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Perspective

Medicinal Chemistry of Neuronal Voltage-Gated Sodium Channel Blockers

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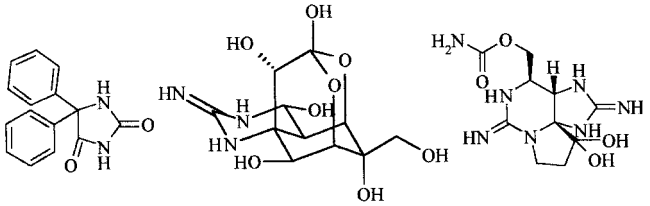
Introduction

Sodium channels play an important role in the neuronal network by transmitting electrical impulses rapidly throughout cells and cell networks, thereby coordinating higher processes ranging from locomotion to cognition. These channels are large transmembrane proteins, which are able to switch between different states to enable selective permeability for sodium ions. For this process an action potential is needed to depolarize the membrane, and hence these channels are voltage-gated.

It was not until the landmark studies of Hodgkin and Huxley[1] in the early 1950s that the mechanism and function of sodium channels were revealed. Since then the development of the patch-clamp technique, which enables single-channel recordings, and the first cloning of a voltage-gated ion channel by Noda[2] have enhanced our knowledge of sodium channel function and structure. In a similar manner, the antiepileptic drug phenytoin (**1**) and the neurotoxin tetrodotoxin (TTX) (**2**) were milestones in the recognition of the potential of neuronal sodium channel blockers in CNS-related disorders. It became apparent that voltage-gated sodium channels could be targeted, either selectively or in combination with other cellular processes, for the treatment of stroke, epilepsy, and several types of neuropathic pain. This review focuses on the latest developments in these areas, but we also refer the reader to several excellent reviews which cover the older literature more comprehensively.[3−5]



Phenytoin, **1** Tetrodotoxin, **2** Saxitoxin, **3**

Molecular Biology

We now know that the fully functional sodium channel is a heteromeric complex consisting of an α-subunit and at least one auxiliary *β*-subunit. However, prior to the identification and sequencing of the voltage-gated sodium channel α-subunit from the electric eel electroplax,[2] electrophysiology and binding studies with neurotoxins were the only techniques available to characterize sodium channel activity. Since then a further 11 distinct sodium channel α-subunits, as well as associated isoforms,[6,7] have been identified in mammalian brain and peripheral tissues (Table 1). The amino acid sequence identity between the brain sodium channel genes *SCN1A*, *SCN2A*, and *SCN3A* is greater than 90%, and it has been postulated that these three genes are recently diverged.[5] These three genes show more identity with brain *SCN8A* (75%) than with peripheral tissue and sensory neuron genes (60%). However, comparison of amino acid sequences in key regions of all the sodium channel genes, such as the inactivation gate, voltage sensor, and selectivity filter, reveals a much higher degree of conservation with substitutions restricted to those with a chemical similarity.[8]

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Table 1. Cloned Sodium Channel α-Subunit Genes and Their Location[a]

gene	chromosome human	chromosome mouse	cDNA	major expression sites	ref
SCN1A	2q24	2 (36)	rat brain I	CNS, PNS	11
SCN2A	2q23-q24	2 (36)	rat brain II/IIA	CNS, glia	12
SCN3A	2q24	2 (36)	rat brain III	CNS, glia	13
SCN4A	17q23-q25	11 (64)	μ1, SkM1	skeletal muscle	14
SCN5A	3p21	9 (70)	μ2, SkM2, H1	cardiac muscle	15
SCN6A	2q21-q23		hNav2.1	heart, uterus	16
SCN7A		2 (41)	NaG, SCL-11	heart, glia	17
SCN8A	12q13	15 (64)	Nach6, CerIII, PN4, CSC-1	CNS, PNS, glia	18
SCN9A	(2q24)	2 (36)	PN1, hNe-Na, NaS	PNS, glia	19
SCN10A	3q22-q24	9 (67)	PN3, SNS	PNS	20
SCN11A			NaN, SNS2	PNS	21
SCN12A	3p23-p21.3			DRG, CNS, glia	22

[a] Human data taken from online Mendelian inheritance in humans[9] and mouse data from the Jackson Laboratory Bioinformatics Server.[10]

Blot hybridization experiments have revealed differential expression of mRNAs for *SCN1A*, *SCN2A*, and *SCN3A* sodium channels in both stage of development and location throughout maturation of the rat CNS.[23] Thus *SCN3A* mRNA is expressed predominantly during fetal and early postnatal stages of development, whereas *SCN1A* mRNA is predominantly expressed during late postnatal stages. In adult CNS *SCN1A* mRNA shows a rostral-to-caudal gradient, which is in direct contrast to the caudal-to-rostral gradient exhibited by *SCN2A* mRNA. While *SCN3A* mRNA is not detectable in normal adult rat CNS, moderate-to-high levels of expression are observed in dorsal root ganglion (DRG) following axotomy.[24]

Posttranslational modification of the sodium channel is complex and may be influenced by both phosphorylation and glycosylation.[25] The intracellular linker between domains I and II may be either long (neuronal and cardiac) or short (skeletal muscle and eel), and phosphorylation of the five consensus cAMP-dependent kinase sites of neuronal and cardiac sodium channels reduces current amplitude without affecting gating. In contrast protein kinase C alters the function of all mammalian channel isoforms by phosphorylation of a highly conserved serine in the domain III−IV linker.[26] Phosphorylation of this site reduces maximal channel conductance and alters gating in an isoform specific fashion. Both α- and β-subunits are modified by glycosylation, which predictably affects voltage-dependence of gating through alteration of the surface charge of the channel protein. Glycosylation is also essential for cell surface expression as inhibition of glycosylation by tunicamycin, a mixture of several nucleoside antibiotics, decreases the density of saxitoxin (**3**) binding to neuroblastoma cells.[27]

In a 'typical' mammalian neuron sodium channel, density is much greater in the myelinated axon nodes of Ranvier than in the initial axon segment, with much lower densities in the cell body and nerve terminals.[28] Different isoforms show different distribution patterns within the cell: e.g. *SCN1A* channels show a greater density in cell bodies and dendrites than in axons, whereas rat forebrain *SCN2A* channels show a greater density in axons and proximal dendrites than in cell bodies. Sodium channels may also migrate within the cell as the accumulation of sodium channels within the tips of injured axons exposed to the neurotoxin potassium tellurite has demonstrated.[29]

Structure

Direct chemical identification of the multimeric nature of sodium channels was first demonstrated following polyacrylamide gel electrophoresis of synaptosomal proteins employing covalent labeling with a photoreactive derivative of *Leiurus quinquestriatus* toxin.[28] Although three components were identified (a 270-kDa α-subunit, a 39-kDa β1-subunit, and a 37-kDa β2-subunit), the α-subunit alone demonstrates the basic pharmacology and physiology of the native channel.[30] Currently, no drugs are known which interact directly with β-subunits. Their role appears to be that of a modulator of α-subunit function, although they may be especially important during expression, localization, and folding of the native channels.[5,31] Recently, an additional β-subunit, with homology to β1 and labeled the β3, has been reported and shown to have different distribution and kinetic characteristics compared to β1.[32] While the three-dimensional structure of sodium channels has not been solved, models have been derived to explain how the sodium channel may be embedded in the membrane (Figure 1). Thus the α-subunit has a modular architecture of four homologous domains (DI−DIV) each consisting of six transmembrane segments (S1−S6). Voltage-dependent potassium channels exist as tetramers of four identical subunits, which are thought to form a closely packed group of transmembrane helices around the central ion-conducting pore. Extending this analogy to voltage-gated sodium channels, the regions between the S5 and S6 segments (the P segment) in all four domains of the protein should fold to form part of the extracellular entrance of the channel with the S6 segments lining the ion-conducting pore. There is a marked asymmetry in the contribution of each domain to the permeation pathway, and the S5 and S6 regions from DIII and DIV have been shown to play a particularly prominent role in the determination of sodium ion selectivity.[25]

With respect to the three-dimensional structure of the sodium channel, although attempts to obtain crystallographic information have so far been unsuccessful, the availability of a crystal structure for the pore-forming region of a bacterial potassium channel has proved useful.[33] This model comprises the transmembrane M1 and M2 α-helical regions of a bacterial KcsA channel which form a tetrameric array around the pore of the channel. The P loops that connect the four sets of M1 and M2 helices form the selectivity filter toward the larger mouth of a 'teepee'-like structure.

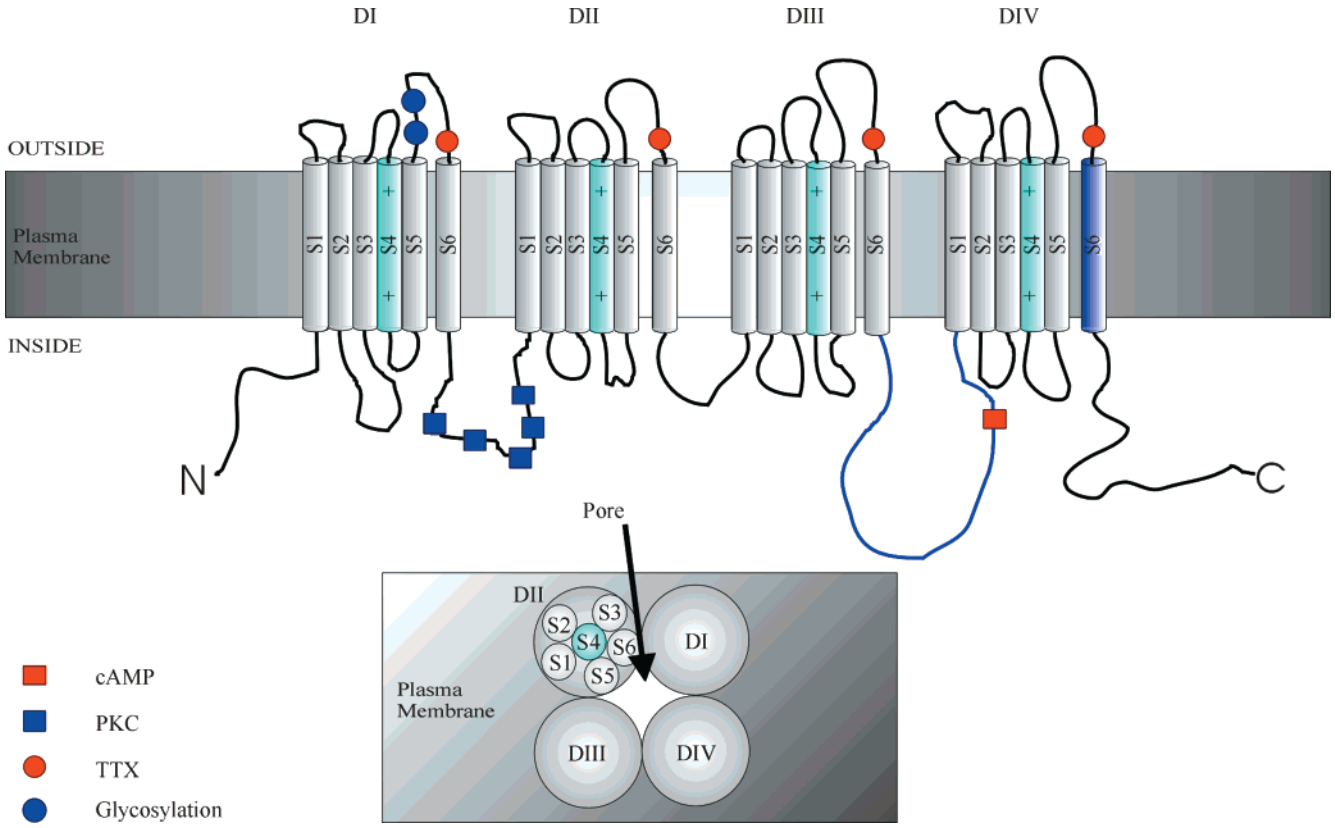


Figure 1. Schematic structure of the α-subunit of the sodium channel and the pore-forming unit. The voltage-sensing S4 is highlighted light-blue, the binding site for anticonvulsants and local anesthetics on DIV S6 dark-blue, and the inactivation loop between DIII and DIV blue.

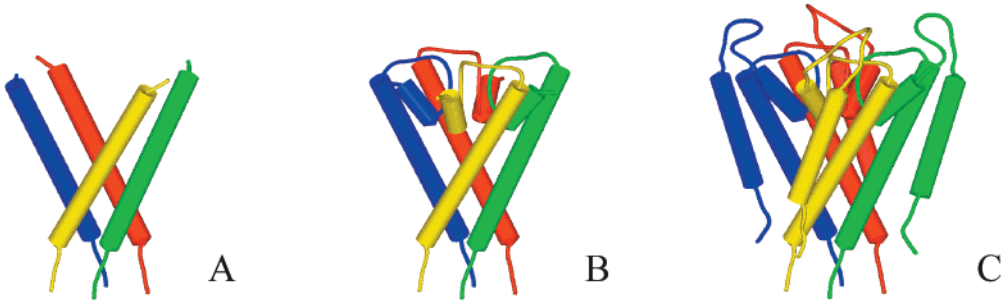


Figure 2. Schematic of the pore-forming region of the KcsA potassium channel: (A) pore-lining M2 segments only, analogous to the sodium channel S6; (B) M2 segments with the P loops attached; (C) adding the membrane-facing M1 segments, analogous to the sodium channel S5 segments.

It is likely that channels such as the KcsA potassium channel, with its two membrane-spanning regions, are the evolutionary predecessors of the six-transmembrane sodium and potassium channels. In this model the M1 and M2 segments of these channels are analogous to the S5 and S6 segments of the sodium channel (Figure 2). This information has been used to construct a model of the pore-forming region of the voltage-gated sodium channel.[34] This model is consistent with most of the current mutational and biophysical properties of the sodium channel. In particular this model, which places the S6 segments facing the pore of the channel and the S5 segments facing the lipid portion of the membrane, predicts a binding site for local anaesthetics and anticonvulsants on DIV S6. This conforms to the observed effects of mutation at Phe-1764 and Tyr-1771 and also predicts additional key residues in this area.[35]

Function

Voltage-gated sodium channels are responsible for generation of the action potentials of axonal nerve fibers via fast, selective transport of sodium ions across the cell membrane leading to the rapid transmission of depolarizing impulses throughout cells and cell networks. At its simplest it has been postulated that sodium channels can exist in at least three different states.[1] Thus at resting membrane potentials (less than −60 mV) sodium channels exist predominantly in a 'resting' (closed) nonconducting state. As the membrane potential increases the probability of the channel conducting sodium (i.e. activation) increases resulting in an inward flow of sodium ions across the cell membrane. This leads to a further increase in membrane potential, which itself increases the probability of channels opening so that at a critical membrane potential an action potential is

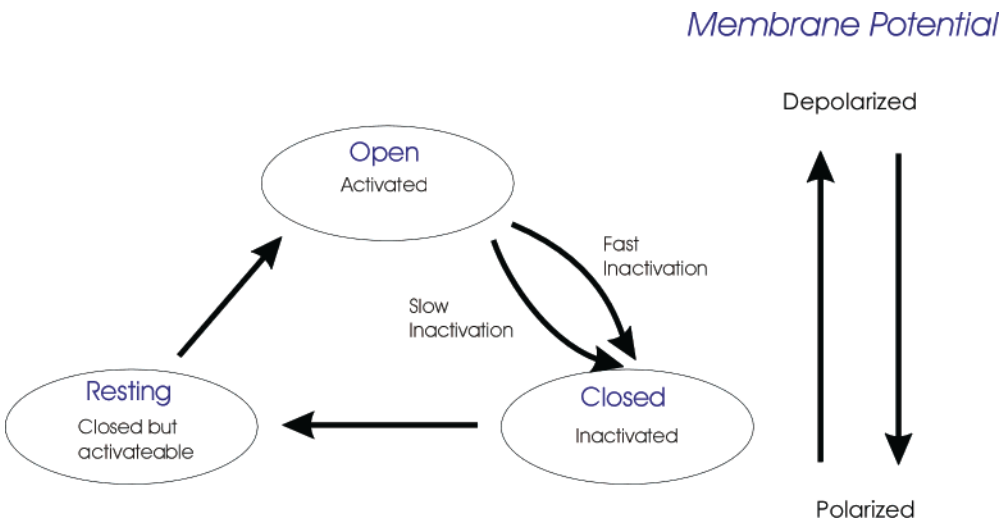


Figure 3. Relationship between open, closed, and resting state of sodium channels.

initiated. These open channels, however, close rapidly (within around 1 ms) by transformation to a rapidly 'inactivated' state, which terminates the inward current. Reactivation of the inactivated channels by transformation back to the resting state is membrane potential-dependent as repeated depolarizations delay the transformation back to the resting state (Figure 3).

Drugs that interact with sodium channels to block ion flux cause the channels to inactivate to a greater extent and with smaller depolarizations than normal. The relatively slow off-rate of drugs such as phenytoin (**1**) means that there is an accumulated block following repeated depolarization (termed use-dependency). This means that, in the case of therapeutically relevant concentrations of phenytoin (8 μM), channel block is only significant if cells remain depolarized for at least 5 s.[36] This may explain why most sodium channel blockers do not alter normal action potentials or excitatory synaptic potentials, as these typically last less than 200 ms. Sustained depolarization, during ischemia or seizures, greatly enhances the blocking action of this type of drug. This kinetic model has been described by Kuo and Bean[37,38] and is supported by most biophysical data, while the often mentioned 'slow inactivation state' for the interaction between sodium channels and drugs is still the subject of some controversy.[39]

Voltage Sensing

Depolarization is the signal for activation of ion channels, and to detect these changes the protein must possess a charged structure within the hydrophobic portion of the membrane. The highly conserved and highly charged S4 regions of the protein are now widely accepted as forming the voltage sensors. The contributions of each S4 sensor to activation is not equivalent, and in the process of activation charged residues traverse the membrane through a narrow cuff formed by other, as yet unidentified, regions of the channel.[40] Furthermore, gating current and mutagenesis studies have revealed a direct coupling of inactivation to activation with the time course of current decay reflecting the voltage dependence of activation.[25] In potassium channels the mouth of the inner channel can be physically occluded by S6 in response to voltage.[41] As mutations in S6 alter sodium gating, this segment would appear to be the primary candidate for the physical activation gate.[42]

Inactivation

The mechanism by which sodium channels are able to inactivate has been the subject of extensive study. It is clear that these channels are able to inactivate through both a fast (milliseconds) and slow (seconds to minutes) pathway and that the interplay between activation and inactivation pathways is held in a delicate balance. Internal perfusion of the squid axon with Pronase, a proteolytic enzyme, prevents the fast inactivation of sodium currents, implicating the cytosolic region of the channel in this inactivation mechanism.[43] Mutation and expression studies also suggest a role for the cytosolic linker between DIII and DIV in the fast inactivation process. More specifically fast inactivation is almost completely blocked by mutations to the hydrophobic peptide sequence IFM within this linker.[44] Thus the IFM residues appear to form part of an inactivation particle, which occludes the intracellular mouth of the pore by binding to a 'receptor' formed by the linker between S4 and S5 in DIV (Figure 4).[45]

It is now becoming more apparent that channel activation and inactivation are inextricably linked, and a primary role of the movement of the S4 segments in the voltage sensitivity of fast inactivation as well as activation is clear. Recent studies, using site-directed fluorescent labeling of each S4 domain, have demonstrated that the S4 segments in DIII and DIV are responsible for voltage-sensitive conformational changes linked to fast inactivation.[46] Very little is known of the structural requirements and mechanisms of slow inactivation, although mutation studies in DII S4[47] and DIV S4[48] suggest a role for these segments in the voltage dependence of slow inactivation. Suppression of slow inactivation has been reported in paramyotonia and other skeletal myopathies.[49] It is clear, however, that defects in slow inactivation underlie the pathophysiology of many channelopathies (see later).

Neurotoxin Binding Sites

Studies using a wide range of neurotoxins have identified at least eight distinct neurotoxin binding sites

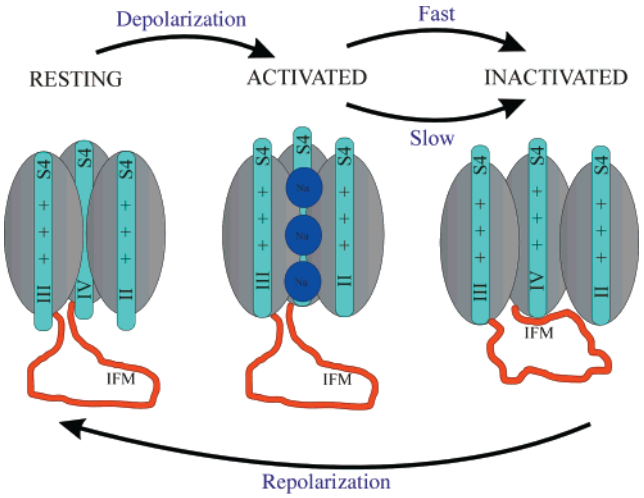


Figure 4. Inactivation of the sodium channel and role of the channel blocking IFM motif. The intracellular loop connecting DIII and DIV is shown in red with the residues known to play a role in inactivation (Ile-1488−Met-1490) highlighted. The 'receptor' for this motif is thought to lie on the intracellular loop between S4 and S5 in DIV.

Table 2. Neurotoxin and Insecticide Binding Sites on the Voltage-Gated Sodium Channels[a]

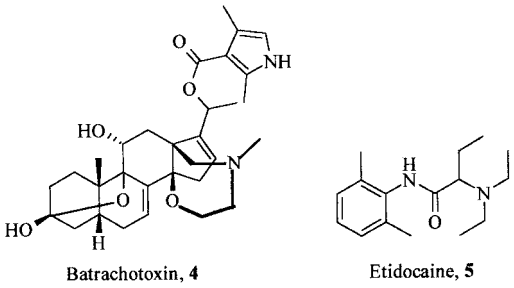
site	neurotoxin	physiological effect	ref
1	tetrodotoxin (TTX, **2**)	inhibition of transport	65
	saxitoxin (STX, **3**)		66
	μ-conotoxin		67
2	batrachotoxin (BTX, **4**)	persistent activation	68
	veratridine (**6**)		69
	aconitine (**8**)		70
	grayanotoxin (**7**)		71
	N-alkylamides		72
3	α-scorpion toxins	inhibition of activation	73
	sea anemone II toxin (ATXII)	enhancement of persistent activation	74
	δ-atracotoxins	slow sodium current inactivation	75
4	*β*-scorpion toxins	shifting of voltage dependence	76
5	brevetoxins (**9**)	repetitive firing	77
	ciguatoxins (**10**)	shifting of voltage dependence	78
6	*δ*-conotoxins (*δ*-TxVIA)	inhibition of activation	79
7	*DDT* and analogues	inhibition of activation	80
	pyrethroids	shifting of voltage dependence	81
8	goniopora coral toxin	inhibition of activation	82
	conus stratius toxin		83
9	local anesthetics		84
	anticonvulsants	inhibition of ion transport	85
	dihydropyrazoles		86

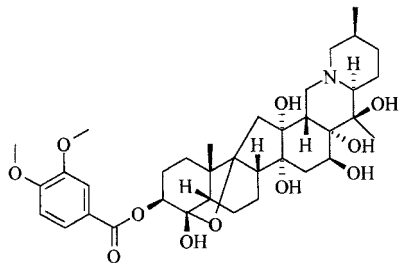
[a] Insecticides are shown in italic type. Substances are considered to belong to a given site if they compete with each other in binding assays and induce similar electrophysiological effects.

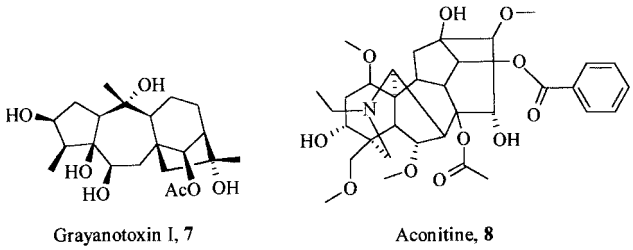
and a 'drug' binding site (Table 2). These binding sites either affect ion transport directly (sites 1 and 9) or modify the gating process (sites 2−8). Allosteric interactions between these sites are also common, and such interactions may be related to the 'state-dependence' of binding.[50] For example, batrachotoxin (BTX) (**4**) (site 2) preferentially binds to the open state of the channel, and this binding is enhanced by ligands which bind to sites 3, 5, and 7 and inhibited by ligands that bind to site 1 as well as by local anesthetic, anticonvulsant, and antiarrhythmic drugs.[51]

The small, polar, heterocyclic guanidines, TTX (**2**) and saxitoxin (**3**), bind at site 1 and are thought to block ion transport by physical occlusion of the channel. Using site-directed mutagenesis, a single-point mutation of Glu-387 (in the extracellular loop formed between transmembrane S5 and S6 of DI) into glutamine in rat brain *SCN2A* showed a drastically reduced sensitivity to these toxins with only marginal effects on macroscopic current properties.[52] While this residue may play a key role in determining the binding of **2**, mutagenesis of glutamate residues in the corresponding areas of the other three domains also reduces binding affinity.[53] These results are consistent with a model in which TTX (**2**) binds to the external mouth of the pore thus preventing the access of sodium ions to the channel.

Although the sodium channel voltage sensors (S4 regions) are located within the membrane, many neurotoxins modify voltage-dependent activation/inactivation by binding to receptor sites on the extracellular surface of the channel. Cestele et al.[54] have proposed a voltage sensor trapping mechanism by which polypeptide toxins can modulate activation/inactivation through a molecular interaction with the S3 and S4 loops in the appropriate domains of the sodium channel. Thus α-scorpion toxin (site 3) slows sodium channel inactivation by preventing the outward movement of the DIV S4 segment and 'trapping' it in an inward position. This allows activation but prevents fast inactivation, whereas *β*-scorpion toxin (site 4) traps the DII S4 segment in the outward, activated position thus enhancing channel activation.







Ragsdale et al.[42] have previously shown that mutations at positions 1764 and 1771 in the DIV S6 of *SCN2A* segment significantly reduced the affinity of inactivated channel states for the local anesthetic etidocaine (**5**). More recent studies with these mutated sodium channels also showed a reduced binding affinity for both a range of sodium channel blocking drugs[42] and

the binding of tritiated BTX.[51] Thus it was concluded that the BTX binding site (site 2) shares overlapping but not identical molecular determinants with the local anesthetic/anticonvulsant binding site (site 9). One model of how this may occur places the DI S6 segment adjacent to the DIV S6 segment in the BTX/local anesthetic/anticonvulsant binding area.[55] In support of this model, mutations in DI S6 (N434K and L437K) were found to reduce the affinity of BTX-resistant sodium channels to local anesthetics.[56]

While most neurotoxins, such as TTX (**2**), saxitoxin (**3**), and veratridine (**6**), distinguish between sodium, potassium, and calcium channel binding, many neurotoxic peptides can also discriminate strongly between different subtypes of sodium channels. The group of μ-conotoxins, for example, bind to sites 2 and 3 (Table 2), but most of its members have a much higher affinity toward skeletal muscle sodium channels than neuronal channels.[67] The μ-conotoxin GIIIA has been synthesized and used as a probe for selective binding of these types of sodium channels in electrophysiological experiments and for structure−activity relationship (SAR) studies. Recently, another member of this family, μ-conotoxin PIIIA, was isolated from *Conus purpurascens* (an East Pacific fish-hunting species) and found to have novel binding properties.[57] PIIIA distinguishes between different subtypes of neuronal sodium channels[58] and therefore may serve as a very useful tool in specific binding studies. Similarly, several members of the α-scorpion toxins have been shown to discriminate between mammalian CNS sodium channel subtypes.[59]

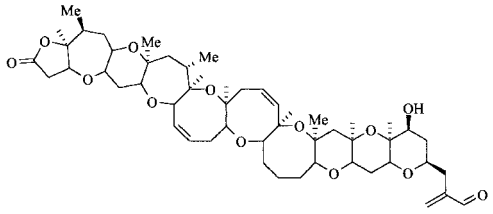
Sodium Channelopathies

It will be clear from the preceding sections that the degree of regulation imposed on, and the complexity of, sodium channels provides enormous scope for genetic mutations leading to pathophysiological changes in channel function. Several forms of generalized epilepsy have been linked to sodium channel mutations. In GEFS$^+$ type 1 patients mutations in the sodium channel β1-subunit gene *SCN1B* causes generalized epilpesy with febrile seizures. This mutation, C121W, disrupts an extracellular disulfide bridge, altering the ability of the β1-subunit to regulate sodium channel gating.[60] GEFS$^+$ type 2 has been mapped to chromosome 2q24-q33, a region encoding a sodium channel α-subunit cluster containing *SCN1A*, *SCN2A*, and *SCN3A*.[61,62] The actual mutation has recently been shown to be on *SCN1A*, with one group showing a C2624T mutation, leading to methionine replacing threonine at position 875 in the encoded protein, and a second group showing G4943A, leading to histidine replacing arginine at position 1648.[63] In both cases the mutated residues reside in the voltage-sensing S4 transmembrane segments of the sodium channel. The mutation corresponding to Arg1648His in the *SCN2A* channel (Arg1638His) causes a decrease in the rate of channel inactivation, and this would be expected to cause the observed increased neuronal excitability and susceptibility to seizures.[64]

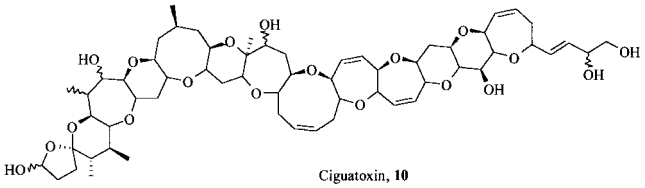
Familial mutations in the skeletal and cardiac sodium channels have also been demonstrated, leading to various periodic paralysis syndromes and hyperexcitability such as inherited long QT syndrome, respectively.[62]

Structure and Function Outlook

The problems associated with experimental determination of three-dimensional structures of ion channels, as with any transmembrane protein, are very difficult to overcome, and it is likely to be many years before high-resolution structures are available for the sodium channels. However, recent success with the KcsA potassium channel structure (although only a small portion thereof) is encouraging and more importantly provides us with a template to build better sodium channel models around. The huge volume of electrophysiological, mutational, and drug binding information available for sodium channels serves to refine and test these models such that they can now be considered very useful tools. Particularly we can now begin to study the role of individual residues in these structures and to look at the influence of the subtle sequence differences between the neuronal sodium channel subtypes that might predict different drug sensitivity. Such studies, coupled to our increasing ability to define the precise sequence outcome of familial genetic disorders of sodium channels, will eventually enable the design of exquisitely selective drugs.



Brevetoxin A PbTx-1, **9**



Ciguatoxin, **10**

In Vitro Sodium Channel Assay Technologies

The methods applied to measurement of ion channel function, and detection of compounds that modulate this function range from the low-throughput (but high-information-content) techniques such as electrophysiology to the high-throughput, lower-information-content assays such as simple ligand binding experiments. In practice a combination of different assay techniques have been applied in most ion channel-related drug discovery programs, with most groups opting for a high-throughput system as the primary point of detection of active species, with a low-throughput/high-information-content assay as a followup. Typically this combination might be an initial binding assay (where the target site of action is known and a suitably labeled ligand is available) or a flux assay (where any means of modulating ion flux through the target channel is of interest). These primary assays may be followed perhaps by patch-clamp electrophysiology to provide direct evidence of a functional effect at the required target. Increasingly

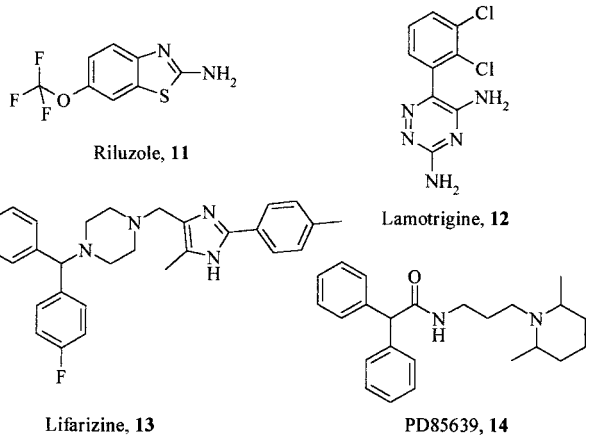
the former two techniques, which have formed the core of drug discovery efforts in the sodium channel area for many years, are being replaced by high-throughput methods based on fluorescence detection of membrane potential changes.

The role of sodium channels in epilepsy, ischemia, pain, drug dependency, and many other areas is well-documented.[3−5] In each case it is the application of blockers of the activity of particular channel populations that is of therapeutic use, and the assay systems that have been developed to study sodium channels reflect this.

One of the key developments in this area is the growing availability of cloned individual sodium channels. The ability to target a particular population of sodium channels by building an assay around cell lines expressing only this channel makes the assays easier to run and interpret and also allows the use of exclusion assays based on channels whose modulation is undesirable. The neuroprotective agent riluzole (**11**), for example, has been shown to act at the rat brain IIA sodium channel α-subunit.[87] As more discrete channels become available in cloned cell lines, it is inevitable that this will become a more useful assay system. However, there are certain drawbacks to this methodology as the cloned cell lines may express different channel subunits or handle regulatory processes such as phosphorylation differently. The following sections describe the assay technologies in common use in the sodium channel area.

Radioligand Binding Assays. Binding studies have revealed the presence of multiple neurotoxin binding sites on sodium channels, as well as additional (or overlapping) sites for drug classes such as anticonvulsants, local anesthetics, and the pyrethroid class of insecticides (Table 2). The action of the different classes of neurotoxins varies from persistent channel block (site 1, TTX (**2**)) to stabilization of the channel in the open state (site 2, BTX (**4**)). The situation is made more complex by the presence of an extensive network of allosteric interactions between these binding sites. It is generally considered that the neurotoxin binding sites, because of the lack of subtlety in their action, make poor drug targets. However, these ligands are used extensively in drug discovery as tools to artificially change the sensitivity of sodium channels or as a means of characterizing particular populations of channels. Of more interest is the ability of some drugs to identify and bind selectively to channels that are at a particular stage of the activated/inactivated/resting cycle of channel activity. This permits the more desirable option, given the correct binding kinetics, of targeting rapidly cycling channels such as occur in convulsant episodes rather than channels functioning at the normal level. The anticonvulsant lamotrigine (**12**) has been shown to act in this way, targeting and stabilizing channels in the inactivated state and thus slowing and reducing the undesirable excitatory responses characteristic of a convulsant episode.[88]

Taking advantage of this kind of selectivity should enable the development of channel blockers with a better side-effect profile. Consequently the preferred option for a binding assay is the use of a radiolabeled derivative of a known, state-selective, high-affinity channel blocker. Such ligands are starting to appear in the literature, principally as tools for investigating site-(s) of action of sodium channel blockers.



Lifarizine (**13**) has been shown to have neuroprotective activity in models of global and focal ischemia[89] and has been radiolabeled and shown to target a site allosterically linked to neurotoxin binding site 2.[90] Similarly the local anesthetic receptor on the sodium channel has been labeled with a tritiated derivative of the benzeneacetamide blocker PD 85639 (**14**).[91] It is reasonable to assume that the use of such compounds in high-throughput binding assays should lead to the identification of novel sodium channel blockers with a similar therapeutic profile. It is surprising that, now identified, these tools have not been incorporated into more detailed sodium channel studies, such as photoaffinity labeling.

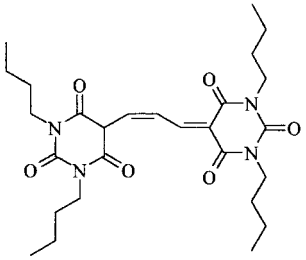
Radioactive Flux Assays. The radioactive [^{22}Na]- and [^{14}C]guanidinium ions are in common use as tracers for sodium channel activity, both in whole cells and in synaptosomal preparations. Such assays are based on the ability of neurotoxins such as veratridine (**6**) to stabilize the channels in the open state and therefore allow flux of the tracer into the cells. In the presence of a channel blocker flux is reduced and therefore a measurable reduction in the uptake of the radiolabel is observed. These methods traditionally rely on the use of separation stages to remove radiolabel that is not incorporated into the cell/synaptosome followed by application of liquid scintillant and subsequent signal measurement on a scintillation counter.[92] However recent advances in microtiter plate technology allow the culturing of cells into a monolayer over a transparent, scintillant-laced well bottom. Incorporation of radiolabel into the cell monolayer accumulates radioactivity adjacent to the scintillant and therefore generates a light signal, which can be measured without recourse to separation or manipulation of liquid scintillant, providing an assay more suited to high-throughput.[93]

The availability in some laboratories of individual sodium channels, expressed in stable cell lines, means that assays of flux through particular channel subtypes can now be carried out. Such tools will allow a clearer correlation between activity of a particular channel and therapeutic potential to be established, as has been shown for the type IIA sodium channels and neuroprotective activity.[94] More importantly, availability of these cell lines provides the tools to identify small molecules which act selectively on particular channel subtypes.

Fluorescence-Based Assays. Fluorescence-based assays are rapidly becoming established as the method of choice in high-throughput screening environments. The combination of high sensitivity, homogeneous medium, and ability to measure true equilibrium conditions provides a level of information content unavailable in other screening techniques. The development of new fluorescent ligands, fluorescence resonance energy-transfer (FRET) systems, and advances in detection systems suggest that these techniques will form the mainstay of future high-throughput assay methodology. The application of this technology to ion channel studies has taken two main forms: measurement of membrane potentials with voltage-sensitive dyes and measurement of the concentration of particular ions with ion-selective fluorescent dyes.

Voltage-sensitive dyes are by definition insensitive to the mechanism by which the membrane potential is being modulated. This lack of selectivity can be overcome in some instances either by employing cell lines expressing particular ion channels or by judicious use of neurotoxins to slow the reaction kinetics (and thus lengthen the response period) for ion movements mediated by particular channels. In the former case care is required to select cell lines which provide a minimum of interference from endogenous ion channels.

Voltage-sensing dyes can de divided into those with a response time measured in minutes, such as the lipophilic oxonol dyes typified by bis(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$) (**15**),[95] and the



Bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DIBAC_4(3)$), **15**

fast-responding (milliseconds or less) styryl dyes. Fast-responding dyes generally work through changes in fluorescence due to a direct interaction between the excited state of the dye and the transmembrane electric field (the electrochromic mechanism). Such dyes are particularly suited to high-resolution imaging of neurobiological processes and are not generally applied to screening efforts because of their relatively poor voltage sensitivity. The slow, oxonol-based dyes are negatively charged and tend to accumulate inside cells that are depolarized (Figure 5). In the aqueous extracellular medium these compounds have a low fluorescent yield, but when bound to hydrophobic intracellular sites this yield increases and changes in membrane potential can thus be visualized. These dyes, having relatively high voltage sensitivity, are particularly suited to high-throughput screening, and many of the early applications of fluorescence imaging employed systems such as $DiBAC_4(3)$ (**15**).

FRET has yielded improvements in the usefulness of voltage-sensing dyes through coupling of the dye to a voltage-insensitive donor fluorophore. In this system the donor fluorophore is designed to remain in the outer

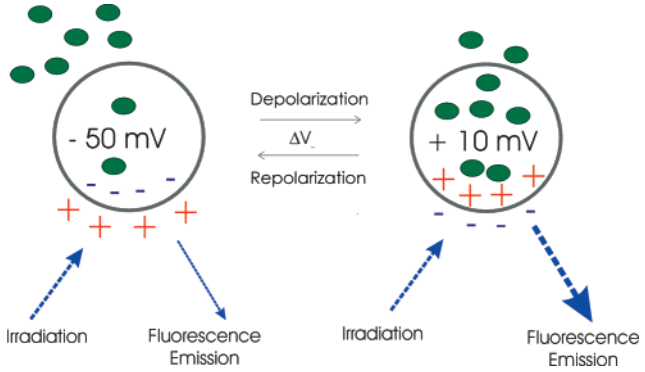


Figure 5. Redistribution of membrane potential-sensitive dyes. As the cells depolarize the amount of (negatively charged) fluorescent dye within the cell accumulates and the fluorescent signal rises.

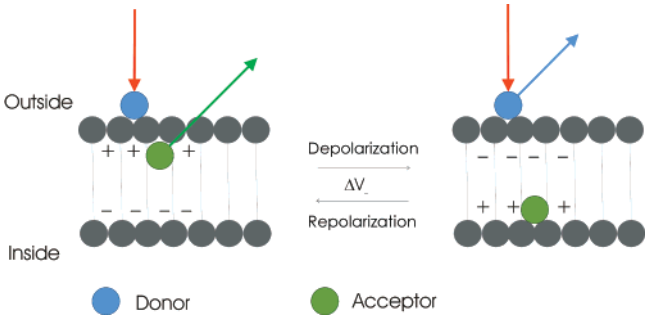
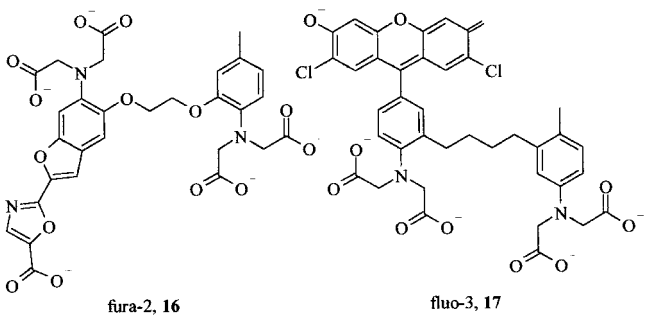


Figure 6. FRET applied to membrane potential measurement. The mobile acceptor species (green) moves away from the donor (blue) in response to membrane depolarization, and thus fluorescence quenching is reduced.

leaflet of the cell membrane and the oxonol dye is designed to move within the cell membrane in response to changes in membrane polarization. Thus at negative membrane potentials the dye resides at the outer leaflet of the membrane and the resonance energy transfer with the donor is efficient. Upon membrane depolarization, however, the dye moves to the inner leaflet of the membrane, at a distance from the donor, which precludes FRET, and a signal is observed (Figure 6).

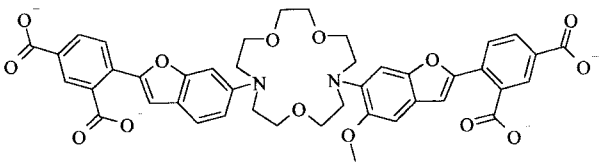
Ion-selective dyes for measuring calcium levels in cells have become established through the availability of compounds such as fura-2 (**16**) and fluo-3 (**17**).[95]



fura-2, **16** fluo-3, **17**

Advances in plate-reading technologies, particularly the advent of charge-coupled devices, now allows the measurement of rapid changes in fluorescence and thus the observation of reaction kinetics. Typical among measuring devices for such techniques is the fluorescence imaging plate reader (FLIPR) from Molecular Devices. Fluorescent dyes specific for sodium ions have been somewhat slower to appear; selectivity for sodium over

potassium is still problematic with many of the dyes available. The most widely applied sodium-selective dye is the sodium-sensitive benzofuran isophthalate (SBFI) (**18**) dye.[96] Measurement of intracellular sodium with this dye has been possible in adrenal chromaffin cells,[97] ventricular myocytes,[98] and neocortical neurons.[99] Dyes such as SBFI can clearly be incorporated into high-throughput systems for identifying modulators of sodium channel activity, although few reports of this have appeared to date.



SBFI, **18**

Electrophysiology. The assay methods described above have been optimized to provide a reasonably high-throughput of test compounds at the expense of sensitivity and the level of detail of the information gathered. This is reasonable considering the availability of large compound libraries and the desirability of rapid lead generation. However, ion channel targets are notorious for demonstrating multiple binding sites and multiple activation states. It is often important to determine the precise binding mode of active species, and it is in this kind of situation that low-throughput/high-information techniques such as electrophysiology are applied. Sodium channels cycle through several stages of activation, and it has been shown to be desirable to target those channels which have become inactivated and to slow the rate at which they become available for activation again.[100] This has the affect of reducing the excitability of neurons without compromising basal levels of activity. Deciphering the mode of action of sodium channel blockers at this level can only be achieved through the application of electrophysiological techniques. Electrophysiology has also been used to identify common binding sites for sodium channel blockers.[85]

Several attempts have been made to automate the patch-clamp testing of ion channel ligands to provide increases in throughput. The most successful to date is probably the system employed by NeuroSearch A/S (Ballerup, Denmark) where compounds are delivered automatically by adapted HPLC injectors to excised patches from HEK cells which have been transfected with the channels of interest.[101]

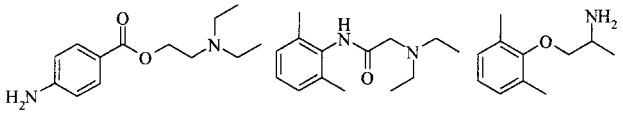
Cell Viability. Assays based around the ability of compounds to modulate cell growth or survival, in cells manipulated to have a requirement for modulation of the flux of a particular ion, are becoming established, particularly for potassium channels.[102] The need for an efficient screening process to test for the presence of neurotoxins in seafood has driven the development of useful assays for both sodium channel blockers and activators. Sodium channel blockers acting at site 1, particularly the saxitoxins, are known to accumulate in molluscan shellfish feeding on toxic dinoflagellate blooms. Ingestion of these compounds causes paralytic shellfish poisoning. Similarly, accumulation of members of the family of site 5 sodium channel neurotoxins, the brevetoxins, by shellfish causes neurotoxic shellfish poisoning on ingestion in humans. The brevetoxins operate by disturbing normal membrane responses and causing prolonged activation of sodium channels. An assay based on the rescue of neuroblastoma cells treated with veratridine and ouabain (to block sodium efflux and inhibit sodium/potassium ATPase, respectively) by sodium channel blockers was initially developed[103] and modified to provide higher throughput.[104] Further modifications to this procedure have added sensitivity to sodium channel-activating compounds as well as channel blockers, and enabled a colorimetric readout based on the metabolism of a tetrazolium salt by mitochondrial dehydrogenase.[105] Assays such as this offer a distinct advantage over a simple binding assay by incorporating the functional activity of the compounds under investigation into the assay.

Assay Technologies Outlook

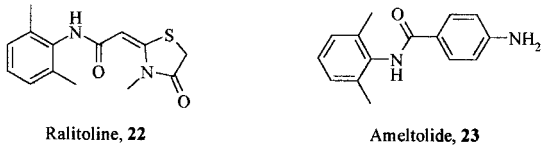
Over the last 5−10 years the potential therapeutic indications for compounds which modulate sodium channels has increased enormously, and crucially, some of these indications are in valuable markets which are currently poorly served. The corresponding pharmaceutical industry interest has seen an area which was previously mainly the domain of the electrophysiologist move into the world of high-throughput screening for drug discovery. This trend is set to continue as new channels are discovered and their physiological or pathophysiological roles characterized. In this regard it will be the development of techniques that allow the study of individual channel types which will be of most interest over the next few years.

Therapeutic Applications

In the past few years a much better understanding of sodium channels and drugs interacting with them has been developed. Particularly it has become clear that a number of drugs of previously unknown mechanisms of action actually act by modulating sodium channel conductance. These include local anesthetics such as procaine (**19**), class I antiarrhythmics such as lidocaine (**20**) and mexiletine (**21**), whose structural motifs have appeared in more recent drugs such as ralitoline (**22**) and ameltolide (**23**), and anticonvulsants such as carbamazepine (**24**) and phenytoin (**1**). In fact common structural motifs occur widely across the various sodium channel blockers in the literature, and attempts to identify these analogies will be made in several sections. The importance of drugs such as phenytoin and carbamazepine is that they enabled characterization of the role of sodium channels first in epilepsy, then in neuroprotection, and, more recently, in analgesia.



Procaine, **19** Lidocaine, **20** Mexiletine, **21**



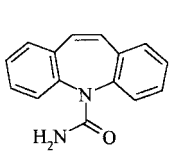
Ralitoline, **22** Ameltolide, **23**

The structural similarities between sodium, calcium, and potassium channels mean that selectivity is a serious issue. Many sodium channel blockers also block calcium channels and vice versa. Furthermore, to treat CNS-related diseases, any new drugs have to be significantly more active at neuronal sodium channels compared to those in cardiovascular tissue as well as demonstrating a lack of effect on normal action potentials. All these problems need to be considered in the successful development of any new sodium channel blocker.

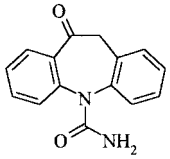
Most compounds described here have been applied to more than just one of the possible disorders related to sodium channels: i.e. certain types of epilepsy, several kinds of pain, neuroprotection, etc. Compounds with multiple functionality are described and discussed in the appropriate sections concerning the activity with which they are mainly associated or where the mechanisms of action are investigated most comprehensively.

Anticonvulsant Therapy. Epilepsy is a disorder of brain function characterized by recurrent seizures that have a sudden onset. For many years it was assumed that epilepsy could be treated with just one drug, but it is now apparent that this is not the case as more than one mechanism may be responsible for the various types of seizures. Although most seizures can be treated to some extent, 30% of all patients suffering from epilepsy do not respond to any current drug, either in monotherapy or in combinations.[106]

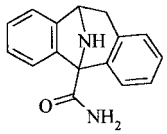
Neuronal sodium channel blockers found their first major application with the discovery of their potential as new treatments for epilepsy. The frontrunners of this development were phenytoin (**1**) and carbamazepine (**24**), both long established as anticonvulsants, developed without a clear understanding of their mechanism of action. Carbamazepine (**24**) has been examined extensively, and several derivatives[107] have been introduced for the treatment of epilepsy, such as oxcarbazepine (**25**), an analogue of an active metabolite of **24**, and ADCI (**26**). Similarly, development of phenytoin led to the identification of fosphenytoin (**27**, Cerebyx).



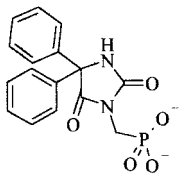
Carbamazepine, **24**



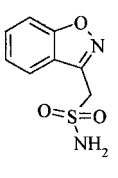
Oxcarbazepine, **25**



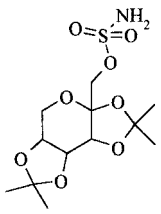
ADCI, **26**



Fosphenytoin, **27**



Zonisamide, **28**



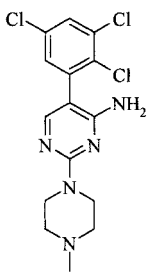
Topiramate, **29**

More recently, lamotrigine (**12**, marketed under the tradename Lamictal), zonisamide (**28**, Excegran), and topiramate (**29**, Topamax) have entered clinical use, and their modes of action suggest an involvement of sodium channel blockade. This assumption was supported by the similar behavior of these anticonvulsants and TTX (**2**) in rat hippocampal slices.[108] Of course, it is possible that other pharmacological actions may also play a role in their activity.

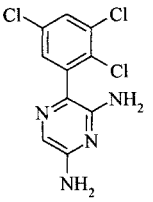
After this discovery more extensive studies have been carried out into the involvement of sodium channels in certain types of epilepsy to develop novel anticonvulsants. Particularly, displacement of tritiated BTX- and veratridine-induced release of neurotransmitters have been widely used as rapid in vitro assays. In rodents, these compounds and others prevent seizures from maximum electroshock (MES), and this model is widely in use as an in vivo assay for further evaluation of potent anticonvulsants.[109] Many of the current drugs suffer from various side effects, particularly sedation and neurological impairment. Some measure of these effects can be made using a rotarod assay to measure alertness and dexterity, the results from which can then be compared to the compounds' in vivo efficacy to generate a therapeutic ratio for a new compound.

Many analogues of phenytoin (**1**) have been synthesized in order to achieve better activity as well as selectivity. Most derivatives of phenytoin (**1**) share its hydantoin moiety or a bioisostere thereof. In recent years computational chemistry such as comparative molecular field analysis (CoMFA) has helped to build a model of the binding site of this structure.[110]

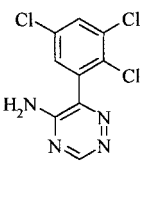
The phenyltriazine lamotrigine (**12**) appears to have been a particularly successful starting point with several analogues under development for a variety of indications. Studies on this compound have led to a series of related compounds, such as the phenylpyrimidine BW619C89 (**30**) (discussed in Neuroprotection). This compound shows improved side-effect liability, particularly reduced dihydrofolate reductase (DHFR) inhibition (IC_{50} rat liver DHFR > 100 μM),[131] which seemed to be a minor problem with comparable compounds. Analogous phenylpyrazines and phenylpyridines have also been interesting, leading to GW273293X (**31**),[129] GW261C89 (**32**),[130] and GW273295 (**33**). It will be interesting to monitor the development of this class of compounds over the next few years.



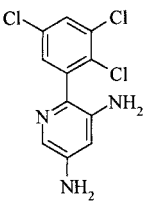
BW619C89, **30**



GW273293X, **31**



GW261C89, **32**



GW273295, **33**

Another series containing lipophillic aromatic rings connected to a group containing a basic nitrogen (the heterocycle in the case of **30**−**33**) is represented by CNS1237 (**34**).[111] This compound shows good in vitro anticonvulsant activity and sodium channel inhibition (IC_{50} [^{14}C]guanidine flux = 1.64 μM)[112] but has also been reported to be a calcium channel blocker. Both activities may contribute to its potent neuroprotectant activity. To avoid this dual mode of action, a whole family of conformationally constrained analogues of *N*,*N*′-diaryl- and *N*-aryl-*N*-arylalkylguanidines has been designed.[113] Structurally demanding substituents, i.e. aryl rings, as

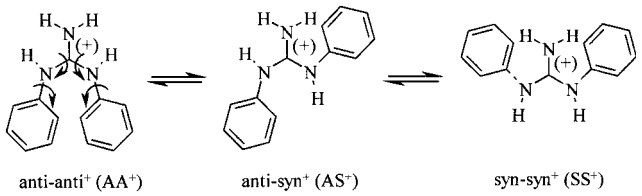
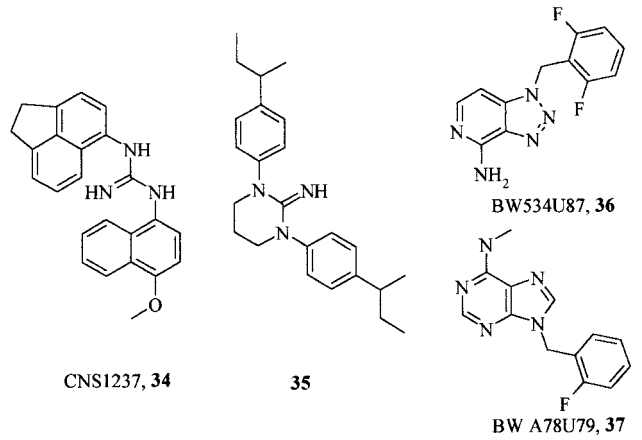


Figure 7. Conformations of *N*,*N*′-diarylguanidines. The arrows show rotation around key bonds.

well as ring closure around the guanidine group might force these compounds into a single conformation. Through selective changes several possible 'lockamers' (Figure 7) were synthesized and tested for sodium and calcium channel inhibition. The most active compound appeared to be 1,3-bis(4-*sec*-butylphenyl)tetrahydropyrimidin-2-ylideneamine (**35**), locked in the SS^+-conformation, with very good in vitro activity for sodium channel blocking (IC_{50} [^{14}C]guanidinium flux = 0.06 μM) but potency against calcium channels is retained (IC_{50} [$^{45}Ca^{2+}$] flux = 0.39 μM). However, its selectivity for sodium versus calcium channels was 17 times higher, a 3-fold improvement compared to its parent compound **34**.



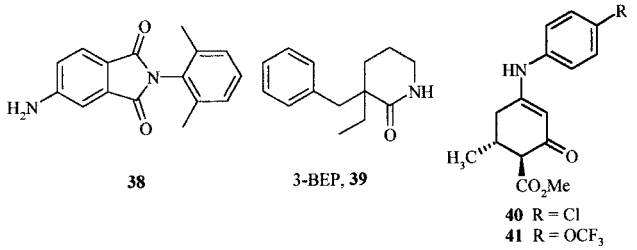
Similarly, BW534U87 (**36**) with its unique pyridinotriazole moiety, shows very good activity in a variety of assays against seizures.[128] The clinical development of an earlier compound in this series, BW A78U (**37**), had been abandoned due to emesis and nausea in phase I trials. Preclinical evidence indicates that **36** would be free of these side effects while retaining significant activity in the MES model of anticonvulsant activity.[128]

Ameltolide (**23**) is a potent anticonvulsant with structural similarities to lidocaine (**20**). It was first developed by Clark and co-workers in the early 1980s and further investigated by Eli Lilly. The 4-aminobenzamide unit was identified as a key structural feature in this compound. This information was built into a SAR with phenytoin and led to the design of hybrids between the two compounds, and one of these, 4-amino-*N*-(2,6-dimethylphenyl)phthalimide (**38**), shows increased activity both in vitro and in vivo (IC_{50} [^3H]BTX = 0.11 μM, MES ED_{50} = 6.7 mg/kg).[124]

In the early 1980s a series of γ-butyrolactones were synthesized as noncompetitive $GABA_A$ antagonists. Over the years, derivatives of the original structure with improved anticonvulsant activity have been developed, one of them being 3-BEP (**39**).[114] However, electrophysiological studies (holding potential of −60 mV; IC_{50} = 487 μM) revealed no enhanced activity against the GABA receptor, and therefore its mechanism of action remained unclear. **39** has now been found to modulate sodium channels in a use-dependent manner.[115] The anticonvulsant activity of an extensive series of enaminone esters such as ADD 196022 (**40**) has also been reported,[116] along with detailed SAR interpretations which led to the identification of **41** as a useful improvement on the initial leads in this series.[117]

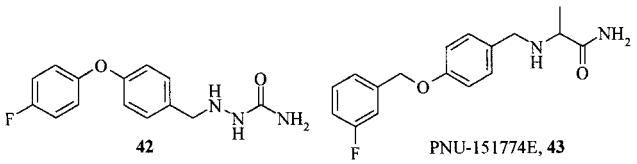
Table 3. In Vivo Activity of Some Anticonvulsants (administered ip or iv), Their Side-Effect Liability, and Additional Sites of Action

compd	additional targets	MES ED_{50} (mg/kg)	rotarod TD_{50} (mg/kg)	ref
ADCI (**26**)	NMDA receptor	8.9		123
ADD-196022 (**40**)		26.2	254.8	117
ameltolide (**23**)		13		124
AWD 140-190 (**47**)		2.5	>500	125
42		4.2		126
BIIR 561 CL (**46**)	AMPA receptor	2.8		127
BW534U87 (**36**)		6.3		128
carbamazepine (**24**)		3.4	27.4	107
GW273293X (**31**)		1.13	18.8	129
GW261C89 (**32**)		1.7	40	130
lamotrigine (**12**)		2.2	84	131
lidocaine (**20**)		87	41	132
mexiletine (**21**)		2		127
phenytoin (**1**)	calcium channels	10 (iv)	66	133
PNU-151774E (**43**)	calcium channels	4.1		134
45		18.1		135
ralitoline (**22**)		2.8		136
RP 66055 (**50**)		3.9		137
topiramate (**29**)	AMPA and GABA receptors	39		138
U-49524E (**51**)		35		139



Arylsemicarbazones have also shown good activity against epilepsy in in vivo seizure models,[118] although their mechanism of action was never completely studied. Recently, it was found that similarly substituted semicarbazides have comparable efficacy and furthermore seem to be acting by inhibition of sodium channels.[126] One of the most active of this series of compounds (Table 3) is 1-[4-(4-fluorophenoxy)benzyl]semicarbazide (**42**).

In the past few years several compounds have been developed from the classical anticonvulsant drugs in order to achieve a better therapeutic index. One of these is PNU-151774E (**43**),[119,120,134] a propanamide derivative developed from milacemide (**44**), with very good separation over neurological impairment.[135] On the basis of computational analysis, a hybrid between **43** and dextromorphan, a morphine-type anticonvulsant with so far uncertain mechanism of action, was synthesized in order to achieve greater rigidity. This hybrid (**45**) showed much better separation in the rotarod assay then dextromethorphan, although it does not seem to be as potent as **43**.



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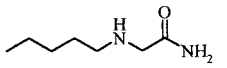
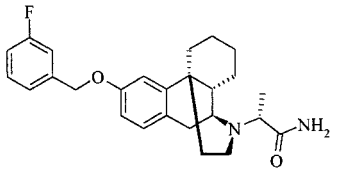
PNU-151774E, 43



Milacemide, 44



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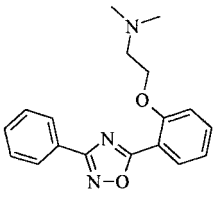
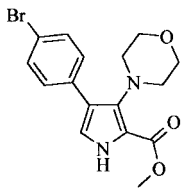
Antagonists acting on glutamate receptors such as the AMPA subtype also exhibit antiepileptic activity. Recently, a novel mixed sodium channel blocker/AMPA antagonist, BIIR 561 CL (**46**), has been described with promising anticonvulsive (Table 3) as well as neuroprotective properties (44% infarct reduction at 60 mg/kg in the middle cerebral artery occlusion model).[127] Although a single mechanism of action would be preferred, its unique structure with a 1,2,4-oxadiazole and trisubstituted amino group as a possible proton donor (under physiological conditions) makes it very attractive for further development.

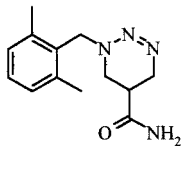
Another novel and potent sodium channel blocker with good anticonvulsant activity and weak activity in ataxia models such as the rotarod is AWD 140-190 (**47**), a 3-aminopyrrole derivative.[121,125] An extensive SAR study around AWD 140-190 has been undertaken, taking into account the structures of other blockers such as lamotrigine (**12**), carbamazepine (**24**), and rufinamide (**48**), resulting in another series, 3-amino- and 5-aminopyrazoles, being synthesized.[122] In particular, 4-chlorophenyl-3-(morpholin-4-yl)-1*H*-pyrazole (**49**) was active in the MES model (ED_{50} = 47 mg/kg ip) and showed no effect in the rotarod assay at 100 mg/kg. It will be interesting to follow these potent drug candidates into clinical trials. Finally, RP-66055 (**50**) and U-49524E (**51**) have also been shown to have sodium channel blocking and anticonvulsant activity (Table 3).



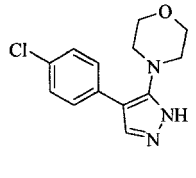
BIIR 561 CL, 46



AWD 140190, 47



Rufinamide, 48



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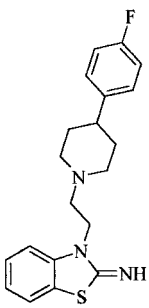
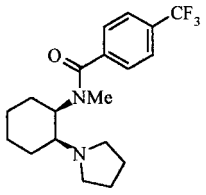


RP66055, 50



U-49524E, 51

Neuroprotection. Stroke and other brain injuries are major causes of mortality in the adult population for which no efficient therapy is presently available.[140] In the developed countries stroke is the third most common cause of death and is responsible for severe disabilities.[141] The only therapy generally licensed for the treatment of ischemic stroke is Genentech's thrombolytic recombinant tissue plasminogen activator (rt-PA; Activase). Unfortunately, this therapy is complicated, expensive, and only effective against certain types of stroke. A widely applicable medical treatment for acute stroke would have an enormous public health impact.

Although the mechanisms causing irreversible brain damage after ischemia are not fully understood yet, there is now substantial evidence from animal models that sodium channel blockers are capable of neuroprotection.[5] The findings that TTX alone significantly decreases the infarct area (the area of tissue damaged by an ischemic episode) in several models suggest an important role of voltage-sensitive sodium channels in global (whole brain) and focal (local) ischemia.

The development of a model of the ischemic cascade (Figure 8) has led to a far better understanding of the complex nature of cellular damage following stroke or other brain trauma and the involvement of changes in cellular ion homeostastis.[142] It is now generally accepted that hypoxic (oxygen-deprived) neurons are rapidly depolarized and thus sodium channels and other voltage-sensitive mechanisms such as calcium and potassium channels are activated. High levels of intracellular calcium lead to release of glutamate and other excitatory amino acids that in turn activate AMPA and NMDA receptors to allow even more sodium and calcium ions pass through the cell membrane. However, it may be that glutamate release during ischemia is fundamentally different from the normal physiological vesicular mechanism. Some studies indicate the relative independence of glutamate release from calcium levels and suggest the mediation of a sodium-dependent glutamate transporter.[143,144] Nevertheless, continued sodium influx elevates cytosolic sodium concentration to abnormally high levels, causing detrimental changes such as reversal of sodium/calcium exchange, bulk inflow of chloride, and changes in neurotransmitter transport. Eventually, all these events activate further calcium-dependent intracellular processes, leading to depletion of ATP stores and cell death.[5]

This biochemical pathway describes the role of sodium and sodium channels during hypoxic depolarization and helps to explain the neuroprotective properties of neuronal sodium channel blockers. Of course, other neuroprotective therapies have been developed each targeting different mechanisms of the ischemic cascade, particularly NMDA-coupled ion channel blockers and excitatory amino acid antagonists (Table 4), although the latter group has been hampered by dose-limiting side effects.[145] However, cerebroprotective sodium channel blockers could have some substantial advantages, making them more attractive as new agents for the treatment of stroke and other brain trauma. Such compounds would act more effectively during conditions of cellular depolarization sustained for seconds (use-dependency). This should result in the compounds having little effect

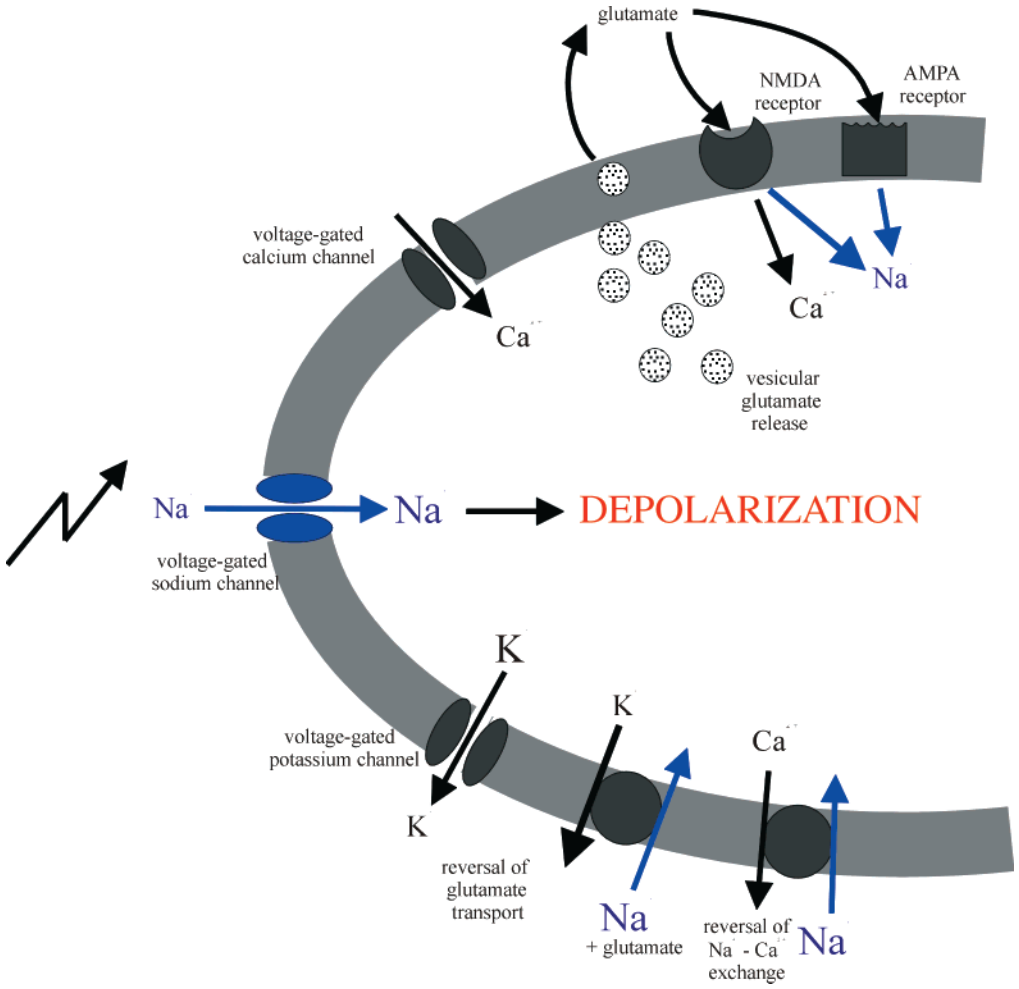
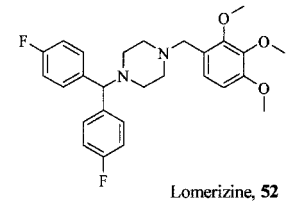


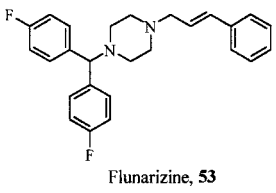
Figure 8. Neurotoxic cascade in ischemic brain injury.

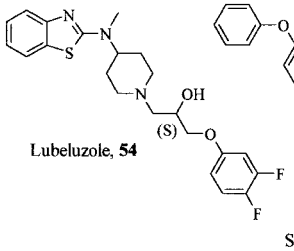
on normal neuronal signaling but allow the blockade of sodium channels during pathological conditions such as seizures or ischemia. Furthermore, sodium channel blockers also prevent injury from hypoxia in isolated optic nerve, a model of ischemic white matter damage in the brain,[146] while glutamate antagonists only protect gray matter.

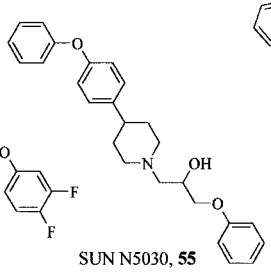
The most common animal model for ischemia is the rat middle cerebral artery occlusion (MCAO) model of focal stroke.[147,148] In this model compounds are tested for their ability to reduce the size of the damaged area following interrupted blood flow to the brain (Table 5). Most of these sodium channel blockers are very selective and do not show significant cardiovascular activity, although their ability to cause ataxia (as measured in the rotarod model) is considerable. However, the extent of neuroprotection in models of focal ischemia seen with phenytoin (**1**), lamotrigine (**12**), or BW619C89 (**30**) is certainly comparable to or better than that seen with noncompetitive NMDA antagonists or other treatments for stroke.[5]

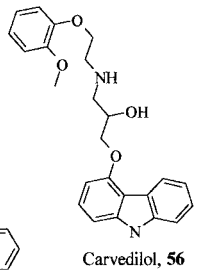
There are several promising sodium channel blockers currently in clinical trials for the acute treatment of cerebral stroke (Table 4). Piperazines such as lifarizine (**13**) and its analogues lomerizine (**52**) and flunarizine (**53**) are showing promise, as are a family of compounds with a β-blocker-like core such as lubeluzole (**54**), SUN N5030 (**55**), and carvedilol (**56**). Like lubeluzole, SUN N5030 is a mixed calcium/sodium channel blocker, although it is lacking the significant dopamine D_2 receptor affinity which commonly occurs in this class of structure.[149]


Lomerizine, **52**


Flunarizine, **53**


Lubeluzole, **54**


SUN N5030, **55**


Carvedilol, **56**

One of the most recently reported sodium channel blockers is a benzazocine structure identified by a group at Boehringer Ingelheim. This compound, BIII-890-CL (**57**), is already in preclinical trials. Interestingly, the authors could not find any significant affinity

Table 4. Compounds in Development for the Treatment of Stroke[a]

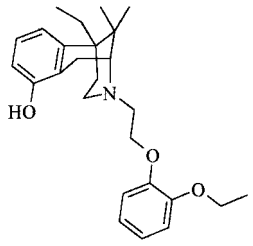
compd	mechanism	company	status
idebenone	antioxidant	Takeda	launched (Japan)
enlimomab	WBC adhesion blocker	Boehringer Ingelheim	launched
cerestat	noncompetitve NMDA antagonist	Cambridge Neuroscience	phase III
chlormethiazole	$GABA_A$ agonist	Astra	phase III
citicholine	phospholipid/membrane stabilizer	Interneuron	phase III
GW150526A (gavestinal)	NMDA antagonist	GlaxoWellcome	phase III
lubeluzole (**54**)	NOS inhibitor	Janssen	phase III
	sodium channel blocker		
BAYx3702	5-HT_{1A} agonist	Bayer	phase III
trazec	competitive NMDA antagonist	Novartis	phase III
ARL 15896AR	noncompetitive NMDA antagonist	Astra	phase II
raxofelast	antioxidant	Biomedica Foscama Industria	phase II
remacemide (**60**)	NMDA antagonist	Astra	phase II
	sodium channel blocker		
CPC-211	glutamate reduction	Cypros Pharmaceuticals	phase II
MS-153	glutamate reduction	Mitsui	phase II
pro-urikinase	fibrinolytic	Neurex	phase II
trofermin	BFGF ligand	Scios Nova	phase II
pergorgotein	PEG-SOD	Sterling-Winthrop	phase II
lifarizine (**13**)	calcium and	Syntex	phase II
	sodium channel blocker		
BW619C89 (**30**)	**sodium channel blocker**	CeNeS	phase II
MCI-186	antioxidant	Mitsubishi	phase II
CPI 1233	antioxidant	Centaur/Astra	phase I
NPC 17742	competitive NMDA antagonist	Guildford	phase I
propentiofzilline	adenosine reuptake blocker	Hoechst Marion Roussel	phase I
BTS-72-664	$GABA_A$ ligand	Knoll	phase I
NPS-1506	NMDA antagonist	NPS Pharma	phase I
NIF	antiinflammatory	Pfizer	phase I
RG2716	novel	Richter/Takeda	phase I
unknown	AMPA antagonist	Schering AG	phase I
SB-21742	endothelin antagonist	SmithKline Beecham	phase I
YM 90K	AMPA antagonist	Yamanouchi	phase I
ACEA 1021	NMDA glycine antagonist	ACEA Pharmaceuticals	phase I
BIII-890-CL (**57**)	**sodium channel blocker**	Boehringer Ingelheim	phase I

[a] Sodium channel blockers are highlighted in bold type.

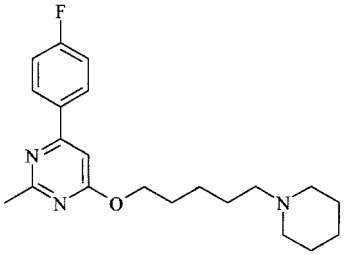
Table 5. Minimum Effective Doses (MED) for Selective Sodium Channel Blockers in the MCAO Model and Their Side-Effect Liability in the Rotarod Assay When Administered ip or iv

	MED (mg/kg)		
compd	neuroprotective	rotarod	ref
BIII-890-CL (**57**)	2 × 0.3 (rat)		150
BW1003C87 (**59**)	20 (rat)		155
BW619C89 (**30**)	30 (rat)	60	156
carbamazepine (**24**)	2 × 50 (rat)		137
CNS1237 (**34**)	30 (gerbil)		157
lamotrigine (**12**)	20 (rat)		158
lubeluzole (**54**)	0.3 (rat)		159
NS-7 (**58**)	0.5 (rat)		151
phenytoin (**1**)	2 × 100 (rat)		137
remacemide (**60**)	10 (rat)	100	156
riluzole (**11**)	4 (rat)	30	156
RP66055 (**50**)	2 × 8 (rat)		160
SUN N5030 (**55**)	3 (rat)		149
zonisamide (**28**)	100 (rat)		161

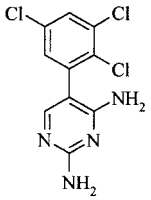
toward NMDA receptors even though similar compounds show activity in that field. **57** replaces BTX, blocks sodium channels use-dependently when studied by patch-clamp, inhibits veratridine-induced glutamate release, and shows neuroprotection in the MCAO model.[150] Another recent example of a potent sodium channel blocker is NS-7 (**58**)[151] with the more familiar structure of a pyrimidine core unit, as found in BW619C89 (**30**) and BW1003C87 (**59**). **58** shows good activity against veratridine-induced glutamate release and also high affinity in BTX binding with a good selectivity (13-fold) over cardiac sodium channels (Table 6). In the MCAO model NS-7 (**58**) reduced the infarct area significantly.[152] Surprisingly this effect was seen in the cerebral cortex but not in striatum.



BIII-890-CL, **57**



NS-7, **58**



BW1003C87, **59**

As mentioned earlier (see Neurotoxin Binding Sites) several neurotoxins have binding sites on sodium channels. TTX (**2**) and saxitoxin (**3**) are two examples, both blocking the channel voltage independently which makes them unusable as drugs. However, they are useful tools for drug discovery because of their specifity for sodium channels. Through site-directed mutagenesis the bind-

Table 6. Activity of Various Sodium Channel Blockers Against Veratridine-Induced Guanidine Flux and [^{3}H]BTX Binding

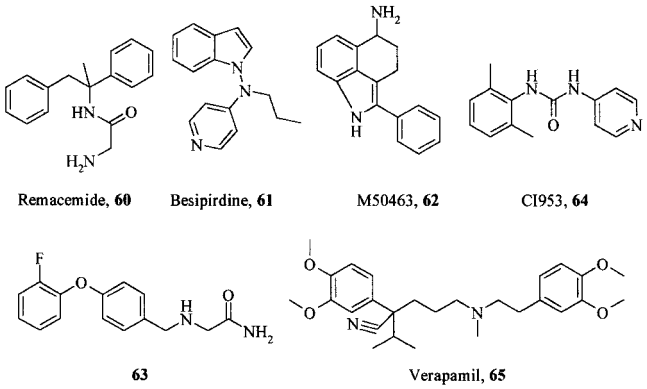
compd	veratridine block (IC_{50}, μM)	BTX binding (IC_{50} or K_i, μM)	ref
besipirdine (**61**)	30 (vs Na rel)	5.5	162
BW1003C87 (**59**)	1.6 (vs Glu rel)		155
BW619C89 (**30**)	5.3 (vs Glu rel)		163
carbamazepine (**24**)	30 (vs Glu rel)	131	164, 165
carvedilol (**56**)	0.306 (vs cell death)		166
	1.7 (vs Asp rel)		
CI 953 (**64**)		29	167
flunarizine (**53**)	0.12 (vs cell death)	0.35	168
lamotrigine (**12**)	21 (vs ion flux)	114	42, 169
			170
lidocaine (**20**)	97 (vs ion flux)	113, 265	70, 171
lifarizine (**13**)	0.3 (vs cell death)	0.119	89
lomerizine (**52**)		0.026	172
lubeluzole (**54**)		0.28	153
M50463 (**62**)	4.1 (vs Glu rel)	1.1	153
mexiletine (**21**)		18	173
PD 85639 (**14**)	5 (vs cell death)	0.046	132, 91
NS-7 (**58**)	7.7 (vs Glu rel)	1	151
phenytoin (**1**)	21 (vs Glu rel)	40	169, 164
ralitoline (**22**)		25	167
riluzole (**11**)	4.0 (vs ion flux)		174
SUN N5030 (**55**)	0.22		149
verapamil (**65**)	2.5 (vs cell death)	2.5	168

ing site of these two neurotoxins has been established as being at the mouth of the channel. This is probably due to their highly charged guanidinium moiety, mimetics of which are present in some small-molecule sodium channel blockers such as lamotrigine (**12**) and its analogues.

Other neurotoxins, such as veratridine (**6**), a lipid-soluble alkaloid, alter channel activation and inactivation by binding to the intracellular side of the channel. **6** has been used as a selective high-affinity probe of the binding of several drugs such as flunarizine (**53**), PD 85639 (**14**), or besipirdine (**61**), which act on voltage-dependent sodium channels. In a similar fashion BTX (**4**) influences sodium channel functionality and therefore is a useful probe for in vitro studies of sodium channel inhibition. Both of these neurotoxins have been used as tools for in vitro assays to screen potential compounds as sodium channel blockers (Table 6).

One of the more recently explored sodium channel blockers found using the procedures described above is M50463 (**62**).[153] This compound, a tetrahydrobenzindole, does not relate to any of the known sodium channel blockers. **62** displaces tritiated BTX (**4**) and also prevents veratridine-induced glutamate release. A group of scientists at CoCensys Inc. have recently published[154] data showing another new class of compounds, one of them being 4-(2-fluorophenoxy)benzylaminoacetamide (**63**). These are potent sodium channel blockers, shown by binding assays at site 1 (displacing tritiated saxitoxin) and at site 2 (displacing tritiated BTX), respectively, as well as by patch-clamp recordings, and are clearly close analogues of the semicarbazides such as **42**. Another aminoacetamide, remacemide (**60**) also shows activity in the MCAO model of neuroprotection, while the pyrdinylurea CI 953 (**64**) and verapamil (**65**) are inhibitors of BTX binding.

Head Trauma. The necrosis that follows a blow to the head appears to develop as a result of an interruption in blood flow causing edema (large amounts of fluids in the intercellular tissue spaces) and ischemia.



Several sodium channel blockers including BW619C89 (**30**),[175] BW1003C87 (**59**),[155] and riluzole (**11**) have shown activity in animal models of head trauma such as the rat fluid percussion model.[176] Although few other sodium channel blockers have been tested in this model, it is very likely that this activity will be a general feature of this class of drugs.

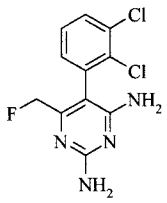
Amyotrophic Lateral Sclerosis. Amyotrophic lateral sclerosis (ALS; a motor neuron disease) is a neurodegenerative disease for which sodium channel blockade has been suggested as a possible treatment. ALS causes gradual death of spinal and neocortical motorneurons, resulting in paralysis of skeletal muscles and death. Afflicted patients die from respiratory failure within 3−5 years of diagnosis. Until recently, no efficient treatment was available for ALS. However, riluzole (**11**) is now approved for this indication by the FDA (U.S.A. Federal Drug Administration) under the tradename Rilutek. Although **11** has many pharmacological actions, such as blocking of glutamate release,[177] reduction of nonvesicular (calcium-independent) transmitter release,[5] blocking of potassium channels,[178] inhibition of certain NMDA-dependent responses,[179] and reduction of neurotransmitter release in a pertussis toxin-sensitive manner,[180] it appears that neuroprotection in ALS arises primarily from sodium channel blockade. Riluzole (**11**), as well as TTX (**2**), prevented neurodegeneration in vitro in cultured spinal cord neurons and in vivo in a transgenic mouse model, both established to mimic ALS.[181] Clinical studies with riluzole (**11**) for the treatment of ALS are showing promising results, and it is likely that other sodium channel blockers will also be investigated for this condition.[182,183]

Analgesia. Neuropathic pain is believed to be caused by nerve injury after inappropriate impulse generation within injured axons and their corresponding DRG neurons. This process has been shown to be dependent on sodium channels, and several sodium channel blockers reduce pain from trigeminal neuralgia, diabetic neuropathy, post-herpetic neuralgia, neuroma pain, and phantom limb pain. In particular, carbamazepine (**24**) was originally approved in the United States for the treatment of trigeminal neuralgia before it was used against epileptic seizures. Other anticonvulsants such as lidocaine (**20**), phenytoin (**1**), and lamotrigine (**12**) have also been found to be useful in the treatment of neuropathic pain or are active in animal models of this disorder. Significant progress is expected from these

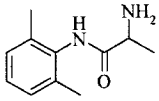
types of compounds, particularly with regard to their side-effect liability.

An interesting fact about the properties of sodium channel blocking analgesic agents is their ability to give relief from pain over longer periods than might be expected. This might be due to the drugs' interruption of neuronal hyperexcitability in pain-sensitive neurons, decreasing the inflammation caused by neuropathic pain and abnormal pain sensations. These neurons have a high proportion of TTX-insensitive (TTXi) sodium channels that show slightly different properties to those in the brain. Hyperalgesic agents such as prostaglandin E_2 (PGE_2) and serotonin seem to stimulate expression of TTXi channels and also increase their excitability, indicating a modulation of TTXi channels directly by inflammation. This type of sodium channel makes an ideal target for a selective sodium channel blocker to treat pain.

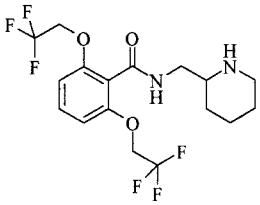
Lamotrigine (**12**) in particular has been shown to act on TTXi channels and therefore has been used as a lead structure in this area. Based upon its triazine moiety, a novel compound, GW4030W92 (**66**), has been described in the literature as a voltage- and use-dependent sodium channel inhibitor[184] with potential for the treatment of chronic inflammatory[185] and neuropathic pain.[186] Besides blocking TTXi channels, **66** also inhibits TTX-sensitive channels, recorded from rat DRG small diameter neurons in vitro, showing a preferential affinity for the slow inactivated state of the channel. Interestingly, GW4030W92 (**66**) possesses a stereocenter due to restricted rotation around the two aromatic rings with the *R*-(−)-conformation being preferred. The opposite enantiomer appears to be 20 times less active in models of acute and chronic pain.[187]



GW4030W92, **66**



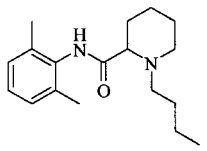
Tocainide, **67**



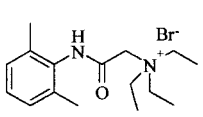
Flecainide, **68**

Although local anesthetics have only had restricted use in acute pain, primarily due to safety concerns over potential CNS and cardiac toxicity, they give much more promising results in chronic pain, especially for syndromes which are unresponsive to treatment with standard opiates and nonsteroidal antiinflammatory drugs (NSAIDs).[188] Several studies indicate that local anesthetics such as lidocaine (**20**), tocainide (**67**), and flecainide (**68**) are most effective against neuropathic pain originating in the peripheral nervous system rather than in the CNS[189] and that their activity arises from sodium channel blockade.

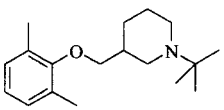
In clinical studies lidocaine (**20**) has been successfully applied topically to relieve spontaneous, ongoing, and stimulus-evoked pain,[190−192] and similar in vivo results have been achieved with bupivacaine (**69**)[193,194] and QX-314 (**70**),[195] a quaternized derivative of **20**. This evidence gave rise to the assumption that sodium channels located in peripheral sensory neurons might be responsible for several types of acute pain and therefore make an ideal target for selective drugs.



Bupivacaine, **69**



QX-314, **70**



71

The class Ib antiarrhythmic drug mexiletine (**21**) shows similar activity in in vivo models and, indeed, has been successfully used as an analgesic both in monotherapy and following an initial lidocaine (**20**) infusion in the clinic. The core structure of **20**, a phenoxypropanamine, can also be found in a recently reported sodium channel blocker developed by a group at Hoffmann-La Roche.[196] These phenoxymethylpiperidine derivatives (**71**) have been reported to be active in both in vitro and in vivo assays for the treatment of chronic neuropathic pain. However, the promising activity of local anesthetics and class I antiarrhythmics against chronic pain is still tempered by a poor side-effect profile in chronic pain, and it remains to be seen whether a successful drug will emerge from this class of compound.

Pharmacophore Modeling

It is quite clear from the preceding sections that there are several common structural motifs which occur on a regular basis in sodium channel blockers. It is also becoming increasingly clear that the same motifs can often give activity at other ion channels, particularly calcium channels. In some instances this can be advantageous: blockade of excitatory sodium and calcium channels in the treatment of reperfusion injury following stroke for example. The downside to this, however, is that where more precise modulation of particular channel populations is required, for example in the treatment of neuropathic pain, the correct selectivity may not be available. In addition, we sometimes need to re-interpret pharmacological (and clinical) data in the light of newly discovered channel activities of old drugs.

There have been several attempts to create SARs for discrete classes of sodium channel blockers, but it is only recently that attempts to compile models from diverse classes of compounds into a pharmacophore have been reported. The advantages of such an approach are many; a better understanding of the existing data would be useful, but the real value of such a model would be as a predictive tool in the design of better compounds. However, such an approach is always fraught with difficulties, and in this case the complexity of the target protein is the main problem. We have already outlined the diversity of binding sites discovered for sodium channels, and it is also clear that many of these sites share allosteric interactions so common radioligand displacement data does not always indicate a common binding site for different groups of compounds. Consequently the value of any derived pharmacophore depends entirely on whether the structures chosen to derive it share an identical binding site.

Phenytoin and its analogues have been comprehensively investigated for structural features predictive of sodium channel blocking activity. A strong influence of log *P* on the activity of this series,[197] as well as a sensitivity to intracellular pH,[198] has been reported.

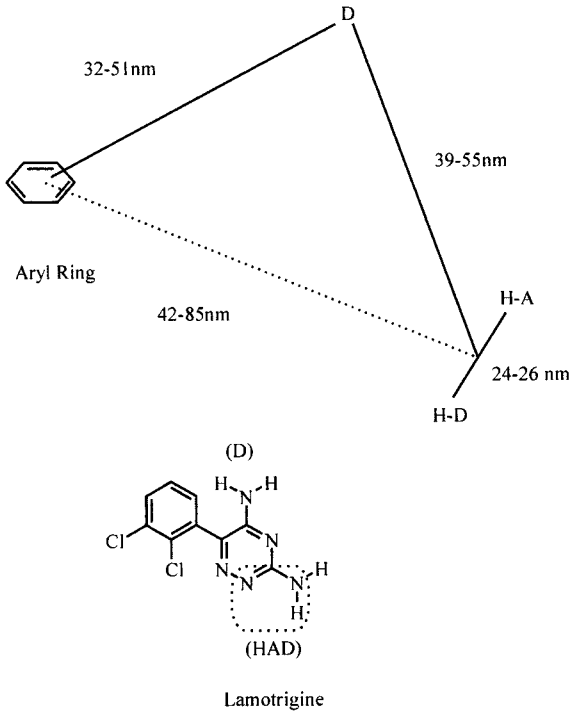
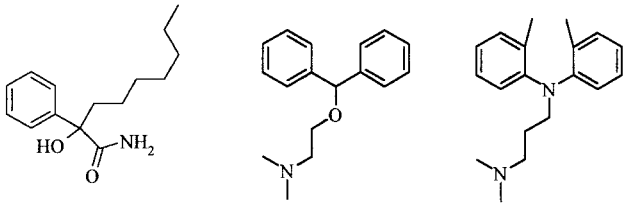


Figure 9. Pharmacophore model of the interaction of several known anticonvulsants with a common sodium channel binding site. This pharmacophore incorporates an aromatic ring with a hydrogen bond donor (H-D) moiety and a separate hydrogen bond donor/acceptor (H-A and H-D) group, as illustrated for lamotrigine (**12**).

Further development in the form of a CoMFA study led to a three-dimensional model of the optimum properties associated with the binding of hydantoins. This model was then used to successfully predict the activity of a test set and then to design a novel blocker (**72**) whose activity was confirmed following synthesis.[199]

A molecular dynamic simulation with AWD 140-190 (**47**) and several known anticonvulsants such as carbamazepine (**24**), phenytoin (**1**), and zonisamide (**28**) also led to the development of a pharmacophore model (Figure 9). All these molecules have at least one aryl ring, one electron donor atom, and a second donor atom in close proximity to the NH group, forming a hydrogen bond acceptor/donor unit. Further investigation into the conformation of these structures gave a distance relation between the selected structural elements, and this was used to explain the activity of the morpholinopyrroles being investigated, as well as giving a good fit with other channel blockers such as remacemide (**60**).[125]



72 Diphendryamine, **73** Imipramine, **74**

An additional complication to the development of pharmacophore models is the way in which changes in membrane potential affect the efficacy of different classes of drugs to different extents. Thus some drugs (phenytoin, carbamazepine) block channels more effectively as the membrane depolarizes, whereas others such as diphenhydramine (**73**) and imipramine (**74**) have some activity even against hyperpolarized channels.[200] It is clear from this work that the only common feature between a large set of sodium channel blockers is the presence of two lipophillic groups and a hydrogen bond donor. It is also clear that many commonly prescribed drugs conform to this model and might be expected to have effects on sodium channels in addition to the target for which they were developed. Indeed several members of the histamine H1 antagonist group of drugs have shown sodium channel blocking activity.[201]

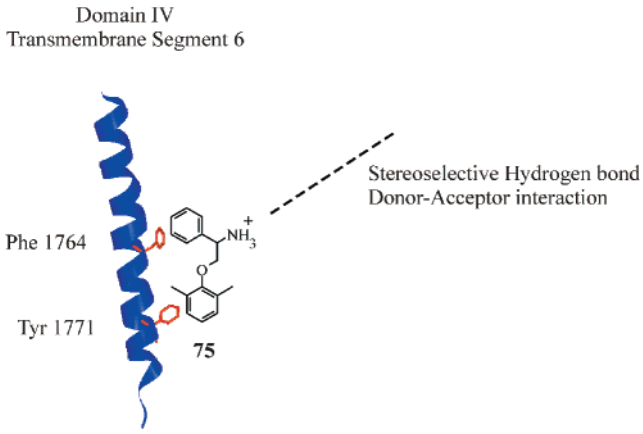


Figure 10. Model of the interactions of mexiletine analogues with the DIV S6 segment of the neuronal Type II sodium channel.

One clue to how such a simple pharmacophore might arise is given in a recent report outlining SARs for a series of analogues of the local anesthetic mexiletine (**21**).[202] In this study replacing the methyl group in **21** with a phenyl group (to give **75**) gave improved channel blocking activity. The authors propose a model in which the two aromatic rings of **75** bind to Phe-1579 and Tyr-1586 in the skeletal muscle sodium channel investigated, analogous to Phe-1764 and Tyr-1771 in the brain Type II channel (Figure 10). Crucially, these are the two residues found in earlier work to reduce the affinity of local anesthetics when mutated.[42] Also of interest is the observation of a stereoselective role for the amino group in the mexiletine analogues, indicating that this substituent is being recognized by the protein. A basic (protonated at physiological pH) amino group is present in the vast majority of compounds found to have activity at sodium channels.

In conclusion, we now have a working model, at least in the case of local anesthetics, of both the basic structure required for activity and how this may interact with the target protein. The next big development in this area will probably be detailed structural information for the protein, either through crystallization studies or molecular modeling of relevant segments, allowing much more accurate design of small-molecule blockers.

Therapeutic Outlook

Despite the remarkable progress that has been achieved in our knowledge of the pharmacology and physiology of sodium channels, providing a closer insight into channel gating, cycling, subtype distribution, and binding affinities, the most prescribed drugs are still

phenytoin (**1**), carbamazepine (**24**), lamotrigine (**12**), and riluzole (**11**). These compounds represent the first generation of sodium channel blockers. To date, by far the best progress, with several compounds in the later stages of clinical trials or even on the market, has been made in the area of anticonvulsants. The family of 'lamotrigine-like' compounds, such as GW273293X (**31**), GW261C89 (**32**), or GW273295 (**33**), is just one example of the improvements to be gained through the development of first-generation compounds. Furthermore, the development of a pharmacophore model based on a whole series of known anticonvulsants, including AWD 140-190 (**47**), as well as structural analysis of several *N*-alkyl-*N*′-aryl- and *N*,*N*′-diarylguanidines have enhanced the knowledge of drug interactions with sodium channels.

Ischemic brain injury is a fairly common cause of death in the developed world, and it has emerged over the past few years that voltage-gated sodium channels play a major role in its aetiology. Research on neuroprotective sodium channel blockers is a new field, which is receiving increasing interest. Furthermore the participation of sodium channels at the start of the neurodegenerative cascade and the acceptable side-effect profile resulting from their selective blockade make them an attractive target. Again, compounds such as phenytoin (**1**), lamotrigine (**12**), and riluzole (**11**) are the frontrunners, but a new generation of more selective and active drugs has been developed, most notably lubeluzole (**54**), BW619C89 (**30**), and lifarizine (**13**), which are already in later stages of clinical trials. Also RP66055 (**50**), SUN N5030 (**55**), BIII-890-CL (**57**), NS-7 (**58**), and BW1003C87 (**59**) are giving good results in in vivo tests.

The early groups of sodium channel blockers have provided the tools to establish a role for these channels in many disorders and act as starting points for the development of new drugs. Lamotrigine (**12**), originally designed as an anticonvulsant but found to have analgesic activity, led to GW4030W92 (**68**), with an improved profile, for the treatment of different types of neuropathic pain. Structural analogues of the antiarrhythmic mexiletine (**21**) have been found to be local anesthetics, while others, such as lidocaine (**20**) and its analogues, are analgesics.

The missing part of the equation in the development of sodium channel blockers for new indications is the extent the selectivity of their action needs to be considered. Clearly a lack of effect on basal sodium conductance is essential to prevent loss of motor control. But is selectivity for neuroprotective potential over analgesic potential a requirement? Is it important for anticonvulsants to be neuroprotective? Or should they be selective in their activity? In this regard the sodium channel area as a source of therapeutic targets is in its infancy; we do not have enough information on the physiological and pathophysiological roles of the different channel subtypes to make decisions on selectivity requirements at the molecular level. At the moment we can only decide on preferred profiles of activity on the basis of clinical observation. This is fraught with complications, however, as many sodium channel blockers have additional targets such as calcium channels which serve to confuse our interpretation of their clinical profile. There is a hierarchy of selectivity, which is only just beginning to be addressed in this area. Once selective sodium channel blocking activity is achieved (compared to other ion channels or receptors) − many of the compounds discussed here fail at this first hurdle − it is necessary to evaluate selectivity within the different groups of sodium channels (cardiac, skeletal, and neuronal). Few studies on these compounds have addressed this basic point, and it is highly likely, given the homology between these channel families, that many of the reported compounds are poorly selective even at this level. Even further selectivity, between individual members of these channel families, will probably turn out to be desirable for a truly selective therapeutic endpoint.

Conclusion

Over the last 10 years neuronal voltage-gated sodium channels have come under scrutiny as the target for an increasing number of therapeutic endpoints. The usefulness of first-generation drugs for indications such as anticonvulsant therapy has been significantly improved upon with new compounds having better selectivity and side-effect profile. This trend will continue as our understanding of these channels increases and more single-channel-expressing cell lines become available. More interesting is the way in which sodium channel block as a means of achieving neuroprotection has become established; over the next few years we will see new drugs being identified and developed in this area. The success of such agents will probably depend on changes in the way stroke patients are dealt with by the emergency services before the benefit of such drugs is fully realized. A more straightforward therapeutic endpoint is the treatment of painful neuralgic and other hyperalgesic conditions with sodium channel blockers. This can possibly be achieved much more quickly through the additional development and licensing of exisiting compounds and their analogues. In conclusion, in the past decade neuronal voltage-gated sodium channels have emerged as useful targets in medicinal chemistry and much progress toward new therapies can be expected over the next few years.

Biographies

Thorsten Anger received his Ph.D. in organic chemistry from Göttingen University, Germany, in 1995 and completed postdoctoral training at York University, England. He joined the Wolfson Institute for Biomedical Research in 1998 and is currently a Senior Research Fellow in the Department of Biological and Medicinal Chemistry.

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Mushtaq Mulla received his M.Sc. in organic chemistry from Bangor University, Wales, in 1997. He joined the Wolfson Institute for Biomedical Research in 1998 and is currently a Research Fellow in the Medicinal and Biological Chemistry Department.

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