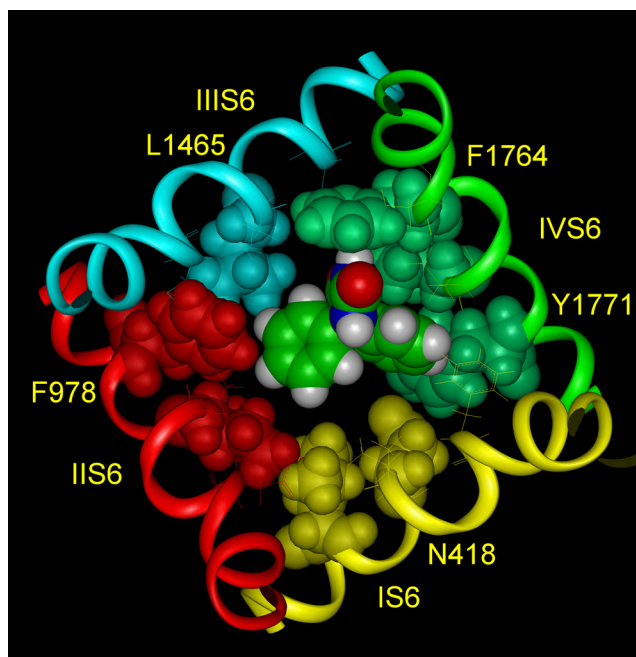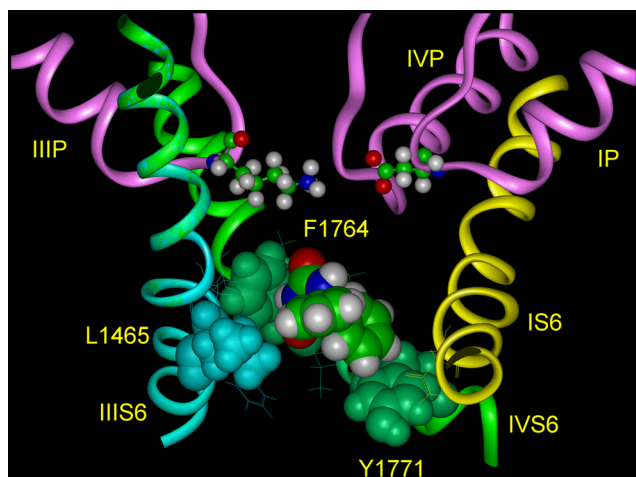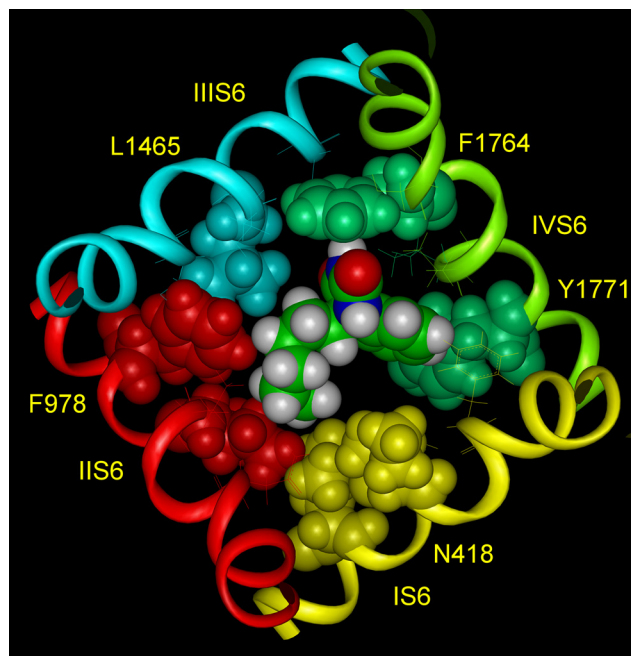

Fig. 5. 5-Hexyl-5-phenyl-hydandoin inside the inner pore (top view). The 5-hexyl alkyl chain participates in hydrophobic interactions with the nonpolar side chains of IS6–IIIS6 helices.

state relative to the high affinity with the open/inactivated state of the Na^+ channel.

Carbamazepine. Carbamazepine also limits repetitive firing of nerve action potentials (McNamara, 2006), and it shows neuronal Na^+ channel blocking characteristics similar to those of phenytoin (Brown et al., 1999): low-affinity resting block, high affinity for the inactivated state ($K_I \sim 25 \mu M$; Kuo, 1998) and weak use-dependent block. Both drugs belong to the family of tricyclic anticonvulsants, and contain practically the same pharmacophore segment—the aromatic ring and the amide group in the middle. For carbamazepine, the amide group is part of the carbamyl at the central iminostilbene ring, which is flanked by two aromatic rings (Fig. 1). As in the case of phenytoin, where the pharmacologically important amine at N3 is divided by three chemical bonds from its aromatic ring, the amino group of carbamazepine is also divided from each aromatic ring by three chemical bonds. For this analysis the spacial structure of carbmazepine was energy-minimized with the Discover module of Insight II (Fig. 1). This optimal structure is very close to the X-ray determined structure of carbamazepine (Lowes et al., 1987). In both cases the side-chain carbamyl group at the *N*-5 position is almost perpendicular to the plane of the central 7-member ring. Superposition of two aromatic rings—one from phenytoin and the other from carbamazepine—leads to the near superposition of two important amino groups: the N3-H3 bond of the hydantoin ring of phenytoin and the N-H bond of the side-chain carbamyl of carbamazepine (Fig. 6).

Assuming that the pharmacophore segments of carbamazepine and phenytoin are similar, our initial location of carbamazepine inside the pore was by superimposition with the location of phenytoin (Fig. 4) by fitting the corresponding aromatic rings and amide groups. From two possible superpositions of aromatic rings of carbamazepine with the aromatic ring of phenytoin that interacts with Tyr-1771 of DIV-

S6, we have chosen the one with optimal superposition of N3-H3 of phenytoin and N-H of carbamazepine, including also both C-β atoms of the two aromatic rings (Figs. 7 and 8). As in the case of phenytoin, one aromatic ring interacts with the side chain of Tyr-1771, whereas the other ring, at an approximately 120° angle with the first, is located in the center of the pore, closer to III-S6 and its Leu-1465, and to DII-S6 (Phe-978), practically occluding the pore lumen. The energies of van der Waals interactions with each of these side chains (and also Asn-418) are approximately -1 to 2 kcal/mol. At the same time, the amino group of the carbamyl is directed to the center of the aromatic ring of Phe-1764, where it can form an "amino-aromatic hydrogen bond." The energy of electrostatic interactions with this side chain, taking into account the partial charges of the amino group (Creighton, 1993), is ~ -1 kcal/mol. According to this structural analysis, the presence of similar pharmacophore segments causes similar binding of carbamazepine and phenytoin and probably the same mechanism of block of Na^+ permeation, despite very different chemical structures,

Very low resting state block (Kuo, 1998) implies that the drug binds poorly to the closed pore. When we attempted to dock carbamazepine into the inner pore of our model of the closed channel (Lipkind and Fozzard, 2000), it was apparent that the wide and bulky tricyclic carbamazepine fits closely to the size of the inner cavity and participates in van der Waals repulsion with the walls. Consequently, the rigid structure of the drug effectively prevented its binding in the energy-minimized form seen in binding to the open state. This fit required transformation of carbamazepine to a more energetically strained structure (approximately 1.5 kcal/mol).

Lamotrigine. The binding affinity of lamotrigine for the inactivated state of $Na_v1.2$ is reported to be 31.9 μM (Liu et al., 2003). Alanine scanning mutagenesis of DIV-S6 identified F1764A and Y1771A as important to the binding affinity
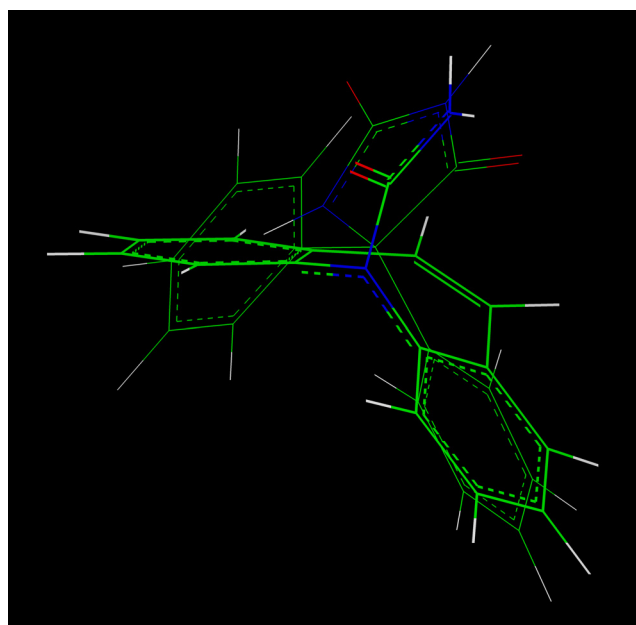

Fig. 6. Superposition of molecular structures of phenytoin (thin lines) and carbamazepine (thick lines). Superposition of one aromatic ring from each structure leads to the coincidence of the amino groups of phenytoin (N3-H3) and carbamazepine (its amide) with separation not more than 0.5 Å.
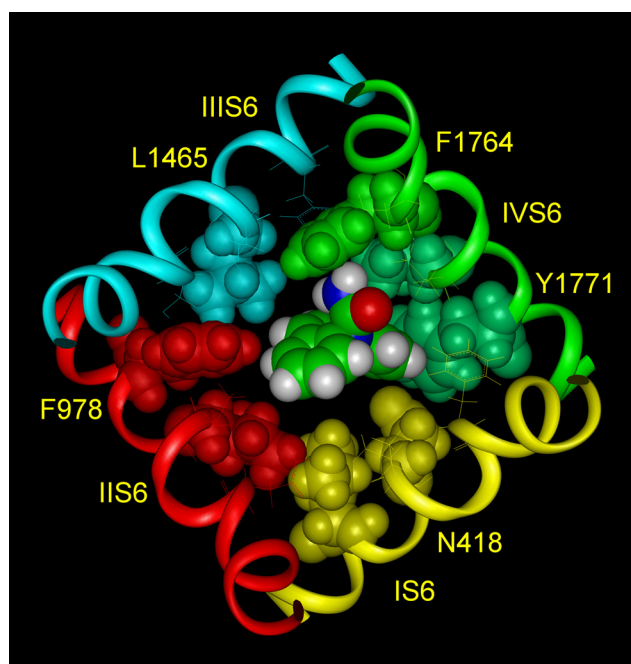

Fig. 7. Carbamazepine. Optimal location of carbamazepine inside the inner pore of the $Na_v1.2$ channel (top view).

of lamotrigine—reducing IC_{50} values for the WT channel by 7- and 12-fold, respectively (Liu et al., 2003). Therefore, lamotrigine binding to Phe-1764, so crucial in local anesthetic binding and action, is only approximately 1 kcal/mol, consistent with our suggestion of an amino-aromatic interaction. In contrast, its interaction with Tyr-1771 is stronger (~1.5 kcal/mol). In addition, lamotrigine interacts with Leu-1465 and is consequently bound in the interface between DIV-S6 and DIII-S6, because L1465A also decreased its binding 6-fold (Yarov-Yarovoy et al., 2001). Drug affinity for the resting state is very low ($K_d = 641 \ \mu M$) (Xie et al., 2001).

Lamotrigine contains a triazine ring connected to another aromatic ring (a dichlorophenyl in this case). The triazine ring (Fig. 1) is substituted by two amino groups in the 3- and 5-positions (*para*- and *ortho*-, correspondingly), a structure maintained for all effective analogs of lamotrigine (Clare et al., 2000). Although both amino groups are important, only the 5-amino group is obligatory (Clare et al., 2000). The asymmetric heteroaromatic nature of the triazine ring is likely to confer high levels of polarity of its atoms, with partial negative charges on N1, N2, and N4, whereas hydrogen atoms the amino groups will bear partial positive charges (Janes, 1999). Energetic optimization of lamotrigine led to a structure with almost perpendicular arrangement of the triazine and phenyl rings (~70°; Fig. 1), as found in the crystal structure of lamotrigine (Janes, 1999).

In principle, either the 3- or the 5-amino group could participate in the amino-aromatic hydrogen bond with the side chain of Phe-1764. However, using the common pharmacophore concept for phenytoin, carbamazepine, and lamotrigine with superposition of the essential structural elements (Fig. 1), we designate the 5-amino group, which is also divided from the phenyl ring by 3 chemical bonds. This is the amino group that is experimentally obligatory for block. If we assume the adequate location of their aromatic rings at the side chain of Tyr-1771, the best fit for interaction of the aromatic ring of Phe-1764 with the N3-H3 group of the hydantoin of phenytoin corresponds to direct interaction of the amino group of lamotrigine at the
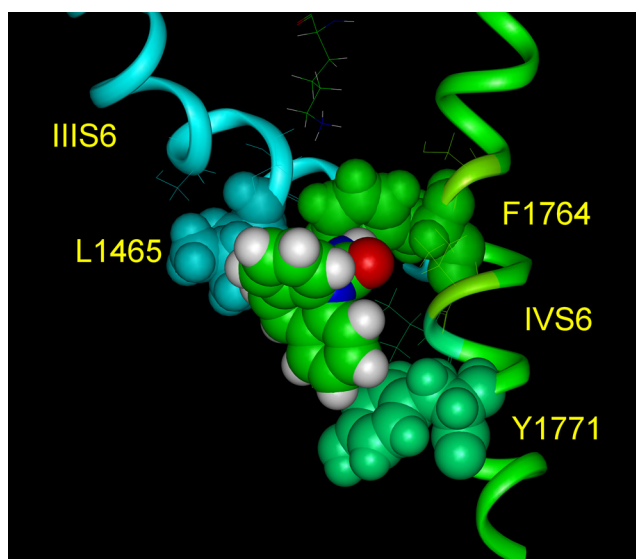
5-position. Optimization of localization of lamotrigine inside the pore based on this initial approximation is shown in Fig. 9. In this location, the phenyl ring interacts with the side chain of Tyr-1771, the 5-amino group makes immediate contact with the center of the aromatic ring of Phe-1764, whereas the triazine ring itself, like the second aromatic rings in phenytoin and carbamazepine, is directed to the center of the pore and physically occludes the lumen. The same role could be played by the two voluminous chloro atoms of the dichlorophenyl ring (Fig. 9). It is also possible that the partial positive charge of the 3-amino group, now located closer to the center of the pore, could contribute an electrostatic barrier to Na^+ permeation. Figure 10, top, illustrates the way in which the somewhat different bulky structures of carbamazepine and lamotrigine extend into the lumen of the channel in such a way that they could block permeation.
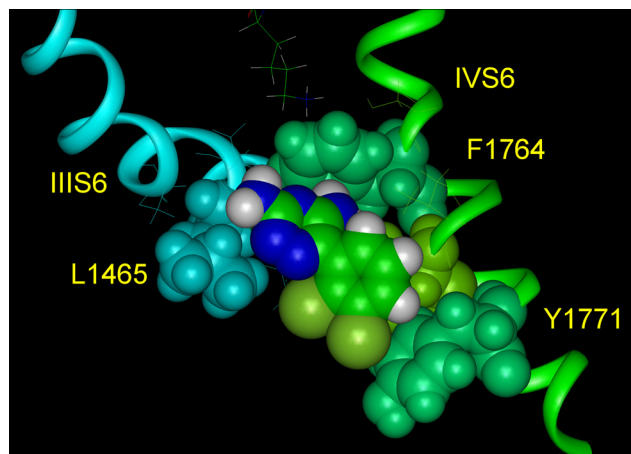

Fig. 9. Lamotrigine. Optimal location of lamotrigine inside the inner pore (side view). In accordance with the common pharmacophore core of anticonvulsants, the amino group in the 5-position of the triazine ring interacts with Phe-1764.

Fig. 8. Carbamazepine in the interface between IIIS6–IVS6 of the inner pore of the Na^+ channel (side view). The amide group of carbamazepine interacts with the side chain of Phe-1764, whereas the approximately perpendicular location of the two aromatic rings provides physical occlusion of the pore.
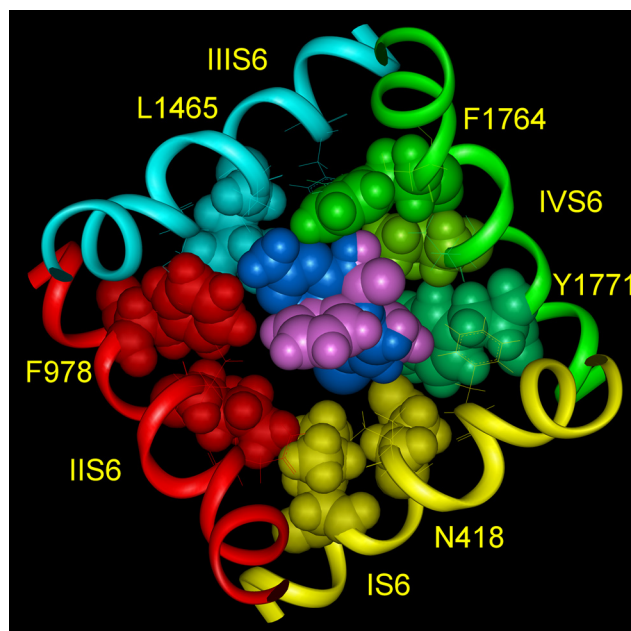

Fig. 10. Superposition of structures of carbamazepine (pink color) and lamotrigine (blue color) inside of the inner pore (top view). Different bulky anticonvulsants interact with the common receptor of the Na^+ channel and extend into the lumen of the pore in such a way that they could block conductance.

Discussion

Energetic analysis of binding of representative members of the anticonvulsant drugs - phenytoin, carbamazepine, and lamotrigine- has led us to similar structures for their complexes with the two domain IV S6 residues in the open inner pore of the Na^+ channel that are known from mutagenesis studies to be critical for their blocking action. Anticonvulsants are too wide and rigid to fit optimally into the modeled closed state, but they do fit easily into the open/inactivated state. All anticonvulsants of this family contain a common pharmacophore core—one obligatory aromatic ring available for an aromatic-aromatic interaction with the side chain of Tyr-1771 and a very special amine, located such that it could interact with π-electrons of the aromatic ring of DIV-S6 Phe-1764. It is important for this interaction that the amine is located in the molecular fragments of anticonvulsants with a high level of polarity, connected with electronegative atoms (or groups) CO-NH, $CO-NH_2$, $CN-NH_2$ (Fig. 1), specifically raising the high partial positive charges on the hydrogen atoms of their amino groups. The electron donor groups in the anticonvulsant structures play only a subsidiary role and do not participate in direct interactions with the channels, contrary to the proposition by Unverferth et al. (1998). In the crystal structure of phenytoin, carbamazepine, and lamotrigine, these amines form hydrogen bonds (NH. . . O or NH. . . N). However, in the inner pore of the Na^+ channel, the tendency of the amino groups to form hydrogen bonds is transformed to interactions with the aromatic ring of Phe-1764 to form an amino-aromatic hydrogen bond. Anticonvulsants, in contrast to local anesthetics and antiarrhythmics, are neutral, but this high permanent polarity of the pharmacophore amino groups compensates partially for the local anesthetic equilibrium between uncharged and charged forms. The positive charge per se is not obligatory for the block of Na^+ permeation.

The energies of interaction of anticonvulsants with the side chains of Phe-1764, Tyr-1771, and Leu-1465 are not high. Nevertheless, alanine-scanning mutagenesis has clearly shown that these three amino acid residues are important, allowing us to propose that they are essential to the channel's recognition of the anticonvulsant structures and that they orient the rigid drug structures at the interface cleft between domains III and IV. Hydrophobic interactions also undoubtedly contribute an important energetic component to the anticonvulsant interaction energy in the open/inactivated state. For example, the 5-alkyl-5-phenyl-hydantoins are as effective as phenytoin itself. This suggests that the second aromatic rings of the tricyclic phenytoin, carbamazepine, and lamotrigine are positioned so that they participate in hydrophobic interactions with other segments of the inner pore: DI-S6 and DII-S6. Hydrophobic interactions by dense packing with the walls of the inner pore play a crucial role for binding of anticonvulsants, compensating for lower energy interactions resulting from the absence of a positive charge that is so important for the local anesthetics. The drug structures are so bulky and wide that they could easily occlude the central part of the inner pore and obstruct Na^+ permeation sterically. On the other hand, in the resting closed inner pore, the bulky drugs can neither interact normally with Phe, Tyr,

and Leu on domains III and IV nor use hydrophobic forces, reducing their affinity.

The prediction of our modeling suggests that scanning study of anticonvulsant interaction with the inner segments of DI-S6 and DII-S6 helices would identify additional residue interactions. The high aromaticity of anticonvulsants suggests a strategy other than alanine scanning. Substitutions by aromatic residues (Phe and Trp) in domain IID6 (Val-974 and Leu-975) and domain IS6 (Asn-418 and Leu-421) might be expected to improve drug binding. In this context, we note that substitutions of Asn-434 of $Na_v1.4$, homologous to Asn-418 of $Na_v1.2$, by phenylalanine increased the binding of the local anesthetic bupivacaine by 6-fold (Nau et al., 1999). Molecular modeling of this interaction has shown that one side of the aromatic ring of anticonvulsants located at the interface of III-S6 and IV-S6 interacts with the side chain of Tyr-1771, whereas the flat side of the ring can interact with a phenylalanine substituted for Asn-418 by forming a perpendicular stack and thereby stabilize the interaction. Therefore, our models are compatible with the N418F/Y mutations.

Analysis of mutational data and molecular modeling of the interaction and binding of local anesthetics (Lipkind and Fozzard, 2005) and this model of anticonvulsant binding reinforce the idea that the potassium channel structures provide a reasonable structural template for location of the S6 α-helices of domains III, IV, and I of Na^+ channels. However, it is still unclear whether DII-S6 can also be modeled in this way. Alanine-scanning mutagenesis of DII-S6 did not change binding of either etidocaine or lamotrigine (Yarov-Yarovoy et al., 2002). Consequently, modulation of binding of the rigid structures of phenytoin and carbamazepine, which should protrude into the inner pore closer to DI-S6 and DII-S6, by substitution with bulky neutral aromatic amino acid residues (Phe, Trp) and even Arg could provide useful information on possible asymmetry of the S6 segments in the inner pore of the Na^+ channel.

The amino-aromatic interaction with the side chain of Phe-1764 is probably key to the voltage dependence of high-affinity block by the anticonvulsants. We have previously shown that local anesthetic affinity is greatly reduced, and use dependence is abolished when the equivalent phenylalanine in $Na_v1.5$ is mutated to a nonaromatic residue (McNulty et al., 2007), and the characteristic immobilization of gating charge by the charged lidocaine is abolished by mutation of the phenylalanine (Sheets et al., 2008; Hanck et al., 2009). Furthermore, replacement of the aromatic ring of the equivalent Phe-1579 ($rNa_v1.4$, equivalent to Phe-1759 in $hNa_v1.5$) by cyclohexane reduced local anesthetic use-dependent block (Ahern et al., 2008).

The structural differences between anticonvulsants and local anesthetics determine the differences in inhibition of the neuronal Na^+ channels: local anesthetics have a high tendency for use-dependent block at modest stimulus rates, whereas neutral anticonvulsants show a low level of use-dependent block. Anticonvulsants have high off-rate upon repolarization, similar to the local anesthetic benzocaine, but still enough to suppress very high action potential rates. Anticonvulsant drug binding is clearly voltage-dependent, not as a result of charge movement directly associated with entry of the drug into its binding site in the pore, but as consequence of the higher affinity for the open/inactivated configuration of the channel, probably through their interac-

tion with DIV-S6 Phe-1764, which is lost upon repolarization. On balance, depolarization of the resting potential may be more important to their effectiveness in Na^+ channel block, rather than frequency of action potentials. These different functional profiles allow the drugs to distinguish their target organs. Treatment of cardiac tachyarrhythmias benefits from high use dependence of local anesthetics, whereas their use dependence in the central nervous system is an undesirable side effect. In contrast, anticonvulsants do not interfere with normal rapid action potential rates in the central nervous system because of their poor use dependence, but they have a preferential high affinity for block in depolarized regions that characterize epilepsy.

Acknowledgments

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