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The bidirectional effects of scorpion's toxins and sodium channels in convulsant

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Abstract

Nowadays, there is a tremendous scope for development of safe and effective drug for the management of epilepsy with decreased adverse effects. Scorpion has been used to treat epilepsy for several of years, while symptomatic acute epileptic seizures may occur in up to 5% of individuals, especially children, with scorpion stings. Na_vs are composed of a pore-forming α and auxiliary β subunits, and scorpion venom are classified into α and β which both can active on Na_vs. Herein, we briefly describe the roles of scorpion's toxins and its bidirectional effects to the pathogenetic mechanism and therapeutic target of epilepsy.

Introduction

Epilepsy is a serious and chronic neurological disorder that affects no fewer than 50 million population globally [1]. The highest risk of epilepsy is in the neonatal period [2], while the highest incidence is in the elderly population across the lifespan [3]. Although great advances have been made in the development of new antiepileptic drugs, like vigabatrin, levetiracetam, topiramate, lamotrigine, zonisamide, lacosamide, rufinamide and stiripentol have been developed for epilepsy treatment [4]. However, seizures in 20-30% of patients remain refractory to therapies using conventional antiepileptic drugs [5], and all the currently available synthetic anticonvulsant drugs are prone to cause one or more side/adverse effects such as neurotoxicity, dizziness, impaired concentration and cognition function, mental slowing, ataxia, mental confusion, sleep disturbance, anorexia, somnolence, aggression and so on [6]. Therefore, there is a tremendous scope for development of safe and effective drug for the management of epilepsy with decreased adverse effects.

In the past few years, hundreds of polypeptide toxins with multipharmacological effects have been purified from the venom of scorpions, spiders, and wasps, and the scorpion's venom are most widely studied [7]. Some of these polypeptides have either convulsant or anticonvulsant activity and the latter have been considered to be potential candidates for antiepileptic drugs [8]. On the one hand, scorpion has been used to treat various neurological disorder symptoms for over two thousand years and it is the foremost choice for epileptic treatment as a traditional Chinese medicine [9]. On the other hand, symptomatic acute epileptic seizures may occur in up to 5% of individuals, especially children, with scorpion stings. Hence, a comprehensive description of scorpion's toxins and its bidirectional effects to the pathogenetic mechanism and therapeutic target of epilepsy has the potential and profound impetus function.

Voltage-gated sodium channels (Na_vs) are crucial components in neurotransmission, which are responsible for the generation and propagation of action potentials (AP) along neurons [10]. Based on the opening in response to membrane depolarization Na_vs allow sodium entry and thus the continuation of depolarization along the plasma membrane[11]. Na_vs are composed of a pore-forming α and auxiliary β subunits, the pore forming α subunit is a single-polypeptide chain which consists of DI–DIV structural domains, each domain has a voltage-sensing domain (VSD; S1–S4 segments) and a pore-forming domain (S5–S6 segments). The related research suggested that DI–III VSDs govern the pore opening of the Na_vs, whereas the DIV VSD controls its fast inactivation [11-14]. Scorpion venom is one of these toxins or (and) drugs active on Na_vs, is formed by cysteine-stabilized α-helix and β-sheet (CSαβ) fold cross-linked by four disulfied bridges [15], and the scorpion toxins are classified into two distinct type (α and β, both are gating modifier toxins) based on the pharmacological effects and channel binding properties [16,17]. Alpha-scorpion toxins mainly bind to the loop connecting S3 and S4 in Na_v domain IV and inhibit the fast inactivation of Na_v channels without dramatically affecting activation of the channels [18-24]. Beta-scorpion toxins mainly binds to the Domain II VSD and holds that VSD in the activated state and increase Na^+ currents by shifting the threshold of Na_v activation in the hyperpolarized direction by more than 20 mV [25-29].

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So far, $Na_v1.1$ to $Na_v1.9$, nine sodium homologous structures have been shown [30]. Among these Navs, mutations in the genes encoding Na_v 1.1, 1.2, and 1.6 are highly associated with epilepsy: mutations of SCN2A (encoding $Na_v1.2$) [31-33] and SCN8A (encoding $Na_v1.6$) [34,35] are the major causes of genetic epilepsy, the heterozygous loss of function mutation of SCN1A (encoding $Na_v1.1$) is associated with Dravet syndrome spectrum disorders [36,37], but the connection between $Na_v1.3$ and epilepsy is unclear. These toxins and drugs which can modify sodium channel activity have been widely used as tools to study physiological and/or pathological synaptic mechanisms including seizures occur, and at least six sites of toxin binding have been identified [38]. Herein, we further summarized the bidirectional mechanism of scorpion's toxins in convulsant and anticonvulsant as well as the roles of Na_v mutations in the scorpion toxins effects.

The convulsant effects of scorpion's toxins

Venomous animals are spread throughout the globe, most species of these animals use their venom for predation or defense, especially concerning scorpion's species [39]. Scorpions are a rather depauperate group within the class Arachnida with approximately 2200 known species up to the present [40]. The scorpion poisoning syndrome is a public health problem tropical region of the world, around 8000 scorpion envenomation accidents are reported every year in Brazil [41], and 14,569 cases were notified in the USA in 2001 [42]. Concerning the capacity of scorpion venom to induce convulsion and the highest risk of epilepsy in the neonatal period, severe intoxication in children with the presence of seizures were reported [43-45], which even secondary to an extensive destructive brain lesion [46]. Recent study suggesting that these deleterious effects induced by scorpion toxins may be a consequence of neuronal damage, also could be due to the results of seizures interfere with developmental processes of immature brain [47]. Moreover, that deleterious effects on the brain play a major role on the lethality induced by scorpion envenoming [48]. Furthermore, Nencioni ALA, *et al.* [49] reviewed the main effects caused by scorpion venoms, including myocardial damage, cardiac arrhythmias, pulmonary edema and shock-are mainly due to the release of mediators from the autonomic nervous system, also the central nervous system and inflammatory response participated in the process.

So far, sorts of polypeptide scorpion toxins with multipharmacological effects have been purified, which part of these polypeptides have been performed in the rodent studies. Scorpion venom is formed by mucopolysaccharides, hyaluronidase, phospholipase, serotonin, histamine, and protease inhibitors. The clinical manifestations of scorpion venom intoxication exclusively result from the action of α- and β-toxins [15-17]. The α-toxin is present in the venom of Androctonus australis Hector, Androctonus mauretanicu mauretanicus, Buthus eupeus, Buthus occitanus tunetanus, Leiurus quinquestriatus, and T serrulatus, whereas the β-toxin can be found in the venom of Centruroides sculpturatus, Centruroides suffusus suffusus, and T serrulatus.

Alpha-type scorpion toxin (TsTX) are peptides of 60-76 amino acid residues in length and tightly bound by four disulfide bridges, animal experiments have shown that these toxins modified the gating mechanism of the Na^+-channel function affecting either the inactivation (-toxins) or the activation (-toxins) kinetics of the channels suggesting TsTx is Na^+ channel specific scorpion toxin of the α type [38,50]. TsTX causes incremental overall internal concentrations of sodium and calcium ion, increases channel depolarization time and consequently induces excessive neurotransmitters release such as glutamate in a dose dependent manner. The earliest study of related TsTX, Carvalho FF, *et al.* intrahippocampal administration of TS-8F toxin, a neuropeptide isolated from Tityus serrulatus scorpion venom, which caused neuronal damage in CA1 and CA2 pyramidal cells and granular cells of the dentate gyrus, induced high-frequency and high-voltage spikes that evolved to seizure activity in the hippocampus and cortex, and resulted in epileptic seizure behavior, suggested the TsTx could lead to changes of neuronal excitability affecting the susceptibility of the central nervous system to convulsions induced by various agents [15,50].

Compared with TsTX, beta-type scorpion toxin (TiTX-gamma) caused little increase of internal sodium and calcium ion concentrations at low doses while evoked a significant increased release of glutamate [51]. Actually, one of the earliest studies found tityustoxin, an active venom component of the Brazilian yellow scorpion Tityus serrulatus, induced specific release of the glutamate, GABA and aspartate neurotransmitter in the synaptosomes of rats superfused sensori-motor cortex due to a depolarising action [52,53]. Also, AF Bicalho found tityustoxin which binds to sodium channel toxin site 3, have an effect in the increase of glutamate release, Na^+ influx, $[Ca^{2+}]i$, depolarization and exocytosis at steady state in the rat cerebrocortical isolated nerve endings [54]. These provides an interesting perspective concerning modulation of neurotransmitter release via pharmacological manipulation of Na^+-channel properties, that may lead to a better comprehension of its physiological and pathological roles.

The anticonvulsant effects of scorpion's toxins

Epilepsy treatment remains challenging in the clinic, and many treatments for epilepsy are still in the exploration stage. Scorpion has been the foremost choice for epileptic treatment as a traditional Chinese medicine [9], exhibiting strong anti-epileptic effects, and the anti-epileptic peptide isolated from the scorpion is more potent [55]. Earlier study that Zhou XH isolated and purified an anti-epilepsy peptide (AEP) from venom of the scorpion Buthus martensii Karsch, showing strongly inhibited epilepsy induced by coriaria lactone and cephaloridine [56]. Later research that Miguel Corona, *et al.* isolated a novel toxin from the venom of the scorpion Centruroides limpidus limpidus Karsch, named Cll9, which immediately induced sleep when i.c.v. injected in the rat, suggesting a neurodepressant effect and the inhibiting effect of Na^+ permeability in (cultured) rat peripheral ganglia further supports its neurodepressant actions. However, this peptide did not affect other Na^+ channels such as those from cerebellum granular cells in culture or the rSkM1 Na^+ channels expressed in HEK293 [57].

Buthus martensii Karsch (BmK) is a widely distributed scorpion species in Asia, which has been used to treat epilepsy for a long time. Even the scorpion components remain difficult to determine and its anti-epileptic mechanisms remains poorly understood, it has been speculated that the inhibition of hippocampal astrocyte activity are associated with its anti-epileptic effects [58]. Yi Liang, *et al.* discovered that the ethanol extracts of scorpion show anti-epileptic effect through decreasing hippocampal glial fibrillary acidic protein expression in a rat model of lithium chloride-pilocarpine induced epilepsy [58]. Moreover, scorpion extract plays a neuroprotective role and inhibits neuronal apoptosis following seizure, prevents glial cell scar formation in epileptic rats, and downregulate transcription factors associated with GFAP gene expression [59-63].

Up to now, several neurotoxins, BmK M1, M4, M8 (mammalian specific) and BmK IT and IT2 (insect specific) have been identified. All the cDNAs of BmK M1, BmK M9 [14] and BmK IT have been cloned,

while the genomic structures of BmK P01, P03 and P05, BmKTX, BmTX1 and TX2 , and Bm-12 have also been elucidated [64]. As far as can be determined, the α-toxins bind to receptor site 3 of voltage-gated Na^+ channels of vertebrates when the membranes are polarized. BmK I, an α-like neurotoxic polypepytide , has been shown to have potent nociceptive actions and to prevent epileptic seizures [65-69]. The β-toxins bind to receptor site 4 of vertebrate Na^+ channels causing the opening of Na^+ channels at more negative potentials. BmK IT2, BmK AS, and BmK AS-1 have been identified as β-type neurotoxins [70] and has been shown to inhibit peak tetrodotoxin sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium currents, while only BmK IT2 has been detected as an effective anticonvulsant in animal seizure models by modulating sodium channels [71].

The different roles of Na_vs in epilepsy

Na_vs mediate the generation and propagation of electrical signals (AP) in excitable tissues such as brain, spinal cord and peripheral nerve, and muscle [72-74]. The α and β subunits form the Na_vs structure, and that α subunits consist of four domains (I-IV), each with six transmembrane segments (S1-S6,S1-S4 forming the voltage sensor; S5-S6 contributing to the central ion-conducting pore) [72]. In mammals, nine α subunits (Na_v1.1-1.9) have been identified which are encoded by the genes SCN1A-5A and SCN8A-11A) [75]. At present the gene (SCN1A-5A and SCN8A-11A) mutation of sodium channels (Na_v1.1-1.9, except Na_v1.3) is a significant cause of abnormal excitability underlying human disease including epilepsy (especially with Na_v1.1,1.2 and1.6), periodic paralysis, cardiac arrhythmia, and pain syndromes.

SCN1A

SCN1A is recognized as the most important epilepsy gene discovered to date. It encodes the alpha 1 subunit of the voltage gated sodium channel. Mutations in SCN1A have been reported in patients with different types ofepilepsy, including generalized epilepsy with febrile seizures plus, severe myoclonic epilepsy in infancy, malignant migrating partial seizures in infancy [76]. With Computational analysis of single nucleotide polymorphisms in SCN1A gene of epilepsy, which implicates sodium voltage gated channel function play a key role in epilepsy, the most commonly mutated gene in epilepsy being SCN1A. SCN1A alleles cause protein truncation either by nonsense or frameshift mutation, and a large proportion of missense mutations studied in vitro confer a loss-of-function phenotype to the channel protein [77]. Na_v1.1, managed by SCN1A, was clustered predominantly at the axon initial segments of parvalbumin-positive (PV) interneurons and involved in sustained high-frequency firing of neocortical fast-spiking interneurons. For knock-in mouse line with a loss-of-function nonsense mutation in the SCN1A gene, both homozygous and heterozygous knock-in mice developed epileptic seizures within the first postnatal month. In heterozygous knock-in mice, trains of evoked action potentials in these fast-spiking, inhibitory cells exhibited pronounced spike amplitude decrement late in the burst [78].

SCN2A

In adult cerebral cortex of wild-type mice, most Na_v1.2 is expressed in excitatory neurons with a steady increase and redistribution from proximal (i.e., axon initial segments) to distal axons. Mutations in the SCN2A gene encoding a voltage-gated sodium channel Na_v1.2 are associated with epilepsies, intellectual disability, and autism [79]. SCN2A mutation of patients cause atypical generalized epilepsy with febrile seizures plus [80]. SCN2A gain-of-function (increased or accelerated, but not toxic) has been recognized as a cause of early infantile-onset severe epileptic encephalopathies such as ohtahara syndrome, whereas loss-of-function SCN2A mutations underlie ASD or intellectual disability with later-onset mild epilepsy or without epilepsy [81,82]. Focal epilepsy phenotype is caused by transgenic expression of an engineered Na_v1.2 mutation displaying enhanced persistent sodium current in Scn2aQ54 mouse [77,78]. It has been reported that the α-like scorpion toxin BmK I can enhance membrane excitability via persistent sodium current by preventing slow inactivation and deactivation of rNa_v1.2a expressed and induce the I_{NaP}, which may be involved in the BmK I-induced epilepsy.

SCN8A

SCN8A encodes the voltage-gated sodium channel Na_v1.6, and SCN8A-related epilepsies are associated with developmental and epileptic encephalopathies (DEE) including West Syndrome and Lennox-Gastaut Syndrome, as well as benign familial infantile epilepsy (BFIS) and patients with intellectual disability (ID) without epilepsy [83,84]. The SCN8A-related DEEs are severe epilepsies, often with refractory seizures, severe cognitive impairment and features such as cognitive visual impairment and spontaneous bone fractures [85]. Alleles that reduce the activity of *Scn8a* are known to increase resistance to acute seizures, reducing seizure severity and improving survival of *Scn1a* epileptic mutant m*ice [86-88]. However, reduced Scn8a activity also leads to non-convulsive absence epilepsy in mice [89] and humans [90]. Na_v1.6 is the principal sodium channel implic*ated in the generation of resurgent current in cerebellar Purkinje and dorsal root ganglia neurons [90], and spontaneous mutations of Na_v1.6 in the mouse result in neurological disorders including tremor, dystonia, ataxic gait, paralysis, and juvenile lethality [91,92]. The β-toxins purified from scorpion venoms of the Centruroidesspecies affect several voltage-gated sodium channels (VGSCs), which more affected resulted to be Na_v1.6 > 1.1 > 1.2 and induced resurgent current also in isoforms different from Na_v1.6, suggested that the scorpion toxins play Antiepileptic effects with Na_v1.1, Na_v1.2, play the opposite role with Na_v1.6 [93]. Moreover, CssII is another β-scorpion peptide that modifies preferentially sodium currents of the voltage-dependent Na^+ channel (Na_v1.6) sub-type by the amidated C-terminal of the CssII toward its interaction to the Na_v1.6 receptor [94].

Conclusion

Scorpion venoms have been used to treat epilepsy, while scorpion toxins also induce epileptic seizures. Na_vs are composed of a pore-forming α and auxiliary β subunits, and scorpion venom are classified into α and β which both can active on Na_v 1.1, 1.2 and 1.6 (Na_v1.6 > 1.1 > 1.2), which may further participant in the pathogenesis or treat mechanism of epilepsy. Hence, further study should be performed to detect the bidirectional effects of scorpion venoms to the pathogenetic mechanism and therapeutic target of epilepsy.

Conflict of interest and statement

The research was conducted in the absence of any commercial or financial relationships that could be misconstrued as a potential conflict of interest. We confirm that all authors contributed to this manuscript and have approved the final article.

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