Acta Physiologica Sinica, August 25, 2022, 74(4): 621–632
DOI: 10.13294/j.aps.2022.0064 http://www.actaps.com.cn

Review

Anti-epileptic/pro-epileptic effects of sodium channel modulators from *Buthus martensii* Karsch

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Abstract: The East Asian scorpion *Buthus martensii* Karsch (BmK) is one of the classical traditional Chinese medicines for treating epilepsy for over a thousand years. Neurotoxins purified from BmK venom are considered as the main active ingredients, acting on membrane ion channels. Voltage-gated sodium channels (VGSCs) play a crucial role in the occurrence of epilepsy, which make them become important drug targets for epilepsy. Long chain toxins of BmK, composed of 60–70 amino acid residues, could specifically recognize VGSCs. Among them, α-like neurotoxins, binding to the receptor site-3 of VGSC, induce epilepsy in rodents and can be used to establish seizure models. The β or β-like neurotoxins, binding to the receptor site-4 of VGSC, have significant anticonvulsant effects in epileptic models. This review aims to illuminate the anticonvulsant/convulsant effects of BmK polypeptides by acting on VGSCs, and provide potential frameworks for the anti-epileptic drug-design.

Key words: *Buthus martensii* Karsch; toxin; epilepsy; voltage-gated sodium channels; receptor site

产自东亚钳蝎的钠通道调节剂的抗癫痫/促癫痫作用

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摘 要：东亚钳蝎(*Buthus martensii* Karsch, BmK)是治疗癫痫的经典中药之一，已有上千年的历史。从BmK毒液中分离纯化的神经毒素被认为是作用于膜离子通道的主要活性成分。电压门控钠通道(voltage-gated sodium channels, VGSCs)在癫痫发生中起着重要的作用，使其成为重要的癫痫药物靶点。BmK的长链毒素由60~70个氨基酸残基组成，能特异性识别VGSCs，其中用于建立癫痫模型的α-样神经毒素与VGSC受体位点3结合，可诱发鼠类癫痫。而β或β-样神经毒素则与VGSC受体位点4结合，对癫痫模型有显著的抗惊厥作用。本综述旨在阐明BmK多肽作用于VGSCs的抗惊厥或惊厥作用，同时也为抗癫痫药物设计提供潜在的框架。

关键词：东亚钳蝎；毒素；癫痫；电压门控钠通道；受体位点

This work was supported by grants from the National Natural Science Foundation of China (No. 82074162, 81903995), the Young Elite Scientists Sponsorship Program by CACM (No. CACM-2019-QNRC2-C10), Science and Technology Innovation Project of Putuo District Health System (No. ptkwws201902, ptkwws201908, ptkwws202009, ptkwws202107) and Research Project (No. 2019307, 2020308A) and the One Hundred Talents (No. 2022-RCLH-02) of Putuo Hospital, Shanghai University of Traditional Chinese Medicine.

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1 Introduction

Voltage-gated sodium channels (VGSCs) are key transmembrane proteins that consist of two different types of subunit: the pore-forming α subunit associated to one or two β auxiliary subunits (β1–4). As a single polypeptide chain, the α subunit folds to four homologous repeats (domain I–IV, D I–IV). Each domain contains six transmembrane helices designated S1 to S6. The segments S1–S4 in each domain constitute the voltage-sensing domain (VSD) that regulate the switch of the central ion-conducting pore domain (PD) enclosed by S5 and S6 helices [1, 2]. Due to the contribution to the cell membrane potential, action potential (AP) initiation and propagation [3, 4], VGSCs play crucial roles in cell excitability and the tissue-specific physiology as well as pathology. In view of this, VGSCs are considered to become important therapeutic targets for treatment of a range of diseases, including epilepsy, pain, myotonias, cardiac arrhythmias and insufficiencies, as well as cancer [5, 6].

Nine subtypes of VGSC α subunits have been found in humans, including Nav1.1–Nav1.9, encoded by the genes *SCN1A–SCN5A*, and *SCN8A–SCN11A*, respectively. A common human inherited epilepsy, generalized epilepsy with febrile seizure plus (GEFS+), has been proved to be caused by variants within α or β subunits from multiple sodium channel subtypes of the central nervous system. Genetic studies in patients with epilepsy have also confirmed that the gene encoding VGSC produces a large number of mutations, which could be divided into the gain of function and loss of function mutations. The mutation of the Nav1.2 gene *SCN2A* is associated with various epilepsies, such as GEFS+, Dravet syndrome (DS), and other stubborn childhood epileptic encephalopathies. As reported that, the gain of function missense mutations in *SCN2A* was often associated with benign familial neonatal-infantile seizures (BFNIS) [7]. For the loss of function mutation, the Nav1.1 gene *SCN1A* is the clinically most relevant SCN gene for epilepsy. More than 1 200 mutants have been identified to be associated with epilepsy, most of them are febrile seizures [8]. Even when the complete loss of function mutations occurred in Nav1.1, myoclonic epilepsy of infancy would appear [9]. The *SCN3A* gene, which is located in the soma of neurons, is widely expressed in adult brain. It is reported that loss-of-function of *SCN3A* may lead to increased seizure susceptibility [10]. Moreover, for the missense mutation of *SCN8A*, decreasing Scn8a expression in cortical excitatory neurons could reduce seizures. On the contrary, the decreasing expression of SCN8A in the thalamic reticular nucleus (RT) leads to absence seizures [11]. A little number of sodium channel mutations associated with epilepsy were found in *SCN9A* [12]. From inherited epilepsy models, the steady-state inactivation of sodium currents of CA1 neurons shifted towards depolarization and the value of VGSC currents increased. Similar results also have been observed in the local seizure model, such as the epileptic seizure caused by hippocampal injury [13]. In the convulsion model induced by pentylenetetrazol (PTZ), the AP duration of hippocampal neurons was significantly extended and the sodium conductance was increased. Though VGSCs are considered as therapeutic targets for anti-epileptic drugs, such as carbamazepine, phenytoin, oxcarbazepine and so on, these drugs are found to act on multiple-targets, which could cause cognitive side effects [14], neurological disorders and miscellaneous side effects [15]. Therefore, it is necessary to develop VGSC subtype modulatory agents with high specificity for treating epilepsy.

During the long-term evolution of species, VGSCs have become target receptors for many exogenous toxins, such as spider toxins [16], ciguatoxins [17], conotoxins [18], and sea anemone toxins [19]. There are at least six major types of exogenous toxin receptor sites on VGSCs [20]. Most VGSC toxins are gating modifiers that trap the channel in a particular stage of the gating segments through interacting with one or more VSDs [21]. Toxins alter the normal delivery of electrical signals ultimately leading to paralysis and even death in animals by regulating various functional activities of VGSCs, including the ion permeability and gating [22]. In recent years, with the development of cryo-electron microscopy technology, the crystal structure of VGSCs and the interaction with toxins have been deeply investigated, which could illuminate the interaction mode between drug and target, also could be used in novel drug-design based on the VGSC structures [23]. As reported, the structures of a eukaryotic Nav channel alone and in a complex containing an α-scorpion toxin, AaH2, could be observed by electron microscopy at 3.5-Å resolution [24].

The *Buthus martensii* Karsch (BmK) scorpion is capable of treating nervous system diseases such as epilepsy, pain and hemiplegia in China from ancient to modern times [25, 26]. The main active ingredient of scorpion is considered as neurotoxins [27]. According to the

length, scorpion toxins are mainly divided into two types: long-chain peptides acting on VGSCs and short-chain peptides targeting voltage-gated potassium channels [28]. Based on the pharmacological effects and the binding abilities of receptor sites on VGSC gating, long-chain toxins can be further classified into two categories: α/α-like scorpion toxins, which bind to the receptor site 3, can inhibit the rapid inactivation of VGSCs; β/β-like scorpion toxins, binding to site 4, can reduce the current amplitude and alter the threshold of the activation to more negative membrane potentials (Fig. 1 and 2, and Table 1).

There are a little number of natural toxin peptides, like Botulinum neurotoxin E [29], with anti-epileptic activity. As reported, Parawixin 2, a compound isolated from the venom of the spider *Parawixia bistriata*, has been defined as a novel non-selective GABA uptake inhibitor, with anticonvulsant effects on temporal lobe

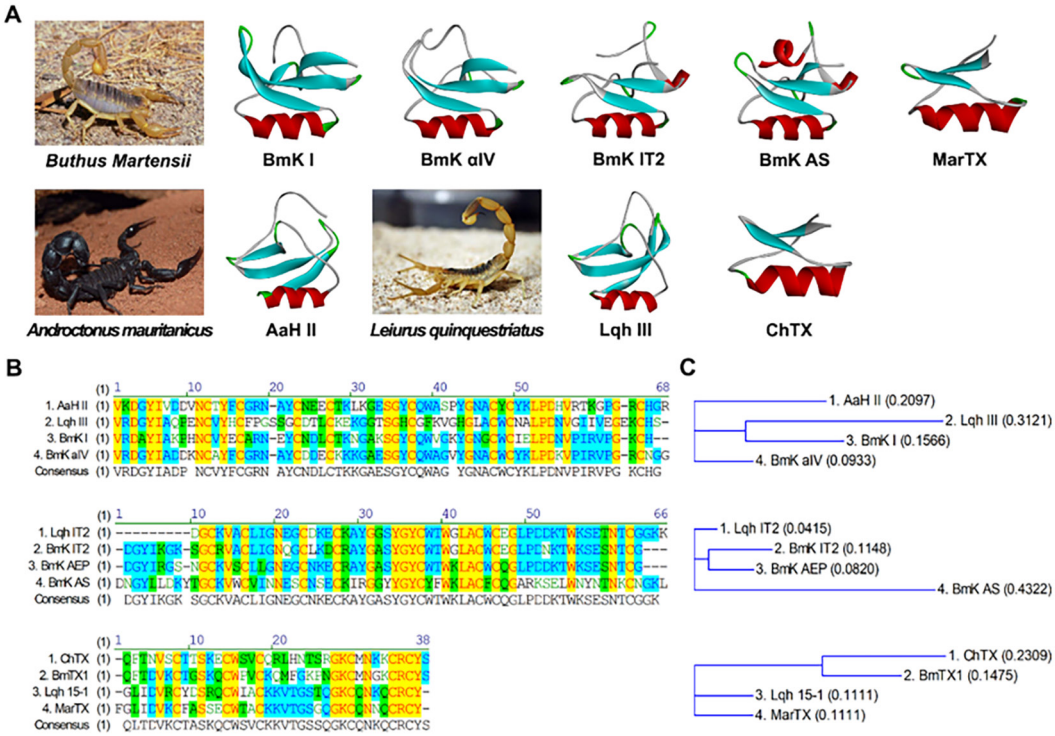

Fig. 1. The structures of scorpion toxin peptides. *A*: The α/α-like scorpion toxins BmK I (PDB: 1SN1) [35] and BmK αIV [36–38] (using LQQ III, PBD: 1LQQ; BmK I, chimera Lqh αIT/AaH II, PBD: 1SEG; BmK α2, PDB: 2KBJ as templates) isolated and purified from *Buthus martensii* Karsch, AaH II [33] (PDB: 1PTX) isolated and purified from *Androctonus mauritanicus*, Lqh III [40] (PDB: 1BMR) isolated and purified from *Leiurus quinquestriatu*s. The β/β-like scorpion toxins BmK IT2 [36, 41–43] (using Lqh IT2, PBD: 2I61; LQQ III, PBD: 1LQQ; Lqh αIT A39L, PDB: 2YEO; Kurtoxin, PDB: 1T1T as templates), BmK AEP (using the same templates as BmK IT2), and BmK AS [38, 41, 43–45] (using Kurtoxin, PDB: 1T1T; Lqh IT2, PBD: 2I61; CsE-V, PBD: 1NRB; Ts3, PBD: 5CY0; BmK α2, PDB: 2KBJ as templates) isolated and purified from *Buthus martensii* Karsch. Lqh IT2 [41] isolated and purified from *Leiurus quinquestriatu*s. The short chain scorpion toxins acting on K^+ channels regulating the function of VGSCs. The toxins MarTX [46] (PDB: 1M2S) isolated and purified from *Buthus martensii* Karsch. ChTX[38] isolated and purified from *Leiurus quinquestriatu*s. Sequence homology comparison is obtained by using PSI-Blast, and homology modeling of scorpion toxins is acquired by using Discovery Studio 2017 R2. *B*: Upper, multiple sequence alignment of α/α-like scorpion toxins. Middle, multiple sequence alignment of β/β-like scorpion toxins. Below, multiple sequence alignment of toxins acting on K^+ channels regulating the function of VGSCs. Conserved residues and cysteines formatting intrachain disulfide bonds are in red and shadowed in yellow; residues conserved in most of the peptides are shadowed in blue; residues with same type of charge in most of the peptides are shadowed in green. The species of toxins are mentioned above, except for Lqh 15-1[47] isolated and purified from *Leiurus quinquestriatu*s; BmTX1[48] isolated and purified from *Buthus martensii* Karsch. *C*: The guide tree is constructed by ALIGNX, a component of the VECTOR NTI 11.0 software suite. Scores in the brackets are based on the identity of the amino acids chemical properties. Upper, the guide tree of α/α-like scorpion toxins. Middle, the guide tree of β/β-like scorpion toxins. Below, the guide tree of short chain toxins.

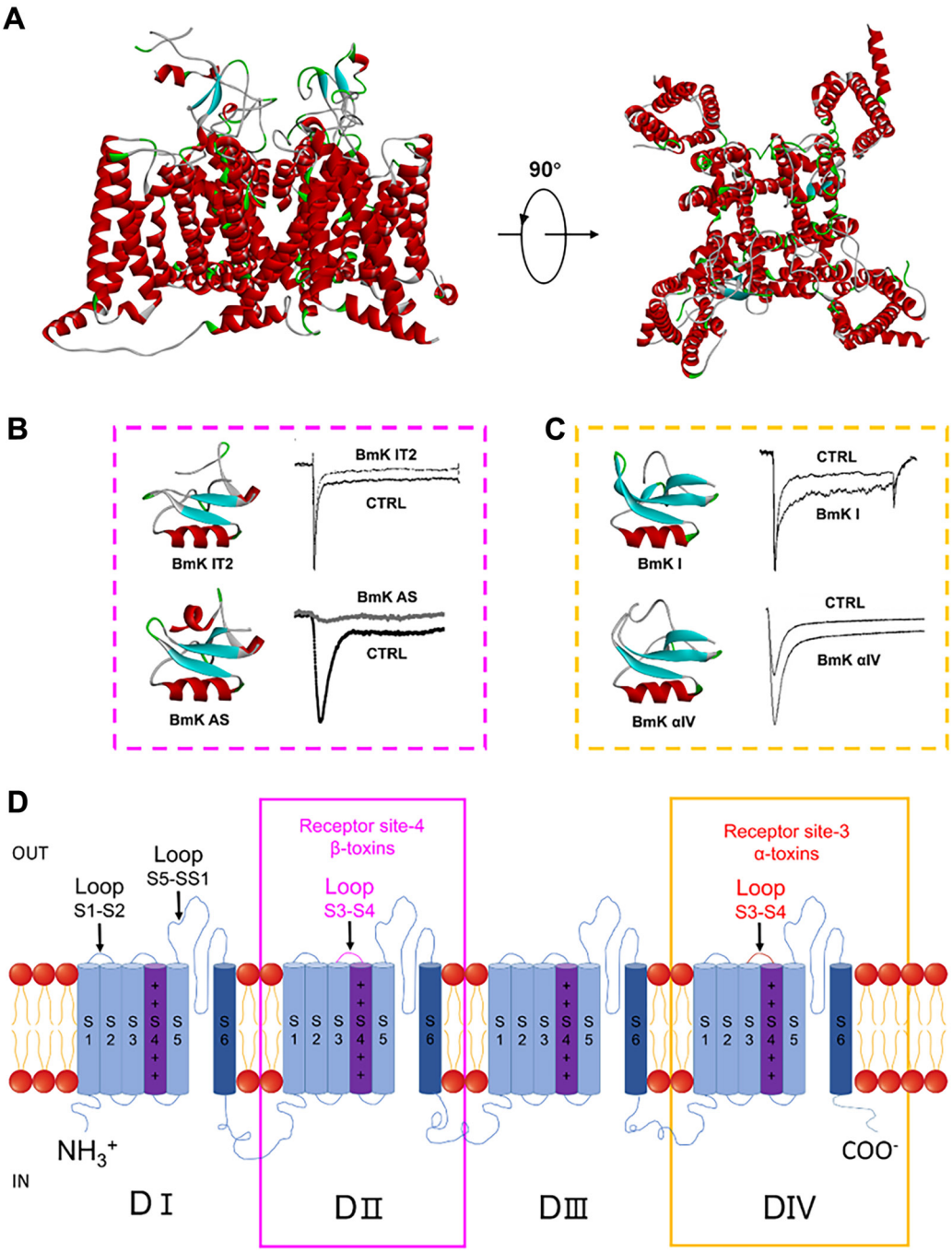

Fig. 2. The structure of VGSC and its pharmacological characterization modulated by α/α-like or β/β-like scorpion toxins. *A*: The structure of the cardiac sodium channel Nav1.5 (PDB: 6UZ3), as an example of VGSC structure. *B*: The β/β-like toxin BmK IT2 suppresses persistent currents of VGSCs on hippocampal pyramidal neurons by targeting the receptor site-4. BmK AS inhibits not only transient but also persistent currents of VGSCs on hippocampal pyramidal neurons via acting on the site-4. *C*: The α/α-like toxin BmK I delays the inactivation of VGSCs on hippocampal pyramidal neurons by targeting the receptor site-3. BmK αIV increases both transient and persistent currents of Nav1.2 via acting on the site-3. *D*: Topological diagram of sodium channel, and the receptor site-3 as well as -4.

epilepsy (TLE) in rats [30]. Also, the venom from the ant *Dinoponera quadriceps* (Kempf) has the potential pro- and anticonvulsant effects on Swiss mice model. The pre-administration of the denatured venom AbDq

Table 1. Examples of venom peptides from scorpion *Buthus martensii* Karsch acting on epilepsy-related ion channels

Peptide	Number of residues	Disulfide pattern	Target channels	IC_{50} or Kd	Binding domain (Sites)
BmK IT2	61	C^{I}-C^{VIII}, C^{II}-C^{V},	Hippocampal Nav [42]	NR	Domain II Site 4,
		C^{III}-C^{VI}, C^{IV}-C^{VII}	DmNav1 [43]	(2.96 ± 0.36) μmol/L	Glu896, Leu899, Gly904
BmK AEP	61	C^{I}-C^{VIII}, C^{II}-C^{V},	Cortical Nav [28]	~2.12 μmol/L	Domain II Site 4
		C^{III}-C^{VI}, C^{IV}-C^{VII}	Nav1.1	~3.20 μmol/L	
			Nav1.2	>10 μmol/L	
			Nav1.3	~1.46 μmol/L	
			Nav1.6	~0.39 μmol/L	
BmK AS	66	C^{I}-C^{VIII}, C^{II}-C^{V},	Nav1.2 [44]	NR	Domain II Site4, ps.
		C^{III}-C^{VI}, C^{IV}-C^{VII}	Nav1.3 [45]	NR	Domian IV Site 3
BmK I	64	C^{I}-C^{VIII}, C^{II}-C^{V},	Nav1.5 [46],	500 ± 30 nmol/L	Domain IV Site 3
		C^{III}-C^{VI}, C^{IV}-C^{VII}	Nav1.6 [47],	565 ± 16 nmol/L	E1613
			Nav1.2	252 ± 60 nmol/L	
			Nav1.3	214 ± 30 nmol/L	
BmK αIV	66	C^{I}-C^{VIII}, C^{II}-C^{V},	Nav1.2 [48]	~100–500 nmol/L	Domain IV Site 3
		C^{III}-C^{VI}, C^{IV}-C^{VII}			
MarTX	37	C^{I}-C^{IV}, C^{II}-C^{V}, C^{III}-C^{VI}	BK (α+β4)	(78.01 ± 5.86) nmol/L [49]	ps. pore and β4 [50]

IC_{50}, half maximal inhibitory concentration; K_d, dissociation constant; NR, not reported; MarTX, martentoxin; BmK, *Buthus martentsii* Karsch; ps., perhaps.

protected the animals against tonic-clonic seizures and death induced by administering bicuculline [31]. For the toxins acting on sodium channel regulators, BmK AEP, which was a scorpion peptide purified form the venom of BmK, displayed anti-epilepsy activity through inhibiting the current of Nav1.6 gating [32]. Hm1a, a spider *Heteroscodra maculata* venom peptide, was mainly distributed in inhibitory interneurons. It could selectively enhance the activity of Nav1.1 channels [33], by increasing the excitatory activity of Nav1.1 interneurons, but did not affect the firing of excitatory neurons. Thus intracerebroventricular infusion of Hm1a could rescue DS mice from seizures and premature death [34].

2 Anticonvulsant effects of β-toxins via targeting site-4 of VGSCs

2.1 BmK AS

BmK AS, a β-like scorpion neurotoxin, is extracted from the venom of BmK. It consists of 66 amino acid residues, which is stabilized by four intrachain disulfide bonds [58]. BmK AS not only inhibits tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na^+ currents dose-dependently, but also causes a hyperpolarization shift in the steady-state inactivation of TTX-R and TTX-S Na^+ currents [59]. BmK AS was also observed to increase [^{3}H]-noradrenaline release from hippocampal slices of rat brain and enhance the conductance of Na^+ in NG108-15 cells [60]. BmK AS facilitates steady-state activation and inhibits slow inactivation by stabilizing both the closed and open states of the Nav1.3 channel, which might result from an integrative binding to two receptor sites on the VGSCs [52].

Intrahippocampal injection of BmK AS produced dose-dependent anticonvulsant activity in the PTZ-induced epileptic model [61], with inhibited seizure-associated behavior as well as reducded amount and extent of high-amplitude, high-frequency discharges (HAFDs) on the electroencephalogram (EEG). The peak sodium currents were significantly inhibited by BmK AS at the cellular level [25, 51]. In addition, administering the high dose of BmK AS in hippocampus potently suppressed the increase of c-Fos expression and extended the latency to status epilepticus induced by pilocarpine [25].

2.2 BmK IT2

BmK IT2, a β-scorpion toxin polypeptides composed of 61 amino acid residues with 4 disulfide bond, is strongly relaxing and paralyzing to poisonous insects, but has no obvious toxicity to mammals [62]. Injection of BmK IT2 at hippocampal CA1 region could inhibit PTZ-induced epileptic like behavior in a dose-dependent manner and reduce the number, duration of HAFD components. Similarly, BmK IT2 significantly prolongs

the incubation period of status epilepticus onset, reduces the severity of status epilepticus and inhibits the expression of c-Fos in the hippocampus in the pilocarpine-induced epileptic model [49].

BmK IT2 relieves epileptogenesis and is thought to inhibit the activity of VGSCs. However, previous studies have found that BmK IT2 has no significant inhibitory effect on the peak currents of Nav1.2, Nav1.3 and Nav1.6 expressed in oocytes. However, binding experiments have found that BmK IT2 could bind to neuronal synaptosome membranes. The patch-clamp experiment also demonstrated that BmK IT2 could inhibit the persistent sodium current of hippocampal pyramidal neurons [49]. The possible mechanism was that the voltage sensor toxin needed to affect the interaction between the channel and the membrane to perform its function [63]. Channel toxicology is dependent on the surrounding plasma membrane environment, and the lipid bilayer would disturb the pharmacological sensitivity of VGSCs for the first time [61]. Thus the results from mammalian cells are more reliable than those from oocytes.

Studies have found that BmK IT2 and AS could inhibit TTX-S and TTX-R sodium currents in DRG [59, 64, 65]. Their main physiological significance is analgesia, but may cause mild pain-insensitive side effects. In the future, analysis of VGSC structure information [2], combined with the interaction of BmK IT2 and AS with peripheral sodium channel Nav1.7–1.9 is good for constructing novel selective VGSC anti-epileptic drugs.

2.3 BmK AEP

BmK AEP, which was composed of 61 amino acid residues with 4 disulfide bonds, was less toxic to mice and insects but had an anticonvulsant activity in rats, and is thus named as BmK AEP (BmK anti-epilepsy peptide) [66]. In a rodent model of epilepsy induced by coriaria lactone, BmK AEP could reduce the seizure rate, prolong the latent period of epileptic seizure, relieve seizures degree and reduce the average duration time of status epilepticus. BmK AEP, to a certain extent, had an effective anti-epilepsy activity [67].

The recent studies have shown that BmK AEP suppresses neuronal excitability in primary cultured cortical neurons in a concentration-dependent manner and inhibits Na peak current in cortical neurons by modulating the half-maximal voltage of the activation of VGSCs to hyperpolarized direction without affecting the steady-state inactivation. In addition, BmK AEP dose-dependently inhibits the currents of Nav1.1, Nav1.3, and Nav1.6, which is heterologously expressed in HEK-293 and shifts the steady-state activation of them in the hyperpolarizing direction, with minimal effect on steady-state inactivation [32].

3 Epileptic seizures models constructed by α-toxins via targeting site-3 of VGSCs

At present, dozens of animal models have been applied in the study of epilepsy [68], such as PTZ, KA and pilocarpine-induced models. These chemical agents have been shown to act mainly on voltage or ligand-gated channels to induce epilepsy. Scorpion peptide toxins, as specific sodium channel modulators, could also be used to construct epileptic seizures models for studying sodium channel-related epilepsy [69, 70].

3.1 BmK I

BmK I, one of the α-like toxins, is composed of 64 amino acid residues, which is identified as specifically binding to receptor site-3 and prolonging the inactivation phase of VGSC [71]. The intrahippocampal injection of BmK I could induce convulsion behavior of rats and the expression of c-Fos is increased in the hippocampus after BmK I injection [70]. Nissl staining showed that BmK I caused significant morphological changes in the hippocampus of rats, resulting in the reduction of the number of neurons in different regions of the hippocampus. Calcium imaging revealed that BmK I significantly increased the concentration of calcium and sodium in the synaptic membrane of rat brain, and this phenomenon could be completely inhibited by TTX [70]. The results suggested that BmK I could induce the increase of intracellular sodium and calcium concentration by modulating VGSCs, enhance the excitability of neurons and induce the convulsion in rats. The epileptic model of BmK I could be used as a novel experimental animal model for the study of VGSCs associated-epileptic seizure and the development of novel anti-epileptic drugs.

3.2 BmK αIV

BmK αIV, a novel VGSC modulator, was cloned from BmK venomous glands and heterologously expressed in *Escherichia coli*. The mature polypeptide of BmK αIV, containing 66 amino acid residues, has forceful toxicity in mice and cockroaches. The study showed that BmK αIV could bind to both cerebrocortical synaptosomes of rat brain and neuronal membranes of

cockroach, and shared similar binding sites with AaH II, a classical α mammal neurotoxin from *Androctonus australis* Hector [55]. Intracerebroventricular injection of BmK αIV could induce rat behavioral seizure, which has a similar pharmacological effect to BmK I. Unlike BmK I, BmK αIV has been found to bind to cerebrocortical synaptosomes of rat brain, suppressing the inactivation phase and increasing the steady-state and pick currents of rNav1.2 [72]. BmK αIV also could increase the intracellular calcium and sodium concentration and induce the release of glutamate from rat cortical synaptosomes, and its effect could be completely inhibited by TTX. Therefore, BmK αIV is expected to be a useful tool for studying neurological diseases such as epilepsy caused by abnormal sodium channel function and the neuroexcitatory imbalance [73].

4 Anti-epileptic effects of potassium channel toxin by regulating the function of VGSCs

BK channels, Ca^{2+} and/or voltage activated K^+ channels with the large conductance [74], have been demonstrated to regulate the rapid spike repolarization and the fast after hyperpolarization (fAHPs) in many classes of neurons [75]. However, in particular cases, by limiting the inactivation of Nav channels, BK channels induce neuronal spike shortening, increase firing rate and excitatory transmitter release, which could exacerbate seizure bursts [76].

In human beings, mutations in BK channels that lead to a gain of function phenotype are implicated in the pathophysiology of idiopathic-generalized epileptic seizures. In experimental studies, the BK β4 subunit knockout (KO) mice display TLE behavior associated with a gain of function phenotype in BK channels, with both sharpening APs and higher firing frequency in hippocampal DG granule cells [77, 78].

It is noteworthy that martentoxin, a polypeptide consisting of 37 amino acid residues, can selectively block iberiotoxin-insensitive neuronal BK channels (α+β4) [79] and has no significant effects on BK channels with α subunits alone [56]. In animal model experiments, recombinant martentoxin (rMarTX) [80] could prolong the latency, decrease the duration time, and the number of seizures, especially the high stage seizure, induced by PTZ. The amplitude and the duration of epileptic discharge were both decreased [57, 81]. In addition, martentoxin could significantly increase the latency time of seizure, reduce seizure duration and numbers in PTZ-treated rats, inhibit hippocampal hyperexcitability, and display neuroprotective effects in hippocampal neurons [57].

5 Perspectives

Up to now, 15 toxin-derived drugs have been used to treat a variety of diseases in clinic, including hypertension, diabetes and pain. Many lives have been saved by them. Moreover, at least 30 animal-derived toxins are considered to be drug candidates, which have entered clinical trials [82]. Among them, scorpion toxin chlorotoxin (CTX), isolated from *Leiurus quinquestriatus*, is under phase II clinical trial. It was reported that Iodine-131-chlorotoxin (TM-601) is a targeted drug candidate for the treatment of gliomas because it could cross the blood-brain barrier as well as some tissue barriers and specifically bind to malignant brain tumor cells without influencing the function of normal cells [83]. ShK derivatives, ShK-186 and ShK-192, are mainly used to treat autoimmune diseases, including neuroinflammatory multiple sclerosis by targeting Kv1.3 channels. However, the mechanism of epilepsy is still unclear, and the effect of clinical treatment is limited. After the treatment of common anti-epileptic drugs, about 30% of patients still develop into intractable epilepsy (IE) [84]. But scorpion and scorpion venom have remarkable effects in treating IE, and our previous study had showed that compound Dingxian pill, which containing scorpion, has been used as an anti-epileptic agent in China from ancient to modern time [85]. It was worth noting that the side effects of current common anti-epileptic drug could not be ignored, for instance, hematotoxicity [86], low bone mineral density [87] and liver injury [88] of the treatment of sodium valproate (VPA), nausea and vomiting of the carbamazepine [89, 90] and so on.

In addition, a good specificity of scorpion toxins has been also exhibited. Not only the epileptic seizure model, but also the chronic epileptogenesis model could be established by BmK I (or BmK αIV) [70, 73]. BmK I (or BmK αIV) as a sodium channel-specific modulator [91], could simulate the clinical symptoms of human GEFS+ by mediating the sodium channel function acquisition type mutation [92, 93].

In this review, we discussed the possibility of BmK scorpion toxins for clinical treatment on ion channel-relevant epilepsy. It is shown that long-chain scorpion toxins, such as BmK IT2 and BmK AS, and short-chain

scorpion toxin MarTX could effectively suppress neuroexcitability in epileptic seizure via VGSCs or BK channels. This brings the dawn to the effective control of intractable epilepsy or epileptogenesis suspected to be overcome. However, it is still a challenge for BmK toxins to be used to the treatment of this neurological disorders. The first problem underlying the application of these peptides is that they could not be taken orally, mainly because they are difficult to penetrate the intestinal mucosa. Due to their molecular size, polarity, hydrophilicity, and chargeability, the cell membrane penetration of BmK toxins is hampered. The second obstacle is that BmK toxins cannot cross the blood-brain barrier. Different from multiple sclerosis, the myelin and blood-brain barrier are not destroyed in other neurological diseases [94]. Clinical application of BmK toxins for treating these diseases will encounter difficulties. Fortunately, the situation is not unsolvable, and we still have a glimmer of light. A few years ago, scientists at the Sunnybrook Health Science Center in Canada used focused ultrasound technology to successfully pass chemotherapy drugs across the blood-brain barrier in a non-invasive manner [95] and reach the location of the tumor, which is of great significance in the field of neuropharmacology. In addition, the cell penetrating peptide (CPP) [96] with a strong cell membrane penetration, could be used as a drug carrier to assist the passage of polypeptide drugs across the cell membrane [94]. The fusion protein consists of CPP and BmK toxin might be developed as an oral drug for treating epilepsy. In short, finding suitable, safe, and efficient ways to promote the clinical use of BmK toxins are most valuable points to be solved.

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