The study of sodium channels involved in pain responses using specific modulators

JI Yong-Hua1,*, LIU Tong2
1School of Life Sciences, Shanghai University, Shanghai 200444, China; 2Graduate School of the Chinese Academy of Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

Abstract: Voltage-gated sodium channels (VGSCs) are transmembrane proteins responsible for generation and conduction of action potentials in excitable cells. Physiological and pharmacological studies have demonstrated that VGSCs play a critical role in chronic pain associated with tissue or nerve injury. Many long-chain peptide toxins (60-76 amino acid residues) purified from the venom of Asian scorpion Buthus martensii Karsch (BmK) are investigated to be sodium channel-specific modulators. The α-like neurotoxins that can bind to receptor site 3 of sodium channels, named as BmK I and BmK abT, could induce nociceptive effects in rats. On the contrast, the β-like neurotoxins that can bind to receptor site 4 of sodium channels, named as BmK AS, BmK AS-1 and BmK IT2, could produce potent anti-nociceptive effects in animal pain models. BmK I could strongly prolong the fast inactivation of tetrodotoxin (TTX)-sensitive Na+ currents on the rat dorsal root ganglia (DRG) neurons together with the augmentation of peak current amplitude. However, BmK IT2 and BmK ASs, potently suppressed both the peak TTX-resistant and TTX-sensitive Na+ currents on rat small DRG neurons. Moreover, BmK ASs could decrease the excitability of small DRG neurons. Thus, the nociception/anti-nociception induced by scorpion neurotoxins may attribute to their distinct modulation on sodium channels in primary afferent sensory neurons. Therefore, the sodium channel-specific modulators from BmK venom could be used as not only pharmacological tools for better understanding the roles of VGSCs in pain signal conduction, but also lead molecules in the development of ideal analgesics targeting VGSCs.

Key words: voltage-gated sodium channel; sodium channel-specific modulator; nociception; anti-nociception

1 Introduction

Voltage-gated sodium channels (VGSCs) are kind of transmembrane proteins responsible for generation and conduction of action potentials in excitable cells[1]. In addition, VGSCs are also found to influence subthreshold electrical activity through persistent or resurgent sodium currents[2]. Thus, VGSC can be considered as a molecular integrator that controls neuronal firing pattern.

Generally, VGSC consists of one α subunit (approximately 260 kDa) and one or more β subunits (33-36 kDa). The α subunit consists of four homologous domains (I-IV), each domain containing six transmembrane segments (S1-S6) and a pore-forming loop between segments V and VI. The fourth segment in each domain contains a voltage sensor. The loop between segments V and VI lines the channel pore and confers ion selectivity. A unique hydrophobic sequence, the IMF motif, located in the intracellular loop between domains III and IV, has access to and can block the inner mouth of the pore when the channel is open. The IMF-mediated block of open channels induces channel inactivation[3]. β subunit regulates the expression level of channels, modulates gating properties of α subunit and anchors channels at specific sites in the plasma membrane[4]. So far, nine subtypes of VGSC α subunit (Nav1.1-1.9) and four different auxiliary β subunits (β1-4) have been identified in mammal. The tenth α subunit (NaX) has been identified, but it does not appear to function as a voltage-gated channel[5]. On the basis of their susceptibility to blockade by tetrodotoxin (TTX), VGSCs are separated into TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) channels[6].

Many of the subtypes of VGSCs have specific developmental, tissue and cellular distributions. Nav1.4 is almost exclusively expressed in skeletal muscle. Nav1.5 is...
is predominantly expressed in cardiac muscle, though message and current of Nav1.5 are also detectable in neonatal and some adult dorsal root ganglion (DRG) neurons at very low levels. Adult central nervous system (CNS) neurons co-express Nav1.1, Nav1.2 and Nav1.6. Adult DRG sensory neurons express combinations of Nav1.1, Nav1.6, Nav1.7, Nav1.8 and Nav1.9. Nav1.3 is expressed in neonatal neurons predominantly and at very low levels in adult neurons. Nav1.2, one of the predominant isoforms in CNS, is also detectable in adult DRG neurons at low levels\(^6\).

So far, at least 6 distinct receptor sites have been characterized in a subunit of sodium channel\(^7\). By binding to specific receptor sites of sodium channel, a variety of toxins have effects ranging from pore blocking to modification of the gating kinetics of sodium channel. TTX, saxitoxin and \(\mu\)-conotoxin can bind to receptor site 1, blocking sodium conductance. Lipid-soluble neurotoxins e.g. batrachotoxin, veratridine, aconitine and grayanotoxin can bind to receptor site 2, then cause persistent activation at resting membrane potential. Receptor site 3 of sodium channels can be occupied by \(\alpha\) scorpion toxins, sea anemone toxins and some spider toxins. These toxins slow or block sodium channel inactivation. \(\beta\) scorpion toxins can bind to receptor site 4 to induce both a shift in the voltage dependence of sodium channel activation in the hyperpolarizing direction and a reduction of the peak sodium current amplitude. Lipid-soluble brevetoxins and ciguatoxins can act on receptor site 5 to cause a shift in activation to more negative membrane potentials and a block of inactivation. \(\delta\)-conotoxins have been proposed to bind to receptor site 6 to cause inhibition of sodium current inactivation resulting in a marked prolongation of action potentials\(^9\).

Genetic alteration in VGSCs gene and changes in their functional expression levels clearly illuminate the involvement of VGSCs in pathological conditions. In last decade, it was found that membrane ion channel mutations could cause numerous inherited disorders, which are termed “channelopathies”\(^9\). Among them, the diseases caused by the mutations within \(\alpha\) subunit or \(\beta\) subunit of VGSCs are named as “sodium channelopathies”, including muscle diseases such as paramyotonia congenita, cardiac diseases e.g. different forms of arrhythmias and neuronal diseases e.g. a variety of epilepsies\(^9\). Altered expression profile and level of VGSCs in pathological states are demonstrated in kinds of disorders, such as multiple sclerosis, spinal cord injury, diabetes, and neuropathic pain\(^11-13\). Modulation of VGSCs by different ligands can combat a series of disorders such as cardiac arrhythmias, chronic pain, epilepsy, and demyelinating diseases. Therefore, the modulators targeting VGSCs may represent therapeutic potential in many pathological conditions\(^14\).

## 2 VGSCs and pain

Physiological and pharmacological evidence has demonstrated a critical role of VGSCs in many types of chronic pain syndromes. VGSCs blockers, including anticonvulsants, antiarrhythmics and local anesthetics, are effective in the treatment for pathological pain, providing clinical evidences for the importance of these channels in pain states\(^15\). The human genetic studies and knock-out mice confirm the specific roles of different sodium channel isoforms\(^6,16\). Tissue and nerve injury lead to changes in expression profile and function of sodium channel subtypes, in turn which can increase the excitability of sensory neurons. The roles of Nav1.3, Nav1.7, Nav1.8 and Nav1.9 in different pain states were well delineated, using antisense oligonucleotides (ODNs) technique and knock-out mice\(^6\). The distribution in nociceptors and the critical roles in pain signals of VGSCs make them ideal targets for relieving pain without central side effects.

### 2.1 Nav1.3 and neuropathic pain

The expression of Nav1.3 is dramatically up-regulated in DRG following peripheral nerve damage, while the expression of both TTX-S channels, Nav1.1, Nav1.2, Nav1.6 and Nav1.7 included, and TTX-R channels, Nav1.8 and Nav1.9 included, are down-regulated\(^3\). Neuropathic pain behavior was reversed by administration of glial cell-derived neurotrophic factor (GDNF) and this effect was concomitant with a block of a fiber ectopic discharge and normalization of Nav1.3 expression in the injured DRG\(^17\). Unexpectedly, intrathecal injection of antisense ODNs targeting Nav1.3 failed to reverse tactile hypersensitivity in spared nerve injury (SNI) model\(^18\) and ectopic discharges from damaged nerves were unaffected by the absence of Nav1.3 in global knock-out mice\(^9\). Thus, it suggested that besides Nav1.3, multiple VGSC subtypes contributed to the development of neuropathic pain.

### 2.2 Nav1.7 in acute and inflammatory pain

Nav1.7 is expressed in peripheral sensory and sympathetic neurons and located at the terminal of sensory neurons\(^20\). Loss-of-function of Nav1.7 can result in a complete inability to sense pain in multiple populations\(^21,22\). Several mutations of gain-of-function in Nav1.7 can cause clinical pain symptoms\(^23,24\). Global deletion of Nav1.7 is lethal,
but nociceptor-specific Nav1.7 knock-out mice had increased mechanical and thermal pain thresholds. Meantime, inflammatory pain responses evoked by a range of formalin, carrageenan, complete Freund’s adjuvant, or nerve growth factor, were reduced and even abolished. However, in nociceptor-specific Nav1.7 knock-out mice or Nav1.7 and Nav1.8 double knock-out mice, it was found that establishment of neuropathic pain does not require the presence of either or both of Nav1.7 and Nav1.8. Thus, it strongly indicates that Nav1.7 plays a crucial role in acute and inflammatory pain and might be an ideal target for the development of novel analgesics.

### 2.3 Nav1.8 and pain

It has been demonstrated that Nav1.8 expression was increased in the rat DRG following carrageenan treatment and in human patients with chronic neurogenic pain. Antisense ODNs targeting Nav1.8 could decrease inflammatory hyperalgesia, neuropathic pain and visceral pain in rats. Nav1.8 null mutant mice have deficits in inflammatory and visceral pain, but not in neuropathic pain. In addition, A-803467, a conotoxin selectively blocking Nav1.8, can relieve inflammatory pain responses. Thus, Nav1.8 is regarded as an important player in acute, inflammatory, visceral and possible neuropathic pain.

### 2.4 Nav1.9 and inflammatory pain

Nav1.9 expresses selectively in small diameter DRG neurons in peripheral nervous system. It mediates a TTX-resistant persistent current. The study on Nav1.9 knockout mice reveals that it contributes to thermal hyperalgesia and spontaneous pain behaviors associated with peripheral inflammation. Inflammatory mediators, including PGE2 and serotonin, can modify the function of Nav1.9 via G-protein-dependent mechanism to mediate hyperalgesia related to inflammation. However, knock-down Nav1.9 by an antisense ODNs was ineffective on either thermal hyperalgesia or tactile hypersensitivity in the neuropathic rat. It indicated that Nav1.9 mainly contributed to inflammatory pain, but not neuropathic pain.

Finally, although sodium channel blockers, such as local anesthetics, antiarrhythmics, and anticonvulsants, are effective in the treatment of chronic pain, these drugs lack selectivity for VGSC subtypes. So development of specific modulators targeting VGSC subtypes related to pain has become efficient strategy for developing novel analgesics.

### 3 Sodium channel-specific modulators and pain

Venomous animals evolve many toxins for prey capture and defense, and many of these toxins could produce analgesic effect. Excitingly, the analgesic conotoxin, ω-MVIIA (Ziconotide), a selective blocker of neuronal N-type voltage-sensitive calcium channels, was recently approved for treatment of chronic intractable pain. Because of the critical roles of VGSCs in pain signal conduction, many neurotoxins targeting VGSCs could also produce potent anti-nociceptive effects. Thus, animal venoms become an valuable source for potential analgesic drugs.

Although Asian scorpion *Buthus martensi* Karsch (BmK) is not considered as a dangerous species, the envenomation of BmK could cause intense pain at the site of the sting and also at distant sites, skin edema and burning sensation. On the other hand, whole scorpions, the tails or the venom extracts have been used as one of the indispensable materials in Chinese traditional medicine to treat some neural diseases such as apoplexy, epilepsy, facial paralysis and especially chronic pain for thousands of years. However, the molecular mechanisms underlying this paradox are unclear so far.

Biochemical and pharmacological studies have documented that the most of long-chain scorpion neurotoxins composed of 60-70 amino acid residues. The scorpion toxins purified from BmK venom are sodium channel modulators. They are divided into two groups: α/α-like scorpion toxins that can bind to VGSC receptor site 3 such as named as BmK I and BmK abT, β/β-like scorpion toxins that can bind to VGSC receptor site 4 such as named as BmK IT2 and BmK AS. Our studies found that they could produce nociception/anti-nociception by the distinct modulation of VGSCs on primary afferent sensory neurons in animals.

### 3.1 Pain-related responses and inflammation induced by BmK venom/BmK I

Aimed to characterize pain-related responses and analyze mechanisms of scorpion sting pain, a pain animal model was developed by intraplantar injection of BmK venom into one side of rat hind paw. It was found that BmK venom-induced pain-related behaviors in rats could represent several manifestations of clinical pathological pain, such as spontaneous pain, cutaneous thermal and mechanical hypersensitivity and mirror-image mechanical hypersensitivity. In addition, BmK venom could induce paw edema that persist for 24-48 h. The results indicated BmK venom test may be an available tonic inflammatory pain model displaying various pain-related behaviors, including spontaneous pain behaviors, primary thermal or mechanical.
hypersensitivity and mirror-image mechanical hypersensitivity. Subsequently, peripheral and central mechanisms underlying scorpion sting pain was further investigated. In periphery, plasma extravasation, recruitment of immune cells, degranulation of mast cells and histamine release at injury site had been demonstrated to contribute to BmK venom-induced peripheral sensitization and inflammatory responses in rats[43,44,46]. The functional depletion of capsaicin-sensitive primary afferent fibers by systemic administration of resiniferatoxin (RTX) attenuates rat pain-related behaviors and paw edema induced by BmK venom[47]. In spinal cord, dynamic release of excitatory amino acids from dorsal horn could be triggered by peripheral administration of BmK venom[48]. The increase of c-Fos expression, which is a functional marker of neurons activation, could be evoked spatially and temporally in rat spinal cord by intraplantar injection of BmK venom[49]. Recently, it was found that bilateral up-regulation of neuronal nitric oxide synthase (nNOS) expression in spinal cord was involved in BmK venom-induced pain[50]. Intracellular phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in spinal cord dorsal horn was also activated by peripheral administration of BmK venom[51]. Taken together, it indicated that nociceptive responses induced by BmK venom may attribute to activation of multiple signaling pathway associated with peripheral and central sensitization. The supraspinal mechanisms underlying scorpion sting pain remain to be further investigated.

However, which components of BmK venom contribute to its nociceptive effects? BmK I, an α-like neurotoxin and major toxic component in BmK venom, was found to replicate nociceptive effects of BmK venom[40,45]. Peripheral administration of BmK I could also induce excitatory amino acid release from spinal cord and increase of spinal c-Fos expression in rats[48,52]. The results demonstrate that BmK I is one of contributors to BmK venom-induced pain-related behaviors. Besides BmK I, BmK abT, another neurotoxin from BmK venom, also displays nociceptive effects and inflammatory responses in rats (unpublished data).

3.2 Anti-nociception of VGSC receptor site 3- and 4-specific neurotoxins

Many long-chain scorpion neurotoxins acting on VGSCs from BmK venom such as BmK IT2 and BmK ASs were considered as analgesic peptides[55-57]. Intraplantar injection of BmK IT2 inhibited thermal hyperalgesia in carrageenan-treated rats and significantly prolonged paw withdrawal latency in normal rats[56]. BmK IT2 also displayed a biphasic inhibitory effect on C component of the rat nociceptive flexion reflex by subcutaneous injection[55]. Peripheral or spinal delivery of BmK IT2 suppressed formalin-induced nociceptive behaviors and c-Fos expression in spinal cord[58,59].

Similar with BmK IT2, intraplantar injection of BmK AS also inhibited thermal hyperalgesia in carrageenan-treated rats and prolonged paw withdrawal latency in normal rats[53]. Peripheral or spinal delivery of BmK AS significantly suppressed formalin-induced nociceptive behaviors and c-Fos expression in spinal cord[41,54]. Intrathecal injection of BmK AS-1, a polypeptide with high sequence identity to BmK AS, increased the rat paw withdrawal latency to radiant heat and intraplantar injection of BmK AS-1 reduced the level of C components in rat nociceptive flexion reflex[57].

4 The distinct modulation on VGSCs by receptor site 3- and 4-specific neurotoxins

The primary sensory neurons isolated from the L4-L6 of adult rats were chosen to investigate the distinct modulation of receptor site 3/4-specific modulators on VGSCs. It was found that BmK I could strongly prolong the fast inactivation of TTX-S Na+ currents in DRG neurons together with the augmentation of peak current amplitude[60]. BmK abT also displays the similar effects in DRG neurons[61]. The modulation of BmK I or BmK abT on sodium currents in DRG neurons may result in augmentation of the excitability of neurons. In contrast, a biphasic inhibition by BmK IT2 of total Na+ currents was observed in some small DRG neurons. The inhibition of TTX-R Na+ currents by BmK IT2 was found to be more potent than that of TTX-S Na+ currents, but the TTX-S Na+ currents were inhibited more quickly than the TTX-R Na+ currents[55]. BmK AS or BmK AS-1 also depressed the peak TTX-S and TTX-R Na+ currents[54,57]. Furthermore, BmK AS could decrease excitability of small DRG neurons[51]. The results suggest that peripheral or central anti-nociception of BmK IT2 and BmK ASs may be attributed to the distinct modulation of TTX-R and TTX-S Na+ channels located at the peripheral or central terminals of small DRG neurons (Fig. 1).

5 Perspectives

Chronic pain seriously impairs human’s health and life quality. VGSCs have emerged as novel molecular targets in the treatment of chronic pain states. Due to their diversified pharmacological functions and exquisite target specificities, animal toxins seem to be highly perspective for the development of
novel analgesics. BmK venom is a rich source of sodium channel-specific modulators, which may be developed as novel analgesic peptides with therapeutic potential. However, for therapeutic applications of toxins, a lot of issues associated with safety, pharmacokinetics, immunogenicity, and the way of delivery remain to be further addressed.

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