Unique interactions between scorpion toxins and small conductance Ca\(^{2+}\)-activated potassium channels

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Abstract: Small conductance Ca\(^{2+}\)-activated potassium channels (SK channels) distributing in the nervous system play an important role in learning, memory and synaptic plasticity. Most pharmacological properties of them are determined by short-chain scorpion toxins. Different from most voltage-gated potassium channels and large-conductance Ca\(^{2+}\)-activated potassium channels, SK channels are only inhibited by a small quantity of scorpion toxins. Recently, a novel peptide screener in the extracellular pore entryway of SK channels was considered as the structural basis of toxin selective recognition. In this review, we summarized the unique interactions between scorpion toxins and SK channels, which is crucial not only in deep-researching for physiological function of SK channels, but also in developing drugs for SK channel-related diseases.

Key words: protein-protein interaction; peptide screener; potassium channel; α-KTx

Potassium channels are the largest and most diverse superfAMILY of ion channels. They are tetrameric membrane proteins and play a key role in cellular excitability and signal transduction pathways \(^1\), \(^2\). Among the potassium channel family, small conductance Ca\(^{2+}\)-activated potassium (SK) channels are expressed in a wide range of excitable and non-excitable cells, especially in the central nervous system. Apart from regulating...
somatic excitability, SK channels can also affect learning and memory when they are expressed in the postsynaptic membrane of glutamatergic synapses [3, 4]. Since scorpion toxins can selectively act on different subtypes of potassium channels, they are widely used as molecular probes to differentiate these potassium channel members [5, 6].

Scorpion potassium channel-blocking toxins (KTxs) are short-chain peptides that are cross-linked by two, three or four disulfide bridges, which have recently been divided into five subfamilies, called α-, β-, γ-, κ-, and δ-KTxs [6–8]. The α-KTxs have been proven to be powerful tools for investigating the pharmacological, physiological, biochemical, biophysical and structural characteristics of potassium channels and their associated ionic currents [9–11]. Interestingly, the SK channels were only inhibited by a small quantity of scorpion toxins with conserved structures, such as scorpion toxins P05, BmP05 and ScyTx instead of numerous Kv channel-sensitive and BK channel-sensitive toxins [12–14]. Recently, the novel peptide screener was found in SK3 channels [15], which well unmasked the unique interaction mechanisms between scorpion toxins and SK channels. In this review, the common structural features of SK-sensitive scorpion toxins were summarized. Meanwhile, novel peptide screener in three SK channels was further presented, which would promote the development of peptide inhibitors specific to different channel subtypes.

1 Common structural features of scorpion toxins acting on SK channels

Scorpion toxins such as ScyTx, BmP05 and P05 can interact with the SK channels through using their basic residues as critical residues. Structure-functional relationships indicate that the interacting surfaces of these scorpion toxins with SK channels mainly locate in the α-helix domain with several positively charged residues [12, 15, 16] (Fig. 1). When we count the number of the basic residues that play important roles in toxin pharmacology, it is easy to find that those toxins present only two or three basic residues at their binding interfaces. SK3 channels are almost insensitive to many Kv channel-sensitive and BK channel-sensitive scorpion toxins, which possess more than three basic residues at close vicinity of the channel pore-blocking basic residues [17, 18]. Such distribution comparison of critical basic residues among different types of scorpion toxins unMASKs the common structural features of SK channel-sensitive scorpion toxins which hold not more than three functional basic residues in their binding interfaces. To support this conclusion, Feng et al. introduced one or two basic residues for respectively constructing BmP05-S14R, BmP05-L15R, BmP05-N4R/S14R and BmP05-N4R/L15R mutant. Although BmP05 and its mutants adopted the similar overall structural topology, all mutants could not effectively block SK3 channel currents [15]. In addition, the decreased potency was stronger when two extra arginyl residues were incorporated into the BmP05 channel-binding interface (BmP05-N4R/S14R and BmP05-N4R/L15R) than a single residue (BmP05-S14R and BmP05-L15R). These common structural features of SK channel-sensitive scorpion toxins would accelerate the findings of more toxins blocking SK channels in the future.

2 Novel peptide screener in SK channels for controlling toxin recognition

Among different potassium channels targeted by scorpion toxins, different structural features in the pore region have been illustrated by computational and experimental studies. As shown in Fig. 2A, the channel pore region mainly includes three parts: Turret, Pore helix and Selectivity filter. So far, Kv1.2 and hERG channel turrets form an open state conformation in the pore region when interacting with scorpion toxins mautherotonin, BeKm-1 and BmKKx2, respectively [19–21]. In the pore region of BK channel, its turrets switches from an open state conformation for scorpion toxin charyb-
dotoxin binding to a compact “helmet” conformation for charybdotoxin insensitivity when BK channel interacting with its auxiliary β4 subunit. As for the distinct pharmacological properties of SK channels, a unique structural feature of peptide screener has been found in SK channels. As shown in Fig. 2A, SK channels have two highly conserved arginine residues (Arg-302 and Arg-306 of SK1, Arg-330 and Arg-334 of SK2, and Arg-485 and Arg-489 of SK3). These conserved basic residues form the peptide screener composed by two rings of basic residues in the pore region: The large ring is formed by the first conserved arginine residue, and the small ring is formed by the second conserved arginine residue (Fig. 2A–B). In this peptide screener, the salt bridges between conserved basic and adjacent acidic residues in channel pore region play a critical role in stabilizing the screener structure and maintaining the screener function, which was detailedly elucidated by the pharmacological changes of many designed mutant SK3 channels. The affinity of mutant channels for BmP05 reduced by 88-fold (SK3-R485H), 101-fold (SK3-R489H), 38-fold (SK3-R485K), and 51-fold (SK3-R489K), suggesting that the basic nature of the amino acid residues at SK3 positions 485 and 489 (e.g. Arg, Lys, or His) is key to maintain scorpion toxin BmP05 selectivity for the SK3 channel, whereas the presence of arginyl residues at these positions is mandatory for maintaining high BmP05 affinity. An additional step in significantly weakening the electrostatic interactions in the peptide screener is to substitute the SK3 channel dyad of arginyl with acidic or polar amino acid residues. This was achieved by replacing Arg-485 or Arg-489 with a glutamic acid (acidic) or serine (polar) residue in the SK3 channel. In agreement with the importance of Arg-485 and Arg-489 in the high affinity of scorpion toxin BmP05 for the SK3 channel, BmP05 produced much less inhibition of the SK3-R485E, SK3-R489E, SK3-R485S, and SK3-R489S mutant channels compared with the inhibition observed for the wild-type SK3 channel. Once the structure of peptide screener was destroyed, the mutant SK channels become susceptible to the “new” blocking activity by SK channel-insensitive scorpion toxin charybdotoxin with IC50 values of 381 nmol/L, 110 nmol/L, 84 nmol/L and 30 nmol/L for SK3-R485E, SK3-R489E, SK3-R485S, and SK3-R489S mutant channels, respectively. These experimental data strongly support the essentiality of the
electrostatic interactions in structure and function of SK channel peptide screener. Together, the novel peptide screener well underlies the unique pharmacological properties of SK channel controlling toxin recognition.

3 Unique molecular mechanisms of scorpion toxin-SK channel interactions

It is understandable that there are unique molecular mechanisms of scorpion toxin-SK channel interactions due to both two factors: One is the common structural features of SK channel-blocking scorpion toxins (Fig. 1), and the other is the novel peptide screener in the SK channel pore region (Fig. 2). Firstly, we simply summarize the mechanism progress of scorpion toxins acting on SK channels (Fig. 3A). As for scorpion toxins with the conserved structures composing by one α-helix and two anti-parallel β-sheets, the SK channel-insensi-

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Fig. 3. Mechanism of SK3 channel selectively interacted with scorpion toxins that use α-helix with 2–3 basic residues as binding interface. A: Schematic diagram of SK3 channel selectively interacted with scorpion toxins. B: Top view of the BmP05-SK3 complex and the amino acid residues form the peptide screener rings and two basic residues in BmP05 α-helix interact with SK3 channel, respectively. C: Top view of the ScyTx-SK3 complex and the amino acid residues form the peptide screener rings and two basic residues in ScyTx α-helix interact with SK3 channel, respectively.
tive scorpion toxins usually adopt their anti-parallel β-sheets as the binding interfaces to recognize Kv and BK channels. However, they can not bind SK channels due to the stronger electrostatic repulsion forces between scorpion toxins with more than three basic residues in their binding interfaces and peptide screener (Fig. 3A). Interestingly, SK channel-sensitive scorpion toxins usually use α-helix domains as the binding interfaces with only 2 or 3 basic residues (Fig. 1), and they will lose its binding capability when more than three basic residues locate in their binding interfaces (Fig. 3A). Fig. 3B and 3C further showed such unique molecular mechanisms of scorpion toxin-SK channel interactions at the level of modeled complex structures. In order to minimize the electrostatic repulsion force between scorpion toxin BmP05 and SK3 channel, the side-chain residue of Lys-6 is plugged into pore region of the SK3 channel (Fig. 3B). Several hydrogen bonds are formed to stabilize the binding. Meanwhile, the rest two basic residues can orient their own suitable position at the interface. Arg-7 of BmP05 is located between two Arg-489 residues that form the small ring of basic residues, whereas Arg-13 of the toxin hangs just above the two rings of Arg-485 and Arg-489. In the ScyTx-SK3 complex, instead of Lys-6 in BmP05, Arg-13 of ScyTx is the channel-blocking residue (Fig. 3C). Just as the Arg-13 of BmP05, Arg-6 of ScyTx also hangs above the two rings and interacts with the YHD-QQ (Aa490–Aa494) region in turret of SK3 channel. Clearly, these two toxins adopt a different pattern to recognize SK channels, which is likely caused by the location and number of toxin acidic residues [23]. All these unique molecular mechanisms of scorpion toxin-SK channel interactions will provide the insights into the pharmacological differences between SK channel-sensitive and SK channel-insensitive scorpion toxins.

4 Discovery prospects of scorpion toxins specific for potassium channel subtypes

The relationship between potassium channels and its toxin inhibitors will be helpful to develop peptide drugs targeting disease-related channels. Its in-depth research can accelerate the research and application of the numerous peptide toxins from natural venomous animals and help to design the highly selective diagnostic and therapeutic inhibitors [24]. It is clear that the shortage of compounds discriminating between potassium channel subtypes is still hampering the understanding of the roles of many channel subtypes in different tissues [25]. The novel peptide screener in SK channels actually narrow the exploring range of animal toxins inhibiting SK channels. Undoubtedly, they provide structural and mechanistic insights for the design of selective peptide inhibitors specific to each subtype of SK channel. Indeed, subtype specific SK channel blockers would be useful to treat neurological disorders more effectively [26], and pharmacological intervention targeting a particular SK channel subtype could reduce potential side effects that a non-selective compound might produce [25].

REFERENCES


