Potassium Channel Blocking Peptide Toxins from Scorpion Venom

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Abstract

In the last three decades, numerous peptides isolated from scorpion venom have been identified as members of the KTx, or potassium channel-blocking group of toxins. This chapter provides an overview of the four families of KTx, named α, β, γ, and κ, discussing characteristic structural features and K^+ channel selectivity of these peptides. Methods of KTx peptide identification and isolation, as well as techniques for the assessment of the efficacy of potassium channel blockade, are described. With the advancement of molecular biology, molecular dynamics simulations, and nuclear magnetic resonance (NMR) techniques, many details of the toxin-channel interaction have been clarified and models of different modes of toxin binding have emerged. A table summarizing all currently known 133 members of the KTx group of peptides is presented, including their systematic and common names, along with their affinities for the major target K^+ channels, which may be in the low picomolar range.

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P. Gopalakrishnakone et al. (eds.), *Scorpion Venoms, Toxinology*,
DOI 10.1007/978-94-007-6404-0_30

These peptides have provided vital information about the topology of the external pore region of K^+ channels highlighting similarities and even minute differences. In addition to being valuable exploratory molecular tools, peptide blockers of K^+ channels with high affinity and selectivity offer great potential for therapeutic use in a wide variety of diseases as was illustrated by several successful trials in animal models.

Introduction

The venom of scorpions contains a rich mixture of various compounds including many peptide components with a wide range of molecular weights. The biologically active constituents are often small peptide toxins that modulate the ion channels in the plasma membrane of a variety of cells. Some of these toxins alter the operation of Na^+-, Cl^--, or ryanodine-sensitive Ca^{2+} channels, but the largest and best-studied group consists of toxins that block K^+ channels (KTx).

Potassium channels represent the largest and most diverse ion channel type in the human organism with very wide tissue distribution and functional roles (Shieh et al. 2000). Peptide toxins that bind to specific K^+ channels have proven to be very valuable for two reasons:

1. They can be used as efficient molecular tools to learn about ion channel structure and function. The ability to examine how mutations in the toxin and/or channel affect the interaction offers great flexibility in the use of these peptides. Docking simulations with toxins of known structure make it possible to pinpoint minor structural differences in the topology of closely related channels, which may then explain observed functional differences between them. Blocking a certain subset of K^+ channels by high selectivity toxins can distinguish between the functions of similar channels expressed by the same cell.
2. Considering the enormous variety of the physiological and pathophysiological roles of K^+ channels and their cell-/tissue-specific expression distribution, they are attractive pharmacological targets in the therapy of several diseases. Successful experiments in animal disease models with potassium channel-blocking toxins have provided proof of concept for the feasibility of these efforts.

Primary Structure of KTxs

A large number of scorpion toxins have been identified by the isolation of mRNA from the venom gland. The reverse-transcribed cDNA sequences can be used to predict the amino acid sequences of the peptides expressed in the venom gland. These sequences are generally 50–60 amino acid-long precursor sequences containing signal peptides and other residues that are removed after translation. Many of such KTx peptides have not been tested on K^+ channels yet; however, all

long-chain peptides have putative or confirmed mature, short-chain derivatives in the α-KTx group. Other toxins were directly isolated from scorpion venoms and purified with high-performance liquid chromatography (HPLC) method, and their amino acid sequences were obtained by Edman degradation or MS/MS. In most of the cases, the amount of peptide isolated from the venom is not sufficient to determine the sequence, disulfide pairing, 3D structure, and the receptor specificity of the toxin. Knowing the amino acid sequences, toxins can be synthesized with recombinant techniques or chemical synthesis methods which allow the production of the peptides in large amount.

Secondary and Tertiary Structures and Classification of KTxs

The group of scorpion toxins targeting K^+ channels comprises short-chain (23–43 residues) and long-chain (42–84 residues) peptides, whose structure is stabilized by three or four disulfide bridges. At present, KTx toxins are classified into four families, α, β, γ, and κ, based on structural similarities and their specificities for various K^+ channels. Except for the κ-KTxs, all members of the other three families share a characteristic structural motif, called cysteine-stabilized α/β motif (CS-αβ), in which the α-helix is connected to a strand of the β-sheet (consisting of at least two strands, i.e., an αββ topology) by two disulfide bridges in C_i–C_j and C_{i+4}–C_{j+2} configuration. Although the CS-αβ-fold is a dominant structural feature among KTxs, it is not exclusive for this class of molecules, as peptides with different functions also share this motif (Dimarcq et al. 1998; Thomma et al. 2002; Caldwell et al. 1998; Zhao et al. 2002). Thus, the K^+ channel-blocking property should not be assumed solely based on the presence of this fold.

A highly conserved pair of residues, dubbed “the functional dyad” in many KTxs, was found to be important for high-affinity block of various K^+ channels. It consists of a lysine, whose positively charged side chain protrudes into the negatively charged environment of the selectivity filter of the potassium-conducting pore, and a hydrophobic (often aromatic) residue often situated nine positions downstream in the sequence, sterically separated by about 7 Å from the lysine. The dyad is found on the β-sheet side of the toxins. The dyad performs the same function even in toxins from sea anemone that have folds different from the signature CS-αβ. Besides the dyad, other residues also play crucial roles in forming the contact surface with the channel, thus determining selectivity (see below). Moreover, KTxs without the dyad that still block K^+ channels with high affinity have also been described, suggesting that the dyad is not an essential element for blockade (Batista et al. 2002).

Members of the KTx group of scorpion toxins that were discovered the earliest and shared high sequence and structural homology were classified into the α family, and the nomenclature α-KTxm.n was introduced to denote the *n*th member of the *m*th subfamily among the α-KTx toxins. The toxins included in the original classification were short (<40 residues) and contained six conserved cysteines. Since then the α-KTx family has vastly expanded, now including 133 members, and

ranging from 23 to 43 residues in size. Most of them have 3 disulfide bonds; however, all members of families 6, 12 (except 12.5 and 12.7), and 23 are stabilized by 4 disulfide bonds. α-KTx toxins are generally known to block *Shaker*-type Kv channels and Ca^{2+}-activated potassium channels.

Toxins of the β-KTx family are longer than the α-KTx toxins (47–84 residues stabilized by 3 disulfide bonds) and originate from the Buthidae, Caraboctonidae, and Scorpionidae families. These toxins contain two functionally different domains: a freely moving, possibly α-helical N-terminal segment, and a more compact cysteine-rich C-terminal segment that contains the signature CS-αβ structural motif. The N-terminal segment confers cytolytic activity to the toxin, while the C-terminal domain is responsible for K^+ channel-blocking ability. These toxins are further divided into three classes based on sequence similarity.

A separate family, γ-KTx, has been devoted to KTx toxins interacting with K^+ channels of the *ether-á-go-go*-related gene (ERG) family. γ-KTxs are all stabilized by four disulfide bonds except members 2.1 and 2.2. Their length ranges from 36 to 47 amino acids. The topography of the outer pore/turret region of the ERG family of channels is quite different from that of most other Kv channels; therefore, it is not surprising that a particular toxin is not likely to block both groups of channels. However, there has been one toxin, BmTx3, found, which belongs to subfamily α-KTx15 and yet blocks hERG channels (Huys et al. 2004a). A more detailed study revealed that two basic residues on the α-helix side of the toxin interact with the hERG channel, most likely with residues in the turret region, distant from the selectivity filter. On the other hand, BmTx3 also possesses the K_i-Y_{i+9} functional dyad on the β-sheet side, via which it was shown to block A-type K^+ currents, and thus was suggested to have two interaction surfaces, each acting on different channels. Interestingly, despite the presence of the dyad, the toxin does not block *Shaker*-type channels. Some of the γ-KTxs were found to be selective among human and rat ERG1, ERG2, and ERG3 channels, such as γ-KTx1.1, γ-KTx1.7, γ-KTx1.8, and γ-KTx2.1, which blocked these channel subtypes with varying affinities and therefore can be employed for the discrimination of these channels (Restano-Cassulini et al. 2006, 2008). There are currently 29 toxins that belong in the γ-KTx family.

The newest family of K^+ channel-blocking toxins from scorpion venom is the κ-KTx family of peptides consisting of 22–28 residues. They originate from scorpions in the Scorpionidae and Liochelidae families. Unlike members of the other KTx families, which are based on the CS-αβ scaffold, κ-KTx toxins adopt a structure that is formed by two parallel α-helices linked by two disulfide bridges. Although the presence of the functional dyad in hefutoxins, the first κ-KTxs to be described, and Om-toxins suggested that their targets would be K^+ channels, their affinities were found to be very low on the assayed channels. At present 18 κ-KTxs are listed in UniProt.

The systemic and common names of all currently known KTx toxins are listed in Table 1.

Table 1 List of KTx toxins isolated from scorpion venoms (main reference: http://www.uniprot.org/docs/scorpktx). The columns contain the Swiss-Prot accession number, the systemic and other names of the toxins, and the number of amino acids and disulfide bridges. The biological activity if determined is represented by the inhibition of a given channel (IC_{50} or Kd in brackets) if not stated otherwise (activation, cytotoxic effect, etc.). The abbreviated terms in the Method column indicate the principle of the measurement of the toxin-channel interaction (electrophysiology: voltage-clamp on insect or vertebrate cells; radio ligand: radioactive ligand binding assay; Xenopus: voltage-clamped Xenopus laevis oocytes; Rb^+ flux: measurement of radioactive Rb^+ efflux through K^+ channels).

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
P13487	α-KTx 1.1	Charybdotoxin, ChTX, ChTX-Lq1, ChTx-a	37	3	Kv1.2 (14 nM), Kv1.3 (2.6 nM) (Grissmer et al. 1994), Shaker (120 nM) (Goldstein et al. 1994), KCa1.1 (3 nM) (Meera et al. 2000), KCa3.1 (5 nM) (Wulff and Castle 2010)	electrophysiology
P45628	α-KTx 1.2	Charybdotoxin-2, ChTX-Lq2, ChTx-d	37	3	KCa (Lucchesi et al. 1989)	radio ligand
P24663	α-KTx 1.3	Iberiotoxin, IbTx	37	3	KCa1.1 (1 nM) (Meera et al. 2000)	*Xenopus*
P0C167	α-KTx 1.4	Limbatotoxin, LbTx, Limbatustoxin	37	3		
Q9NII6	α-KTx 1.5	Neurotoxin TX1, BmTX1	37	3	Kv1.3 (0.6–1.6 nM) (Romi-Lebrun et al. 1997a)	
Q9NII5	α-KTx 1.6	Neurotoxin TX2, BmTX2	37	3	Kv1.3 (0.6–1.6 nM) (Romi-Lebrun et al. 1997a)	
P45660	α-KTx 1.7	Toxin 15–1, Lqh 15-1	36	3		
	α-KTx 1.8	Reclassified as alpha-KTx 16.1				
P59848	α-KTx 1.9	Hongotoxin-2, HgTX2	36	3	Kv1.1 (30 pM) Kv1.2 (170 pM) Kv1.3 (86 pM) (Koschak et al. 1998)	Rb^+ flux

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
P83112	α-KTx 1.10	Parabutoxin-3, PBTx3	37	3	Kv1.1 (79 μM) Kv1.2 (547 nM) Kv1.3 (492 nM) (Huys et al. 2002)	*Xenopus*
P0C182	α-KTx 1.11	Slotoxin, SloTx	37	3	KCa1.1 (1.5 nM) (Garcia-Valdes et al. 2001)	*Xenopus*
P59943	α-KTx 1.12	Charybdotoxin b, ChTx-b	37	3		
P59944	α-KTx 1.13	Charybdotoxin c, ChTx-c	37	3		
H2ETQ6	α-KTx 1.14	Kcug1	37	3		
H2ER23	α-KTx 1.15	Kcug2	37	3		
P08815	α-KTx 2.1	Noxiustoxin, NTx, toxin II.11	39	3	Kv1.2 (2 nM), Kv1.3 (1 nM) (Grissmer et al. 1994)	electrophysiology
P40755	α-KTx 2.2	Margatoxin, MgTX	39	3	Kv1.1 (1.7–4.7 nM), Kv1.2 (6.4 pM), Kv1.3 (11.7 pM) (Bartok et al. 2014)	electrophysiology
P45629	α-KTx 2.3	Toxin-1, toxin I, toxin II.10.9.1, CllTx1	38	3	Rat brain K^+ channels (Martin et al. 1994)	radio ligand
Q9TXD1	α-KTx 2.4	Noxiustoxin-2, NTx2, NTx-2	38	3	Rat brain K^+ channels, bovine endothelial KCa (Nieto et al. 1996)	radio ligand, electrophysiology (KCa)
P59847	α-KTx 2.5	Hongotoxin-1, HgTX1	39	3	Kv1.1 (31 pM), Kv1.2 (170 pM), Kv1.3 (86 pM) (Koschak et al. 1998)	radio ligand
P59849	α-KTx 2.6	Hongotoxin-3, HgTX3	34 (partial sequence)			
P45630	α-KTx 2.7	Toxin II, toxin II.10.9.2, CllTx2	36	3	Rat brain K^+ channels (Martin et al. 1994)	radio ligand

P0C161	α-KTx 2.8	Toxin Ce1	39	3	Kv1.3 (0.71 nM) (Olamendi-Portugal et al. 2005)	electrophysiology
P0C162	α-KTx 2.9	Toxin Ce2	39	3	Kv1.3 (0.25 nM) (Olamendi-Portugal et al. 2005)	electrophysiology
P0C163	α-KTx 2.10	Toxin Ce3	38	3	Kv1.3 (366 nM) (Olamendi-Portugal et al. 2005)	electrophysiology
P0C164	α-KTx 2.11	Toxin Ce4	39	3	Kv1.3 (0.98 nM) (Olamendi-Portugal et al. 2005)	electrophysiology
P0C165	α-KTx 2.12	Toxin Ce5	39	3	Kv1.3 (69 nM) (Olamendi-Portugal et al. 2005)	electrophysiology
P85529	α-KTx 2.13	Toxin Css20	38	3	Kv1.2 (1.26 nM), Kv1.3 (7.1 nM) (Corzo et al. 2008)	electrophysiology
	α-KTx 2.14		37	3		
P24662	α-KTx 3.1	Kaliotoxin-1, KTX-1	38	3	Kv1.1 (0.1 nM), Kv1.2 (25 nM), Kv1.3 (1.5 nM) (Mourre et al. 1999), KCa (*H. pomatia* 2.5 nM) (Romi et al. 1993)	*Xenopus*/*Helix pomatia*
P46111	α-KTx 3.2	Agitoxin-2, AGTX-2, AgTx2	38	3	Kv1.1 (44 pM), Kv1.3 (4 pM), Kv1.6 (37 pM), Shaker (640 pM) (Garcia et al. 1994)	electrophysiology
P46112	α-KTx 3.3	Agitoxin-3, AGTX-3, AgTx3	38	3	Shaker 0.64 nM (Garcia et al. 1994)	*Xenopus*
P46110	α-KTx 3.4	Agitoxin-1, AGTX-1, AgTx1, leiurotoxin-2, leiurotoxin II, LeTx II	38	3	Kv1.1 (136 nM), Kv1.3 (41.7 nM), Kv1.6 (149 nM), Shaker (0.16 nM) (Garcia et al. 1994)	electrophysiology
P45696	α-KTx 3.5	Kaliotoxin-2, KTX-2	37	3	Rat brain K^+ channels, KCa (*H. pomatia*) (Laraba-Djebari et al. 1994)	radio ligand, electrophysiology

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
Q NII79	α-KTx 3.6	Kaliotoxin-1, KTX-1	38	3	Kv1.1 (203 pM), Kv1.2 (8.9 nM), Kv1.3 (171 pM) (Gao et al. 2010)	electrophysiology
P55896	α-KTx 3.7	Toxin OsK1, OsK-1	38	3	Kv1.1 (0.6 nM), Kv1.2 (5.4 nM), Kv1.3 (14 pM), KCa3.1 (225 nM) (Mouhat et al. 2005)	electrophysiology
P59886	α-KTx 3.8	Charybdotoxin-like peptide Bs 6, Bs6	38	3		
P59290	α-KTx 3.9	Kaliotoxin-3, KTX-3	37	3	Rat brain K^+ channels (Meki et al. 2000)	radio ligand
P0C908	α-KTx 3.10	BoiTx1	37	3	Shaker (3.5 nM) (Kozminsky-Atias et al. 2007)	electrophysiology
P0C909	α-KTx 3.11	OdK2	38	3	Kv1.3 (7.2 nM) (Abdel-Mottaleb et al. 2008)	*Xenopus*
P0C8R1	α-KTx 3.12	Kaliotoxin analog, Aam-KTX	38	3	Kv1.2 (10.4 nM), Kv1.3 (1.1 nM) (Abbas et al. 2008)	*Xenopus*
P46114	α-KTx 4.1	Tityustoxin K-alpha, TsTX-K-alpha, TSK4, toxin II-9, Ts7	37	3	Rat brain K^+ channels (Rogowski et al. 1994)	Rb^+ flux
P56219	α-KTx 4.2	Neurotoxin Ts-kappa, TsKapa, TsK	35	3	Rat brain K^+ channels (Legros et al. 1996)	radio ligand
P59925	α-KTx 4.3	Toxin TdK1	37	3	Shaker (280 nM) (D'Suze et al. 1999)	electrophysiology
P60210	α-KTx 4.4	Toxin Tc30	37	3	Kv1.3 (16 nM), Shaker (4.7 μM) (Batista et al. 2002)	electrophysiology
Q5G8B6	α-KTx 4.5		37	3		

P0CB56	α-KTx 4.6	Tst23	37	3	Kv1.2 (19 nM), Kv1.3 (10.7 nM) (Papp et al. 2009)	electrophysiology
P16341	α-KTx 5.1	Leiurotoxin-1, leiurotoxin I, LeTx I, scyllatoxin, ScyTx	31	3	Rat brain K^+ channels (Auguste et al. 1990)	radio ligand
P31719	α-KTx 5.2	Leiurotoxin I-like toxin P05, AmP05	31	3	Rat brain K^+ channels (Sabatier et al. 1993)	radio ligand
Q9TVX3	α-KTx 5.3	Neurotoxin BmP05, potassium ion channel blocker P05	31	3	Rat brain K^+ channels (Romi-Lebrun et al. 1997b)	radio ligand
P59869	α-KTx 5.4	Tamapin	31	3	Rat brain K^+ channels (Pedarzani et al. 2002)	radio ligand
P59870	α-KTx 5.5	Tamapin-2	31	3	Rat brain K^+ channels (Pedarzani et al. 2002)	radio ligand
Q10726	α-KTx 6.1	Potassium channel-blocking toxin 1, Pi1, Pi-1, PiTX-K-gamma	35	4	Rat brain K^+ channels, Kv1.2 (1.3 nM) (Mouhat et al. 2004), Kv1.3 (11.7 nM) (Peter et al. 2000), Shaker (32 nM) (Gomez-Lagunas et al. 1997)	radio ligand, electrophysiology
P80719	α-KTx 6.2	Maurotoxin, MTX	34	4	Kv1.1 (45 nM), Kv1.2 (0.8 nM), Kv1.3 (180 nM) (Kharrat et al. 1997), KCa3.1 (14 nM) (Castle et al. 2003)	*Xenopus*/Rb^+ flux
P59867	α-KTx 6.3	Neurotoxin HsTX1	34	4	Kv1.1 (7 nM) (Regaya et al. 2004), Kv1.1 (12.5 pM) (Lebrun et al. 1997)	electrophysiology
P58498	α-KTx 6.4	Potassium channel-blocking toxin 4, Pi4, Pi-4	38	4	Kv1.2 (8 pM) (M'Barek et al. 2003), Shaker (8 nM) (Olamendi-Portugal et al. 1998)	electrophysiology
P58490	α-KTx 6.5	Pi7, Pi-7, toxin-7	38	4		
Q6XLL9	α-KTx 6.6	OcKTx1	37	4		
Q6XLL8	α-KTx 6.7	OcKTx2	37	4		

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
Q6XLL7	α-KTx 6.8	OcKTx3	37	4		
Q6XLL6	α-KTx 6.9	OcKTx4	38	4		
Q6XLL5	α-KTx 6.10	OcKTx5	37	4		
P0C194	α-KTx 6.11	Male-specific potassium channel inhibitor IsTX	41	4		
P0C166	α-KTx 6.12	Anuroctoxin	35	4	Kv1.2 (6.14 nM), Kv1.3 (0.73 nM) (Bagdany et al. 2005)	electrophysiology
P84094	α-KTx 6.13	Spinoxin	34	4		
P84864	α-KTx 6.14	HgeTx1	36	4	Shaker (52 nM) (Schwartz et al. 2006)	electrophysiology
P85528	α-KTx 6.15	Hemitoxin	35	4	Kv1.1 (13 nM), Kv1.2 (16 nM), Kv1.3 (2 nM) (Srairi-Abid et al. 2008)	electrophysiology
C5J896	α-KTx 6.16	OcyC12	43	4		
P86116	α-KTx 6.17	Toxin OcyKTx2	34	4	Kv1.3 (18 nM), Shaker (52 nM) (Schwartz et al. 2013)	electrophysiology
	α-KTx 6.21	Urotoxin	37	4	Kv1.1 (253 nM), Kv1.2 (160 pM), Kv1.3 (91 nM), KCa3.1 (70 nM) (Luna-Ramirez et al. 2014)	electrophysiology
P55927	α-KTx 7.1	Toxin PiTX-K-alpha, pandinotoxin-alpha, potassium channel-blocking toxin 2, Pi2, Pi-2	35	3	Kv1.2 (32 pM) (Rogowski et al. 1996), Kv1.3 (44 pM) (Peter et al. 2001), Shaker (8.2 nM) (Gomez-Lagunas et al. 1996)	electrophysiology

P55928	α-KTx 7.2	Toxin PiTX-K-beta, pandinotoxin-beta, potassium channel-blocking toxin 3, Pi3, Pi-3	35	3	Kv1.3 (795 pM) (Peter et al. 2001), Shaker (140 nM) (Gomez-Lagunas et al. 1996)	electrophysiology
P56215	α-KTx 8.1	Neurotoxin P01, AmP01	29	3	Rat brain K^+ channels (Zerrouk et al. 1996)	radio ligand
Q9U8D2	α-KTx 8.2	Neurotoxin BmP01, potassium ion channel blocker P01	29	3	Rat brain K^+ channels (Romi-Lebrun et al. 1997b)	radio ligand
P80670	α-KTx 8.3	Toxin GaTx2, gating modifier of anion channels 2, leiuropeptide II, LpII, leiuropeptide-2	29	3	ClC-2 Cl^- channels (20 pM) (Thompson et al. 2009)	electrophysiology
P80671	α-KTx 8.4	Leiuropeptide-3, leiuropeptide III, LpIII	29	3		
P0CC12	α-KTx 8.5	OdK1	29	3	Kv1.2 (183 nM) (Abdel-Mottaleb et al. 2006)	electrophysiology
Q9NJP7	α-KTx 9.1	Neurotoxin BmP02, potassium ion channel blocker P02, toxin Kk6, BmKK6	28	3	Rat brain K^+ channels (Romi-Lebrun et al. 1997b)	radio ligand
Q9U8D1	α-KTx 9.2	Neurotoxin BmP03, potassium ion channel blocker P03	28	3	Rat brain K^+ channels (Romi-Lebrun et al. 1997b)	radio ligand
P80669	α-KTx 9.3	Leiuropeptide-1, leiuropeptide I, LpI	28	3		
P60209	α-KTx 9.4	Toxin BTK-2, Bt BTK-2	32	3	Kv1.1 (4.6 μM) (Dhawan et al. 2003)	electrophysiology
P84744	α-KTx 9.5	Neurotoxin Kbot1	28	3	Kv1.1 (145 nM), Kv1.2 (2.5 nM), Kv1.3 (15 nM), rat brain K^+ channels (Mahjoubi-Boubaker et al. 2004)	*Xenopus*/radio ligand

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
O46028	α-KTx 10.1	Cobatoxin-1, CoTx1, gtIX	32	3	Kv1.1 (24 μM), Kv1.2 (27 nM), Kv1.3 (5.3 μM), Shaker (1 μM), KCa3.1 (7.5 μM) (Jouirou et al. 2004), rat brain K^+ channels (Selisko et al. 1998)	electrophysiology
P58504	α-KTx 10.2	Cobatoxin-2, CoTx2	32	3	Kv1.1 (1 μM), Shaker (4.1 μM), rat brain K^+ channels (Selisko et al. 1998)	electrophysiology
P60164	α-KTx 11.1	Parabutoxin-1, PBTx1	37	3	Kv1.1 (150 nM) (Huys et al. 2004b)	electrophysiology
P60165	α-KTx 11.2	Parabutoxin-2, PBTx2	37	3	Kv1.1 (1 μM) (Huys et al. 2004b)	electrophysiology
Q6WGI9	α-KTx 11.3	Parabutoxin-10, PBTx10	36	3	Kv1.1 (1 μM) (Huys et al. 2004b)	electrophysiology
P59936	α-KTx 12.1	Butantoxin, BuTX, TsTX-IV, Ts6	40	4	KCa (mouse) (Novello et al. 1999)	electrophysiology
P0C168	α-KTx 12.2	Butantoxin, BuTX, TtBut	40	4	Shaker (660 nM) (Coronas et al. 2003)	electrophysiology
P0C185	α-KTx 12.3	Butantoxin-like peptide, Tco30	40	4		
P0C8L1	α-KTx 12.4	Butantoxin, BuTX, TstBut	40	4		
P0CH12	α-KTx 12.5	Neurotoxin KTx10	38	3	Kv1.1 (1.7 μM), Kv1.2 (12.6 μM), Kv1.3 (28 nM) (Liu et al. 2009)	electrophysiology
P0CI47	α-KTx 12.6		43	4		
P0CI48	α-KTx 12.7		38	3		
P83243	α-KTx 13.1	Toxin Tc1	23	3	Shaker (65 nM), mouse brain K^+ channels (Batista et al. 2000)	electrophysiology/ radio ligand

P83244	α-KTx 13.2	Toxin OsK2, OsK-2	28	3	Kv1.2 (97 nM) (Dudina et al. 2001)	electrophysiology
P84630	α-KTx 13.3	Toxin Tpa1	23	3	Shaker (200 nM) (Barona et al. 2006)	electrophysiology
P0C8L2	α-KTx 13.4	Toxin Tst-17	23	3	Shaker (3 μM) (Batista et al. 2007)	electrophysiology
Q967F9	α-KTx 14.1	Toxin Kk1, BmKK1	31	3		
Q95NK7	α-KTx 14.2	Toxin Kk2, BmKK2, BmTXKS3, neurotoxin BmP07, potassium ion channel blocker P07, KK1	31	3		
Q9BJX2	α-KTx 14.3	Toxin Kk3, BmKK3, neurotoxin SKTx2	31	3		
Q9BKB4	α-KTx 14.4	Neurotoxin SKTx1, BmSKTx1	31	3		
P60233	α-KTx 15.1	Peptide Aa1	37	3	Rat brain K^+ channels (Pisciotta et al. 2000)	electrophysiology
Q8I0L5	α-KTx 15.2	Toxin BmTX3, neurotoxin TX3, BmTX3A	37	3	hERG (1.9 μM) (Huys et al. 2004a), rat brain K^+ channels (Vacher et al. 2001)	electrophysiology
P60208	α-KTx 15.3	Toxin AmmTX3	37	3	Rat brain K^+ channels (Vacher et al. 2002)	electrophysiology
Q867F4	α-KTx 15.4	Toxin AaTX1, toxin Aa1	37	3	Rat brain K^+ channels (Pisciotta et al. 2000)	electrophysiology
Q86SD8	α-KTx 15.5	Toxin AaTX2	37	3		
P84777	α-KTx 15.6	Discrepin	38	3	Rat brain K^+ channels (D'Suze et al. 2004)	electrophysiology
Q5K0E0	α-KTx 15.7	Neurotoxin AamTX	37	3		
Q86BX0	α-KTx 15.8	Neurotoxin Kk4, BmKKx1	38	3		

(continued)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
D9U2A8	α-KTx 15.9	Neurotoxin KTx9	38	3		
P0C173	α-KTx 16.1	Tamulotoxin, TmTX	36	3		
Q9NBG9	α-KTx 16.2	Martentoxin, BmK 622, BmTx3B	37	3	KCa1.1 (80 nM) (Shi et al. 2008)	electrophysiology
Q8MQL0	α-KTx 16.3	Charybdotoxin-like toxin 1, Kchtx1, KTX1	37	3		
Q95NJ8	α-KTx 17.1	Toxin Kk4, BmKK4, toxin TXKs4	30	3	Rat brain K^+ channels (Li et al. 2003)	electrophysiology
P0CI46	α-KTx 17.2		31	3		
P60211	α-KTx 18.1	Toxin Tc32	35	3	Kv1.3 (10 nM), Shaker (74 nM) (Batista et al. 2002)	electrophysiology
P0C1X5	α-KTx 18.2	Toxin TdK2	34	3		
P0C1X6	α-KTx 18.3	Toxin TdK3	36	3		
P83407	α-KTx 19.1	Neurotoxin BmBKTx1, BmK37	31	3	Insect BK channels (Xu et al. 2004)	electrophysiology
P0C183	α-KTx 20.1	Toxin Tt28	29	3		
P86270	α-KTx 21.1	Tityustoxin-15, TS15	36	3	Kv1.2 (196 nM), Kv1.3 (508 nM) (Cologna et al. 2011)	electrophysiology
Q8MUB1	α-KTx 22.1	Toxin Kcugx, neurotoxin BmK38	40	3		
P0DJ31	α-KTx 23.1	Toxin Vm24, toxin alpha-KTx 21.1	36	4	Kv1.1 (30–40 nM), Kv1.2 (5–10 nM), Kv1.3 (2.9 pM), KCa3.1 (14–30 nM) (Varga et al. 2012)	electrophysiology
P0DJ32	α-KTx 23.2	Toxin Vm23, toxin alpha-KTx 21.2	35	4		

A7KJJ7	α-KTx 26.1	Neurotoxin BmK86	35	3	Kv1.3 (150 nM) (Mao et al. 2007)	electrophysiology
D9U2B2	α-KTx 26.2		39	3		
R4GUQ3	α-KTx 28.1	Toxin ImKTx104	27	3	Kv7.1 (11.7 μM) (Chen et al. 2012)	electrophysiology
D9U2A6	α-KTx 29.1	Neurotoxin-F, toxin LmKTx2	32	3		
R4GUQ1	α-KTx 29.2	Neurotoxin KTx3, toxin LmKTx71	32	3		
P0CI86	α-KTx 29.3	Neurotoxin-E, neurotoxin LmKTx95	32	3		
P0DL33	α-KTx 30.1	Toxin StKTx23	42	3	Kv1.3 (>1 μM) (Chen et al. 2012)	electrophysiology
P0DL34	α-KTx 30.2	Toxin SjKTx32	42	3		
P0DL35	α-KTx 30.3	Toxin SjKTx51	42	3		
β-KTx class 1 subfamily						
P69939		AaTXK-beta, AaTXKbeta, beta-KTx 2	64	3	Activation of Kv7.x channels (Landoulsi et al. 2013)	electrophysiology
B8XH40		BuTXK-beta, BuTXKbeta, Tx690	64	3		
Q9N661		BmTXK-beta-2, BmTX K-beta2, BmTXKbeta2, BmTX K beta2′, beta-KTx 4	64	3		
Q5G8A6		Tco-beta-KTx, TcobetaKTx, Tco 41.46-2, Tco 42.14	60	3		
Q0GY44		Tdi-beta-KTx, TdibetaKTx	60	3		
P69940		TsTXK-beta, tityustoxin K-beta, TsTX-K beta, TsTX K beta, TsTXKbeta, TSK2, beta-KTx 1	60	3	Rat brain K^+ channels (Rogowski et al. 1994)	Rb^+ flux

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
P0C2F3		Tst-beta-KTx, TstbetaKTx	60	3	Kv1.1 (96 nM) (Diego-Garcia et al. 2008)	electrophysiology
Q0GY46		Ttr-beta-KTx, TtrbetaKTx	60	3		
P0CI49		Neurotoxin beta-KTx 31.1	67	3		
A9XE60		MeuTXK-beta-1, MeuTXKbeta1	72	3		
A9XE59		MeuTXK-beta-2, MeuTXKbeta2	72	3		
D9U2A7		Neurotoxin beta-KTx 7	67	3		
β-KTx class 2 subfamily						
Q0GY41		Hge-beta-KTx, HgebetaKTx	58	3	Cytolytic (Diego-Garcia et al. 2008)	electrophysiology
P0CH57		MeuTXKbeta3, BeL-5, BeL-69, Meucin-24, MeuTXKbeta3	47	3		
Q9NJC6		BmTXK-beta, BmTX K-beta, BmTXKbeta, BmKLK, beta-KTx 3	61	3	Rabbit myocyte K^+ channels (Cao et al. 2003)	electrophysiology
Q0GY42		TcoKIK	47	3		
Q0GY43		TdiKIK, TdKIK	47	3		
P0C8W4		TstKMK, toxin 5536	47	3		
P86822		Beta-Ktx 2	64	3		
Q0GY45		TtrKIK	47	3		
P0CJ45		Neurotoxin beta-KTx 52.1	50	3		
D9U2B1		Neurotoxin beta-KTx 12	50	3		
P0CI42		Neurotoxin beta-KTx 14.3	48	3		
C6ZH27		Neurotoxin beta-KTx 17	48	3		

β-KTx class 3 subfamily						
Q0GY40		Hge-scorpine, Hg-scorpine-like 1, Hgscplike1, HgeScplp1, Hge36	48	3	HGE36 fragment on Kv1.1 (158 nM), cytolytic (Diego-Garcia et al. 2008)	electrophysiology
P0C2F4		Heteroscorpine-1, HS-1	76	3	Antibacterial (Uawonggul et al. 2007)	
C5J891		Scorpine-like, OcyC7	53	3		
P56972		Scorpine, scorpin, panscorpine	75	3	Antimicrobial (*Plasmodium*) (Conde et al. 2000)	
P86121		Scorpine-like	15	0		
Q5WR03		Opiscorpine-1	76	3	Antimicrobial (Zhu and Tytgat 2004)	
P0C8W5		Hg-scorpine-like 2, Hgscplike2, HgeScplp2	84	3		
Q5WR01		Opiscorpine-2	76	3		
Q5WQZ7		Opiscorpine-3	76	3		
Q5WQZ9		Opiscorpine-4	76	3		
Q86QT3	γ-KTx 1.1	Ergtoxin, ErgTx, ergtoxin-like protein 1, ErgTx1, CnErg1, CnErgTx1	42	4	hERG (8.5 nM) (Torres et al. 2003)	electrophysiology
Q86QV6	γ-KTx 1.2	Ergtoxin-like protein 1, ErgTx1, CeErg1, CeErgTx1	42	4		
Q86QV3	γ-KTx 1.3	Ergtoxin-like protein 1, ErgTx1, CgErg1, CgErgTx1	42	4		
Q86QU6	γ-KTx 1.4	Ergtoxin-like protein 1, ErgTx1, CsErg1, CsErgTx1	42	4		
Q86QV0	γ-KTx 1.5	Ergtoxin-like protein 1, ErgTx1, CllErg1, CllErgTx1	42	4		

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
Q86QU1	γ-KTx 1.6	Ergtoxin-like protein 1, ErgTx1, CexErg1, CexErgTx1	42	4		
P0C892	γ-KTx 1.7	CeErg4	42	4	hERG1 (12.8 nM), hERG3 (0.88 nM) (Restano-Cassulini et al. 2008)	electrophysiology
P0C893	γ-KTx 1.8	CeErg5	42	4	Similar effect to gamma-KTx 1.7 (Restano-Cassulini et al. 2008)	electrophysiology
Q9BKB7	γ-KTx 2.1	Neurotoxin BeKm-1	36	3	hERG (3.3 nM) (Korolkova et al. 2001)	electrophysiology
P59938	γ-KTx 2.2	Neurotoxin Kk7, BmKK7, BmKKx2	36	3		
P59939	γ-KTx 3.1	Ergtoxin-like protein 2, ErgTx2, CnErg2, CnErgTx2	43	4		
Q86QV5	γ-KTx 3.2	Ergtoxin-like protein 2, ErgTx2, CeErg2, CeErgTx2	43	4		
Q86QU5	γ-KTx 3.3	Ergtoxin-like protein 2, ErgTx2, CsErg2, CsErgTx2	43	4		
Q86QV2	γ-KTx 3.4	Ergtoxin-like protein 2, ErgTx2, CgErg2, CgErgTx2	42	4		
Q86QU9	γ-KTx 4.1	Ergtoxin-like protein 2, ErgTx2, CllErg2, CllErgTx2	43	4		
Q86QV7	γ-KTx 4.2	Ergtoxin-like protein 5, ErgTx5, CnErg5, CnErgTx5	43	4		

Q86QU0	γ-KTx 4.3	Ergtoxin-like protein 2, ErgTx2, CexErg2, CexErgTx2	43	4		
Q86QT9	γ-KTx 4.4	Ergtoxin-like protein 3, ErgTx3, CexErg3, CexErgTx3	43	4		
Q86QT8	γ-KTx 4.5	Ergtoxin-like protein 4, ErgTx4, CexErg4, CexErgTx4	43	4		
Q86QU8	γ-KTx 4.6	Ergtoxin-like protein 3, ErgTx3, CllErg3, CllErgTx3	43	4		
Q86QU7	γ-KTx 4.7	Ergtoxin-like protein 4, ErgTx4, CllErg4, CllErgTx4	43	4		
Q86QV4	γ-KTx 4.8	Ergtoxin-like protein 3, ErgTx3, CeErg3, CeErgTx3	43	4		
Q86QU4	γ-KTx 4.9	Ergtoxin-like protein 3, ErgTx3, CsErg3, CsErgTx3	43	4		
Q86QU3	γ-KTx 4.10	Ergtoxin-like protein 4, ErgTx4, CsErg4, CsErgTx4	43	4		
Q86QV8	γ-KTx 4.11	Ergtoxin-like protein 4, ErgTx4, CnErg4, CnErgTx4	43	4		
P59940	γ-KTx 4.12	Neurotoxin CsEKerg1	43	4	hERG (232 nM) (Nastainczyk et al. 2002)	electrophysiology
Q86QV9	γ-KTx 4.13	Ergtoxin-like protein 3, ErgTx3, CnErg3, CnErgTx3	43	4		
Q86QU2	γ-KTx 5.1	Ergtoxin-like protein 5, ErgTx5, CsErg5, CsErgTx5	47	4		
Q86QV1	γ-KTx 5.2	Ergtoxin-like protein 3, ErgTx3, CgErg3, CgErgTx3	47	4		

(*continued*)

Table 1 (continued)

Swiss-Prot accession	Systematic name	Other names	Length (amino acids)	Disulfide bonds	Biological effect	Method
P82850	κ-KTx 1.1	Kappa-hefutoxin-1, Kappa-HfTx1	22	2	Kv1.2 (150 μM), Kv1.3 (40 μM) (Srinivasan et al. 2002)	electrophysiology
P82851	κ-KTx 1.2	Kappa-hefutoxin-2, Kappa-HfTx2	23	2		
P83655	κ-KTx 1.3		23	2		
P0DJ33	κ-KTx 1.4	HSP009C	23	2		
P0C1Z3	κ-KTx 2.1	Toxin OmTx1	26	2		
P0C1Z3	κ-KTx 2.2	Toxin OmTx2	27	2		
P0C1Z4	κ-KTx 2.3	Toxin OmTx3	23	2		
P0C1Z3	κ-KTx 2.4	Toxin OmTx4	25	2		
P86110	κ-KTx 2.5	OcyC8, OcyKTx6	28	2	Kv1.1 (217 μM), Kv1.4 (71 μM) (Camargos et al. 2011)	electrophysiology
C5J893	κ-KTx 2.6	OcyC9	24	2		
P0DJ34	κ-KTx 2.7	HSP053C.1, toxin HeTx203, toxin kappa-KTx 2.6	24	2		
P0DJ35	κ-KTx 2.8	HSP053C.2, toxin HeTx204, toxin kappa-KTx 2.7	24	2		
P0DJ36	κ-KTx 3.1	HSP040C.1	27	2		
P0DJ37	κ-KTx 3.2	HSP040C.3	25	2		
P0DJ38	κ-KTx 3.3	HSP040C.4	25	2		
P0DJ39	κ-KTx 3.4	HSP040C.5	27	2		
P0DJ40	κ-KTx 4.1	HSP040C.2	27	2		
P0DJ41	κ-KTx 5.1	HelaTx1	27	2	Kv1.1 (9.9 μM), Kv1.6 (approx. 10 μM) (Vandendriessche et al. 2012)	electrophysiology

Characterization of Toxin-Channel Interactions, Mechanism of Block

To test the affinity of a given peptide to its receptors, several methods are available. At the time of the isolation of the first scorpion toxins (noxiustoxin in 1982 (Carbone et al. 1982)), the availability of cloned ion channel genes was limited. The first ion channel gene cloned in 1982 was the nicotinic acetylcholine receptor (nAChR) of the torpedo ray (Noda et al. 1982) followed by the voltage-gated sodium channel of electric eel in 1984 (Noda et al. 1984). Therefore, the general way to test the efficiency of a peptide in inhibiting K^+ channels was to isolate excitable cells generally from rat nervous system and measure the effect of the test substance on the endogenously expressed channels. Toxin-K^+ channel interactions can be tested on the fast-inactivating A-type current of these cells, which is generated by Kv1.4, Kv3.4, Kv4.1, Kv4.2, and Kv4.3 α-subunits (Vacher et al. 2004; Song et al. 1998; Song 2002) or on delayed-rectifier currents of Kv1.1, Kv1.2, Kv1.5, Kv1.6, Kv2.1, Kv3.1, and Kv3.2 channels (Song 2002). These cells also express Ca^{2+}-activated K^+ channels which makes them suitable to test the inhibitory effect of the toxins on small conductance (SK) channels (Legros et al. 1996; Jouirou et al. 2004). Other primary cell cultures were also used for testing, such as bovine aortic endothelial cells (Nieto et al. 1996) or neurons from snail (Laraba-Djebari et al. 1994) or rabbit (Crest et al. 1992).

After the cloning of individual ion channel genes and the application of heterologous expression systems in *Xenopus* oocytes, insect, or mammalian cells, more precise methods became available to determine the receptors of the toxins (Schwartz et al. 2013; Varga et al. 2012; Lebrun et al. 1997). Measurements can be done by radiography or with electrophysiological methods. Radiography methods can be direct or indirect. Direct measurements require the radioactive labeling (in most of the cases, ^{125}I) of the toxin which may alter the receptor specificity of the labeled toxin compared to the unlabeled form (Koch et al. 1997). Indirect assays are based on the competition of the test substance with a well-characterized radioactive labeled ligand (such as ^{125}I apamin or ^{125}I noxiustoxin) for the binding site (Legros et al. 1996; Pedarzani et al. 2002). The disadvantage of such measurements is that they measure the association and dissociation of the peptides to the targeted receptors at any contact surface. Kd (dissociation constant) values in such measurements do not necessarily represent the pore-blocking ability or the dose dependence of the inhibition of the ionic flux through the channels (IC_{50}). Determination of the radioactive $^{86}Rb^+$ flux is another general tool to test the K^+ channel inhibiting ability. Cells expressing voltage-gated K^+ channels are loaded with $^{86}Rb^+$ and then depolarized by high K^+-containing extracellular solution. $^{86}Rb^+$ flows through open K^+ channels and amount of extracellular $^{86}Rb^+$ can be determined with scintillation counter. Inhibitors of potassium channels decrease the $^{86}Rb^+$ flux in a dose-dependent manner; therefore, the half-inhibiting concentration (IC_{50}) with such method can be determined (Bartschat and Blaustein 1985; Koschak et al. 1998). Electrophysiological methods permit the direct measurement of ionic currents through voltage-clamped

membranes. For these measurements a variety of different cells or membrane preparations can be used. Primary cell lines (neurons, lymphocytes, etc.) expressing specific ion channels endogenously are widely used for the measurements (Schwartz et al. 2013; Varga et al. 2012; Vacher et al. 2001). Recombinant techniques allow the expression of specific ion channels in various cell types (*Xenopus* oocytes, mammalian cells, etc.) which has the advantage of measuring specific inhibitory effect of a toxin on a given ion channel with very low probability of aspecific effect due to the absence of endogenously expressed channels (Schwartz et al. 2013; Bagdany et al. 2005; Romi-Lebrun et al. 1997a).

The receptor site for KTxs is the K^+ channel pore; competition experiments confirmed that the toxins bind to a region that overlaps with the tetraethylammonium (TEA) binding site at the external entrance of the pore and that only a single peptide molecule is able to occupy the binding site at a given time (Varga et al. 2012; Miller 1988).

The relatively small size of KTxs enables them to deeply enter the vestibule of the channels allowing for multiple contact points and also exposes the majority of their residues, which results in highly variable interaction surfaces even due to minor changes in the sequence. These features enable the toxins to bind to channel surfaces in various orientations. There have been three major modes of interaction described between K^+ channels and KTxs. The most frequently identified and best-characterized interaction is via the functional dyad described above (Fig. 1a). In these cases the β-sheet side of the toxin faces the entrance of the channel pore and the lysine side chain in the selectivity filter, and the hydrophobic interaction of the other dyad residue mostly accounts for the high-affinity binding.

A different mode of interaction was described between KCa2.x channels and α-KTx4.2 and members of the α-KTx5 subfamily (Rodriguez de la Vega et al. 2003) (Fig. 1b). In these instances influential residues were localized on the α-helix side of the toxins. Two arginines (for TSκ (α-KTx4.2)) and three arginines (for P05 (α-KTx5.3)) were identified as critical for binding to small conductance calcium-activated potassium channels that made contacts with channel residues on the bottom of the vestibule and the turret region. Thus, compared to typical α-KTx-Kv channel interactions, the contact region is on the opposite side of the toxins and farther away from the selectivity filter.

Members of the γ-KTx family seem to employ yet another way to bind to hERG channels. As described above for BmTx3, γ-KTxs most likely bind to extracellular segments of the extended S5–S6 linker in ERG channels, which may form an extra amphipathic α-helix (Fig. 1c). As exemplified by ErgTx (Pardo-Lopez et al. 2002), this mode of block differs in several respects from the typical α-KTx mode of block, whose characteristics are mainly defined by the critical lysine's interaction with the selectivity filter. Due to the deep penetration of the lysine side chain, it not only interacts with potassium ions in the pore but also senses the electric field, which makes this mode of block by α-KTX sensitive to external K^+ concentration and to the applied membrane voltage. Since γ-KTxs lack the equivalent of the lysine and thus do not interact directly with the pore, the block is insensitive to K^+_{ext}, but not the membrane potential. This was explained by structural rearrangements in the

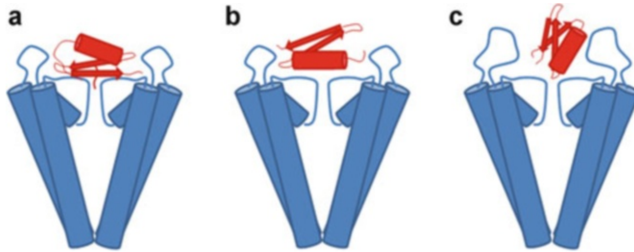

Fig. 1 (**a**) Typical blocking scheme of an α-KTx in the pore of a *Shaker*-related Kv channel. The interaction surface is on the β-sheet side of the toxin forming several close contacts with the bottom of the vestibule, and the side chain of the critical lysine protrudes deeply into the selectivity filter. (**b**) Block of the KCa2.2 (SK) channel by α-KTx5.3 occurs by an inverted orientation of the toxin compared to the typical α-KTx mechanism; the main interaction surface is on the α-helical side of the toxin. Channel residues involved in the interaction are localized in the turret region and the bottom of the vestibule. Brownian dynamics of the recognition of the scorpion toxin P05 with small-conductance calcium-activated potassium channels. (**c**) Interaction of γ-KTx2.1 with the HERG channel occurs mainly between the α-helix of the toxin and the large turret region of the channel. The toxin does not enter very deeply and does not fully block permeation

S5-P linker brought on by strong depolarization, which destabilizes ErgTx binding. Although the overlap of the ErgTx binding site with that of TEA places it at the outer mouth of hERG, the inability of ErgTx to produce total current block suggests an off-center binding position rather than a complete plugging of the pore as known for α-KTxs. γ-KTxs are assumed to bind with their α-helix side in an orientation different from the two previous modes and interact with residues even farther from the selectivity filter (Rodriguez de la Vega et al. 2003; Pardo-Lopez et al. 2002).

The block mechanism of the KTxs has been studied by several methods, the earliest ones involving a large number of mutations both in the toxin and channel sequences. The structure of the toxins was fairly well known from NMR studies (Bontems et al. 1991, 1992), and based on geometric constraints, useful conclusions could be drawn about the topology of the outer pore region of the channels. Using conservative and nonconservative mutations and measuring the binding affinities, the most influential residues were identified (Goldstein et al. 1994). Most KTxs carry a high net positive charge and thus are likely to be attracted toward the negatively charged environment of the selectivity filter by long-range interactions. This involvement of electrostatic interactions is supported by the ionic strength dependence of toxin binding (MacKinnon et al. 1989). However, even charge-neutralizing mutations of toxin residues that drastically affected binding affinity had little effect on association rates, implying that toxin affinity is mostly determined by pairwise close contact interactions with channel residues. Residues forming "close contact" were defined as those whose conservative mutations resulted in great changes in binding affinity and in which the affinity change mostly arose from the dissociation rate of the toxin.

A very influential residue, the mutation of which changed binding affinity by several orders of magnitude, was identified in the "wall" of the vestibule or turret

region of the *Shaker* channel (F425G mutation), whose role was confirmed for the corresponding residue in Kv1.3 as well (position 380) (Aiyar et al. 1995). Strikingly, this residue is very far from the cluster of other critical residues surrounding the entryway of the pore. It was shown that this residue does not contribute to normal binding of the toxin, but can greatly reduce accessibility to the pore by steric hindrance if a bulky residue is situated here. This finding underlines the fact that even residues that are not located on the typical interaction site of the toxin or the channel can have an effect on the formation of a specific channel-toxin complex, which may be a determining factor in the channel selectivity of a toxin.

Applying thermodynamic mutant cycle analysis, the closely interacting residue pairs could be pinpointed with even higher accuracy (Ranganathan et al. 1996). In this technique residues of the toxin and the channel are mutated individually and then simultaneously, and based on the binding affinities of the various combinations, a pairwise coupling energy is calculated, which characterizes the tightness of the interaction of the pair. These early studies established the critical role of the central (dyad) lysine and recognized that it must interact with residues forming K^+ binding sites in the pore based on the external K^+ concentration dependence of the binding. In contrast to the pore-blocking mechanism discussed above, some spider toxins bind to the voltage sensors and modify channel gating instead of plugging the conduction pore (hanatoxin). Chimeric toxins constructed of two other toxins active on different channels were also used to learn about the relevance of various peptide regions in the binding to different K^+ channel subtypes (Regaya et al. 2004). Then, the calculated and hypothesized interaction topology can be further refined by docking simulations that use homology models of the target channel based on known X-ray crystallographic structures of a related channel and typically NMR-derived structures of the toxins. Comparison of the results of docking calculations with different channels can provide clues about which channel residues may allow or prevent high-affinity binding of the toxin.

The identified receptors of KTx toxins can be found in Table 1.

Binding and Selectivity of α-KTxs at the Molecular Level: Docking Simulations and NMR Structure Determinations of the Complexes

From the results obtained using a variety of techniques listed above, the picture of a general blocking mechanism has emerged that is employed by the majority of confirmed high-affinity K^+ channel-blocking toxins. Most toxins carry a high net positive charge and thus are likely to be attracted toward the negatively charged environment of the selectivity filter by long-range interactions. This involvement of electrostatic interactions is supported by the ionic strength dependence of toxin binding (MacKinnon et al. 1989). As described above, many toxins feature the conserved functional dyad that superimposes spatially even in toxins of various lengths and structures (Menez 1998; Dauplais et al.1997) and is a good indicator of high-affinity K^+ channel blockade. However, as sequence comparisons and docking

simulations reveal, the hydrophobic residue of the dyad may have a major influence on the selectivity of a toxin such that the often present tyrosine shows preference for Kv1.2 channels over Kv1.3, while a threonine at that position directs toxin preference toward Kv1.3. Recent studies confirmed these expectations with toxins in which the hydrophobic dyad residue was mutated (Bartok et al. 2013).

A similar strategy was used to convert charybdotoxin (ChTx), which blocks several Kv channels and KCa3.1 into a more selective toxin (Rauer et al. 2000). Docking simulations aided by thermodynamic mutant cycle analyses revealed minor structural differences in the otherwise very similar topology of the external vestibules of Kv and KCa channels. A cluster of negatively charged residues was found in the turret of Kv1.3, not present in KCa3.1. A lysine residue of ChTx, which lies close to this cluster in the bound state, was mutated to negatively charged residues, which significantly reduced the affinity for Kv1.3 and therefore improved selectivity for KCa3.1.

Most models of toxin binding assume rigid topological structures for both the channel and toxin surfaces that must be complementary to a certain extent for the formation of the contact points that establish tight binding. However, recent NMR studies challenged this view and suggested that both structures are capable of flexible rearrangements during the formation of the channel-toxin complex (Lange et al. 2006). Using solid-state NMR spectroscopy (ssNMR), which is performed in a medium with limited mobility compared to the classical liquid-state NMR, the docking of kaliotoxin (KTX, α-KTx3.1) to a KcsA-Kv1.3 chimeric channel was studied. The pore region of Kv1.3, which contains the binding site for KTX, was inserted into KcsA, a bacterial K^+ channel with known crystal structure at the time, and structural changes were investigated upon KTX binding.

The authors observed significant ssNMR chemical shift changes for several KTX residues that are found on one side of the KTX three-dimensional structure bound to the channel and confirmed the general layout of the interaction surface from previous models describing KTx-Kv channel complexes. The results indicated that the structure of the outer and inner helices of KcsA-Kv1.3 was mostly unaffected by KTX binding, but changes were detected in both the pore helix and the selectivity filter, which were quite significant for the GYG signature selectivity filter residues. Their data suggests that the critical lysine side chain is inserted more deeply into the selectivity filter than previous models had assumed and that its methylene groups replace water molecules in the entry region of the pore. This insertion induces a new conformational state of the filter with characteristics of both the conducting and collapsed conformation that was described for KcsA. This reorientation, along with small changes in the toxin itself, is thought to strengthen the binding by allowing a more intimate contact between the toxin and the pore.

A follow-up study by the same group further investigated this phenomenon and found similarities between the structural changes associated with toxin binding and C-type inactivation, a process which makes Kv channels nonconducting during prolonged depolarizations via rearrangement of the external pore region (Zachariae et al. 2008). Using molecular dynamics simulations, ssNMR, and electrophysiological measurements, they showed that upon toxin binding, rotation of external

pore residues widens the pore and increases the number of contacts with the toxin, which both contribute to increased affinity. Thus, the original "lock and key" model of toxin binding was modified to a "hand and glove" or "induced fit" model to account for the mutual flexibility and adaptation of the two partners.

Therapeutic Applications

Many of the toxins of various venomous species are known to exert their harmful effects through interactions with the ion channels expressed by the cells of the prey. With detailed knowledge of the role of an ion channel in a cell's functions and the effects of peptide toxins on the channel, the behavior of cells or even organs can be manipulated in a desired way to achieve therapeutic goals. The high number of potassium channel genes expressed in the human body and the variety of cellular functions that they perform present many potential targets for such medical goals. Although the pharmacological properties of small molecule channel modulators are generally better suited for therapeutic applications, peptide toxins still have some advantages that make them attractive as drug candidates. One important aspect of these is that the greater contact area of the peptides compared to small molecules with the target channel allows a higher-affinity binding; thus, a lower concentration of the blocker is required. The other aspect again arises as a result of the higher number of contact points with the channel, which enables the toxin to differentiate among channels with similar, but still slightly differing structures. As described in previous sections, even minute differences in the topology of the interaction surfaces can lead to great changes in binding affinity. The resulting selectivity is a critical characteristic of drug molecules as this prevents unwanted side effects by avoiding interactions with off-target channels.

Several in vivo experiments in animal disease models have proven the efficacy and applicability of small K^+ channel-blocking peptides (Varga et al. 2012; Koshy et al. 2014). Although some of these experiments were performed with toxins originating from other species (ShK toxins from *Stichodactyla helianthus*), the similar size, structure, and mechanism of action assure that KTxs from scorpions would be just as effective in these applications (Dauplais et al. 1997).

The best-studied target of therapeutic application is the voltage-gated Kv1.3 channel expressed by lymphocytes. In patients with autoimmune diseases, the disease-associated autoantigen-specific T cells were identified as co-stimulation-independent effector-memory T cells, which express a high number of Kv1.3 channels. This was confirmed in multiple sclerosis, type 1 diabetes mellitus, and rheumatoid arthritis patients (Markovic-Plese et al. 2001; Wulff et al. 2003). As the activation and proliferation of the effector-memory T cells responsible for most of the tissue damage can be suppressed by selective Kv1.3 blockers, major improvements can be achieved by the use of such peptides. This concept has been elegantly proven in experiments, in which disease development or progression was prevented in rat models of multiple sclerosis, type 1 diabetes mellitus, rheumatoid arthritis, contact dermatitis, and delayed-type hypersensitivity. An advantage of this approach is that it specifically

suppresses effector-memory T cell activation without compromising the protective immune response. Experiments have shown that at therapeutically relevant concentrations, the toxins did not cause toxicity in the animals (Beeton et al. 2001, 2006) and did not suppress the protective immune response to acute viral and bacterial infections.

Several naturally highly Kv1.3-selective KTxs have been identified, for example, the recently characterized Vm24 (α-KTx21.1) from the venom of *Vaejovis mexicanus smithi*, with very high affinity ($K_d = 2.9$ pM) and exceptionally high (>1,500-fold) selectivity over several other ion channels assayed, including the closest relatives of Kv1.3. It was also shown to reduce delayed-type hypersensitivity in rats; thus, it promises to be a valuable tool for applications requiring selective Kv1.3 blockade (Varga et al. 2012).

A Kv1.3-specific peptide was also found effective in counteracting the negative effects of elevated caloric intake by mice that were fed a diet rich in fat and fructose. It produced effects similar to the effects of Kv1.3 gene deletion, which included a reduction of blood levels of cholesterol, sugar, and insulin and enhanced insulin sensitivity. Overall toxin application resulted in decreased weight gain, adiposity, and fatty liver (Upadhyay et al. 2013).

Another disease where selective KTxs have potential therapeutic value is myotonic dystrophy type 1 (DM1), because voltage-gated K^+ channels are responsible for myoblast proliferation and differentiation.

Comparison of the functional potassium channel expression in myoblasts from healthy individuals to myoblasts from patients with DM1 revealed a switch from KCa1.1 to Kv1 channels. Specifically, Kv1.2 and Kv1.5 channel expression increased, along with a decrease in KCa1.1 expression in DM1 myoblasts. Pharmacological block of Kv1 channels in DM1 myoblasts was found to normalize proliferation and improve other factors of myotube production. In contrast, wound healing and myotube formation were impaired by selective inhibition of KCa1.1 channels in normal myoblasts. Thus, detrimental effects of the switch in K^+ channel expression associated with the early stage of myogenesis in DM1 may be counteracted by selective KTxs (Tajhya et al. 2014).

Besides effector-memory T cells in the synovial fluid, resident joint cells known as fibroblast-like synoviocytes (FLS) are also responsible for many of the pathogenic features of rheumatoid arthritis (RA). FLS in RA (RA-FLS) become invasive and cause joint damage by releasing proteases and proangiogenic and proinflammatory growth factors. RA-FLS were shown to upregulate KCa1.1 channels, which localize on the leading edge of the plasma membrane. Blockade of KCa1.1 inhibited cellular migration and invasion, along with the production of pathogenic factors by interfering with cytoskeletal rearrangements. Pharmacological inhibition of KCa1.1 also improved the clinical symptoms in rat models of RA (Tanner et al. 2014). As in the cases above, the use of a selective KTx inhibitor may render general immunosuppression unnecessary during RA treatment in the future.

Recent results indicate that K^+ channel inhibition may also be a beneficial tool in enhancing antitumor immunity (Koshy et al. 2013). Blockade of KCa3.1 channels was found to increase the degranulation and cytotoxicity of adherent natural killer cells and to increase the ability of these cells to reduce in vivo tumor growth.

Conclusion and Future Directions

The examples above illustrate the wide spectrum of potential applications, in which K^+ channel-specific scorpion toxins of high affinity and selectivity may be used to accomplish therapeutic goals. With the number of identified KTxs growing by the day and the expansion of the body of knowledge on K^+ channel distributions and functions along with details of the toxin-channel interactions, this spectrum is likely to broaden even more, and routine clinical use of these peptides may soon become reality.

Cross-References

► Molecular Description of Scorpion Toxin Interaction with Voltage-Gated Sodium Channels
► Scorpion Venom Interactions with the Immune System
► Scorpionism and Dangerous Species of Mexico

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