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REVIEW

Molecular Diversity of Structure and Function of the Voltage-Gated Na^+ Channels

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ABSTRACT—A variety of different isoforms of voltage-sensitive Na^+ channels have now been identified. The recent three-dimensional analysis of Na^+ channels has unveiled a unique and unexpected structure of the Na^+ channel protein. Na^+ channels can be classified into two categories on the basis of their amino acid sequence, Na_V1 isoforms currently comprising nine highly homologous clones and Na_X that possesses structure diverging from Na_V1, especially in several critical functional motifs. Although the functional role of Na_V1 isoforms is primarily to form an action potential upstroke in excitable cells, recent biophysical studies indicate that some of the Na_V1 isoforms can also influence subthreshold electrical activity through persistent or resurgent Na^+ currents. $Na_V1.8$ and $Na_V1.9$ contain an amino acid sequence common to tetrodotoxin resistant Na^+ channels and are localized in peripheral nociceptors. Recent patch-clamp experiments on dorsal root ganglion neurons from $Na_V1.8$-knock-out mice unveiled an additional tetrodotoxin-resistant Na^+ current. The demonstration of its dependence on $Na_V1.9$ provides evidence for a specialized role of $Na_V1.9$, together with $Na_V1.8$, in pain sensation. Although Na_X has not been successfully expressed in an exogenous system, recent investigations using relevant native tissues combined with gene-targeting have disclosed their unique "concentration"-sensitive but not voltage-sensitive roles. In this context, these emerging views of novel functions mediated by different types of Na^+ channels are reviewed, to give a perspective for future research on the expanding family of Na^+ channel clones.

Keywords: Voltage-gated Na^+ channel, Action potential, Ion channel, Tetrodotoxin, Sensory neuron

1. Introduction

The voltage-sensitive Na^+ channel is a transmembrane protein essential for the generation of action potentials in excitable cells. The mammalian Na^+ channel consists of an α-subunit (approximately 260 kDa) that encodes the core protein of the channel and auxiliary β-subunits (30 – 40 kDa) that modify the channel function (1). A variety of different isoforms of α-subunits have been identified (2, 3). Since these α-subunits were not termed in any systematic way (see the heading "traditional names" in Table 1), the standardized nomenclature has recently been proposed (4).

The amino acid sequence homology and genetic locations for α-subunits known to date are illustrated in Figs. 1 and 2, respectively, and their fundamental properties are summarized in Table 1. Among them, 9 isoforms can be reasonably put into a single family, Na_V1 (3, 4). All of these nine Na_V1 isoforms, except for $Na_V1.9$, whose expression in human embryonic kidney (HEK) 293 cells (5) has been attributed to the endogenous Na^+ current (6), have been successfully expressed in exogenous systems.

Although the functional role of the Na_V1 isoforms is primarily to form an action potential upstroke in excitable cells, recent studies indicate that they can also influence subthreshold electrical activity in neurons through the persistent or resurgent Na^+ currents (7). This has led to a new concept that the Na^+ channels themselves can integrate overall neuronal firing behavior. In addition, some of the Na_V1 isoforms are preferentially expressed in the subset of primary sensory neurons exerting specialized roles in pain sensation (6, 8).

The remaining one isoform, Na_X, has structure diverging from Na_V1 isoforms, especially in several critical functional motifs, such as the S4 voltage sensor or the interdomain III-IV linker (9, 10). Recent investigations suggest that this

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Table 1. Properties of Na^+ channel α-subunits

Formal name	Traditional names	Gene symbol	Chromosomal location*	TTX-sensitivity**	Predominant location	Expression in DRG***
$Na_v1.1$	type I, rat I, Scn1a HBSCI, GPBI	*SCN1A*	M:2 H:2q24	S	CNS, PNS	+++
$Na_v1.2$	type II, rat II HBSCII, HBA	*SCN2A*	M:2 H:2q23–24	S	CNS	+
$Na_v1.3$	type III, rat III	*SCN3A*	M:2 H:2q24	S	CNS (embryonic)	upregulated after axotomy
$Na_v1.4$	SkM1, μ1	*SCN4A*	M:11 H:17q23–25	S	skeletal muscle	–
$Na_v1.5$	SkM2, rH1, H1	*SCN5A*	M:9 H:3p21	R	heart muscle	–
$Na_v1.6$	type IV, NaCh6, Na6 PN4, Scn8a, CerIII	*SCN8A*	M:15 H:12q13	S	CNS, PNS, glia nodes of Ranvier	+++
$Na_v1.7$	PN1, hNE, Nas	*SCN9A*	M:2 H:2q24	S	PNS Schwann cell	+++
$Na_v1.8$	SNS, PN3, NaNG	*SCN10A*	M:9 H:3p22–24	R	PNS (sensory neurons)	+++
$Na_v1.9$	SNS2, NaN, NaT, SCN12A	*SCN11A*	M:9 H:3p21–24	R	PNS	+++
Na_x	$Na_v2.1$, $Na_v2.2$, $Na_v2.3$, SCL11, NaG	*SCN6A* (*SCN7A*)	M:2 H:2q21–23	R	heart, uterus, glia, PNS smooth muscle	+

*M: mouse, H: human. **S: sensitive, R: resistant. ***+++: rich, +: present, –: absent. TTX: tetrodotoxin, DRG: dorsal root ganglion.

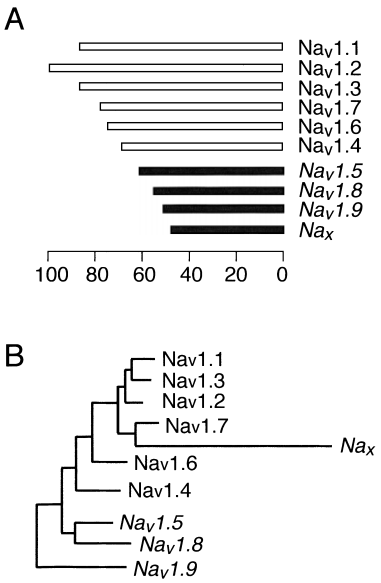


Fig. 1. Schematic presentations of amino acid sequence homology and phylogenetic relationships of rat voltage-gated Na^+ channel α-subunits. A: Amino acid homology is expressed by % identity to $Na_V1.2$. tetrodotoxin (TTX)-sensitive and TTX-resistant isoforms are shown with open and shaded bars, respectively. B: The phylogenetic tree, modified from Goldin (2) and Goldin et al. (3).

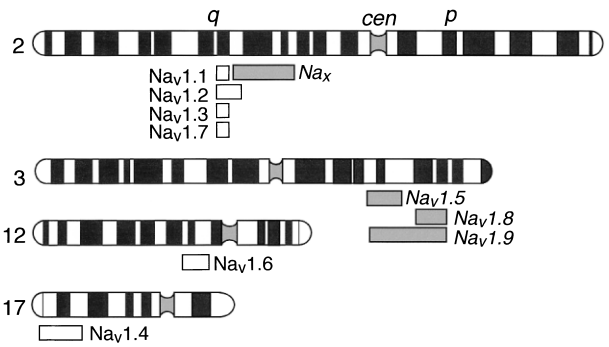


Fig. 2. Human chromosomal karyogram. Locations of genes for Na^+ channel α-subunits in chromosomes 2, 3, 12 and 17 are schematically drawn. Approximate locations of Na^+ channel genes are shown as boxes (exact loci are given in Table 1). Genes expressing tetrodotoxin (TTX)-sensitive and TTX-resistant Na^+ channels are shown by open and shaded boxes, respectively. "*p*", "*q*" and "*cen*" represent short arm, long arm and centromere, respectively.

isoform may be related to transport or absorption of Na^+ ions, by sensing the Na^+ concentration gradient across the membrane (11). Such a "concentration-sensitive" but not "voltage-sensitive" Na^+ channel may produce a variety of important cellular effects, and it undoubtedly adds an additional novel mechanism to diverse Na^+ channel functions.

The aim of this review is to discuss and update a variety of newly reported Na^+ channel forms and functions, focusing on: 1) three-dimensional (3D) image of Na^+ channels, 2) Na^+ channel toxins, 3) persistent and resurgent Na^+ currents, 4) a role of Na^+ channels in pain sensation, 5) emerging views on novel concentration-sensitive Na^+ channels,

and 6) recent progress in genomic analysis of hereditary Na^+ channelopathies.

2. Three-dimensional view of the Na^+ channel

The 3D image of the Na^+ channel studied using helium-cooled cryo-electron microscopy combined with the single-particle image analysis (12) indicates that the Na^+ channel from the eel *Electrophorus electricus* has a bell-shaped outer surface of 135 Å in height, 100 Å in side length at the square-shaped bottom, and a half-spherical top with a diameter of 65 Å (Fig. 3). The upper half-spherical narrower region has four small orifices that probably serve as the extracellular "inlets" for Na^+ ions. These small inlets are connected to the central pore that is thought to represent the ion-permeation pore. The central pore is narrowed at the transmembrane region and splits into four branches at the lower square-shaped region forming four larger orifices that probably serve as the cytoplasmic "outlets" for Na^+ ions. Recently, similar 3D images have been obtained from the *Shaker* K^+ channel (13).

Unexpectedly, in addition to the central ion-permeation pore, four narrow and twisted peripheral pores ("b" in Fig. 3), one in each domain, were found inside the Na^+ channel. These narrow peripheral pores may be involved in voltage-sensing and gating. According to Sato et al. (12), these additional pores may be important for creating the internal hydrophilic environment that enables subtle outward movement of the S4 voltage sensors. The narrow peripheral pores may represent "gating pores" designed to allow the outward translocation of S4 gating charges across the membrane.

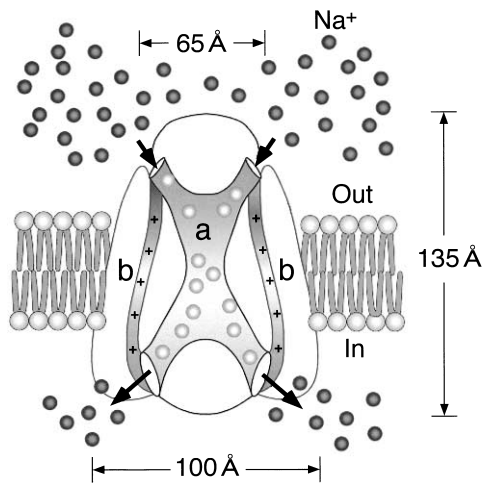


Fig. 3. A schematic structure of the voltage-gated Na^+ channels based on the three-dimensional image of Sato et al. (12). "a" and "b" represent the central ion-permeation pore and gating pore, respectively.

3. Na^+ channel toxins

A variety of toxins and drugs bind to Na^+ channels. Since there have been several excellent reviews on toxins and drugs acting on Na^+ channels (2, 14), this topic will be only briefly summarized here, adding the most recent findings. Table 2 summarizes classification of toxins and drugs acting on Na^+ channels with their fundamental actions and binding sites on α-subunits. Toxins are classified into five groups on the basis of their discrete binding sites. Recently, a novel neuroactive toxin, β-pompilidotoxin, has been purified from the venom of the spider wasp (15). Using chimeric mutants of $Na_V1.2$ and $Na_V1.5$, the binding site for this toxin has been shown to be the S3 – S4 extracellular loop of domain IV (16).

Most of the toxins, except for tetrodotoxin (TTX) and its congeners that occlude the outer pore of the channel, bind to sites that are related to activation and inactivation process (Fig. 4). Some of these binding sites are also the target of mutation in hereditary Na^+ channelopathies (see Fig. 13). For this reason, several toxins can produce abnormal channel kinetics reminiscent of those seen in hereditary Na^+ channelopathies. Various drugs that act on Na^+ channels, such as local anesthetics, analgesics and antiepileptics, bind to the inner surface of the channel pore (domain IV S6 segment), producing resting and use-dependent blocks (17).

4. Subthreshold Na^+ currents in neurons

In addition to the rapidly inactivating Na^+ current associated with action potential generation, the Na^+ channel can give rise to subthreshold types of Na^+ currents. Recently, much attention has been given to the possible functional significance of subthreshold Na^+ currents, since they play pivotal roles in regulating repetitive firing, amplifying dendritic depolarizations, and producing after-depolarizations and plateau potentials (18).

4-1. Persistent Na^+ currents in neurons

Persistent (noninactivating) Na^+ currents flowing at subthreshold potentials have been recorded in various tissues such as frog node of Ranvier (19), cat lumbar motoneurons (20), rat and guinea pig hippocampal neurons (21), rat ventricular myocytes (22), rat optic nerve axons (23), rat and cat cortical pyramidal neurons (24), guinea pig trigeminal motoneurons (25), rat dorsal root ganglion (DRG) neurons (26) and guinea pig cerebellar Purkinje cells (27).

The persistent Na^+ currents may be due to overlapping activation-inactivation kinetics (a window current) or a wide repertoire of gating mode ("mode switching") (24) of the same channels mediating transient Na^+ currents. However, some of the persistent Na^+ currents are most likely to

Table 2. Na^+ channel toxins and drugs

Toxins and drugs	Mechanism of action	Binding site
tetrodotoxin, saxitonin, μ-conotoxins	inhibition of conductance due to pore-occlusion	SS1 – SS2
veratridine, batrachotoxin, aconitine, grayanotoxin	persistent activation due to m_∞ negative shift	I-S6 and IV-S6
α-scorpion toxin, β-pompilidotoxin, sea anaemone toxin	prevent outward movement of IV-S4 voltage sensor	IV-S3 – S4 loop
β-scorpion toxins	Trap II-S4 voltage sensor in outward position	II-S3 – S4 loop
brevetoxins, ciguatoxins	persistent activation due to m_∞ negative shift	I-S6 – IV-S5
local anethetics and their congeners	resting and use-dependent block of the channel	IV-S6

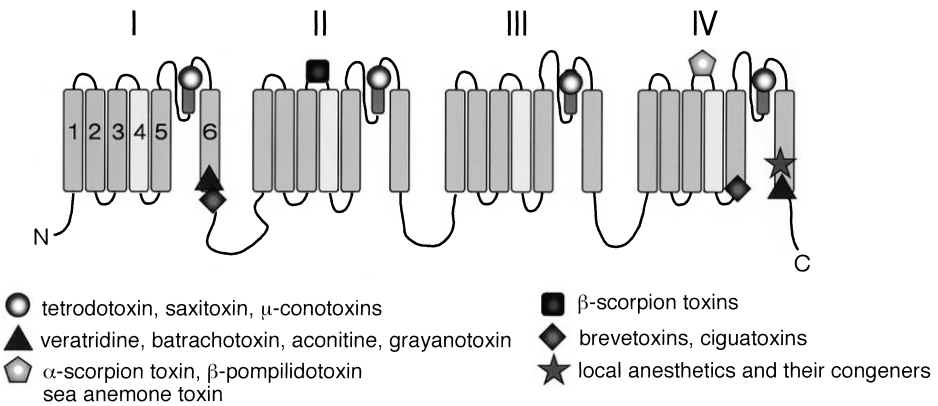


Fig. 4. Topology of toxin and drug binding sites on Na^+ channel α-subunit. Two dimensional molecular map of the α-subunit is schematically shown with its structural parts. Each symbol represents the toxin or drug binding site indicated at the bottom of the figure.

be generated by Na^+ channels distinct from the transient Na^+ channels (28, 29). $Na_V1.6$ has been implicated as a possible mediator of the persistent Na^+ currents in central and peripheral neurons. There is evidence to suggest that the persistent Na^+ current in cerebellar Purkinje cells (29), late Na^+ current in large-diameter neurons of DRG (26) and the "threshold" current at nodes of Ranvier (19) are mediated by $Na_V1.6$. In fact, $Na_V1.6$ is abundantly expressed in these tissues (30). Recently, a novel persistent Na^+ current, which is highly resistant to TTX, has been reported in small nociceptive neurons in DRG (6). This current will be described in the later section on TTX-resistant (TTX-R) Na^+ channels.

The persistent Na^+ currents may also be involved in disorders of neurons and neurites, as suggested from the finding that TTX and saxitoxin exerted a protective effect on the nerve injury produced by anoxic insult (31). Unlike transient Na^+ currents that are immediately inactivated following membrane depolarization, persistent Na^+ currents continue to be activated during prolonged membrane depolarization. The resultant increase in $[Na^+]_i$ through persistent Na^+ influx, coupled with reverse operation of the Na^+-Ca^{2+} exchanger, may cause a damaging increase of $[Ca^{2+}]_i$ (23).

4-2. Resurgent Na^+ current

In addition to the persistent Na^+ current reported by Llinás and Sugimori (27, 32), cerebellar Purkinje cells reveal another type of unusual kinetics, referred to as "resurgent Na^+ current" (7, 28). Following a brief and strong step depolarization, which can be best mimicked by an action potential generation, repolarization to moderately depolarized voltages around –40 mV elicits a slowly rising, slowly decaying Na^+ current. This current is distinct from other subthreshold Na^+ currents, since the current is not "persistent" and apparently differs from so-called tail current following deactivation, since the current is "slowly rising". Resurgent Na^+ current effectively produces transient subthreshold Na^+ current relevant to repetitive firings. The extent and speed of this current depend markedly on the voltage and duration of the preceding depolarization, i.e., larger and faster after brief, large depolarizations (e.g., 5 ms at +30 mV) than after long, smaller depolarizations (e.g., 100 ms at –30 mV).

Figure 5 illustrates the proposed model for the generation of resurgent Na^+ current. The mechanism underlying resurgent Na^+ current has been explained by a model that incorporates, in addition to normal inactivation, an additional "blocked" state in which channels recover by passing

transiently through the open state. The blocked state could represent a voltage-dependent block of the channel by an endogenous particle such as a polyamine that can enter and exit only when channels are open.

Resurgent Na^+ currents were absent in ataxic mice lacking expression of *Scn8a* or from jolting mice with a single amino acid mutation (missense mutation) in *Scn8a* (33), suggesting that *Scn8a* carries resurgent Na^+ current. In these mutant mice, evoked bursts of spikes typical of Purkinje cells were also diminished, indicating that resurgent Na^+ current is important for burst firings. Resurgent Na^+ current flowing immediately after an action potential may effectively depolarize cells reciprocally, promoting the high-frequency firing. Although *Scn8a* is highly expressed also in hippocampal neurons (7) and spinal motoneurons (34), these neurons had little or no resurgent Na^+ current (7, 35). Furthermore, $Na_V1.6$ heterologously expressed in *Xenopus* oocytes were devoid of resurgent Na^+ current (36). Thus, the presence or absence of resurgent Na^+ current appears to depend on cellular factors other than the mere presence of the *Scn8a* transcript.

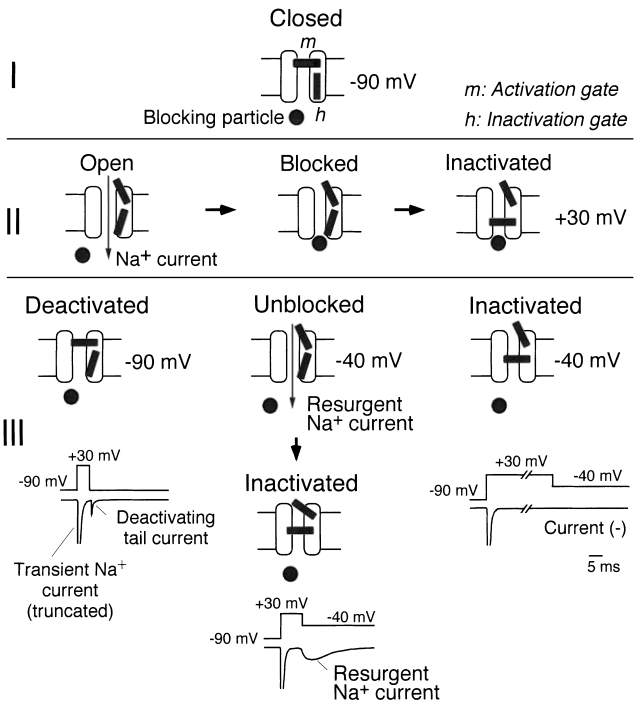


Fig. 5. A state model for resurgent Na^+ current. From "closed" state at –90 mV (I), step depolarization to +30 mV leads to a mixture of "open" state, "blocked" state where a hypothetical blocking particle is trapped in the channel in a voltage-dependent manner, and "inactivated" state (II). Depending on the time elapsed, the open state will shift toward the inactivated state. After varying durations of depolarization at +30 mV, the membrane is repolarized (III). When the membrane is repolarized to –90 mV immediately after the depolarization, open state channels give rise to deactivating tail current (left). When the membrane is repolarized to –40 mV after longer depolarization, where a "blocked" state has developed, the blocking particles will be released, causing reopening of the channel, thus producing resurgent Na^+ current (middle upper). Resurgent Na^+ current decays with time due to the normal inactivation process (middle lower). The repolarization to –40 mV after prolonged depolarization, where all the channels have already shifted into the inactivated state, would not give rise to any detectable current (right).

5. Pain sensation and Na^+ channels

It is now apparent that multiple types of Na^+ channel isoforms play pivotal roles in pain sensation and its pathological changes. Information obtained from recent investigations on Na^+ channels contributed to the understanding of cellular events underlying pathological pain states and to the establishment of a rational basis for a novel evidence-based therapeutic management of painful states.

5-1. Two types of small DRG neurons

DRG neurons are characterized by differences in expressions of neurotrophin receptors and termination within the spinal cord (14, 37, 38), as schematically shown in Fig. 6. Large-diameter Aβ neurons having specialized skin receptors at their peripheral axon terminals are NF200-positive and express p75 / trkB / trkC, receptors for NT-3, and they terminate mainly in layers III and IV. The small-diameter C-fiber neurons can be subdivided into two major subpopulations, based on binding of isolectin IB4 and distinct trophic factor dependence. IB4-positive cells express GFRα1,2 / Ret, receptors for the glial cell line-derived neurotrophic factor (GDNF), terminate mainly in layer II_i, and preferentially express the $P2X_3$ receptors. In contrast, IB4-negative cells are characterized by predominant expression of trkA / p75, receptors for nerve growth factor (NGF), terminate mainly in layers I and II_o, and coexpress the neuropeptides calcitonin gene-related peptide (CGRP)

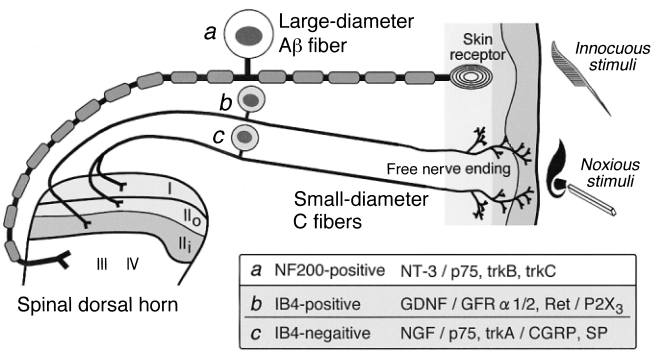


Fig. 6. Principal subgroups of primary sensory neurons with their trophic factor dependence and spinal terminations, modified from Clare et al. (14) and Boucher and McMahon (38). Biochemical properties of myelinated and unmyelinated afferent fibers are shown. GDNF: glial cell line-derived neurotrophic factor, NGF: nerve growth factor, CGRP: calcitonin gene-related peptide, SP: substance P.

and substance P (SP).

An important question is whether the above two populations of small DRG neurons have different functional roles. As yet, there is no definitive answer to this question, but recent two studies (39, 40) might provide suggestive new evidence for their distinct functional roles. Ablation of cells in lamina I that may receive inputs from IB4-negaitive DRG neurons had no effects on baseline behavioral responsiveness to nociceptive thermal and mechanical stimuli. However, hypersensitivity normally induced by capsaicin was almost completely abolished (39). Elimination of protein kinase C in lamina II_i neurons that may receive inputs from IB4-positive DRG neurons abolished hyperalgesic responses induced by nerve injury, whereas the behavioral responses to acute thermo- and mechano-nociceptive stimuli were again unaffected (40). These results suggest that both IB4-negative and IB4-positive DRG neurons may be critically involved in the establishment of chronic pain states. The former may be important in inflammatory conditions, and the latter in neuropathic states.

5-2. TTX-resistant Na^+ channels in DRG

TTX occlude the outer pore of the channel forming hydrogen-bonds with SS1 – SS2 regions of the channel. A single residue in domain I SS2 region is critical for TTX-binding, (6, 8, 41) (Fig. 7). Due to this specific binding, TTX has been widely used as a chemical tool for blocking Na^+ channels. However, it is now well established that several types of Na^+ channel isoforms are resistant to a high concentration of TTX (42).

DRG can express all the Na^+ channel subtypes except for those expressed in muscle ($Na_V1.4$ and $Na_V1.5$), in normal and pathological conditions (43). Five Na^+ channel isoforms, $Na_V1.1$ and $Na_V1.6 – 1.9$, are predominantly expressed in normal adult DRG (44, 45) (see Table 1). Among them, $Na_V1.8$ and $Na_V1.9$ contain a structural motif common to TTX-R Na^+ channels (6, 8, 41). These two types of Na^+ channels appear to constitute an older evolutionary group within the Na^+ channel multigene family (see Fig. 1B). These isoforms are thought to have specialized roles in pain sensation, since they are preferentially expressed in small nociceptive neurons with Aδ/C-fibers (46).

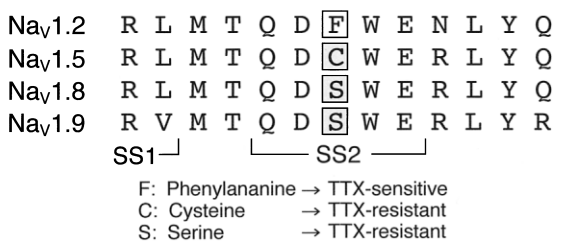


Fig. 7. The amino acid sequence of the SS-2 region of domain I for rat Na^+ channels. Conventional 1-letter abbreviations were used for amino acids. The amino acid residues which are critical for tetrodotoxin (TTX)-sensitivity are framed with boxes.

Although the two TTX-R Na^+ channels, $Na_V1.8$ and $Na_V1.9$, show overlapping distributions within small DRG neurons, $Na_V1.8$ transcripts are expressed in both IB4-positive and IB4-negative neurons, while $Na_V1.9$ transcripts are confined to IB4-positive neurons. NGF, known to be involved in the development of hyperalgesia, regulates $Na_V1.8$ mRNA levels, whereas the down-regulation of $Na_V1.9$ mRNA is not prevented by NGF-treatment (47). Treatment with GDNF up-regulates both $Na_V1.8$ and $Na_V1.9$ mRNA in IB4-positive neurons. These neurotrophin-dependences are consistent with the co-localizations of neurotrophin receptors and Na^+ channel isoforms and suggest that the two types of TTX-R Na^+ channels may play distinct roles in nociception.

Recent studies indicate that the two TTX-R Na^+ channels differ in their electrophysiological properties. Kinetics of $Na_V1.8$ is characterized by slow activation and inactivation gatings, relatively depolarized activation threshold, and small single channel conductance (48 – 50). In contrast to $Na_V1.8$, the Na^+ current mediated by $Na_V1.9$ has not been identified until quite recently, since its expression in exogenous systems has not been successful. Recent patch-clamp experiments on small DRG neurons from $Na_V1.8$-null mutant (knock-out) mice, where $Na_V1.8$-mediated TTX-R current is absent, unveiled an additional TTX-R Na^+ current (6). The demonstration of its dependence on $Na_V1.9$ provided evidence for a novel TTX-R Na^+ current. $Na_V1.9$-mediated current has much slower kinetics and a more hyperpolarized activation threshold than the $Na_V1.8$-mediated current, thus giving rise to a persistent Na^+ current at subthreshold voltages. $Na_V1.9$ may therefore contribute to the regulation of subthreshold excitability of small DRG neurons.

5-3. Nociceptors and Na^+ channels

One important issue to be confirmed in pain sensation is whether the Na^+ channel, which mediates action potential generation in axons of small C-type sensory neurons, is sensitive or resistant to TTX. Although it has been generally accepted that the conducting axonal action potentials are sensitive to TTX, evidence has been accumulating that nociceptive signals are rather dependent on TTX-R propagating action potentials in C-fibers (51 – 53).

Cutaneous receptors are difficult to study, as they are small and physically inaccessible in the skin, so dissociated DRG neuronal somata have been widely used as a model system for functional analyses of events occurring in axons or nerve terminals. However, to study the mechanism of nociception, it is vital to observe directly events at nerve terminals where noxious information is actually sensed by

nociceptors and transduced to action potentials. Recently, two forms of studies attempting this direct approach have been reported (52, 53). These experiments have produced intriguing results indicating that TTX-R but not TTX-sensitive (TTX-S) spikes contribute to the transduction of the nociceptive stimuli at nerve terminals.

Figure 8 schematically outlines the experiment on the nociceptor sensitivity of the corneal membrane (52). Corneal epithelial tissue is particularly advantageous for the above purpose, since this tissue is thin, avascular, transparent and innervated exclusively by Aδ and C fibers; and in addition, thermal, chemical or mechanical stimulation produces "orthodromic" spikes. "Orthodromic" spikes recorded directly from the corneal surface in response to mechanical pressure or topical application of capsaicin were resistant to TTX, whereas "antidromic" spikes evoked by electrical stimulation of optic nerve were abolished by a low concentration of TTX.

5-4. Inflammatory pain and Na^+ channels

Increased hyperexcitability of primary sensory neurons may underlie pathological pain sensation accompanying inflammation. There is strong evidence to suggest that the altered function of Na^+ channels is responsible for this hyperexcitability (54). Inflammatory pain (nociceptor-mediated pain) could be associated with lowered activation thresholds of peripheral nociceptors and resultant up-regulation of Na^+ channels, thus causing hyperalgesia (Fig. 9).

$Na_V1.8$ has been postulated as a possible candidate for the Na^+ channel vital to inflammatory pain, based on its dependence on NGF and other hyperalgesic agents (55, 56) and the analgesic efficacy of $Na_V1.8$-antisense in inflammatory pain models (57). The above idea is supported by evidence demonstrating that selective "knock-down" of $Na_V1.8$ with gene targeting prevents hyperalgesia caused by carrageenan-induced inflammation (58). The rapid repriming property of $Na_V1.8$ (49) suggest that $Na_V1.8$ plays a specialized, pathophysiological role in the repetitive firing of the peripheral neuron during inflammation. Drugs that target $Na_V1.8$ might offer a beneficial treatment of chronic inflammatory pain with minimal side effects, in view of their extremely localized expression confined in a subset of sensory neurons.

5-5. Neuropathic pain and Na^+ channels

Neuropathic pain is characterized by spontaneous burning pain and exquisite pain to innocuous stimuli (allodynia). Neuropathic pain is commonly caused by physical or chemical damage to nerve fibers or diseases affecting the nervous tissue. Unlike nociceptive or inflammatory pain that acts as a defense mechanism, neuropathic pain is an entirely useless and harmful pain. A number of studies have suggested that neuropathic pain is caused by spontaneous firings arising from the injury site of the afferent nerve fiber (ectopic spikes), attributable to abnormal build up of Na^+ channels at an injured site where nerve sprouting or neuroma can be seen (59). Immunocytochemical studies demonstrated that $Na_V1.8$ is up-regulated in nerve sprouts or neuroma, suggesting a role for TTX-R Na^+ channels in neuropathic pain (60). On the contrary, the ectopic spike was blocked by a low concentration of TTX, suggesting a

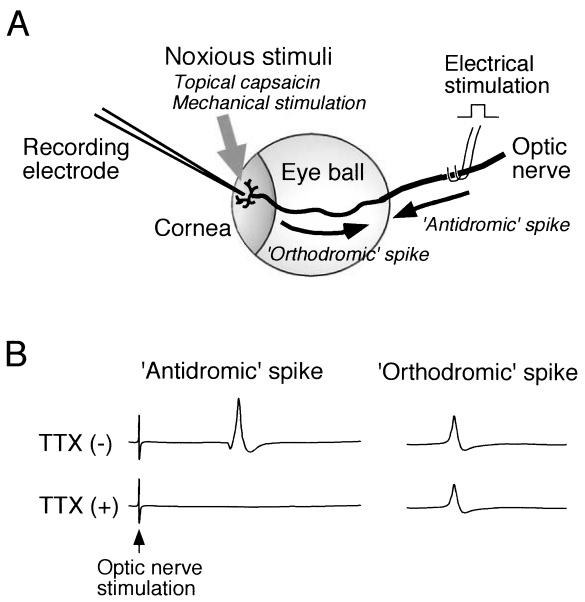


Fig. 8. Schematic diagram of experiments, which enabled direct measurements of nociception-induced unitary spikes. A: Recording set-up. The eye ball and associated optic nerve were mounted in a recording chamber and superfused with physiological saline. The recording electrode was applied to the surface of the corneal epithelium. "Orthodromic" spikes were recorded in response to topical application of capsaicin or direct mechanical stimulation of the cornea. "Antidromic" spikes were evoked by electrical stimulation of optic nerve. B: The orthodromic spike was resistant to tetrodotoxin (TTX), whereas the antidromic spikes were blocked.

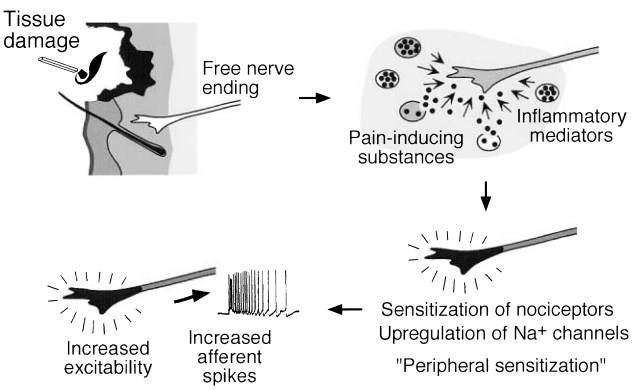


Fig. 9. Diagram showing an involvement of Na^+ channels in the development of inflamatory pain states.

role for TTX-S Na^+ channels (61).

Peripheral nerve injury causes several important changes in Na^+ channel regulation: 1) TTX-R Na^+ currents are attenuated and concomitantly Na^+ channel transcripts mediating TTX-R currents are down-regulated (45); 2) $Na_V1.8$ becomes accumulated at the injured nerve site (17); 3) $Na_V1.3$, which is not normally expressed in adult DRG, becomes significantly up-regulated (45, 47); and 4) the auxiliary β_3-subunit that is expressed preferentially in small nociceptive DRG neurons is up-regulated (62). These alterations in Na^+ channel expression and function might be one of the key events underlying the development of neuropathic pain. Figure 10 summarizes the possible involvement of Na^+ channels in the development of neuropathic pain states.

Recently, it has been demonstrated that intrathecal perfusion of GDNF prevented and reversed the hyperalgesia in animal models of neuropathic pain (63). This analgesic action of GDNF was accompanied by a suppression of abnormal buildup of $Na_V1.3$ accompanied by a reduction in ectopic discharges. The ectopic discharge was totally blocked by a low concentration of TTX, suggesting that the ectopic discharge is mediated by TTX-S. Denervation converts $Na_V1.4$ to embryonic $Na_V1.5$ in skeletal muscle (64). Similar reversion of Na^+ channels to an immature form could occur after nerve injury, resulting in the up-regulation of $Na_V1.3$, which is an embryonic type of Na^+ channel. A rapidly repriming $Na_V1.3$ may represent a critical and necessary Na^+ channel for neuropathic pain.

Unlike the pain-inducing effect of NGF, GDNF does not induce pain (37). GDNF has roles as a potent survival factor for certain classes of neurons and as a protective factor against nerve injury. The mechanical disruption of nerve fibers could result in a decreased supply of GDNF from Schwann cells to neurons. These may partly account for the phenotypic changes in DRG neurons following nerve damage. Thus, the therapeutic actions of GDNF in

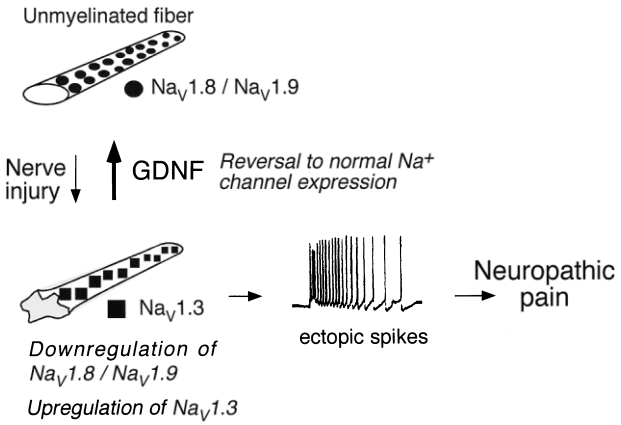


Fig. 11. Diagram showing a sequence of events involved in therapeutic effects of the glial cell line-derived neurotrophic factor (GDNF).

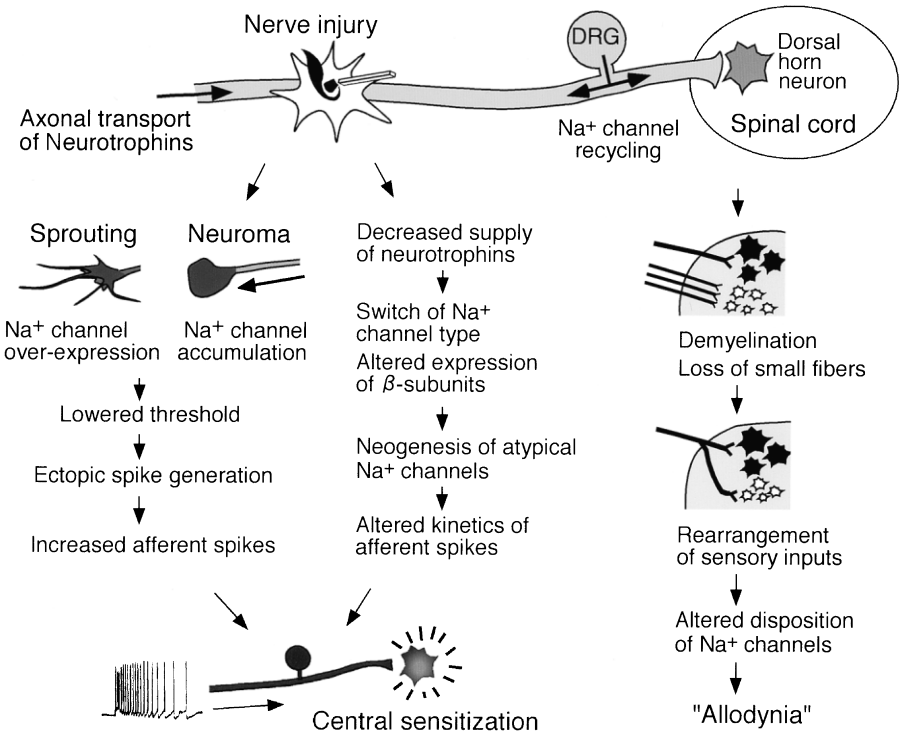


Fig. 10. Diagram showing an involvement of Na^+ channels in the development of neuropathic pain states.

the neuropathic pain model could be primarily attributed to the protection of the TTX-R Na^+ channels by GDNF, as schematically shown in Fig. 11. The actions of GDNF may have important implications for novel pharmaceutical management of neuropathic pain.

5-6. β_3-Subunits and pain

Although the fundamental properties of Na^+ channels are primarily determined by the α-subunit, β-subunits are important modulators of channel function (1). β_1 transcripts are expressed abundantly in large Aβ neurons but are virtually absent in small C neurons of the DRG (65). β_2 is absent in cultured DRG neurons (66). β_3, a novel β-subunit (67), is expressed preferentially in small C neurons, showing a distribution complementary to β_1. Within the spinal cord, β_3 is confined to layers I/II and layer X of the dorsal horn, whereas β_1 appears to be expressed in all layers of the gray matter. Nociceptive C-fibers terminate layers I/II, and the layer X receives inputs from the medial pathway of Lissauer's tract, a fine bundle of fibers that contain primary pain-sensing afferents (68).

β_3 transcripts were significantly up-regulated in small C neurons in DRG following the chronic constriction injury model of neuropathic pain (62). Furthermore, in the *Xenopus* oocyte expression system, co-expression of β_3 markedly augmented $Na_V1.8$-mediated Na^+ current (62). These results, together with the specific localization of β_3 within the nociceptive signaling pathway, indicate a specialized role of β_3 in neuropathic pain. These findings provide an additional insight into the plasticity of Na^+ channel subunits which precipitates pathological pain states and will enable treatment targeting β-subunits.

6. Concentration-gated Na^+ channel, Na_C

NaG found in rat astrocytes (9), $Na_V2.1$ found in human heart (10), $Na_V2.3$ found in mouse atrial tumor cells (69), and SCL11 in rat DRG (70) have been concluded to represent the same isoform; and they are now put together as Na_X ("X" represent "unknown") (71). Na_X is approximately 50% identical to Na_V1 (Fig. 1). The S4 segments, critical for voltage-sensing (72), of the Na_X is markedly different from that of Na_V1 (Fig. 12). The S4 segment of Na_X possesses fewer number of positively charged amino acid residues. Likewise, the interdomain III – IV linker, which is important for the fast inactivation process (73), is poorly conserved. Furthermore, in mouse and human chromosome 2, the Na_X-gene are separate from the Na_V1 genes (71) (Fig. 2).

Na_X	L V Q L L L L S R I I H V L R P G K G P K V F H D L M
$Na_V1.2$	L F R V I R L A R I G R I L R L I K G A K G I R T L L
$Na_V1.5$	L F R V I R L A R I G R I L R L I R G A K G I R T L L
Eel	L F R V I R L A R I A R V L R L I R A A K G I R T L L

Fig. 12. The amino acid sequence of the S4 segment of domain IV for rat Na_X in comparison with those of other rat and eel Na^+ channels. Conventional 1-letter abbreviations were used for amino acids. Amino acid residues of Na_X which are not identical to any of other Na^+ channels are framed with shaded boxes. Positively charged residues are framed with open boxes.

Watanabe et al. (11) found that in the brain, Na_X are abundant in areas belonging to the circum ventricular organs that are involved in the maintenance of body-fluid homeostasis or Na^+ / water balance (74, 75). The Na_X-null mutant mice showed abnormal NaCl intake behavior in both water- and salt-depleted conditions, suggesting that Na_X senses $[Na^+]_o$ and is involved in the regulation of salt intake behavior. Now that the function of Na_X has been identified, it should be renamed as Na_C ("C" stands for "concentration").

7. Na^+ channelopathy

A variety of inherited disorders are caused by mutations in Na^+ channel genes. Most of them are autosomal dominant "gain-of-function" disorders caused by missense mutations. Six muscle diseases linked to human muscle Na^+ channel genes are known. Amongst them, hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PC) and potassium-aggravated myotonia (PAM) occur in the skeletal muscle Na^+ channel gene, *SCN4A*; and the remaining three, long QT syndrome type 3 (LQT3), Brugada's syndrome, and idiopathic ventricular fibrillation (IVF), are associated with the cardiac muscle Na^+ channel gene, *SCN5A*.

Mutations that cause epileptic syndromes have recently been reported. Generalized epilepsy with febrile seizures (GEFS) plus type 1 (GEFS + 1) and GEFS plus type 2 (GEFS + 2) are characterized by febrile convulsion in children and various forms of afebrile seizures in adults. GEFS + 1 carries its mutation in *SCN1B* (gene for β_1-subunit) (76). The mutation in *SCN1B* disrupts a putative disulfide bridge normally stabilizing an extracellular immunoglobulin-like fold in the extracellular domain of the β_1-subunit. GEFS + 2 is due to a mutation in the neuronal type of Na^+ channel gene, *SCN1A* (77).

The positions of the mutations are wide-spread as shown in Fig. 13, locating on regions related to the inactivation process, such as the inactivation particle (III – IV interdomain loop), the inactivation receptor (intracellular loop between S4 and S5 segments in domain III), and the S4 segment in domain IV, the region thought to play a crucial role in the coupling of activation to inactivation (78). A common feature for most of the Na^+ channel mutations is a disruption of the inactivation process that can initiate prolonged and exaggerated inward Na^+ current. As a conse-

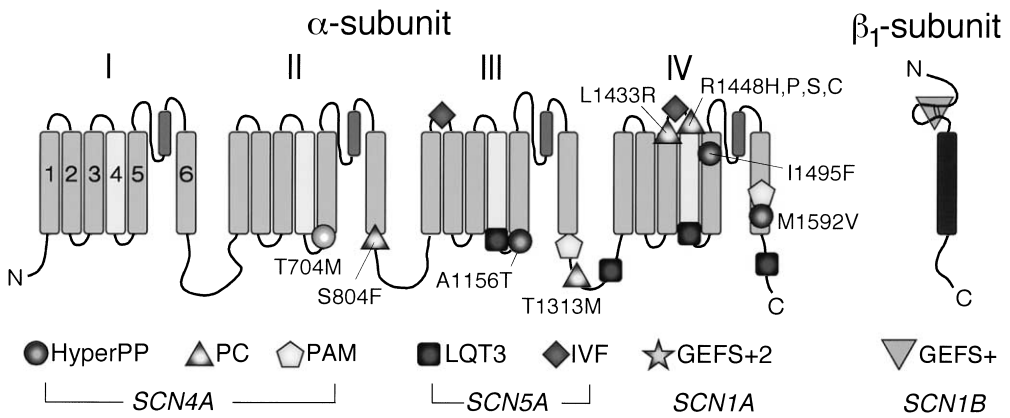


Fig. 13. Topology of mutations in human Na^+ channel genes. Two dimensional molecular map of Na^+ channel α-subunit is shematically shown with its important structural parts. Each symbol represents the Na^+ channelopathy indicated at the bottom of the figure. In cases of missense mutations in *SCN4A* (gene for $Na_V1.4$), the amino acid mutations and their sites are shown. Conventional 1-letter abbreviations were used for normal and replaced amino acids. HyperPP: hyperkalemic periodic paralysis, PC: paramyotonia congenita, PAM: potassium-aggravated myotonia, LQT3: long QT syndrome type 3, IVF: idiopathic ventricular fibrillation, GEFS: generalized epilepsy with febrile seizure.

quence, Na^+ channel mutations can either result in pathological bursts of action potentials (represented as "myotonia") or an eventual refractory unexcitable state (represented as "paralysis").

Hypokalemic periodic paralysis (HypoPP) is caused by missense mutations in the dihydropyridine receptor (79). However, HypoPP arising from Na^+ channel disorders (HypoPP type 2) has recently been reported (80 – 82). Both HypoPP and HypoPP type 2 carry their mutations at functionally equivalent locations within the channel protein, i.e., the outermost arginine residue of the domain II S4 voltage sensor. In addition, these two diseases are clinically indistinguishable from each other. Surprisingly, heterologous expression of the mutant Na^+ channels revealed a hyperpolarizing shift of the steady-state inactivation curve, accelerating slow inactivation, unlike other known hereditary Na^+ channelopathies characterized by a slowed inactivation. Thus, the finding in HypoPP type 2 adds new insight into Na^+ channelopathy.

8. Conclusion and future perspectives

Ten distinct Na^+ channel α-subunits reveal variable degrees of differences in their properties including voltage-dependence, activation and inactivation kinetics, and tissue-specific expression. Our understanding of the structural basis for voltage-dependent gating of Na^+ channels has been notably advanced by the recent identification of mutated Na^+ channel genes that cause Na^+ channelopathies. In addition, the 3D image of Na^+ channels (12) has added novel insights into the functional significance of Na^+ channel structures. This type of innovative structural approach will undoubtedly help to elucidate the detailed mechanistic basis for channel gatings that remain unsolved.

The demonstration of tissue-specific roles of several Na^+ channel isoforms, which implicates their specialized functional roles, is apparently another remarkable finding in recent Na^+ channel research. TTX-R Na^+ channels found in the peripheral pain-conducting sensory neurons (6, 8) may serve as the target for new types of evidence-based drugs with minimal side-effect profiles. In fact, $Na_V1.8$ has been a molecular target for the development of drugs for treatment of neuropathic pain (57).

The mechanism underlying the developmental regulation of Na^+ channels may give clues to the understanding of how tissue-specific expressions of a variety of Na^+ channels are controlled to conform appropriate Na^+ channel deployment in various tissues. It is now clear that expressions of at least several Na^+ channel isoforms are dynamically regulated or modulated by different trophic factors (45) and by a variety of inflammatory mediators (55) as well. These Na^+ channels might be regarded as the "inducible" Na^+ channels that are readily up- or down-regulated in response to a subtle environmental change, unlike "constitutive" Na^+ channels which are regularly expressed and engaged in routine cellular functions.

The "inducible" regulation of the Na^+ channel could be exaggerated under pathological conditions. Moreover, neurotrophins such as GDNF appear to have opposing effects on different types of Na^+ channels, up-regulating one subtype while down-regulating others (63). Such a dynamic and subtype-specific regulation of "inducible" Na^+ channels probably plays a key role in certain types of Na^+ channel disorders including neuropathic pain.

In most of the papers on Na^+ channels, the text begins with the stereotyped phrase; "Na^+ channels are responsible

for action potential generation". However, the recent functional characterization of persistent Na^+ currents has prompted us to revise the above cliche on Na^+ channels. Even the entirely new functional role of the Na^+ channel is now emerging through the investigation of the voltage-insensitive gating of Na_X (11). This concentration-gated Na^+ channel further emphasizes the above notion that the Na^+ channels do not merely conduct the action potential generation. Apparently, the Na^+ channel multigene family plays more global roles than expected, and elucidation of diverse functional roles of Na^+ channels will create new avenues of research in cell biology.

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