MEETING REPORT

Highlights of the Society for Medicines Research Symposium held on September 27, 2001, in London.

The Role of Sodium Channels in Disease

by Alan M. Palmer and Nick Carter

Summary

Native sodium channels exist as polypeptide multimers of an α-subunit (260 kDa) and subsidiary and smaller β-subunits, which are divided into at least three subtypes—β_1, β_2 and β_3. The α-subunits are structurally diverse, arising from multiple sodium channel genes and alternative splicing events. Recent progress has led to a good understanding of the molecular structure of sodium channels, how they work and the significance of their expression in particular cell types. This, coupled with experimental studies linking particular isoforms with particular disease states and the discovery of distinct human sodium channelopathies (specific mutations in specific isoforms that cause a variety of diseases, including paralysis, long QT syndrome and epilepsy), is beginning to reveal how particular sodium channel subtypes underlie specific pathologies. All this provides great potential for the development of new therapies. The first generation of sodium channel blockers has led to a broad-spectrum anticonvulsant that is now widely used (lamotrigine) and an impressive neuroprotective agent that is in clinical trials for stroke (sipatrigine). The development of the next generation of sodium channel blockers will be greatly facilitated by elaboration of the pharmacology of the various isoforms, which itself is dependent upon the existence of reliable, rapid and high-throughput assays for sodium channel activity.

The Society for Medicines Research symposium *The Role of Sodium Channels in Disease* was held at the National Heart and Lung Institute of Imperial College of Science, Technology and Medicine on September 27, 2001. The meeting focused on the role of sodium channels in disease and was organized by Alan M. Palmer (Vernalis, Wokingham, U.K.) and Nick Carter (St. George's Hospital Medical School, London, U.K.), who chaired the proceedings together with David Cavalla (Arachnova, Cambridge, U.K.). The speakers equally represented academia and industry and were from both the United Kingdom and the United States.

Sodium channel activity is intrinsic to neurotransmission and, therefore, sodium channels have a central role in normal neuronal function. Sodium channels are highly selective molecular pores, the opening and closing of which shape membrane potential changes and give rise to characteristic action potentials. Overactivation of sodium channels can lead to neuronal dysfunction. Thus, the depolarization of plasma membranes that occurs in acute brain injury (such as stroke and traumatic brain injury) or overstimulation of certain neurons (such as that occurring in chronic pain states) leads to excessive sodium channel activity.

The pharmacology of sodium channels is currently very rudimentary, since they are distinguished on the basis of sensitivity to a toxin derived from puffer fish, tetrodotoxin. It is now known that native sodium channels exist as polypeptide multimers, comprising a large (260 kDa) α-subunit and smaller (33–36 kDa) modulatory β-subunits (there are at least three different subtypes: β_1, β_2 and β_3). The

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TABLE I: MAMMALIAN SODIUM CHANNEL α-SUBUNITS

TYPE	FORMER NAME	GENBANK NO.[a]	GENE SYMBOL	CHROMOSOMAL LOCATION	SPLICE VARIANTS	PRIMARY TISSUES
$Na_v1.1$	Rat I	X03638 (r)	SCN1A	Mouse 2	$Na_v1.1a$	CNS
	HBSCI	X65362 (h)		Human 2q24		PNS
	GPBI	AF003372 (gp)				
	SCN1A					
$Na_v1.2$	Rat II	X03639 (r)	SCN2A	Mouse 2	$Na_v1.2a$	CNS
	HBSCII	X61149 (r)		Human 2q23-24		
	HBA	X65361 (h)				
		M94055 (h)				
$Na_v1.3$	Rat III	Y00766 (r)	SCN3A	Mouse 2	$Na_v1.3a$	CNS
				Human 2q24	$Na_v1.3b$	
$Na_v1.4$	SkM1, μ-1	M26643 (r)	SCN4A	Mouse 11		Skeletal muscle
	M81758 (h)	Human 17q23-25				
$Na_v1.5$	SkM2	M27902 (r)	SCN5A	Mouse 9		Uninnervated skeletal muscle, heart
	H1	M77235 (h)		Human 3p21		
$Na_v1.6$	NaCh6	L39018 (r)	SCN8A	Mouse 15	$Na_v1.6a$	CNS, PNS
	PN4	AF049239 (r)		Human 12q13		
	Scn8a	AF049240 (r)				
	CerIII	U26707 (m)				
		AF049617 (m)				
		AF050736 (h)				
		AF225988 (h)				
		AF003373 (gp)				
$Na_v1.7$	NaS	U35238 (rb)	SCN9A	Mouse 2		PNS
	hNE-Na	X82835 (h)		Human 2q24		Schwann cells
		PN1 U79568 (r)				
		AF000368 (r)				
$Na_v1.8$	SNS	X92184 (r)	SCN10A	Mouse 9		DRG
	PN3	U53833 (r)		Human 3p22-24		
	NaNG	Y09108 (m)				
		U60590 (d)				
$Na_v1.9$	NaN	AF059030 (r)	SCN11A	Mouse 9	$Na_v1.9a$	PNS
	SNS2	AJ237852 (r)		Human 3p21-24		
	PN5	AF118044 (m)				
	NaT	AB031389 (m)				
	SCN12A	AF126739 (h)				
		AF188679 (h)				
		AF109737 (h)				
		AF150882 (h)				
Nax	$Na_v2.1$	M91556 (h)	SCN7A	Mouse 2		Heart, uterus, skeletal muscle, astrocytes, DRG
	Na-G	M96578 (r)	SCN6A[b]	Human 2q21-23		
	SCL11	Y09164 (r)				
	$Na_v2.3$	L36179 (m)				

[a]The letter in parentheses after each accession number indicates the species of origin for the sequence, as follows: h, human; r, rat; rb, rabbit; m, mouse; gp, guinea pig; d, dog.
[b]This gene was originally assigned symbols SCN6A and SCN7A, which were mapped in human and mouse, respectively. The two most likely represent the same gene, and the SCN6A symbol will probably be deleted.
Abbreviations: DRG, dorsal root ganglion; PNS, peripheral nervous system; CNS, central nervous system. Modified from Goldin et al.[15]

α-subunits are structurally diverse, arising from multiple sodium channel genes and alternative splicing events (Table I). Recent progress has led to a good understanding of the molecular structure of sodium channels and how they work and the significance of their expression in particular cell types. This, coupled with the therapeutic utility of sodium channel blockers for a number of disorders and the elucidation of distinct sodium channelopathies, is beginning to reveal how particular sodium channel subtypes are linked to different disease states. All this provides great potential for the development of new therapies and underlines the attractiveness of this underexploited class of compounds.

The discovery of compounds that block overactivated sodium channels, while permitting normal currents of sodium ions, has provided a basis to develop such sodium channel blockers for the treatment of central nervous system (CNS) disorders, such as stroke, traumatic brain injury and pain; sodium channel blockers may also have utility for other disorders. The

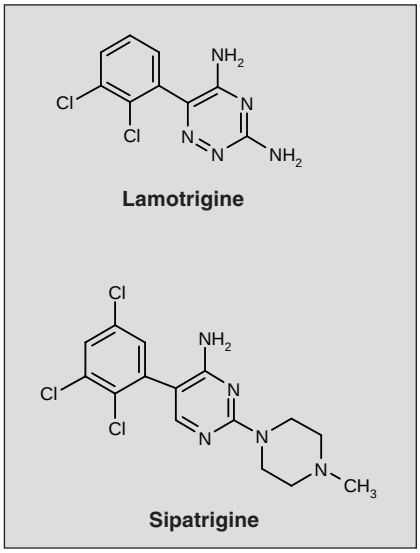

Fig. 1. Prototypic first-generation sodium channel blockers.

ability of sodium channel-blocking drugs to attenuate abnormal activity of sodium channels without affecting normal synaptic transmission probably underlies the good tolerability of sodium channel-blocking drugs. The prototypic example of the first generation of sodium channel blockers is the broad-spectrum anticonvulsant **lamotrigine** (*Lamictal*™; Fig. 1), which is now used widely to treat epilepsy. Lamotrigine may also have utility in the treatment of other disorders (e.g., bipolar disorder). Another prototypic sodium channel blocker, with a different efficacy profile, is **sipatrigine** (BW-619C89; Fig. 1), which is derived chemically from lamotrigine. Sipatrigine is a neuroprotective agent that is in clinical trials for stroke. The development of the next generation of sodium channel blockers will be greatly facilitated by elaboration of the pharmacology of the various isoforms of sodium channel subunits, which itself is dependent upon the existence of reliable, rapid and high-throughput assays for sodium channel activity.

The presentations at this meeting developed three themes: 1) the first generation of sodium channel blockers; 2) relating structure to function; and 3) the prospects for the next generation of sodium channel blockers.

First generation of sodium channel blockers

The two prototypic sodium channel blockers are the phenyltriazine **lamotrigine** and the phenylpyrimidine **sipatrigine**. Both were synthesized and developed by Wellcome scientists at Beckenham, U.K. Lamotrigine was synthesized as part of a program to discover new antifolates and was shown to have anticonvulsant efficacy. Sipatrigine arose from a program to develop analogues of lamotrigine and was subsequently shown to have neuroprotective efficacy. At the time, it was considered that the efficacy of both compounds was mediated by inhibition of the release of the excitatory amino acids aspartate and glutamate. However, John Garthwaite (who joined Wellcome as Head of Neuroscience in 1992 and is now at Wolfson Institute for Biomedical Research, University College London, London, U.K.) reasoned that the mechanism of action was not defined precisely, since both compounds blocked vertadrine-evoked release but not potassium-evoked release of excitatory amino acids. The fact that veratrine evokes release by opening sodium channels suggested that it was sodium channel blockade that was underlying the efficacy of both lamotrigine and sipatrigine. This was then demonstrated directly by electrophysiological studies of cells expressing recombinant $Na_v1.2$ sodium channels.[1]

Prof. Garthwaite went on to describe how sodium channel blockers have great potential as therapies for disorders associated with neurodegeneration, since they protect both gray and white matter. He pointed out how the discovery of ways to protect the brain and spinal cord from degeneration in acute and chronic stress conditions represents one of the major challenges facing neuroscience. Progress is clearly dependent upon understanding the underlying mechanisms, which are likely to be either specific to a particular disease state (e.g., genetic susceptibility, viral infections) or more general (e.g., those leading to cell death by necrosis or apoptosis). A step forward in understanding what might be one of the more general mechanisms was achieved more than two decades ago with the realization that glutamate is not only the main excitatory neurotransmitter in the brain, but also a powerful neurotoxin to many different CNS neurons. This work led to the formulation of the "excitotoxic" hypothesis, wherein excessive glutamate receptor activation contributed to (or caused) neuronal cell death. Demonstrations that antagonists acting on glutamate receptors of both the NMDA and AMPA subclass were protective in animal models of stroke and trauma added weighty evidence in support of this hypothesis. The multiple failures of this approach in clinical trials in stroke victims, however, necessitate a rethink. It can be argued, with some justification, that the hypothesis was never subjected to proper experimental testing in humans (e.g., inadequate dosing or too long a delay prior to drug administration[2]). But another factor is that, to a greater or lesser extent, neurodegenerative disorders affect both neurons in the gray matter and their axons running in the white matter. Moreover, the human brain has disproportionately more white matter than a rodent brain. Hence, a better approach has to be one that is capable of protecting both gray and white matter.

Voltage-gated sodium channels are expressed at the surface of all neuronal elements (dendrites, somata and axons). Maintenance of ionic homeostasis in the face of continuous entry of Na^+ into the cytoplasm represents a major pathway for energy expenditure in the CNS. When energy is lacking (e.g., as a result of poor blood perfusion), failure of Na^+ (and therefore K^+) homeostasis is expected to be one of the first consequences, with many secondary ones to follow should this situation persist. With excitotoxicity in full flurry, little thought was given to the possible importance of this pathway in neurodegeneration, even though there were already clues in the literature that the classic sodium channel inhibitor tetrodotoxin had neuro-

protective properties *in vitro* and *in vivo*. The identification of compounds that act in a voltage- and use-dependent manner on sodium channels (rather like some anticonvulsants) and protect gray matter at least as effectively as glutamate antagonists provided additional evidence. Moreover, these compounds inhibited ischemia-induced glutamate release, consistent with a primary action to preserve ionic homeostasis. Furthermore, investigation into the mechanism of ischemic damage to white matter axons highlighted the central role played by sodium channels in triggering the axonopathy. In this case, irreversible damage appears to be the result of a secondary Ca^{2+} overload of the axoplasm. Hence, through targeting the same protein, it appears possible to provide dual protection of gray and white matter, at least in ischemia. Whether any particular sodium channel subtype dominates in the pathogenesis of the damage remains unknown, but channels that inactivate slowly on depolarization may be especially significant.

Sodium channels cycle between resting, activated and inactivated states. Inactivation proceeds many thousands of times faster when channels are open compared with the resting state. However, inactivation does proceed at a finite rate whenever membranes are depolarized from about –140 mV. Drugs that interact with sodium channels to block ion flux cause the channels to inactivate to a greater extent and with smaller depolarizations than they would normally exhibit. The relatively slow off-rate of drugs such as phenytoin means that there is an accumulated block with repeated depolarizations (use-dependent block). 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. This may explain why phenytoin does not alter normal action potentials or excitatory synaptic potentials—these events last less than 200 ms. Sustained depolarizations, during ischemia or seizures, greatly enhance the blocking action of phenytoin and similar drugs. Hodgkin and Huxley first described the three-state model for sodium channels in 1952,[3] and a theory to explain use-dependent blockade of ion channels, termed the "modulated receptor theory," has been developed on the basis of their observations. This theory assumes that sodium channel blockers bind different channel states with different affinities and that drug binding alters the transition rates between different states.

TABLE II: SODIUM CHANNEL BLOCKERS DISPLAYING NEUROPROTECTIVE EFFICACY IN MODELS OF CEREBRAL ISCHEMIA AND TRAUMATIC BRAIN INJURY

	EVIDENCE OF EFFICACY IN DISEASE MODELS		
COMPOUND	FOCAL ISCHEMIA	GLOBAL ISCHEMIA	TRAUMATIC BRAIN INJURY
Sipatrigine	Yes	Yes	Yes
BW-1003C87	Yes	Yes	Yes
Riluzole	Yes	Yes	Yes

The study by Xie and Garthwaite[1] showed that the inhibition of Na^+ currents by **sipatrigine** is due to modulation of channel gating rather than an open channel block. A selective stabilization of the inactivated state of the sodium channel appears to directly counter Na^+ entry into neurons and, thus, indirectly decreases depletion of ATP stores, calcium influx and excessive release of glutamate and aspartate. State-dependent modulation of sodium channels thus provides a mechanism for selective drug action, since only excessive activation is blocked, leaving normal synaptic transmission unimpaired. Sipatrigine, together with a number of other sodium channel blockers, has been shown to be effective in a number of models of acute brain injury (Table II). The spectrum of activity and the amount of efficacy achieved with these drugs are impressive, particularly when compared with compounds that block the *N*-methyl-D-aspartate (NMDA) receptor. A number of sodium channel blockers that have entered clinical trials for stroke include sipatrigine, **lifarizine**, **fosphenytoin** (a phosphate ester prodrug of phenytoin) and **lubeluzole** (Fig. 2).

Since Terry Smith (CeNeS Ltd., U.K.) was not able to attend the meeting, Alan M. Palmer (Vernalis) presented Dr. Smith's slides describing the clinical development of **sipatrigine** for the treatment of stroke. In addition to blocking sodium channels, sipatrigine has been shown to block a number of subtypes of calcium channels,[4] which may well contribute to the neuroprotective efficacy of this compound.

Several phase II studies with this compound were completed by Wellcome, and preliminary information on safety suggests that the development of visual hallucinations was the limiting factor in a dose-escalation study. Other side effects included nausea, vomiting, agitation, confusion and drowsiness.[5] It is unlikely that these side effects are due to blockade of sodium channels, since they are not observed with **lamotrigine**, which is marketed for the treatment of epilepsy. The phase II trial for **sipatrigine** was terminated when Glaxo merged with Wellcome and a decision was made to develop the NMDA (glycine site) receptor antagonist **gavestinel** (from Glaxo), which showed no efficacy in a randomized, double-blind, placebo-controlled trial,[6] possibly because of failure to cross the blood-brain barrier.[2]

Sipatrigine has undergone extensive preclinical studies leading to a series of clinical safety studies in both healthy volunteers and stroke patients. An efficacy study is planned in stroke patients using diffusion- and perfusion-weighted MRI to determine the effect of sipatrigine on infarct size as a surrogate marker. A dose-escalation study has commenced as a prelude to this study.

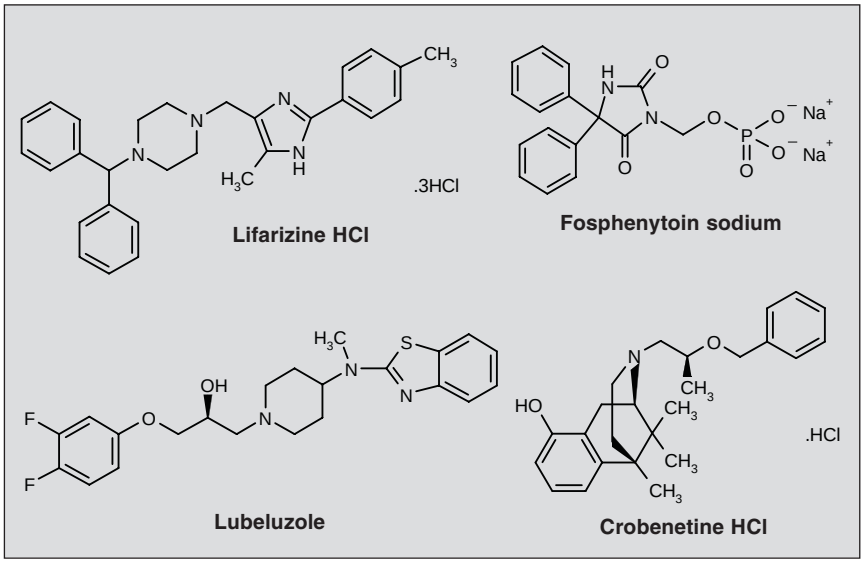

Fig. 2. Sodium channel blockers in clinical trials for stroke. Sipatrigine (Fig. 1) is also in clinical trials for stroke.

Sipatrigine has commenced development for clinical use. Preclinical safety and toxicology studies showed a profile of activity satisfactory for further development. Phase I intravenous studies in healthy volunteers reported no serious adverse events. In a series of safety and tolerance phase II studies in stroke patients who received intravenous infusions of sipatrigine, the main adverse events observed were hallucinations and vomiting. Overall, therefore, sipatrigine has been shown to be clinically acceptable in healthy volunteers and stroke patients. From these data, there is good evidence that sipatrigine crosses the blood–brain barrier. There are insufficient data, however, to identify evidence of efficacy or the optimal dose of sipatrigine with which to establish such efficacy. To try to answer these questions, a clinical trial has commenced utilizing MRI perfusion/diffusion mismatches to investigate the effects of sipatrigine on a surrogate marker, namely, the change in size of ischemic lesion in the cortex.

Dr. Malcolm Nobbs (GlaxoSmithKline, U.K.) gave the slides prepared by Marcus E. Risner (GlaxoSmithKline, U.S.A.), who was not able to attend the meeting. This presentation described **lamotrigine** as a treatment for epilepsy and other disorders. Lamotrigine, a chemically novel phenyltriazine, was synthesized and developed by Wellcome scientists in response to an unmet medical need for the treatment of epilepsy. They examined the possibility that antifolate compounds had anticonvulsant efficacy. Preclinical studies initiated in the 1970s demonstrated that one antifolate (lamotrigine) was, indeed, a potent anticonvulsant in several animal models. Subsequent studies revealed no teratogenic, carcinogenic or mutagenic liability, a wide therapeutic index and a favorable pharmacokinetic profile.

Lamotrigine is thought to act primarily via a use-dependent blockade of voltage-sensitive sodium channels to stabilize the neuronal membrane. It diminishes the overexcitation of neurons displaying epileptiform activity and has no effect on normal neuronal activity. In the clinic, lamotrigine has proved to be a broad-spectrum antiepileptic drug as add-on therapy for adult and pediatric partial seizures and also as add-on therapy for the generalized seizures of the Lennox-Gastaut syndrome. It is also effective as monotherapy, given either to patients with newly diagnosed partial or generalized seizures or to patients converted from other antiepileptic drugs. Lamotrigine's safety profile is generally unremarkable, and most adverse events reported by patients are mild or moderate in intensity, are seen in the first 6–8 weeks of therapy and resolve without the need to discontinue lamotrigine. The adverse event of most concern is skin rash, which is generally simple morbilliform in presentation, although serious cutaneous reactions have also occurred; following the recommended dosing guidelines minimizes this risk. Lamotrigine is currently available for the treatment of epilepsy in more than 90 countries, having first received approval in Ireland in 1990 and in the United Kingdom in 1991, and has been administered to well over 3 million patients worldwide. As a consequence of its widespread use, it has been observed that lamotrigine appears to improve mood, which has been substantiated and extended in a number of clinical trials in patients with bipolar disorder.[7] This work is being taken forward by GlaxoSmithKline. Lamotrigine has also been shown to have efficacy in a number of different types of human neuropathic pain.[8]

Relating structure to function

William A. Catterall (University of Washington, U.S.A.) described elegant studies relating structure to function within sodium channels. The channels are composed of a large α-subunit, together with one or two β-subunits. The α-subunit is the pore-forming subunit. β-Subunits are single membrane-spanning glycoproteins containing immunoglobulin-like folds in their extracellular domains. They interact with the α-subunit through their extracellular domains and modulate channel expression and gating. The immunoglobulin-like folds have the structures of cell adhesion molecules and interact with extracellular proteins, like tenascin, and cell adhesion molecules, like neurofascin. The α-subunits are organized into four homologous domains (I–IV), each of which contain six transmembrane α-helices (S1–S6). The S4 segments

contain positively charged residues, which serve as voltage sensors for channel activation and move outward under the influence of the electric field to initiate activation. The S5 and S6 segments and the short membrane-associated segments between them (SS1/SS2) form the pore. The fast inactivation of the open sodium channel is mediated by closure of a hinged lid-like inactivation gate formed by the intracellular loop between domains III and IV. The hydrophobic motif IFM within this loop serves as the inactivation particle. This motif moves from a cytoplasmic location into the channel structure during inactivation and becomes inaccessible to chemical modification. The three-dimensional structure of the core of the inactivation gate, including the IFM motif, has been determined by NMR spectroscopy and forms the basis for a mechanistic interpretation of site-directed mutagenesis studies of the inactivation process. The inactivation gate folds into a receptor region formed by the IIIS4-S5 loop, the IVS4-S5 loop and the intracellular end of the IVS6 segment.

Local anesthetics and related drugs block the pore of sodium channels by binding to a receptor site formed by amino acid residues in transmembrane segment S6 in domains III and IV. Site-directed mutations of critical amino acids at similar positions in these segments greatly reduce the affinity for local anesthetic block and specifically disrupt high-affinity binding to the inactivated state. Many different structural classes of sodium channel blockers, including anticonvulsant, antiarrhythmic and neuroprotective drugs, interact with this site in the pore. By contrast, peptide scorpion toxins that alter gating of sodium channels bind to the extracellular ends of S4 segments and trap them in either an activated or nonactivated state. α-Scorpion toxins trap the IVS4 segment in its inward position and slow or prevent inactivation; β-scorpion toxins trap the IIS4 segment in its outward position and greatly enhance activation. Voltage sensor-trapping may be a general mechanism of action of peptide toxins that affect ion channel gating. These toxin receptors may provide novel sites for targeting new sodium channel-modulating drugs.

Dr. Catterall presented his sliding helix model of voltage-dependent gating and showed how peptide scorpion toxins alter the gating of sodium channels. β-Scorpion toxins shift activation negatively by binding to the S3-S4 loop in domain II, whereas α-scorpion toxins (and sea anemone toxins) slow coupling of activation to inactivation by binding to the S3-S4 loop in domain IV. In the former case, the channel must be activated for the toxin to have effect and, in the latter case, prolonged depolarization drives the toxin off its receptor site. Other polypeptide toxins may alter voltage-dependent gating by binding tin to the S3-S4 loops of ion channels, for example, hanatoxin binding to potassium channels and binding of both grammotoxin and agatoxin IVA to calcium channels. This suggests that voltage sensor-trapping may be a general mechanism of action of peptide toxins. By contrast, **lamotrigine** and related drugs block the pore of sodium channels by binding to a receptor site formed by amino acid residues in transmembrane segment S6 in domains III and IV. Site-directed mutations of critical amino acids at similar positions in these segments greatly reduce the affinity for local anesthetic block and specifically disrupt high-affinity binding to the inactivated state. The molecular determinants of lamotrigine binding to inactivated sodium channels have established that the amino acids IVS6 F1764A, IVS6 Y1771A and IIIS6 L1465A are critical. The molecular basis for similar drugs having different therapeutic actions (anticonvulsant, antiarrhythmic and neuroprotective) remains to be elucidated. By contrast, it was pointed out that **crobenetine** (BIII-890-CL; Fig. 2), the Boehringer Ingelheim compound that is in clinical trial for stroke,[9] binds to the same site as lamotrigine, but with a 30-fold greater affinity. Compounds that are potent sodium channel blockers are likely to have a reduced probability of interacting with other receptors and channels and can therefore be expected to possess a better side effect profile.

John N. Wood (University College, London, U.K.) described the role of sodium channels in the mechanisms underlying pain.[10] Electrophysiological studies of dorsal root ganglion neurons and the results of polymerase chain reaction, Northern blot and *in situ* hybridization analyses have demonstrated the molecular diversity of sodium channels that operate in sensory neurons. Several subtypes of α-subunit have been detected in dorsal root ganglion neurons and transcripts encoding all three β-subunits are also present. One α-subunit, $Na_v1.8$, is selectively expressed in C-fiber– and Aδ-fiber–associated sensory neurons that are predominantly involved in damage sensing. The production of null mutant mice provides useful information on the specialized functions of particular sodium channels. $Na_v1.8$ null mutant mice are normal, apart from deficits in inflammatory pain (induced by complete Freund's adjuvant or nerve growth factor) and noxious mechanosensation. Neuropathic pain develops normally in the absence of $Na_v1.8$. These data support the idea that selective blockers of $Na_v1.8$ may be useful for treating acute or inflammatory pain. However, at the moment there are no subtype-selective blockers to explore this potentially attractive approach to pain treatment.

An alternative approach to blocking channel function is to manipulate channel expression. Using yeast 2-hybrid cloning, a number of interacting proteins that bind $Na_v1.8$ have been identified. Disrupting essential or permissive interactions that facilitate high-level sodium channel expression may be a route to targeting sodium channel subtypes and disrupting nociceptive processing. The tetrodotoxin channel $Na_v1.3$ is present in sensory neurons during embryonic development but is subsequently down-regulated. It is selectively up-regulated in

dorsal root ganglion neurons in a variety of models of neuropathic pain. The expression of $Na_v1.3$ correlates well with the appearance of ectopic action potentials and allodynia in animal models of neuropathic damage, suggesting that $Na_v1.3$-selective blockers may be useful for the treatment of neuropathic pain. However, overexpression studies using $Na_v1.3$ in normal animals have yet to be carried out to confirm a causative role of $Na_v1.3$ in neuropathic pain syndromes.

Taken together, the evidence supports a selective and significant role for various sodium channel isoforms in different pain states. The development of isoform-specific channel blockers will prove invaluable in determining the role of sodium channel isoforms in pain pathways and are likely to provide a fresh approach to the development of new analgesic drugs.

Nick Carter related sodium channel structure to function by describing how specific mutations in sodium channels cause distinct and deleterious symptoms. He specifically described sodium channelopathies relating to hypertension and arrhythmia.

Hypertension

Essential hypertension is caused by a number of factors, including genetics.[11] Both single- gene and polygenic contribution can alter the clinical phenotype and the majority of genes involved appear to affect renal physiology. Liddle syndrome is a rare monogenic hypertension phenotype relating to mutations in the epithelial sodium channel gene ENaC.

Liddle syndrome is usually caused by truncation mutations in the β-subunit gene, producing increased renal channel activity, sodium absorption, volume expansion and increased blood pressure. A missense mutation in the C-terminus, the "Liddle region," has been demonstrated to be a contributing factor in common polygenic multifactorial high blood pressure. The polymorphism involved (T594M) is frequent in African and Afrocaribbean black people, but is not found in Caucasians. Thus, rare and frequent inherited channelopathies can contribute to hypertension. ENaC is inhibited by **amiloride**, thus, patients with Liddle syndrome may be more effectively treated with this compound, although routine diuretics have proved effective. The molecular changes in ENaC, which cause increased channel activity, are dominant-negative. Other ENaC mutations cause loss of channel function and, hence, sodium loss with concomitant low blood pressure.

Arrhythmia

Sudden cardiac death can be caused by inherited channelopathies, such as long QT syndrome. This is a dominantly inherited condition resulting from mutations in one of several ion channel coding genes important in mediating the cardiac action potential.[12] One gene locus associated with this phenotype is $Na_v1.5$, coding for a sodium channel protein, which, when altered by mutations in key regions of the channel, closes more slowly and causes the QT interval to be lengthened. This phenomenon predisposes to life-threatening arrhythmias. It is clear that any new sodium channel blocker should be devoid of arrhythmia-inducing activity, so knowledge of the extent of interaction at this channel subtype will be important in order to avoid unwanted side effects.

The location of mutations that impair sodium channel activation and cause inherited diseases of hyperexcitability were described by Prof. Catterall. In addition to causing long QT syndrome, such mutations have also been found to cause periodic paralysis and febrile seizures, the latter disorder being caused by mutations on the β_1-subunit.

Prospects for the next generation of sodium channel blockers

Dr. Brian Cox (GlaxoSmithKline, U.K.) described the structure–activity relationships of a series of sodium channel blockers. He pointed out that neuronal sodium channel blockers have been used in therapy for many years, long before a connection with the modulation of sodium channels was made or, indeed, the specific existence of sodium channels was known. The origins of all the neuronal sodium channel blockers lie in two areas: the naturally occurring and complex toxins, such as tetrodotoxin, and the synthetic molecules, such as the local anesthetic **procaine**, the anticonvulsant **phenytoin** and the antipsychotic and anticonvulsant drug **carbamazepine** (Fig. 3). **Tetrodotoxin** (Fig. 3) derives from the puffer fish (conveniently, all its sodium channels are resistant to tetrodotoxin) and binds to the outer pore of the majority of neu-

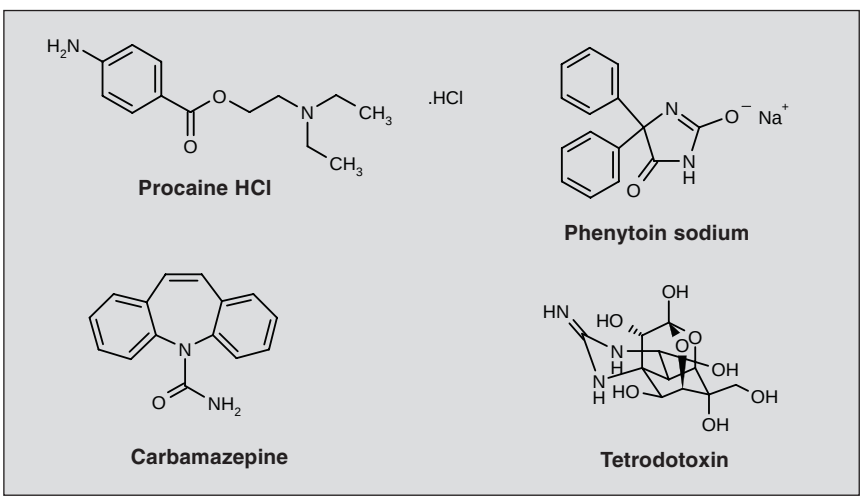

Fig. 3. Miscellaneous sodium channel blockers.

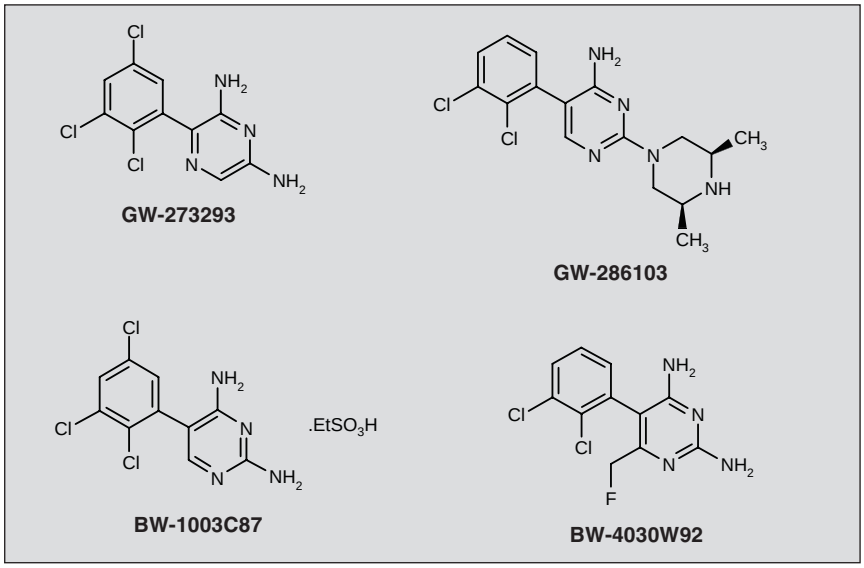

Fig. 4. Phenylpyrimidines with different efficacies.

ronal sodium channels. Both procaine and phenytoin are synthetic compounds and began therapeutic use in 1902 and 1937, respectively, but it was not until 1959, in the case of procaine, and 1983, for phenytoin, that their action on sodium channels was elucidated. Since the discovery of the first sodium channel blockers there has been enormous interest in the field. The search for better local anesthetics and anticonvulsants has continued, as well as the search to find new uses for entirely new indications, for example, as antiarrhythmics for stroke or for bipolar disorder.

Lamotrigine was first synthesized and developed in response to an unmet medical need for the treatment of epilepsy, but has become an important lead for the discovery of further generations of compounds, such as the stroke agent **sipatrigine**, the selective anticonvulsant **GW-273293** and the selective analgesic **GW-286103** (Fig. 4). There were good correlations between 1) tetrodotoxin-resistant currents in dorsal root ganglion neurons (*in vitro*) and analgesia (*in vivo*); and 2) inhibition at cloned type IIA sodium channels and *in vivo* anticonvulsant activity. There was no relationship between anticonvulsant and analgesic activity (carrageenan paw assay), which supports the contention that different blockers have different efficacies.

The foundation establishing structure–activity relationships for sodium channel blockers has now been laid.[13,14] Further elaboration of structure–activity relationships will require the cloning and stable expression of the various subtypes of channel, together with the development of high-throughput assays.

The development of new and high-throughput assays to assess sodium channel activity was described by Dr. Jesús E. González (Aurora Biosciences Corp., San Diego, California, U.S.A.). The assays use voltage-sensitive fluorescence resonance energy transfer (FRET) probes coupled with a Voltage/Ion Probe Reader (*VIPR II*™), a custom fluorescence plate reader. There are two probes. The first is oxonol, a highly fluorescent, negatively charged, hydrophobic ion that is sensitive to changes in membrane potential. In response to changes in membrane potential, it can rapidly redistribute between two binding sites on opposite sides of the plasma membrane. The second fluorescent molecule is coumarin lipid, which binds specifically to one face of the plasma membrane and functions as a FRET donor to the voltage-sensing oxonol acceptor molecule. When the oxonol moves to the intracellular plasma membrane binding site upon depolarization, FRET is decreased and results in an increase in the donor fluorescence and a decrease in the oxonol emission. This approach enables sodium channel function to occur within a time frame of a second or subseconds. The Aurora researchers have screened 125,000 compounds against both the type IIA-like sodium channel endogenously expressed in Chinese hamster ovary (CHO) cells and the human cardiac sodium channel (hH1a) heterologously expressed in HEK293 cells. A large group of compounds were active at both sodium channel types and a few compounds showed subtype selectivity. The functional FRET assays have significant advantages for medicinal chemistry support, rapid prioritization of candidate blockers and exploring drug/channel interactions. This comparative screening approach should be applicable to other members of the sodium channel blocker family.

Conclusion

The discovery of the first generation of sodium channel blockers was made with little dependence on recombinant channels. It was established that such compounds block overactivated sodium channels, while permitting normal currents of sodium ions. This has provided a basis to develop such sodium channel blockers for the treatment of CNS disorders such as epilepsy, stroke, traumatic brain injury and pain. Sodium channel blockers may also have utility for other types of illness, such as bipolar disorder. The ability of sodium channel-blocking drugs to attenuate abnormal activity of sodium channels without affecting normal synaptic transmission probably underlies the good tolerability of sodium channel-blocking drugs. With the discovery of nearly a dozen genes encoding different sodium channels, it is clear that the next generation of sodium channel blockers will have to be made with knowledge of subtype selectivity.

Establishing how the different channels relate to different disease

states is clearly an important challenge. Progress has begun with studies with null mutants for different sodium channels, together with evidence of maladaptive changes in sodium channel gene expression in neurons in certain pathological states. These studies will be greatly complemented by blockers with clear subunit selectivity. Whether such compounds are feasible remains to be seen. It is possible that compounds with different efficacy profiles bind to the same site within transmembrane segments IIIS6 and IVS6 of inactivated sodium channels.

The continued careful elucidation of structure–function relationships within the sodium channel will also facilitate future drug discovery. The relationship between structure and function is also helped by the discovery that mutations in specific isoforms of sodium channel cause a variety of diseases, including paralysis, long QT syndrome and epilepsy. It is likely that more such channelopathies will be discovered in the future. The diversity and dynamic nature of sodium channel expression introduce a high degree of complexity into the nervous system. This not only presents a great challenge for neuroscience, but also provides a golden opportunity for future drug discovery, particularly with the use of recombinant sodium channels and new high-throughput technologies to assess sodium channel function.

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GENZYME AND KIRIN COLLABORATE ON HUMAN MAB DEVELOPMENT

Genzyme Molecular Oncology and Kirin Brewery Co., Ltd. announced November 19, 2001, that they have agreed to collaborate on the development and commercialization of fully human monoclonal antibodies. These will be used as therapies in angiogenesis and vascular targeted cancer drug delivery. Genzyme will utilize its portfolio of proprietary **tumor endothelial markers** (TEMs) as targets to generate product candidates. During the two-year research period, Genzyme will validate a select group of the TEMs and Kirin will generate fully human antibodies to the validated markers using its *KM Mouse*™ technology. Genzyme will receive a USD 2 million up-front payment, two-year funding for the program and milestone payments. Development expenses and worldwide profits will be equally split between the companies for any products that are developed commercially.

Genzyme will receive exclusive marketing rights to the antibodies in North America, with Kirin holding rights in Asia. Genzyme will be the primary marketing party in Europe, but Kirin may co-promote products. Kirin receives the rights to develop and market in Asia for small-molecule drugs targeting those TEMs selected under the research program as antibody targets. All other small-molecule drug rights worldwide are retained by Genzyme.