Voltage-gated sodium channels as therapeutic targets

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Voltage-gated sodium channels (VGSCs) play a central role in the generation and propagation of action potentials in neurons and other cells. VGSC modulators have their origins in empirical pharmacology and are being used as local anaesthetics, antiarrhythmics, analgesics and antiepileptics, and for other disorders. However, the identification of a multigene family of VGSCs, along with tools to study the different subtypes in pathophysiology, is now providing a rational basis for selective intervention. Recent advances have addressed the technical challenges of expressing and assaying these complex proteins, enabling the correlation of empirical pharmacology to subtypes and the screening of individual subtypes for novel inhibitors with increased potency and selectivity.

Voltage-gated sodium channels (VGSCs) are complex membrane proteins that are widely expressed in neuronal, neuroendocrine, skeletal muscle and cardiac cells. They activate in response to membrane depolarization and in most electrically excitable tissues they are responsible for the rapid influx of sodium ions during the rising phase of the action potential. Drugs modulating VGSCs have proven therapeutic value in local anaesthesia, cardiac arrythmia, pain and epilepsy, and are currently under investigation for stroke, bipolar disorder, and other disorders.

The indispensable roles of VGSCs are well illustrated by the lethal effects of their inhibition by an array of highly potent and selective neurotoxins. Remarkably, VGSC-blocking drugs are free from toxicity, owing to their ability to subtly modulate channel function. Many of these drugs were discovered empirically and only subsequently found to block VGSCs. The basis of their therapeutic selectivity and precise mechanism of action, which is an area of active investigation, has yet to be fully elucidated.

The discovery of a multigene family of VGSCs has opened up new opportunities for the development of more selective and disease-specific drugs. Molecular biology has provided the tools to study the roles of different VGSC subtypes in pathophysiology, providing a more rational basis for selective intervention, for example, the sensory neuron-specific VGSCs in pain. Added to this, an increasing number of inherited disorders associated with VGSCs and other ion channel genes (channelopathies) are providing important insights into the role of these proteins in disease (Box 1; Table 1). However, a target-driven approach to VGSC drug discovery has hitherto been hampered by the technical challenges of the cloning and stable expression of these very large, complex genes, and of developing functional assays with sufficient capacity. Recent advances are addressing these challenges, to correlate the rich pre-existing pharmacology to therapeutic selectivity at the level of individual subtypes, and to screen VGSC subtypes for novel inhibitors with increased therapeutic selectivity and potency.

Channel structure and function

Multiple channel states and drug action

The current understanding of VGSC function is based on fundamental biophysical studies that distinguished at least three functional states: (1) open, (2) resting and (3) inactivated. At normal membrane potentials, channels are in a closed, resting state. Membrane depolarization activates channels to the open state allowing the rapid influx of

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Box 1. Sodium channelopathies

An understanding of the diseases associated with mutations in ion channel genes, termed channelopathies, provides a unique insight into the critical role of these proteins in normal cellular physiology and may provide targets for disease intervention strategies. Neurological and neuromuscular channelopathies are usually dominantly inherited and are characterized by episodic incidents of migraine, epilepsy, ataxia, myotonia, weakness and paralysis[53]. To date, voltage-gated sodium channelopathies that are associated with inherited disease in either human patients or mice mutant strains have been identified in four of the α subunits and one β subunit[54].

sodium ions. During prolonged depolarization (>1 ms), the channels inactivate and sodium influx declines; in this state, the channels remain closed and are refractory unless the membrane is repolarized to allow them to recover to the resting state. Sodium channels in the brain can 'cycle' through these states in milliseconds, allowing them to sustain rapid trains of action potentials, which are essential for brain function.

The channels equilibrate between these states depending on the membrane potential. This has important implications for drug action because the therapeutic selectivity of known sodium channel blocking agents such as lignocaine, phenytoin and lamotrigine is a consequence of state-dependent activity. In contrast to simple open channel blockers (e.g. tetrodotoxin, TTX), these drugs preferentially bind and stabilize inactivated states. This accounts for the selective inhibition during sustained depolarization (voltage-dependence) or repetitive firing (use-dependence), allowing control of excitability without compromising normal function, and explaining the safety profile of VGSC drugs.

Functional domains of VGSC polypeptides

Purification of the VGSC revealed it to be a multisubunit complex consisting of a highly glycosylated pore-forming α subunit (260 kDa), with one or two accessory β subunits[1] (33–36 kDa); a third β subunit isoform has also recently been discovered[2]. Cloning and sequence analysis of VGSC α-subunit cDNAs predicts structural features in common with the large superfamily of voltage-gated ion channels, including potassium and calcium channels (Fig. 1). Channels of this type are all formed by the association of four polypeptide domains, each containing six transmembrane regions (S1–S6) and a hydrophobic loop thought to dip into the membrane to line the pore itself (SS1–SS2 or P-region). A single polypeptide chain contains the four domains of sodium and calcium channel α subunits, whereas potassium channels are formed by tetramerization of α-subunit polypetides containing only one domain each. Recently, the structure of the prototypic *Streptomyces lividans* potassium channel (KcsA) has been solved by X-ray crystallography[3], throwing light on the pore structure and the basis of ion selectivity for the whole voltage-gated superfamily including sodium channels[4].

Extensive molecular analysis has delineated conserved regions of VGSCs required for the key channel functions[5] (Fig. 1). The S4 segments of each domain function as voltage

Table 1. Voltage-gated sodium channelopathies

Disease		Sodium channel	Mutations	Phenotype
Long QT syndrome 3		SCN5A (cardiac channel)	Deletion of KQP 1505–1507 causing	Prolonged opening and length of cardiac action potential, persistent current
Potassium aggravated myotinia (PAM)		SCN4A (skeletal muscle channel)	6 point mutations in regions associated with inactivation	Slowed decay of transient sodium current
Paramyotonia congentia (PC)		SCN4A (skeletal muscle channel)	5 point mutations in regions associated with inactivation	Slowed decay of transient sodium current
Hyperkalemic periodic paralysis, type II (hyper PP)		SCN4A (skeletal muscle channel)	5 point mutations in regions associated with inactivation	Slowed decay of transient sodium current
Motor end-plate disease (MED)		SCN8A (type VI channel)	Null	Complete loss of activity, chronic ataxia, dystonia, lethal paralysis
Generalized epilepsy with febrile convulsions (GEFS)		SCN1B (β1 subunit)	Cysteine to glycine	Slowed inactivation of sodium channel α subunits

See Fig. 5 for relevance of symbols.

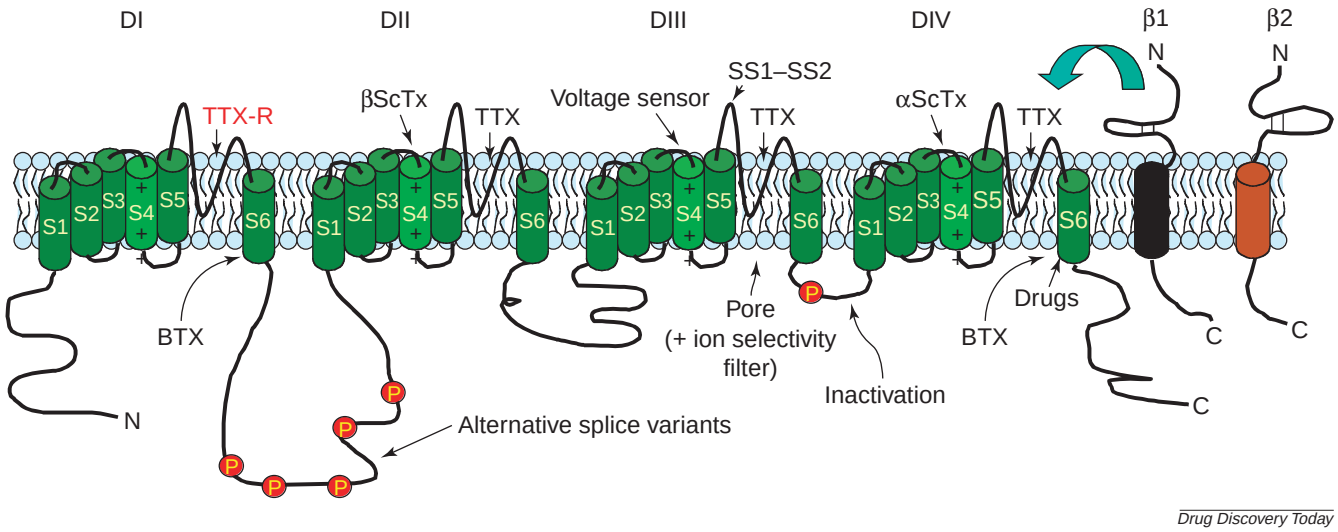


Figure 1. *Structural domains and binding sites on VGSCs. For clarity, the voltage sensor, SS1-SS2, pore and ion selectivity regions are indicated only for domain III but are present in the other domains also. P in a red circle indicates a demonstrated phosphorylation site, the large arrow indicates the α-β1 subunit interaction, and the location of the key amino acid for TTX resistance is indicated in addition to the TTX-binding determinants.*

sensors; they contain positively charged residues that are proposed to move upon depolarization, causing a conformational change that opens the channel. The intracellular loop between domains III and IV contains a hydrophobic motif, IFM, which is thought to occlude the pore during inactivation. In addition to forming the pore, the SS1–SS2 loop contains the ion selectivity filter; mutation of two highly conserved residues (Lys and Ala in domains III and IV, respectively) to Glu residues changes the selectivity of the channel from sodium to calcium[6].

Toxin and drug binding sites

A variety of toxins that modulate VGSCs have been used as tools to probe channel function. They can be classified on the basis of five discrete binding sites (Table 2) and these are beginning to be characterized at the molecular level. VGSCs are often subdivided on the basis of their sensitivity to TTX, the lethal component of puffer fish. A single residue (Cys374 in the cardiac channel or Ser356 in SNS) is critical for TTX resistance[7,8]. The binding site for batrachotoxin (BTX),

Table 2. Toxin and drug binding sites

Site	Modulator	Effect	Mechanism of action	Location of binding site
1	Tetrodotoxin	Inhibits ion conductance	Occludes pore	SS1–SS2 regions of each domain
2	Batrachotoxin	Causes persistent activation	?	IS6 and IVS6
3	α Scorpion toxins (α ScTx)	Delay inactivation	Prevent outward movement of IVS4 voltage sensor	IVS3–S4 loop
4	β Scorpion toxins (β ScTx)	Enhance inactivation	Trap IIS4 voltage sensor in outward position	IIS3–4 loop
5	Brevetoxins	Cause repetitive firing in nerves by enhancing activation and blocking inactivation	?	IS6 and IVS5
6	Local anaesthetics Antiarrhythmics Anticonvulsants	Selectively block repetitive firing by voltage- and use-dependent inhibition	Stabilize inactivated state	IVS6

an alkaloid from poison dart frogs, overlaps the major drug-binding site, consistent with known allosteric interactions with local anaesthetics. The binding sites and the molecular mechanism of scorpion toxins have also been recently characterized[5].

Remarkably, drugs that differ widely in their therapeutic effect and structure have common binding determinants. These are located in IVS6 (i.e. in the S6 region of domain IV) and are proposed to reside on the inner surface of the channel pore. Mutagenesis of brain type II VGSCs identified three residues critical for the action of the local anaesthetic, etidocaine[9]. The single amino acid replacement of aromatic residues 1764 and 1771 reduced both affinity for the inactivated state and use-dependent block without substantially altering normal function, whereas changes at residue 1760 enhanced binding and unbinding from the extracellular side of the channel. These mutations also reduced the sensitivity of the channels to state-dependent block by anticonvulsants (phenytoin, lamotrigine and derivatives) and by anti-arrhythmics (lignocaine, flecainide and quinidine)[10]. The effects differed in magnitude indicating that the drugs interact with these residues in an overlapping but non-identical binding site. Similar findings were obtained for local anaesthetics using comparable IVS6 mutations of the skeletal muscle channel, with further binding determinants identified in domain IS6 (Ref. 11).

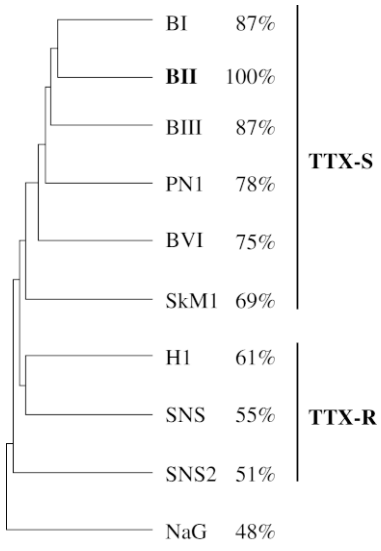
VGSC drug-binding determinants are in an analogous location to the phenylalkylamine-binding site on L-type voltage-gated calcium channels[12], and for substituted tetraethylammonium derivatives on certain potassium channels[13]. Corresponding residues have been mapped to the three-dimensional structure of the KcsA channel, supporting the location of this common drug binding site within the aqueous cavity of the pore. Further characterization of drug binding sites and VGSC structure will assist our understanding of structure–activity relationships, and could enable rational design of novel inhibitors with increased potency and selectivity.

Molecular and functional diversity of VGSCs

Molecular cloning has uncovered a surprising diversity of VGSCs. To date, at least 10 α subunits (Table 3) have been identified, which are highly conserved across different mammalian species. The potential for functional diversity is further increased by alternative mRNA splicing, modulation

Table 3. Voltage-gated sodium channel gene family[a]

Channel	Gene name/ other names	HCL	Key tissue distribution	TTX IC_{50} (nM)	Dendrogram showing percentage identities against hBII VGSC
Pore-forming α subunit					
Brain type I	**SCN1A**	2q24	Brain, spinal cord	6	
Brain type II	**SCN2A**	2q23–24	Brain, spinal cord	13	
Brain type III	**SCN3A**	2q24	Brain (embryonic in rat)	4	
Brain type VI	**SCN8A**/NaCH6/PN4	12q13	Brain, spinal cord, glia, DRG	3	
Skeletal muscle	**SCN4A**/SKM1	17q23–25	Skeletal muscle	5	
Cardiac	**SCN5A**/h1	3p21	Heart muscle	2000	
PN1	**SCN9A**/hNE/NaS	2q22–24	DRG, neuroendocrine cells	4	
SNS	**SCN10A**/PN3	3p21	DRG only	31 000	
SNS2	**SCN11A**/NaN/PN5	3p21	DRG only	1500	
Atypical heart/ glial	**SCN6A**/nav2.3 **SCN7A**/NaG	2q21–23	Heart, uterus, lung, DRG, glia	?	
Auxiliary β subunit					
Beta-1	**SCN1B**	19p13.1	Brain, muscle, DRG	–	
Beta-2	**SCN2B**	11q22	Brain	–	



[a]Including an 'atypical' voltage-gated sodium channel that has been identified in several tissues, including heart, uterus, lung, glia and dorsal root ganglia, but which does not produce sodium currents when expressed in *Xenopus* oocytes[57].

Abbreviations: DRG, dorsal root ganglion; HCL, human chromosomal localization; PN1, Peripheral neuron 1; SN2, sensory neuron specific 2; SNS, sensory neuron specific; TTX, tetrodotoxin.

by cellular factors and assembly with different β subunits. Understanding the consequences of such diversity and elucidating the precise physiological roles of the different variants remain important challenges.

The human α-subunit genes are clustered at four chromosomal locations; phylogenetic analysis suggests that these arose from two ancestral genes, as found in *Drosophila*, with subsequent genomic and tandem duplication events[14]. Intriguingly, electrical communication in the nervous system of *Caenorhabditis elegans* is thought to rely on graded signals rather than sodium-dependent action potentials, and the organism has no known VGSC genes.

Given the structural similarity of the VSCSs, it is conceivable that functional compensation *in vivo* would reduce the efficacy of drugs that selectively target individual subtypes. It is therefore important to characterize the tissue distribution, subcellular localization and biophysical properties of the different subtypes. These studies are beginning to indicate distinct roles for each. For example, although types I, II and VI have an overlapping distribution in the adult CNS, evidence suggests a specialized role in the brain for the type II channel. First, immunological staining of rat brain reveals that it is predominantly localized to projection fibres, whereas types I and VI are localized to the neuronal cell body and dendrites[15,16]. This has been confirmed in humans and it has been shown that type III is more widespread in adult human brain than in rat, but also has somato-dendritic localization (Fig. 2; W. Whitaker *et al.*, unpublished). This suggests a role for type II in action potential propagation and in the processing of synaptic inputs for the other subtypes. Second, studies have indicated that although the cloned human brain α subunits have similar biophysical properties when expressed in mammalian cells, type II has notable differences

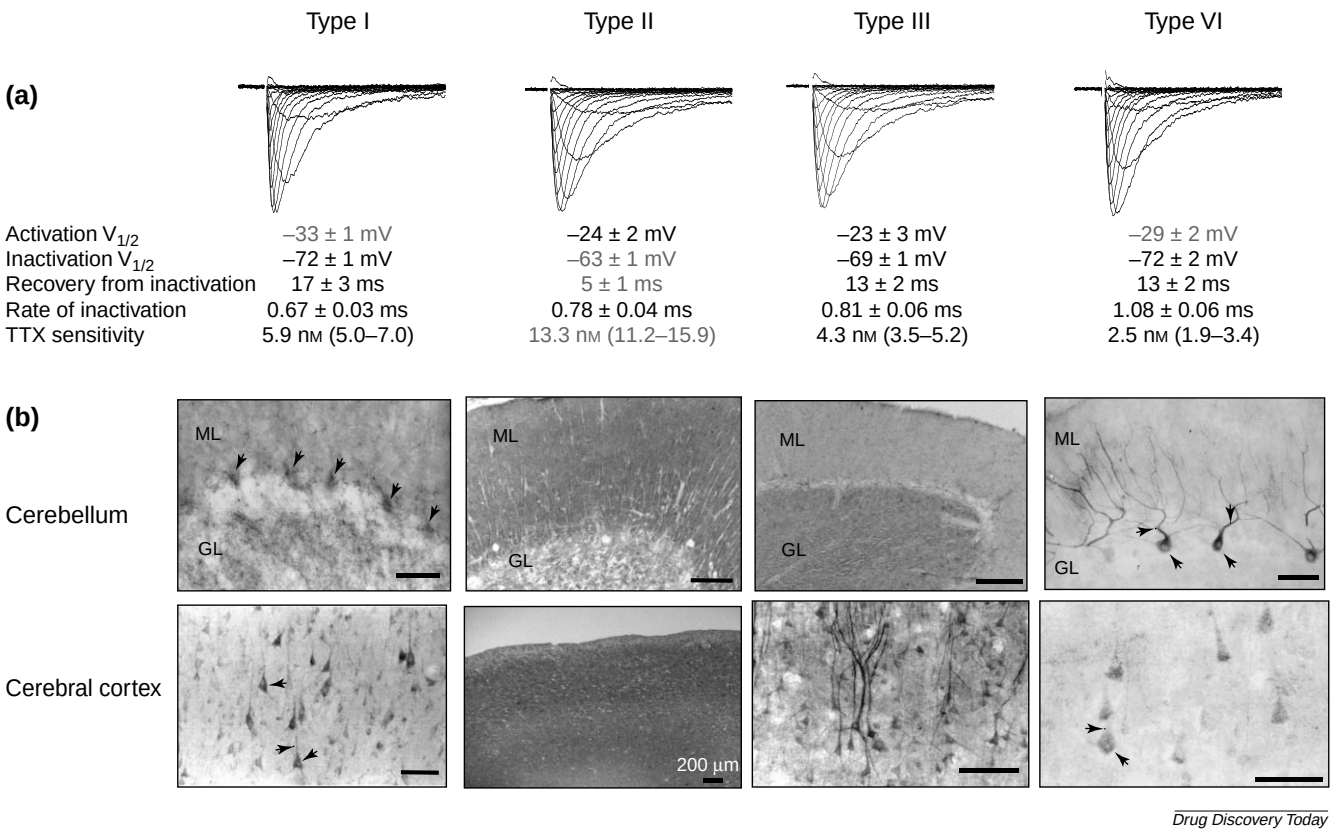


Figure 2. *Biophysical properties and distribution of human brain VGSCs. (a) Human α subunits were cloned and stably expressed in mammalian cell lines and then analysed by patch clamp electrophysiology. The time constants for inactivation (τ) were measured at the voltage that gave maximum currents (−10 mV). The time constants for recovery from inactivation were measured at a test potential of 0 mV. (b) Subtype-specific antipeptide antibodies were used to immunolabel selected human brain regions. Antibody specificity was verified using the recombinant stable cell lines. Scale bars are 100 μm unless indicated. Abbreviations: ML, molecular layer; GL, granular layer. Arrows point to Purkinje (upper panels) or pyramidal (lower panels) cells.*

in inactivation (Fig. 2; T. Dale *et al.*, unpublished), which suggests a greater availability than the other subtypes during depolarization in the brain. Third, gene knockout studies show that type II function is required for viability in the mouse[17]. A role in axonal conduction in myelinated neurons is implicated for the type VI sodium channel because it is localized at the nodes of Ranvier[18,19]. Disruption of the type VI gene in mice causes motor end-plate disease (med), resulting in severe muscular atrophy and death[20].

At least three different VGSC subtypes are primarily expressed in peripheral neurons (PN1, SNS and SNS2). PN1 is highly related to the brain channels and similarly is sensitive to TTX. By contrast, the two sensory neuron specific subtypes, SNS and SNS2, are more distantly related and are resistant to TTX. These two isoforms are restricted to dorsal root ganglion (DRG) neurons, and together are probably responsible for the characteristic slowly activating and inactivating TTX-resistant (TTX-R) currents associated with pain transmission. Recently, a human channel, SCN12A, which has high identity with rat SNS2, has been cloned[21]. This might be the human SNS2 orthologue, although its distribution is reported to be more widespread. The major isoforms found in heart (H1) and muscle (SkM1) share significant identity with the brain channels but have important differences in biophysical properties.

Differential splicing of the VGSC α subunit mRNA is known to occur and could potentially further increase channel diversity. This is thought to be the case for P- and Q-type voltage-gated calcium channels, which differ in sensitivity to Aga-IV toxin and inactivation kinetics, and appear to be alternative splice variants of the α_{1A} subunit[22]. VGSC types II and III have alternatively spliced neonatal (IIN, IIIN) and adult forms (IIA, IIIA), differing by only a single codon in exon 5. The functional consequences are unclear, but it is noteworthy that this splicing event is also regulated by seizures[23]. Alternative versions of exon 5 are also present in the type VI gene. The brain subtypes also show variation in the cytoplasmic loop between domains I and II, which is caused by alternative splicing. The type VI variants show small differences in distribution and in functional properties[24].

Modulation by β subunits, phosphorylation and G proteins

Three accessory β subunits (β1, β2 and β3) modulate the activity of α subunits and hence provide yet more potential for channel diversity. They are non-essential for core channel function and indeed are absent from the *Drosophila* genome. Co-expression of β1 and β2 with α subunits accelerates channel gating to physiological rates, alters the voltage dependence of inactivation and increases peak currents. The magnitude of these effects varies with different α subtypes and with the expression system used. A third isoform, β3, has recently been described that has similar functional effects[2]. The β subunits each contain an immunoglobulin-like motif with structural similarity to neural cell adhesion molecules. They might localize the sodium channel in a particular region of the cell membrane by interaction with the extracellular matrix proteins, tenascin-C and tenascin-R. It has been suggested that these interactions might be involved in the formation of high densities of VGSCs in areas such as the nodes of Ranvier[5,25].

Phosphorylation by protein kinase A (PKA) and C (PKC) is an important mechanism for the modulation of VGSCs and therefore cellular excitability. Induction of protein kinase activity can occur via activation of G-protein-coupled receptors, for example, muscarinic acetylcholine, prostaglandin E2 and dopamine D1, and is strongly linked with inflammatory hyperalgesia[26]. Phosphorylation has different consequences on function depending on the VGSC subtype. For example, phosphorylation by PKA or PKC of sites within the alternatively spliced I–II loop and the III–IV inactivation loop of the brain VGSCs leads to reduced peak currents[27]. By contrast, activation of PKA or PKC leads to an increase in the TTX-R sodium current in sensory neurons in a process that has been linked to nociceptor sensitization[26]. Further molecular studies have identified key serine residues within the I–II loop of SNS that are essential for regulation by PKA[28].

Brain VGSCs are regulated by receptor-activated G proteins causing negative shifts in the voltage dependence of activation and inactivation. G protein modulation of voltage-gated potassium and calcium channels is well established and involves direct interaction with free $G_{\beta\gamma}$ subunits. Recent work has shown that the brain type II VGSC also contains $G_{\beta\gamma}$-binding motifs and that under certain conditions, $G_{\beta\gamma}$ subunits can induce persistent sodium currents[29] that could lead to increased neuronal excitability.

VGSC drugs and VGSCs in disease

Origins of VGSC pharmacology

The empirical origins of VGSC drugs arose in two areas: (1) local anaesthetics, leading to antiarrhythmics, and (2) anti-epileptic drugs. In 1905, following a search for alternatives to cocaine, several synthetic amino-benzoates that had local anaesthetic properties were discovered, for example, procaine. This is hydrolysed rapidly *in vivo* and an attempt to find a superior drug led to the stable analogue

lignocaine (called lidocaine in the USA). It was not until 1959 that these compounds were shown to act by inhibiting VGSCs. Ropivacaine, launched in 1996 for acute pain management and surgical anaesthesia, is claimed to be superior to existing local anaesthetics (more than 40 are currently known) due to its greater selectivity for sensory over motor nerves. In 1936, procaine was shown to protect the heart from arrhythmias, when applied locally during cardiac surgery, and this led to the use of other local anaesthetics for this purpose. From 1979 to 1994, a series of novel VGSC blockers were introduced as class I antiarrhythmics, including propafenone, cibenzoline, moracizine, pilsicainide and pirmenol.

Phenytoin, first synthesized as a potential hypnotic, was identified in 1937 as the best of a series of analogues of phenobarbital that was non-sedating and suppressed electroshock-induced seizures in animals. It was not until 1983 that phenytoin was shown to modulate VGSCs at clinically relevant concentrations. Carbamazepine, first launched in the early 1960s, has a similar spectrum of action; its mode of action was not studied in detail until 1985.

A report that phenytoin and phenobarbital reduce folate concentrations in patients led to the development in 1978 of lamotrigine from antifolate compounds. Although structurally related to antifolate drugs, lamotrigine was shown in 1986 to act by inhibiting VGSCs (Fig. 2), and this initiated an ongoing programme of VGSC biology and medicinal chemistry at GlaxoWellcome. Over a period of years, lamotrigine was also found to be effective in animal models of inflammatory and neuropathic pain, cerebral ischaemia, anxiety, Parkinson's disease and nicotine or alcohol abuse. First-generation compounds were developed and tested *in vivo*, leading to compounds with increased potency in stroke (e.g. 619C90 or sipatrigine, in development by CeNeS), analgesia and epilepsy (e.g. 4030W92). 4030W92, which is an equipotent analgesic and anticonvulsant, was used as the starting point for further lead optimization, attempting to separate these activities and CNS side effects (i.e ataxia). Several hundred compounds were profiled in the relevant animal models, and several were identified that were selective analgesics or anticonvulsants with minimal ataxia side effects (Fig. 3d). The SAR (Fig. 3c) indicated that specific molecular alterations correlate with changes in therapeutic selectivity, strongly suggestive of VGSC subtype specificity. These results have driven a molecular biology programme at GlaxoWellcome that has cloned and expressed all the human VGSC α-subunit genes in order to correlate subtype and therapeutic selectivity.

Although local anaesthetics have conserved structural features generally comprising an aromatic ring and a basic nitrogen separated by a 3–4 atom (ester, amide or ether) linking group, other VGSC modulators have surprisingly diverse structures. Over the past few years, there have been several attempts using computational modelling to develop pharmacophore models from analogues of lignocaine[30], phenytoin[31], and the 3-aminopyrrole AWD-140190 and related anticonvulsants[32]. Further progress in this area is needed to improve our ability to design more potent and selective VGSC modulators in the future.

There is increasing rationale for VGSCs as drug targets for several disorders. The evidence, based on empirical pharmacology, studies of disease mechanism or genetic association, is discussed below along with drugs in clinical use or in development (Table 4).

Cardiovascular indications

Antiarrythmic compounds have been categorized by their effects on the fast sodium current (class I), sympathetic activity of the heart (class II), repolarization currents (class III) and slow inward calcium current (class IV). Class I antiarrythmics act by decreasing the rapid phase of depolarization (phase 0) in conduction and myocardial fibres. Class I antiarrythmics such as lignocaine, tocainide and mexiletine exert a well-documented voltage and frequency-dependent block of cardiac sodium currents; they inhibit the fast sodium current while shortening action potential duration. This class of VGSC blockers is commonly prescribed for ventricular arrythmias, although CNS and gastrointestinal side effects, including bradycardia and hypotension, are well noted[33]. Compounds with more selectivity for the cardiac VGSC might have reduced side effects.

Mutations in the cardiac VGSC gene, SCN5A, have been associated with long QT syndrome 3 (LQT3), a congenital disease in which there is a delay in the repolarization of the cardiac action potential[34]. The mutations occur in the III–IV linker necessary for channel inactivation. Electrophysiological analysis of the mutated gene revealed prolonged inactivation kinetics leading to the generation of persistent current. The use of VGSC blockers in LQT3 is under consideration[34].

Epilepsy

Epileptic seizures are characterized by high-frequency trains of action potentials, resulting in repetitive synchronized burst firing of neuronal populations in the brain. Such seizures are broadly categorized as either partial or generalized. Partial seizures originate from a specific brain region, for example, the cerebral cortex, whereas generalized seizures involve both brain hemispheres.

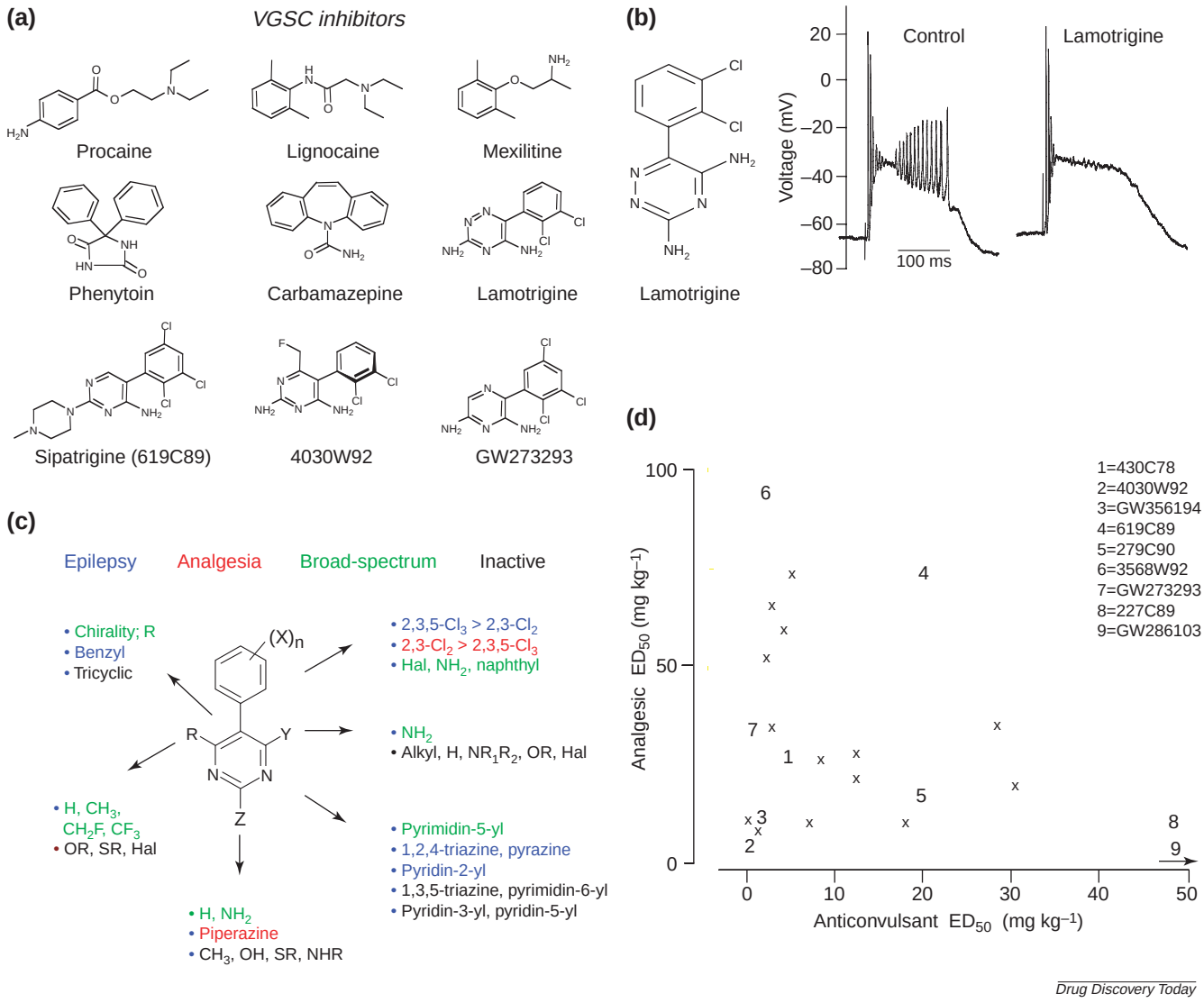


***Figure 3**. Lamotrigine series mode of action, therapeutic selectivity and SAR. (a) Structures for some of the common VGSC inhibitors. (b) Effect of lamotrigine on repetitive firing in hippocampal neurons in rat brain slices. Lamotrigine selectively blocks the epileptiform bursts induced when the slice was superfused in low magnesium and bicuculline, without affecting the initial action potential. (c) Structure-activity relationships for the lamotrigine series. (d) Therapeutic selectivity of lamotrigine analogues in animal models of epilepsy and pain.*

Generalized seizures can be further subdivided into clonic, myoclonic, tonic, tonic-clonic (grand mal) and absence (petit mal).

VGSCs are the molecular targets for the actions of several anti-epileptic drugs including phenytoin, carbamazepine and lamotrigine[35]. Lamotrigine was introduced into the clinic by GlaxoWellcome in 1990 as a broad-spectrum anticonvulsant with an improved therapeutic index compared with phenytoin and carbamazepine. These compounds are effective against partial and generalized tonic seizures but generally not against absence seizures, the notable exception being lamotrigine[35]. They bind preferentially to the inactivated state of the sodium channel and exhibit both voltage- and use-dependence crucial to the clinical efficacy of these drugs, resulting in a remarkable selectivity for pathological burst firing of action potentials[36] (Fig. 3b).

Several VGSC drugs are in development for epilepsy including rufinamide, which is in Phase III development for the treatment of partial and generalized tonic-clonic seizures. GW273293, which is structurally related to lamotrigine, is currently in Phase I clinical trials. It is five times

Table 4. Drugs on the market or in development

Compound	Company	Phase of development	Other indications
Local anaesthetics			
Ropivacaine	AstraZeneca	Launched	Post-operative pain
Levobupivacaine	Celltech	Launched	Post-operative pain
RSD-921	Nortran	Phase II	Arrhythmias
Ciprocaine	IQB	Preclinical	
Antiarrhythmics			
Moracizine	Shire	Launched	
Pilsicainide	Suntory	Launched	
Pirmenol	Warner Lambert	Launched	
E-047/1	Ebewe	Phase II	
Milacainide	Kissei	Phase II	
GYKI-16638	Egis	Preclinical	
Epilepsy			
Lamotrigine	GlaxoWellcome	Launched	Chronic pain, bipolar disorder
Oxcarbazepine	Novartis	Launched	Chronic pain
Fosphenytoin	Warner Lambert	Launched	Stroke
Topiramate	Johnson & Johnson	Launched	Chronic pain, bipolar disorder
Remacemide	AstraZeneca	Phase III	Huntington's/Parkinson's disease
Rufinamide	Novartis	Phase III	Chronic pain
GW273293	GlaxoWellcome	Phase I	
NW-1015	Newron	Phase I	Chronic pain, Parkinson's disease
Co-102862	CoCensys	Phase I	Chronic pain
AWD-33-173	Asta Medica	Preclinical	
Chronic pain			
Lidocaine patch	Endo Pharm	Launched	
(S)-mexilitine	Celgene	Discontinued	
LTA	AstraZeneca	Phase II	(Topical)
GW286103	GlaxoWellcome	Preclinical	
RS-132943	Roche	Preclinical	
WIN 17317-3	Sanofi Winthrop	Preclinical	
4030W92	GlaxoWellcome	Discontinued	Epilepsy
Stroke			
Sipatrigine	CeNeS	Phase II	CNS trauma
Irampanel	Boehringer Ingelheim	Phase II	Anaesthesia, tinnitus
Crobenetine	Boehringer Ingelheim	Phase II	Anaesthesia, tinnitus
NS-7	Nippon Shinyaku	Phase I	
RS-100642	Roche	Preclinical	Chronic pain
SL-651708	Sanofi–Synthelabo	Preclinical	
AM-36	AMRAD	Preclinical	
Bipolar disorder			
GW356194	GlaxoWellcome	Preclinical	

more potent than lamotrigine in a model of generalized tonic-clonic seizures but is only weakly active in the carrageenan pain test. This is consistent with its tenfold higher potency than lamotrigine against human brain type II VGSC. Other compounds in development as anticonvulsants include Co-102862 (CoCensys), NW-1015 (Newron) and RWJ-37947 (RW–Johnson).

Recent human genetic studies have supported the role of VGSCs in controlling epilepsy. A point mutation within the VGSC β1 subunit (SCNIB) has been linked to autosomal dominant generalized epilepsy with febrile seizures plus (GEFS+)[37]. This mutation probably disrupts a disulphide bridge required for maintaining the immunoglobulin-like fold in the extracellular domain and results in loss of

function – the β1 mutant is unable to accelerate inactivation of the brain IIa α-subunit when co-expressed in *Xenopus* oocytes. Mutations in the IIS4 and IVS4 voltage sensor regions of the type I α-subunit are responsible for GEFS+ type II (Ref. 38).

Pain

Pain can be broadly subdivided into three categories: (1) nociceptive (including acute surgical pain), (2) neuropathic and (3) inflammatory. Recently, most attention has focussed on the role of VGSCs in neuropathic pain, perhaps because of the largely unmet need[39], although further opportunities for therapeutic intervention do exist in each of the pain areas.

Local anaesthetics have a unique profile in pain treatment, from topical application to produce cutaneous anaesthesia, through to spinal administration for control of labour and surgical pain, and finally to systemic infusion for debilitating neuropathic pain[40]. The most recent generation of single enantiomer local anaesthetics, such as levobupivacaine and ropivaciane (Table 4), offer improved sensory blockade and reduced cardiovascular side effects; a possible reason for this could be VGSC subtype selectivity. Further improvements in therapeutic index might be achieved by targeting sensory neuron VGSCs.

Over the past few years, it has become increasingly apparent that anticonvulsants such as lamotrigine and carbamazepine, and local anaesthetics and antiarrhythmics such as lignocaine and mexilitine, offer benefit in neuropathic pain. This has led to the investigation of several VGSC inhibitors currently in development for epilepsy, including topiramate, oxcarbazepine, rufinamide, Co-102862 and NW-1015.

Lamotrigine has been shown to be analgesic in rat models of neuropathic pain and in the clinic for trigeminal neuralgia[41], but is ineffective in animal models of nociceptive pain. A systematic search for more potent analgesics from the lamotrigine series led to the identification of 4030W92 and GW286103. Both of these compounds have increased potency over lamotrigine in animal models of neuropathic and inflammatory pain. GW286103, the more potent analgesic, lacks anticonvulsant activity and does not elicit ataxia in animal models. The molecular basis for this selectivity has yet to be established.

Several analogues of the local anaesthetic mexilitine have been developed by Roche (Palo Alto, CA, USA) including RS100642 and RS-132943, the latter showing analgesic effects in a rat chronic model of neuropathic pain (Gogas, K.R. *et al.* Pharmacological characterization of the novel sodium channel blocker RS-132 943 in two animal models of neuropathic pain, *9th World Congress on Pain*, 22–27 August 1999, Vienna, Austria).

The expansion and cloning of the VGSC gene family has made a great impact in pain research. The availability of high-quality reagents to study tissue distribution, including type-specific antibodies and riboprobes, combined with the access to antisense reagents and gene knockouts, has further developed our knowledge of VGSCs in pain pathogenesis. Perhaps the most significant molecular advance has been the cloning of the sensory neuron-specific channels, SNS and SNS2, the coordinated activities of which are likely to comprise the TTX-resistant sodium current highly specific to sensory ganglia and critical to pain transmission[42].

Numerous lines of evidence support SNS and SNS2 as rational pain targets (Fig. 4; Box 2), including their exquisite tissue-specificity, changes in their distribution and phosphorylation seen in induction or maintenance of chronic pain states, and antisense studies using *in vivo* pain models[43] (Fig. 4). Targeting the peripheral VGSCs, SNS and SNS2 offer the potential for highly selective analgesics with no CNS or cardiovascular side effects. We have cloned and expressed rat and human SNS and SNS2 (Ref. 42), and are exploiting advances in plate-based and electrophysiological assay technology, together with expertise in VGSC pharmacology, to identify selective inhibitors of these targets.

Stroke

Ischaemic damage such as stroke or other brain injuries results in delayed neuronal damage in the brain. Although

Box 2. Regulation of SNS1 and SNS2 in injury

The TTX-R current (SNS1 and SNS2) is augmented by inflammatory mediators, such as PGE2, via the coordinated activities of protein kinase A and protein kinase C. It might therefore contribute to the sensitization of nociceptor terminals after inflammation[26]. In rat models, developed to reflect the neuropathic pain state in human patients, SNS1 protein translocates from the cell body to the peripheral axons of the sciatic nerve, accumulating at the site of injury[59]. Antisense oligonucleotides directed against SNS in a rat model of neuropathic pain, reverse the hyperalgesia associated with this model[43]. Studies with SNS and SNS2 have been extended to DRG and peripheral nerve tissue removed from human patients with characterized painful neuropathies. After brachial plexus injury, both SNS and SNS2 α-subunits redistribute in DRG neurones. There is an acute decrease of SNS and SNS2 protein in the sensory cell bodies of DRG whose central axons have been avulsed from the spinal cord and an increase in the staining intensity observed in nerve fibres[60].

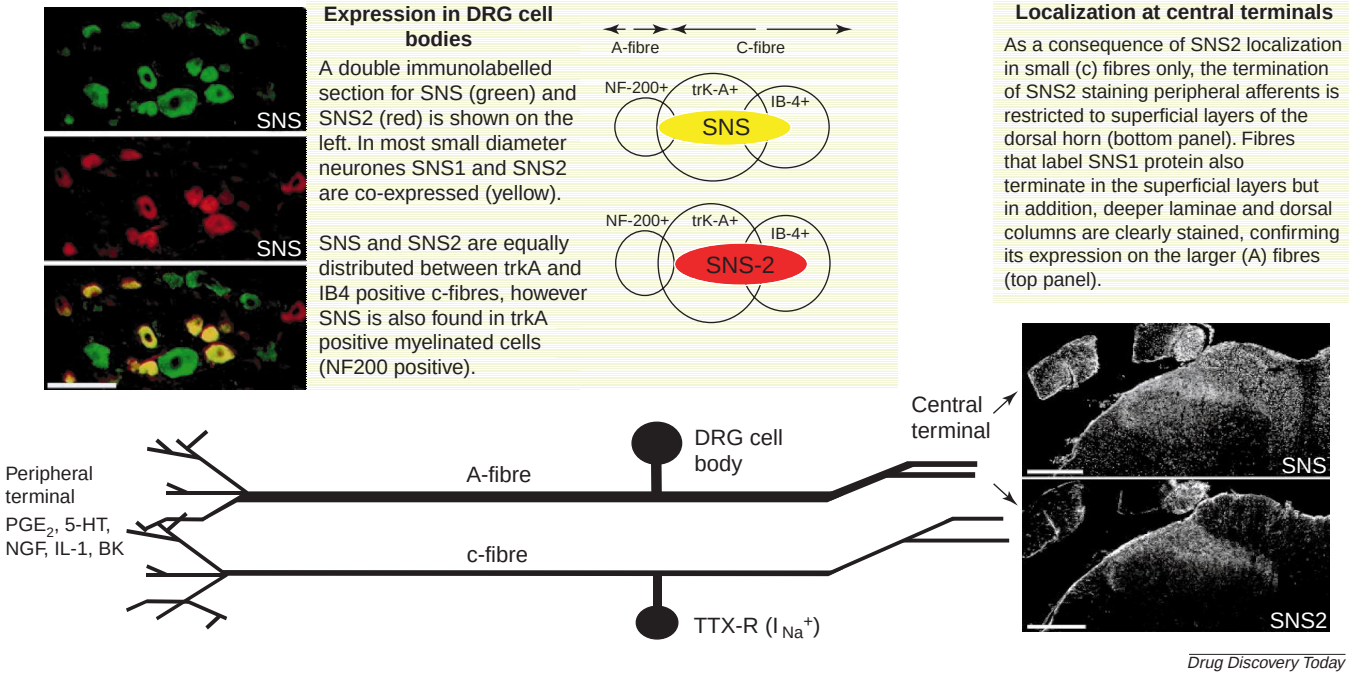


Figure 4. *Distribution of SNS and SNS2 and regulation in pain states. Distribution studies suggest that there is a dynamic expression of voltage-gated sodium channels in DRG neurones, which can change during development, response to injury and upon exposure to inflammatory mediators. Recently, most attention has focussed on the TTX-R channels (SNS1 and SNS2), which are localized in small diameter sensory neurones*[58].

there is currently no effective therapy, interest in VGSC drugs is considerable. VGSC inhibitors of relatively low potency and selectivity have been shown to prevent neuronal cell death in animal models of cerebral ischaemia, probably because of their modulation of glutamate release and neuroprotection during periods of energy depletion[44]. In the rat model of focal ischaemia, lamotrigine, riluzole and phenytoin reduce brain damage at doses that do not show significant ataxic- or cardiovascular-related side effects.

The results of clinical trials with the next generation of more potent and selective VGSC inhibitors will be eagerly awaited. This is exemplified by sipatrigine, which inhibits veratrine-evoked glutamate release from rat cortical slices and provides marked neuroprotection following permanent middle cerebral artery occlusion[45]. Sipatrigine has also been shown to reduce glutamate release and prevent neuronal degeneration in a variety of models of ischaemia or brain injury[46–50].

A search for yet more potent and or more state-selective inhibitors has led to compounds such as 202W92 (GlaxoWellcome), BIIR-561 (Boehringer Ingelheim, Ingelheim, Germany), BIII-890 (Boehringer Ingelheim), RS100642 (Roche) and NS-7 (Nippon Shinkayu, Japan) being developed for the treatment of stroke.

Bipolar depression

Bipolar disorder is a complex disease characterized by recurrent episodes of depression together with one or more episodes of mania. Data from six independent clinical studies, including a Phase III trial[51], over the past four years demonstrate that lamotrigine is an effective treatment. The most recent study was carried out in 324 patients who met specific criteria for rapid cycling bipolar disorder; more than 40% of the patients taking lamotrigine responded to treatment. Improvements were noted in both depressive and manic phases, and it appears that lamotrigine does not cause mood destabilization.

In the absence of any animal models, one can only speculate on the role of VGSCs in bipolar depression. In the manic phases of the disease, one can expect hyper-excitability of the limbic system, resulting in aberrant signalling cascades[52]. The control of neuronal hyper-excitability and synaptic plasticity by VGSC inhibitors might reduce the excessive release of neurotransmitters

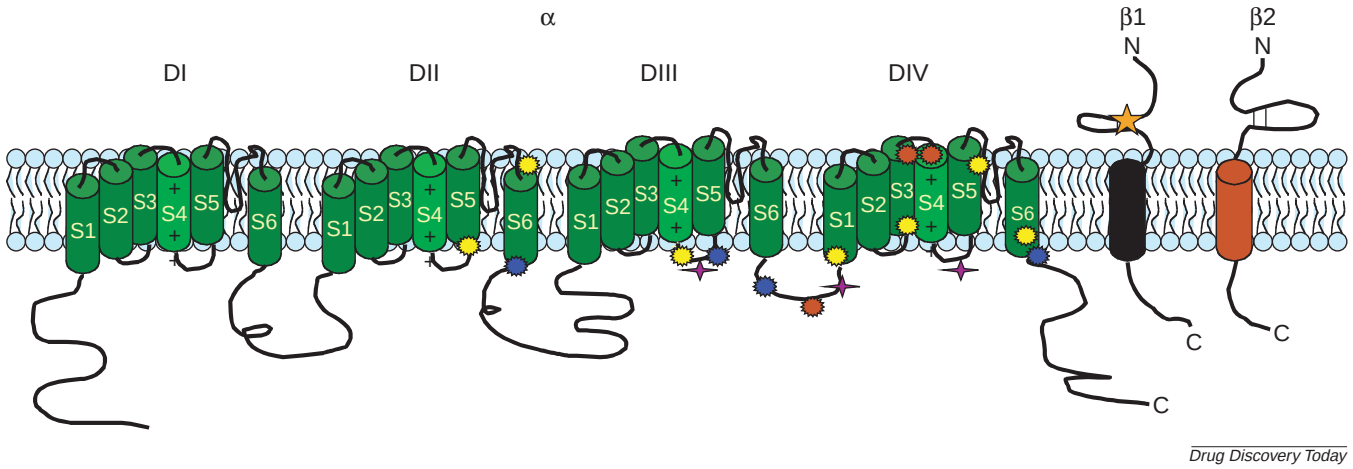


Figure 5. *VGSC channelopathies. Refer to Table 1 for key to symbols.*

and help to control the mania. It is less clear whether or not the effect of lamotrigine on the depressive phases can be attributed to VGSC inhibition because lamotrigine has a range of pharmacological actions at other targets including high-threshold calcium channels. An analogue of lamotrigine, GW356194, is currently in development for this indication.

Hereditary muscle diseases

Genetic analysis has shown the hereditary muscle disorders hyperkalemic periodic paralysis, type II (Hyper PP), potassium aggravated myotonia (PAM) and paramyotonia congentia (PC) to be tightly linked to the human skeletal muscle VGSC on chromosome 17 (Ref. 53). Patients with these disorders show episodic loss of excitability of skeletal muscle. Across these three disorders, 16 disease-causing amino acid changes have been identified (Fig. 5). A variety of these channel mutants have been functionally expressed and the results have always shown a delay in inactivation, leading to a persistent sodium current[54]. These subtle changes in channel function are manifest either as hyper-excitability (myotonia) or hypo-excitability (paralysis), dependent on the degree of membrane depolarization.

Other diseases

Marketed inhibitors of VGSCs continue to be evaluated in conditions such as Parkinson's disease, schizo-affective disorder, tinnitus, migraine and substance abuse, although the multiple actions of these drugs often makes it difficult to clearly associate any clinical benefits with inhibition of VGSCs[55].

Assay technology and target-driven drug discovery

Target-driven drug discovery is still in its infancy for VGSCs, similar to other voltage-gated ion channels. The technical issues that have inhibited it are: (1) stable expression of VGSCs and (2) robust high-throughput assays.

Cloning, manipulation and expression of the VGSC α-subunit genes has proved to be a considerable challenge for a combination of reasons. The very large cDNAs tend to be highly unstable with a high rate of spontaneous rearrangement, deletion and point mutation. This is most likely caused by instability of the repeated domains and toxicity of the gene product. The use of recombination-deficient hosts and propagation at low temperatures, low copy vectors, polycistronic and regulated mammalian vectors have all contributed to solving these difficulties and have allowed the stable expression and functional analysis of the entire family of human α-subunit genes.

Electrophysiology is the gold standard functional assay and the only assay that gives information on the state-dependence of inhibition. However, currently it is a very low-throughput assay that can only be run by highly trained personnel, and despite improvements in liquid handling for automated compound delivery, yields a maximum of ~150 data points per week per electrophysiologist. Fully automated patch clamp assays more amenable to high-throughput screening are currently being developed. In the meantime, there has been a need to develop high-throughput functional assays that correlate with electrophysiology.

The current selection of assays available, including recent advances, are summarized in Table 5. The formats

Table 5. Ion-channel assay technologies

Assay	Detection instrument	Throughput: data points per week	Gating method	Advantages	Disadvantages
Electrophysiology	Patch-clamp rig	150	Voltage/current command protocols	Gold standard assay High information content	Low-throughput assay Not easily amenable to automation
Bis-Oxonol dye re-distribution membrane-potential [e.g. $DiBAC(4)_3$]	FLIPR	96-well (15 000) 384-well (60 000)	Toxin to activate channel or delay inactivation Electrical field stimulation	High-throughput functional assay Amenable to automation	Relatively slow reporting of changes in membrane potential
^{14}C-guanidine flux assay	Scintillation counter	96-well (20 000)	Toxin to activate channel or delay inactivation	Measurement of ion flux through channel, relatively high-throughput, amenable to automation	End-point assay, rather than continuous functional recording
Aurora-FRET–based membrane-potential assay	VIPR	96-well (20 000)	Toxin to activate channel or delay inactivation	High-throughput functional readout, rapid reporter of changes in membrane potential, amenable to automation Ratiometric	Proprietary technology available through corporate alliance
Binding assay	Scintillation counter	>30 000	Non-functional assay	High-throughput	Very low information content, quality membrane preparations needed, several possible binding sites for drug action

Abbreviations: FLIPR, fluorescence imaging plate reader; VIPR, voltage ion probe reader.

available include binding assays, radiolabelled cation flux and voltage-sensitive fluorescent dyes. The latter potentially allow kinetic measurements, although commonly available dyes (e.g. DiBAC) respond very slowly compared with the fast kinetics of VGSCs. The fluorescence energy transfer (FRET) based system developed by Aurora Biosciences (San Diego, CA, USA) largely overcomes this problem by offering a much faster time resolution[56]. All current plate-based assays rely on toxins to activate the channel with the disadvantage of possible allosteric interactions with test compounds. More physiological methods for gating by electric field stimulation are under development; these require fast-responding dyes such as those available from Aurora. None of these assays give information on state-dependence, which currently has to be gained in secondary assays by electrophysiology. Binding assays, although potentially very high throughput, are not considered as a particularly attractive option, given the technical challenges in high level VGSC expression and the possibility of unknown drug-binding sites.

Conclusion

Although empirical pharmacology has yielded many drugs acting at VGSCs, a more rational approach is required to exploit the full therapeutic potential in this area. Current drugs have low potency and it is now possible to screen recombinant VGSCs in order to identify more potent inhibitors with reduced compound-based adverse reactions. Current drugs are also relatively non-specific, targeting VGSCs and other ion channels. It is now feasible to screen for subtype selective drugs that might have greater efficacy with reduced side effects.

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