

## Diversity of Potassium Channel Ligands: Focus on Scorpion Toxins

A. I. Kuzmenkov\*, E. V. Grishin, and A. A. Vassilevski\*

*Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences,  
117997 Moscow, Russia; E-mail: aleksey.kuzmenkov@gmail.com; avas@ibch.ru*

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**Abstract**—Potassium ( $K^+$ ) channels are a widespread superfamily of integral membrane proteins that mediate selective transport of  $K^+$  ions through the cell membrane. They have been found in all living organisms from bacteria to higher multicellular animals, including humans. Not surprisingly,  $K^+$  channels bind ligands of different nature, such as metal ions, low molecular mass compounds, venom-derived peptides, and antibodies. Functionally these substances can be  $K^+$  channel pore blockers or modulators. Representatives of the first group occlude the channel pore, like a cork in a bottle, while the second group of ligands alters the operation of channels without physically blocking the ion current. A rich source of  $K^+$  channel ligands is venom of different animals: snakes, sea anemones, cone snails, bees, spiders, and scorpions. More than a half of the known  $K^+$  channel ligands of polypeptide nature are scorpion toxins (KTx), all of which are pore blockers. These compounds have become an indispensable molecular tool for the study of  $K^+$  channel structure and function. A recent special interest is the possibility of toxin application as drugs to treat diseases involving  $K^+$  channels or related to their dysfunction (channelopathies).

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*Key words:* ion channel, potassium channel, ligand, pore blocker, modulator, venom, scorpion, toxin

Potassium ion ( $K^+$ ) channels comprise a superfamily of integral membrane proteins that provide passive selective transport of  $K^+$  across the cell membrane.  $K^+$  channels have been found in all living organisms from bacteria to higher multicellular animals, including humans. Two major functions of  $K^+$  channels are maintaining the resting membrane potential and forming the action potential in electrically excitable cells. It is difficult to overestimate the role of these proteins in numerous physiological processes, such as ion transport, nerve signal transmission, cell communication and proliferation, neurohu-

moral regulation, and immune response. This makes study of the structure, mechanism of action, and modulation of  $K^+$  channels one of the most important tasks of modern bioorganic chemistry. Moreover, multiple studies have demonstrated that  $K^+$  channels are involved in the development of various pathological states, which makes these channels promising pharmacological targets.

Traditionally, studies of  $K^+$  channels include identification of novel channel ligands and subsequent use of these ligands as specific tools to elucidate the mechanisms of channel action and to search for new members of the superfamily. Based on the mechanism of action,  $K^+$  channel ligands can be classified as either pore blockers or modulators. Channel blockers “plug” the pore, similarly to the cork in a bottle, whereas modulators affect channel functions without mechanically preventing ion flow through the channel. Scorpion venoms are the major source of  $K^+$  channel ligands. These venoms are complex mixtures of tens or even hundreds of compounds, predominantly, short polypeptides. Interestingly, all known  $K^+$  channel ligands isolated from scorpion venoms are pore blockers.

About 250  $K^+$  channel ligands from scorpion venom have been identified by now, which supposedly represent

*Abbreviations:* 4-AP, 4-aminopyridine; BK<sub>Ca</sub>, large-conductance calcium-activated  $K^+$  channels; CS $\alpha/\alpha$ , cysteine-stabilized helix-loop-helix; CS $\alpha/\beta$ , cysteine-stabilized  $\alpha$ -helix  $\beta$ -sheet; ICK, inhibitor cystine knot; IK<sub>Ca</sub>, intermediate-conductance calcium-activated  $K^+$  channels;  $K^+$  channels, potassium channels; K<sub>2p</sub>, two-pore-domain  $K^+$  channels; K<sub>ir</sub>, inward-rectifier  $K^+$  channels; KTx, polypeptide  $K^+$  channel blockers from scorpion venom; K<sub>v</sub>, voltage-gated  $K^+$  channels; RP-HPLC, reversed-phase high-performance liquid chromatography; SK<sub>Ca</sub>, small-conductance calcium-activated  $K^+$  channels; TEA, tetraethylammonium; TM, transmembrane (segment).

\* To whom correspondence should be addressed.

only 0.5% of total number of potentially active compounds. Further studies will broaden our understanding of structural and functional properties of K<sup>+</sup> channels and enable us to find highly selective ligands with potential pharmacological applications.

### K<sup>+</sup> CHANNEL SUPERFAMILY

K<sup>+</sup> channels have been found in all living organisms. In humans, 78 genes code for major ( $\alpha$ -) subunits of these transmembrane proteins (see below). It is now commonly believed that K<sup>+</sup> channels appeared around the time of the origin of life on Earth, a hypothesis that is supported by identifications of more than 200 eukaryotic K<sup>+</sup> channel-related channel-like proteins in Archaea and bacteria [1].

K<sup>+</sup> channels are composed of  $\alpha$ - and  $\beta$ -subunits. The structure and major functions of a channel are determined by the  $\alpha$ -subunits, whereas  $\beta$ -subunits affect the channel kinetics. Human K<sup>+</sup> channels can be subdivided into five structural and functional groups based on the structure of their  $\alpha$ -subunits (Fig. 1).

1. Inward-rectifier K<sup>+</sup> channels (K<sub>ir</sub>) are homo- or heterotetrameric complexes in which each subunit consists of two transmembrane (TM) segments with a pore region (P) between them. Their function is regulated by nucleotides (ATP, ADP), phosphorylation, G proteins, and phosphatidylinositol 4,5-bisphosphate. In humans, K<sub>ir</sub> channels are encoded by 15 different genes [2, 3].

2. Two-pore-domain K<sup>+</sup> channels (K<sub>2p</sub>) contain four TM segments, and their  $\alpha$ -subunits undergo dimerization upon channel formation. These channels are regulated by a broad array of factors: pH, temperature, and cell membrane tension. In humans, 15 genes coding for K<sub>2p</sub> channels have been found [4, 5].

3.  $\alpha$ -Subunits of voltage-gated (voltage-dependent) K<sup>+</sup> channels (K<sub>v</sub>) consist of six TM segments (S1-S6) with one pore region (P) located between S5 and S6. Four  $\alpha$ -subunits form a complete channel. An important structural feature of K<sub>v</sub> channels is the presence of the voltage-sensing domain (VSD) formed by four TM segments (S1-S4). S4 contains regularly positioned positively charged amino acid residues that play the role of a voltage sensor. Voltage-gated K<sup>+</sup> channels are the most abundant group of potassium channels (40 genes for K<sub>v</sub> channels have been identified in humans) [6].

4. Calcium-activated intermediate- and small-conductance (IK<sub>Ca</sub> and SK<sub>Ca</sub>, respectively) potassium channels are formed by subunits consisting of six TM segments (S1-S6) with the pore region (P) located between S5 and S6, similarly to those of K<sub>v</sub> channels. However, in K<sub>Ca</sub> channels, the S4 segment is almost insensitive to changes in potential; the channels are activated by Ca<sup>2+</sup> via a calmodulin-mediated mechanism. In humans, proteins of this family are encoded by four genes [7, 8].

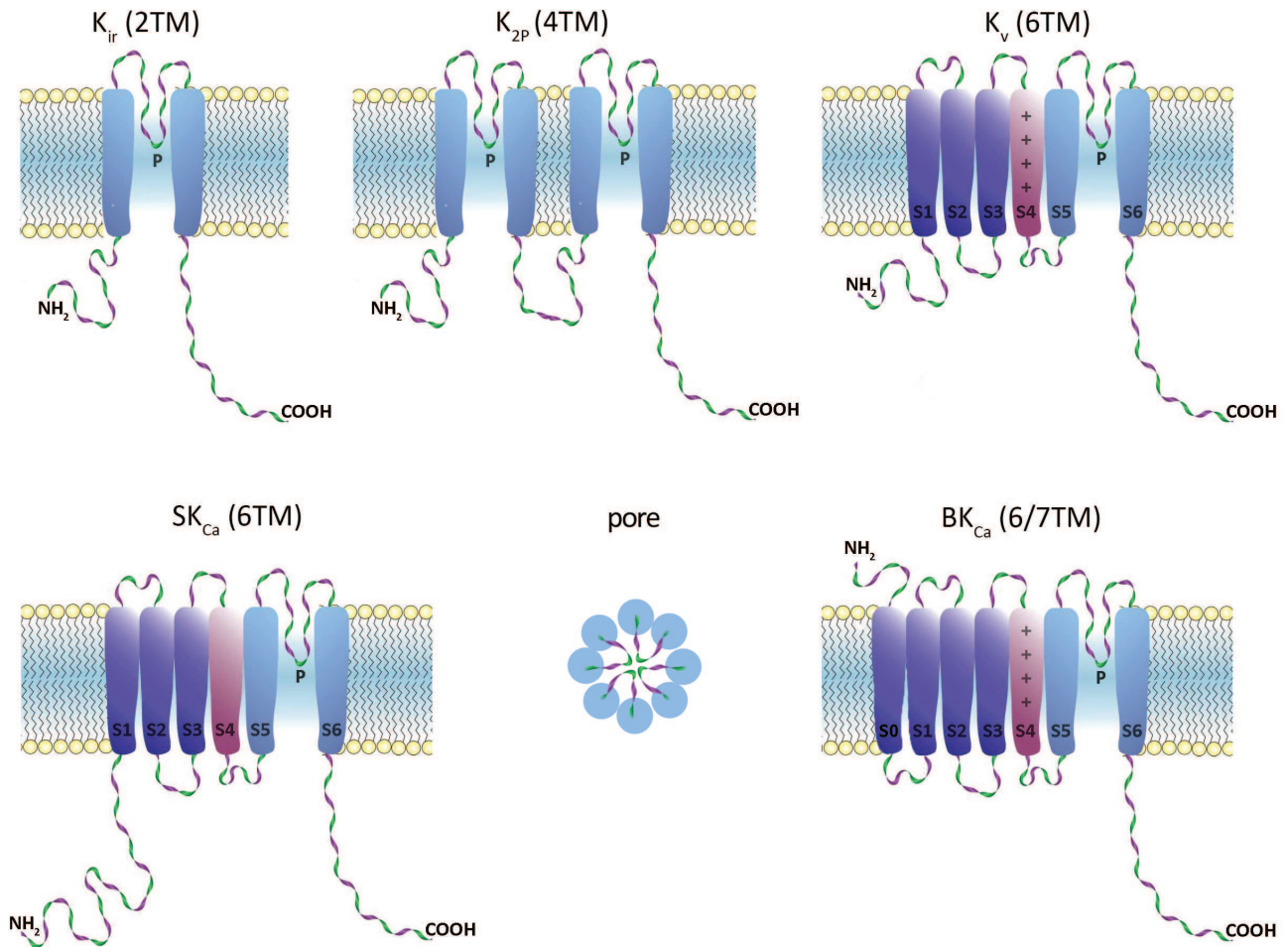
5. Ca<sup>2+</sup>-activated large-conductance channels (BK<sub>Ca</sub>) are encoded by four *slo* genes in humans. Two members of this family contain seven TM segments. An interesting property of these channels is that they can be activated not only by changes in potential, but by a number of ions (Ca<sup>2+</sup>, Na<sup>+</sup>, or Cl<sup>-</sup>, depending on the channel isoform) [9].

K<sup>+</sup> channels of higher plants can be subdivided into three groups: Shaker-like channels, tandem-pore K<sup>+</sup> channels (TPK), and K<sub>ir</sub>-like channels [10]. Similarly to K<sub>v</sub> channels, Shaker-like channel subunits are composed of six TM segments and are activated by changes in membrane potential. TPK and K<sub>ir</sub>-like channel subunits have four and two TM segments and are related to human K<sub>2p</sub> and K<sub>ir</sub> channels, respectively [11, 12].

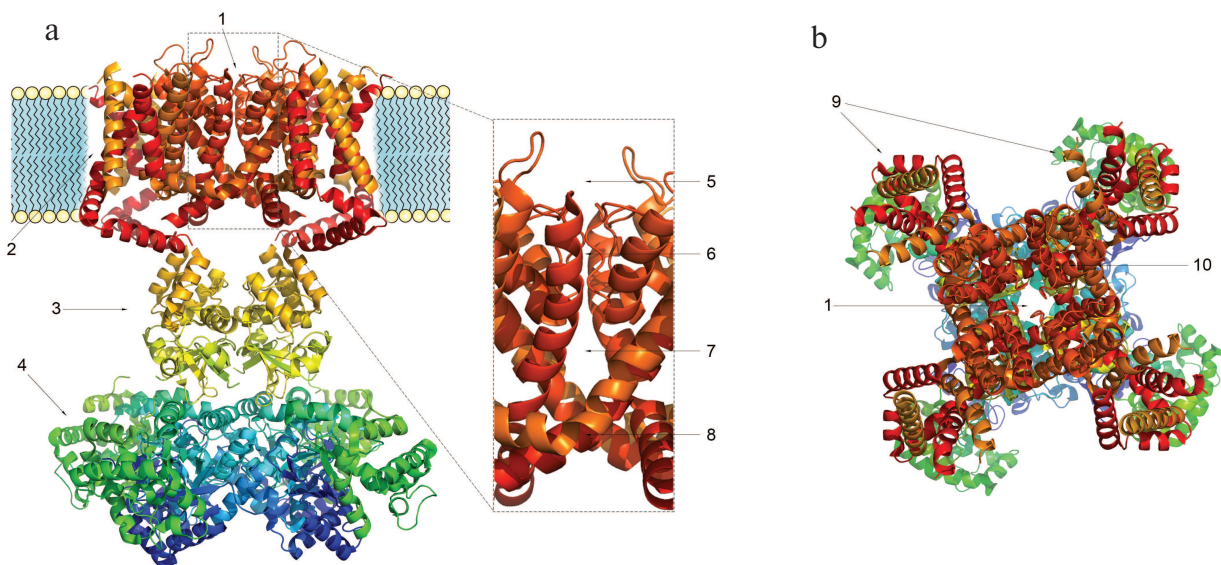
The smallest K<sup>+</sup> channel  $\alpha$ -subunit (94 amino acid residues) was found in PBCV-1 virus parasitizing green algae of the *Chlorella* genus [13]. In prokaryotes, K<sup>+</sup> channel subunits usually have either two (MthK, K<sub>ir</sub>B, and KcsA) or six (Kch, K<sub>v</sub>AP, and Mlo1) TM segments. Quite often, these channels also contain additional Ca<sup>2+</sup>- or nucleotide-binding cytoplasmic domains [14]. Unusual topology of the channel-forming  $\alpha$ -subunit (S1-S2-S3-S4-S5-P-S6-S7-P-S8) was found in fungi; this structure seems to be exclusive to Fungi, and not present in other kingdoms [15]. Interestingly, the genome of infusoria *Paramecium tetraurelia* contains 298 genes for K<sup>+</sup> channels, which is 3.8 times more than in the human genome [14].

**Domain structure of K<sup>+</sup> channels.** The membrane part of a K<sub>v</sub> channel  $\alpha$ -subunit consists of two regions (Fig. 2): the pore region and the voltage-sensing region. The pore region is formed by two transmembrane segments (S5-S6) connected by the pore loop (P). The voltage-sensing region is composed of four transmembrane segments (S1-S4). The mature K<sub>v</sub> channel consists of the pore domain, formed by four pore regions of four different  $\alpha$ -subunits, four voltage-sensing domains, and four cytoplasmic domains [16].

The structure and the mechanism of action of the K<sub>v</sub> channel pore domain are similar to those of voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channels. In the open state, a K<sub>v</sub> channel can conduct 10<sup>6</sup>-10<sup>8</sup> K<sup>+</sup> ions per second [17, 18]. K<sub>v</sub> channels are one of the most diverse family of membrane proteins [19], but its members have a highly conserved seven amino acid sequence TTVGYGD [20] that forms the channel selectivity filter. The filter is responsible for the highly selective transport of K<sup>+</sup>, but not, for example, Na<sup>+</sup>, even though the Na<sup>+</sup> ion radius is only 0.4 Å smaller. This high selectivity is provided by the presence of oxygen atoms inside the pore that coordinate K<sup>+</sup> ions lacking the hydration shell (the radius of a Na<sup>+</sup> ion is too small to form stable coordination bonds). Oxygen atoms of the polypeptide chain carbonyls stabilize dehydrated K<sup>+</sup> ions from four different directions and “substitute” the hydration shell during ion passage through the pore



**Fig. 1.** K<sup>+</sup> channel families (topological diagrams). Center of bottom row, schematic representation of the channel pore formed by pore regions of four individual subunits. TM, transmembrane segment; P, pore loop; S0-S6, TM segments; S4, voltage sensor (in K<sub>v</sub> and BK<sub>Ca</sub>).



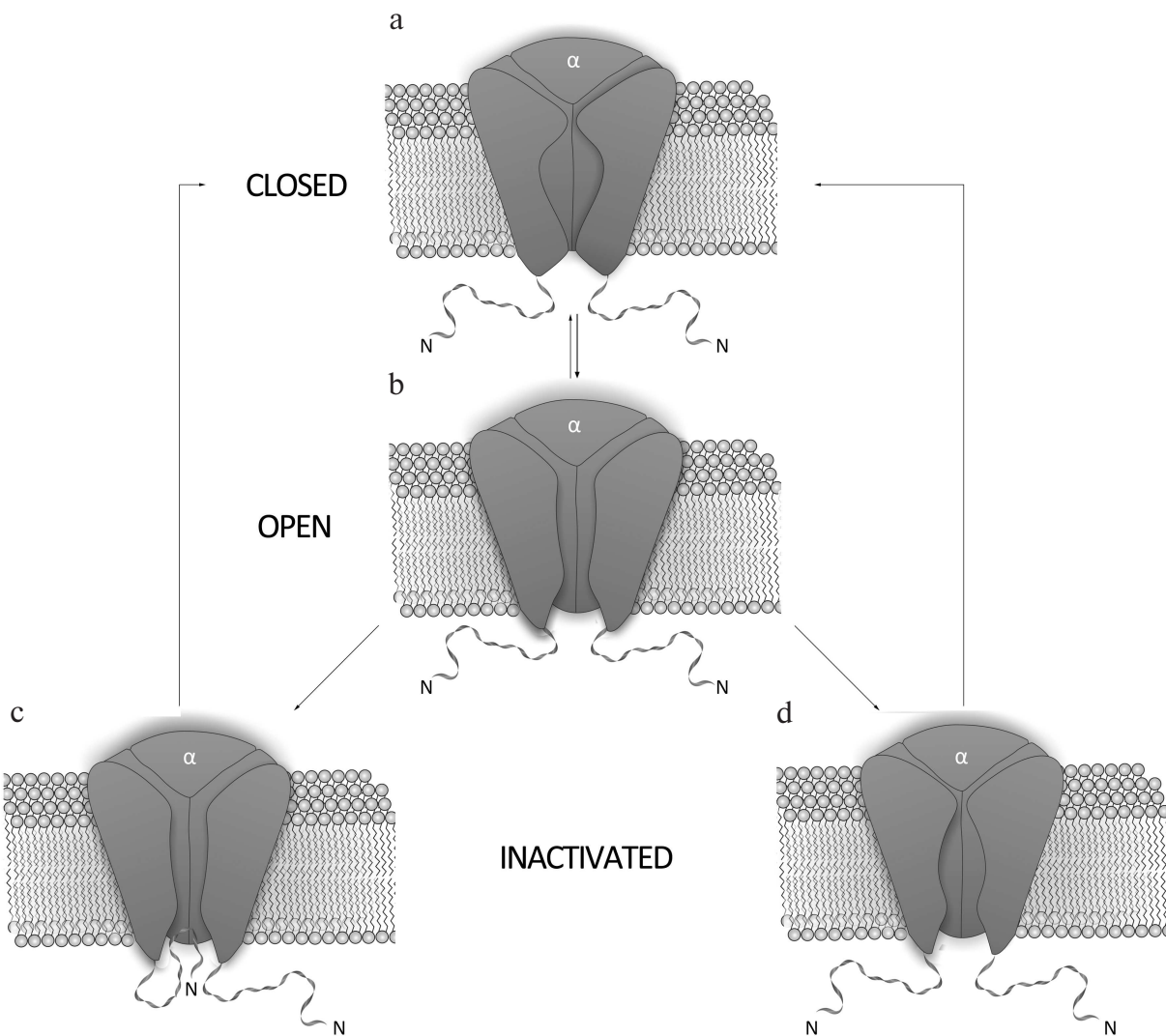
**Fig. 2.** 3D structure of the voltage-gated K<sub>v</sub>1.2 channel (PDB ID: 2A79): a) side view; b) extracellular view. 1) Pore; 2) homotetramer of membrane parts of α-subunits; 3) α-subunit cytoplasmic domain; 4) β-subunits; 5) external vestibule; 6) selectivity filter; 7) internal cavity; 8) inner gate; 9) voltage-sensing domains; 10) pore domain.

[21]. The crystal structure of a bacterial KcsA channel obtained in the laboratory of MacKinnon [22] proved the earlier hypothesis of Hodgkin who studied K<sup>+</sup> flux in squid giant axons and in 1955 suggested that ions pass the channel one after another [23]. When transported into the cell, K<sup>+</sup> ions enter the external vestibule, lose their hydration shell, pass through the selectivity filter, get hydrated in the channel cavity, and exit through the intracellular gate. K<sup>+</sup> ions can also be transported in the opposite direction [24].

The voltage-sensing domain is essential for the K<sub>v</sub> channel response to membrane potential changes. The process involves conversion of the electric field energy accumulated on the membrane into mechanical energy, which is then transferred to the pore domain and causes its conformational changes and pore opening. The S4 segment serves as a voltage sensor due to the high content

of positively charged amino acid residues [25]. Translocation of the voltage sensor necessary for the pore opening requires physical interaction between certain amino acid residues of the transmembrane S6 fragment and the juxtamembrane “linker” helix that connects the S4 and S5 segments [26, 27]. The cytoplasmic domain also plays an important role in the channel activity regulation (e.g. in N-type inactivation (see below) and some cases of  $\alpha$ -subunit tetramerization [28]).

**Mechanism of K<sup>+</sup> channel activity.** The mechanism of K<sub>v</sub> channel function is based on conformational changes of the channel protein (Fig. 3). K<sub>v</sub> channels are closed at resting potential and do not conduct ions. An increase in the membrane potential (depolarization) affects charged amino acid residues of the voltage sensor and can cause channel opening [29, 30]. The transmembrane S4 segment responds to the changes in the mem-



**Fig. 3.** Mechanism of action of a voltage-gated K<sup>+</sup> channel: a) closed state; b) open (activated) state; c) N-type inactivation; d) C-type inactivation.

brane potential, which causes conformational changes in the voltage-sensing domain followed by changes in the pore domain [31]. Due to an extremely high sensitivity of the voltage sensor, the channel can respond even to slight potential changes [32]. The pore opens only when all four voltage sensors are activated [33]; the open channel conducts ions until it enters an inactivation phase.

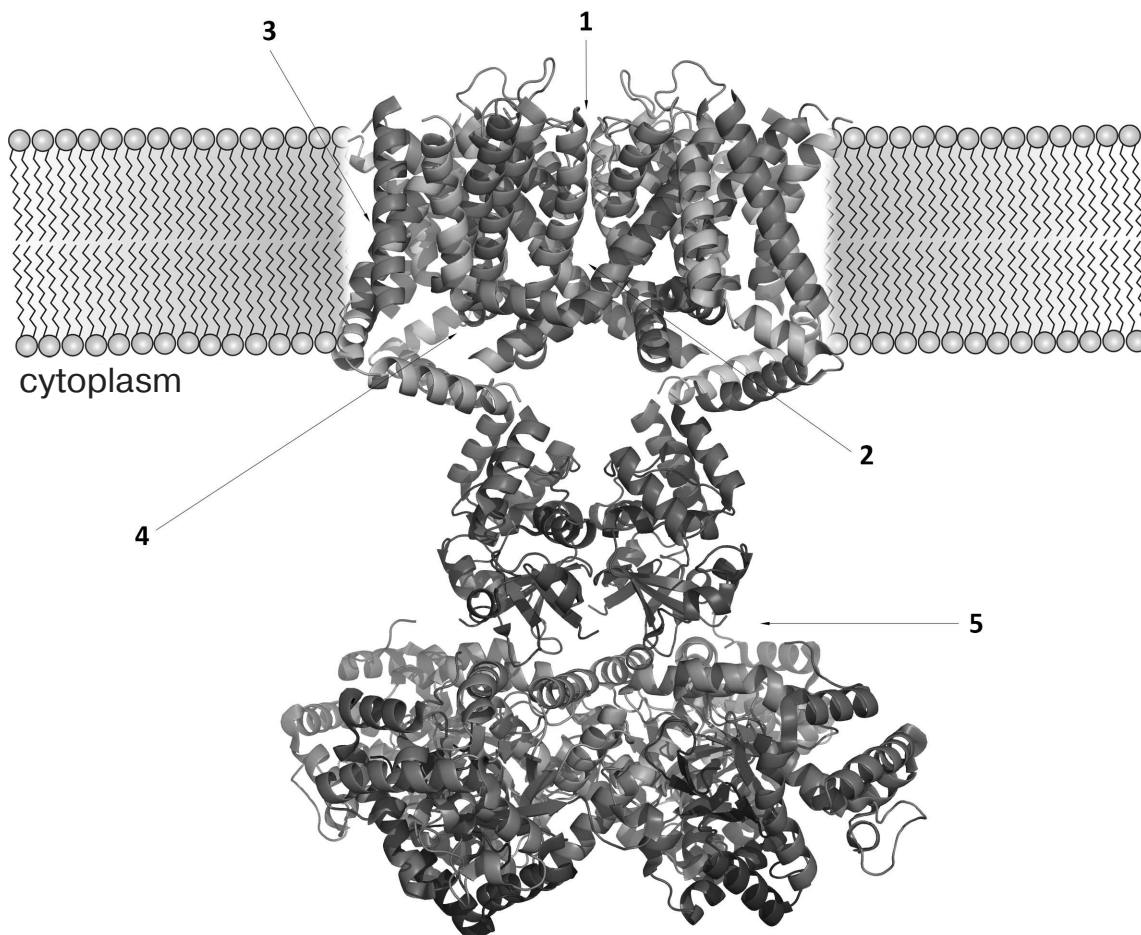
There are two mechanisms for  $K_v$  channel inactivation: the N-type and the C-type. During N-type inactivation, the channel stays in the open conformation, but the pore is blocked from the membrane inner side by the N-terminal fragment of the  $\alpha$ -subunit [34]. The removal of this fragment abolishes channel inactivation via the N-type mechanism, while its addition to the system in a form of a synthetic peptide restores inactivation [35, 36]. The C-type inactivation occurs at a slower rate and does not involve the N-terminus of the channel cytoplasmic domain [37]. Structural elements responsible for the C-type inactivation are located in the external vestibule of the selectivity filter [38] and in the vicinity of the S6 segment N-terminus [39]. The C-type inactivation is caused

by structural changes in the channel external vestibule and by narrowing of the channel mouth, which result in blockade of the ion flux [40]. Decrease in the membrane potential to resting state values (repolarization) causes channel transition from inactivation into the closed state.

### $K^+$ CHANNEL LIGANDS

$K^+$  channel ligands can be divided into several groups, the three major being metal ions [41], small organic molecules [42], and polypeptide toxins [43].  $K^+$  channel ligands can be pore blockers or modulators. Pore blockers prevent ion flow through the channel by binding to the pore region [44]. Modulators do not directly block the ion flow, but rather affect the channel functional properties by interacting with other regions of the channel protein (e.g. voltage-sensing domain or auxiliary  $\beta$ -subunit) (Fig. 4) [45, 46].

In addition to the major ligand groups, isoform-specific antibodies have been obtained for a number of  $K^+$



**Fig. 4.** Regions of ligand interaction with  $K^+$  channels: 1) blockers of the pore outer part; 2) blockers of the pore inner part; 3, 4) modulators acting from the outer and inner side of the membrane, respectively; 5) inactivation inhibitors.

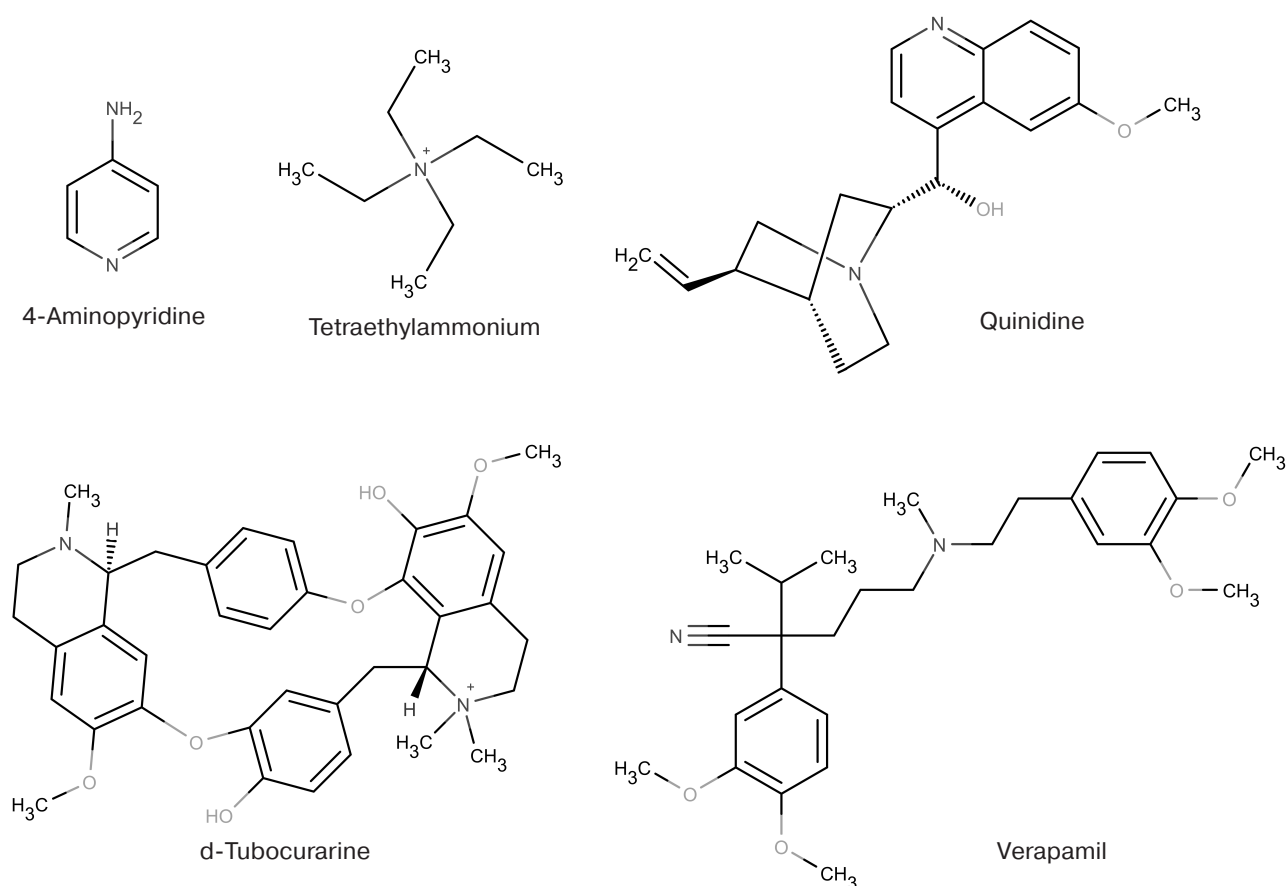


Fig. 5. Nonselective blockers of K<sup>+</sup> channels.

channels that are able to selectively block the ion flow through the corresponding channels [47]. K<sup>+</sup> channel ligands were identified in parasitic worm secretions [48]. Defensins from various organisms have also been shown to affect K<sup>+</sup> flux [49, 50].

Most K<sup>+</sup> channel isoforms can be nonselectively inhibited by Cs<sup>+</sup>, Ba<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and other metal ions in millimolar concentrations [51]. These ions were widely used in early studies of K<sup>+</sup> channel function [52] but later substituted by ligands with higher affinity and selectivity [53].

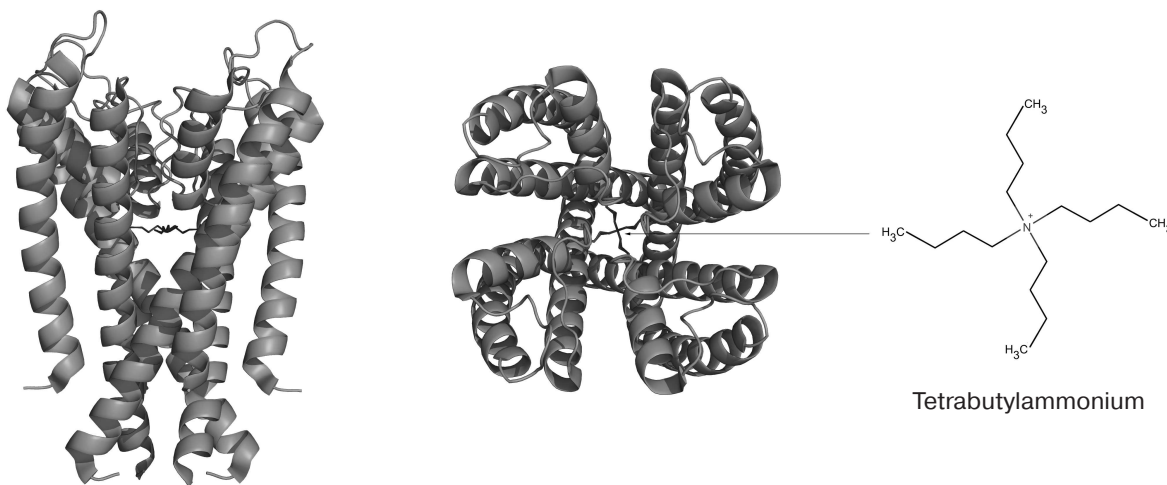
### Small Organic Molecules

At the dawn of the ion flux studies, the two most widely used agents for pharmacological identification of K<sup>+</sup> channels were 4-aminopyridine (4-AP) and tetraethylammonium (TEA). This was due to the fact that they both inhibit (in millimolar concentrations) a majority of K<sup>+</sup> channels without producing any visible effects on Na<sup>+</sup> and Ca<sup>2+</sup> channels [54, 55]. These two compounds remain the only low molecular mass ligands for some K<sup>+</sup> channels [53]. Other organic molecules have been identi-

fied that are capable of binding to the pore inner part and modulating various K<sup>+</sup> channels (quinidine, d-tubocurarine, and verapamil) [56–58]. Some of the low molecular mass ligands of K<sup>+</sup> channels are shown in Fig. 5.

TEA and structurally similar molecules can block K<sup>+</sup> channels from either extracellular or cytoplasmic sides of the membrane [59]. Other hydrophobic cations (d-tubocurarine and verapamil) also “plug” the inner part of the pore with their ammonium or amine groups and block the ion flux (Fig. 6) [60]. Different mechanism of action was proposed for 4-AP that has molecular properties similar to those of the hydrophobic cations, interacts with the channel in the open state, competes with TEA for the binding site on the open channel, and stays bound after channel transition into the closed state. However, the small size of the 4-AP molecule prevents it from plugging the pore. According to recent findings, the site for 4-AP binding is located in a space between the TM S6 fragments [61]. When the channel opens, 4-AP approaches the binding site, binds to the channel, and stabilizes the channel closed state, thereby preventing the ion flux [62].

In addition to nonselective K<sup>+</sup> channel ligands, a significant number of highly selective activators for K<sup>+</sup>



**Fig. 6.** 3D-Structure of tetrabutylammonium complex with the KcsA channel (in the closed state) (PDB ID: 2JK5): side and extracellular views.

channels have been found. These compounds were originally developed as drug prototypes, and some of them are already used in clinics. Table 1 shows the most “popular” blockers and activators of  $K^+$  channels; structures of some of these compounds are shown in Fig. 7.

### Polypeptide Toxins

A great number of polypeptide ligands of  $K^+$  channels have been found in the venoms from bees, snakes, sea anemones, cone snails, spiders, and scorpions. These compounds, often called toxins, are synthesized in the animal venom glands as precursors and then processed into active molecules [63, 64].

Although the number of known venomous animals is huge, only a few of their venoms have been studied, and even fewer have been characterized in details. The lack of information is mostly because venoms are complex mixtures of multiple components, and their comprehensive characterization might take several decades [65, 66]. There are two main strategies for studying animal venoms (Fig. 8). The first, more traditional strategy, involves purification of individual components directly from the venom by various chromatographic methods [67]. The second, more recent strategy, originated due to the rapid development of DNA sequencing techniques and involves construction and analysis of cDNA libraries from mRNAs isolated from the animal venom glands [68, 69].

The first strategy requires sufficient amounts of crude venom that could be obtained, for example, by electrostimulation of venom glands. The crude venom is then subjected to a series of chromatographic procedures, usually starting with size-exclusion chromatography followed by several rounds of ion-exchange or reversed-phase high-performance liquid chromatography (RP-

HPLC). Then the purified components are sequenced by the Edman degradation method [70].

Most often, the main active compounds of animal venoms are peptides. Their amino acid sequences can be determined by isolating total mRNA from the venom gland, reverse transcribing this mRNA into corresponding cDNA, and cDNA sequencing with subsequent analysis of the resulting cDNA library or individual sequences [71]. Amino acid sequences can be deduced by *in silico* translation, and the corresponding peptides can be produced by chemical synthesis or in a recombinant system [72]. Total sequencing of genomes of all venomous animals might provide much valuable information; however, only very few entire genomes of venomous species have been deciphered so far [73-76].

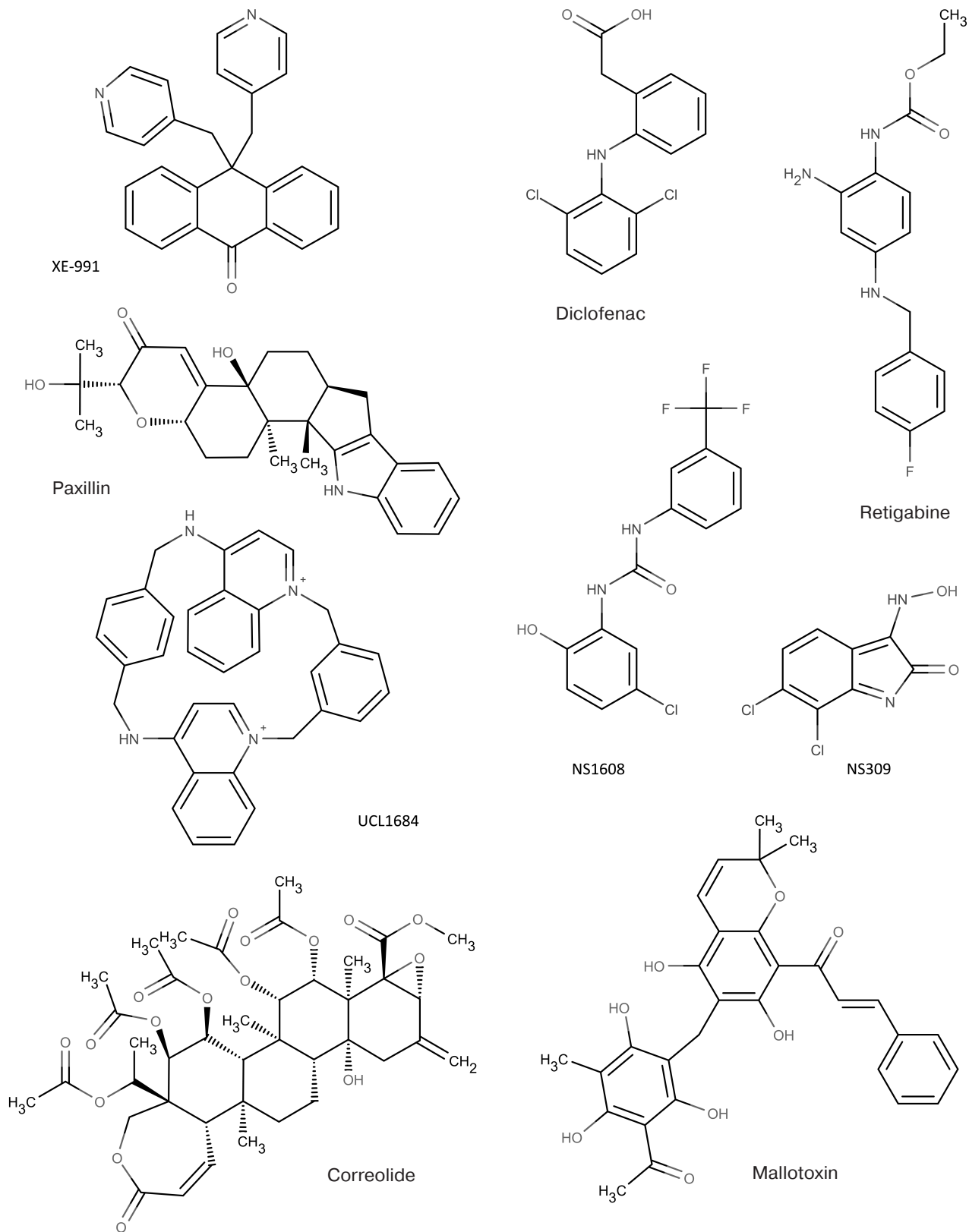
Other traditional approaches to identification and characterization of  $K^+$  channel ligands are electrophysiological measurements (methods of patch clamp and two-electrode voltage clamp) [77, 78] and radioligand binding assay [79, 80]. Electrophysiological studies are traditionally conducted in artificial membranes, eukaryotic cells, and *Xenopus laevis* oocytes expressing  $K^+$  channel genes of interest. Radioligand binding assay uses radioactively labeled channel ligands, and the activity of a particular compound is determined from the substitution of the labeled ligand in its complex with the channel by the studied compound. Recently, new methods have been developed based on the use of fluorescently labeled ligands [81] (e.g. fluorescence-activated cell sorting [82] and single-molecule fluorescence microscopy [83]), that have largely replaced radioligand binding assay [83].

Below, we describe groups of animals whose venoms contain  $K^+$  channel ligands, in the following order: vertebrates, sea invertebrates, and terrestrial invertebrates.

**Snakes.** More than 2650 species of snakes have been described; 500 of these species are considered venomous

## Blockers

## Activators



**Fig. 7.** Low molecular mass blockers and activators of K<sup>+</sup> channels.



**Table 1.** The best known blockers and activators of K<sup>+</sup> channels (according to the International Union of Basic and Clinical Pharmacology, IUPHAR) [2, 4, 6, 7]

K <sup>+</sup> channel	Blockers	Activators
<b>K<sub>ir</sub></b>		
K <sub>ir</sub> 1.1	VU591	–
K <sub>ir</sub> 2.1	spermine, spermidine, putrescine	PIP <sub>2</sub>
K <sub>ir</sub> 2.2	spermine, spermidine	–
K <sub>ir</sub> 2.3	TEA, spermine, spermidine, putrescine, SCH-23390	tenidap, arachidonic acid
K <sub>ir</sub> 2.4	–	–
K <sub>ir</sub> 3.1	AZD2927	ethanol, PIP <sub>2</sub> , ML297
K <sub>ir</sub> 3.2	pimozide, thioridazine, haloperidol, clozapine, halothane, fluoxetine, SCH23390, bupivacaine, F3, dizocilpine, clomipramine, desipramine, imipramine, amitriptyline, maprotiline, nortriptyline, QX-314, verapamil <sup>1</sup>	ethanol, PIP <sub>2</sub>
K <sub>ir</sub> 3.3	–	PIP <sub>2</sub>
K <sub>ir</sub> 3.4	4-AP <sup>1</sup> , TEA <sup>1</sup> , NIP-142, imipramine, AZD2927, desipramine, amitriptyline, clomipramine, maprotiline, nortriptyline	arachidonic acid, fingolimod, ethanol, PIP <sub>2</sub>
K <sub>ir</sub> 4.1	nortriptyline	–
K <sub>ir</sub> 4.2	–	–
K <sub>ir</sub> 5.1	–	–
K <sub>ir</sub> 6.1	glibenclamide, tolbutamide	ATP, uridine diphosphate, nicorandil, diazoxide, guanosine 5'-diphosphate, pinacidil, guanosine-5'-triphosphate, cromakalim, minoxidil diazoxide, pinacidil, nicorandil, cromakalim
K <sub>ir</sub> 6.2	glibenclamide, tolbutamide	diazoxide, pinacidil, nicorandil, cromakalim, minoxidil
K <sub>ir</sub> 7.1	ML-111, VU573	–
<b>K<sub>2P</sub></b>		
K <sub>2P</sub> 1.1	–	–
K <sub>2P</sub> 2.1	quinidine <sup>1</sup>	riluzole, arachidonic acid, halothane
K <sub>2P</sub> 3.1	anandamide	halothane, isoflurane
K <sub>2P</sub> 4.1	–	arachidonic acid, riluzole
K <sub>2P</sub> 5.1	lidocaine, quinidine	halothane
K <sub>2P</sub> 6.1	quinidine	arachidonic acid
K <sub>2P</sub> 9.1	ruthenium red, anandamide	halothane
K <sub>2P</sub> 10.1	quinidine	arachidonic acid, riluzole, halothane, isoflurane
K <sub>2P</sub> 13.1	halothane	arachidonic acid
K <sub>2P</sub> 16.1	quinidine, chloroform	isoflurane
K <sub>2P</sub> 17.1	chloroform	local anesthetics
K <sub>2P</sub> 18.1	quinidine	local anesthetics
<b>K<sub>v</sub></b>		
K <sub>v</sub> 1.1	TEA, 4-AP, diltiazem, capsaicin, flecainide, resineratoxin, nifedipine	–
K <sub>v</sub> 1.2	TEA, 4-AP, diltiazem, capsaicin, flecainide, resineratoxin, nifedipine, anandamide	–

Table 1. (Contd.)

K <sup>+</sup> channel	Blockers	Activators
K <sub>v</sub> 1.3	TEA, 4-AP, correolide <sup>2</sup> , naltrexone, CP339818, quinine, diltiazem, verapamil, UK78282, sulfamidbenzamidoindane, capsaicin, resiniferatoxin, nifedipine, H37	–
K <sub>v</sub> 1.4	TEA, 4-AP, UK78282, riluzole, quinidine, CP339818, nifedipine	–
K <sub>v</sub> 1.5	TEA, 4-AP, AVEO118, S9947, flecainide, quinidine, bupivacaine, resiniferatoxin, nifedipine, diltiazem, clofilium	–
K <sub>v</sub> 1.6	TEA, 4-AP	–
K <sub>v</sub> 1.7	TEA, 4-AP, flecainide, quinidine, verapamil, amiodarone, CP-339818, nifedipine, resiniferatoxin, tedisamil, capsaicin, diltiazem, cromakalim	–
K <sub>v</sub> 1.8	TEA, 4-AP, ketoconazole, pimozone, verapamil	cGMP
K <sub>v</sub> 2.1	TEA, 4-AP, halothane, RY796, DABCO-C <sub>16</sub> , linoleic acid	linoleic acid
K <sub>v</sub> 2.2	TEA, 4-AP, pimozone, phencyclidine, RY796, quinine	–
K <sub>v</sub> 3.1	TEA, 4-AP, flecainide, diltiazem, fluoxetine, norfluoxetine, resiniferatoxin, nifedipine, capsaicin, cromakalim	–
K <sub>v</sub> 3.2	TEA, 4-AP, verapamil, 8-bromo-cGMP	–
K <sub>v</sub> 3.3	TEA, 4-AP	–
K <sub>v</sub> 3.4	TEA, 4-AP, thiopental, DABCO-C <sub>16</sub> , DABCO	–
K <sub>v</sub> 4.1	TEA, 4-AP	–
K <sub>v</sub> 4.2	TEA, 4-AP, arachidonic acid, quinidine	–
K <sub>v</sub> 4.3	4-AP, nicotine, riluzole, sibutramine, bupivacaine	–
K <sub>v</sub> 7.1	XE-991 <sup>2</sup> , linopirdine, L735821, chromanol 293B, chromanol, IKs124, HMR-1556, azimilide	L364373, niflumic acid, mefenamic acid, ML277, R-L3, zinc pyrithione
K <sub>v</sub> 7.2	TEA, XE-991, linopirdine, L735821	retigabine <sup>2</sup> , BMS204352, S-1, ztz240, ICA-27243, QO-58, zinc pyrithione, flupirtine, PIP <sub>2</sub>
K <sub>v</sub> 7.3	TEA, XE-991, linopirdine, bepridil	retigabine, BMS204352, diclofenac <sup>2</sup>
K <sub>v</sub> 7.4	TEA, XE-991, linopirdine, bepridil	retigabine, BMS204352, S-1, ML213, zinc pyrithione, NC00075159, PIP <sub>2</sub>
K <sub>v</sub> 7.5	TEA, XE-991, linopirdine	retigabine, BMS204352, S-1, zinc pyrithione
K <sub>v</sub> 10.1	ICA-105574, LY97241, terfenadine, dofetilide, astemizole, clofilium, E4031, haloperidol, MK-499, quinidine, imipramine	–
K <sub>v</sub> 10.2	quinidine, LY97241	–
K <sub>v</sub> 11.1	astemizole, E4031, sertindole, dofetilide, terfenadine, ibutilide, cisapride, AZD3778, disopyramide	mallotoxin <sup>2</sup> , RPR260243, A-935142, PD-118057, PD-307243, ginsenoside Rg3, ICA-105574, NS1643, VU0405601, KB-130015, NS3623
K <sub>v</sub> 11.2	sipatrigine, E4031	NS1643
K <sub>v</sub> 11.3	sertindole, pimozone, E4031	NS1643
K <sub>v</sub> 12.1	–	–
K <sub>v</sub> 12.2	CX4	–
K <sub>v</sub> 12.3	–	–

Table 1. (Contd.)

K <sup>+</sup> channel	Blockers	Activators
<b>BK<sub>Ca</sub></b>		
K <sub>Ca</sub> 1.1	TEA, paxillin <sup>2</sup>	NS1608 <sup>2</sup> , NS1619, BMS204352, DHS-1, estradiol
K <sub>Ca</sub> 4.1	TEA, quinidine, bepridil	bithionol, NS004, NS1619, loxapine, niclosamide
K <sub>Ca</sub> 4.2	TEA, quinidine, ATP	niflumic acid, NS004, NS1619
K <sub>Ca</sub> 5.1	TEA, quinidine	NS004, NS1619
<b>SK<sub>Ca</sub></b>		
K <sub>Ca</sub> 2.1	TEA, UCL1684 <sup>2</sup> , NS8593, fluoxetine, d-tubocurarine <sup>1</sup> , UCL1848, dequalinium, bicuculline	NS309 <sup>2</sup> , DC-EBIO, riluzole, EBIO
K <sub>Ca</sub> 2.2	UCL1684, NS8593, d-tubocurarine, UCL1848, dequalinium, bicuculline	NS309, DC-EBIO, riluzole, EBIO, CyPPA, chlorzoxazone, zoxazolamine
K <sub>Ca</sub> 2.3	UCL1684, NS8593, NS11757, UCL1848, dequalinium	NS309, DC-EBIO, riluzole, EBIO, CyPPA
K <sub>Ca</sub> 3.1	TRAM-3, TRAM-34, ICA-17043, clotrimazole, nitrendipine	NS309, DC-EBIO, riluzole, EBIO, SKA-31, SKA-121, chlorzoxazone

<sup>1</sup> Nonselective blockers shown in Fig. 5.

<sup>2</sup> Blockers and activators shown in Fig. 7.

[84]. The most dangerous snakes belong to the families Viperidae (vipers and rattlesnakes) and Elapidae (cobras and kraits) [85]. Snake venoms contain a broad array of biologically active compounds: enzymes, polypeptides with anticoagulant, hypotensive, and other activities, various organic molecules (e.g. nucleotides) [86], and polypeptide toxins that are capable of interactions with membrane proteins, including K<sup>+</sup> channels [87]. K<sup>+</sup> channel blockers, named dendrotoxins, have been found in the venom of snakes belonging to the *Dendroaspis* genus (mambas) (Fig. 9a) [88]. Their molecules consist of 57–60 amino acid residues (Table 2), six of which are cysteines that form the Kunitz-type fold with C<sup>1</sup>-C<sup>6</sup>, C<sup>2</sup>-C<sup>4</sup>, and C<sup>3</sup>-C<sup>5</sup> disulfide bond formation pattern [89]. Dendrotoxins undergo a single posttranslational modification – cyclization of the *N*-terminal glutamine into pyroglutamate.

Dendrotoxins are selective toward K<sub>v</sub> channels (K<sub>v</sub>1.1, K<sub>v</sub>1.2, and K<sub>v</sub>1.6) and act at concentrations ranging from pico- to nanomolar and above [90, 91].

**Sea anemones.** Sea anemones (Actiniaria) belong to the coral polyps (Anthozoa). Most sea anemones are solitary sedentary organisms inhabiting solid sea bottom surface [92].

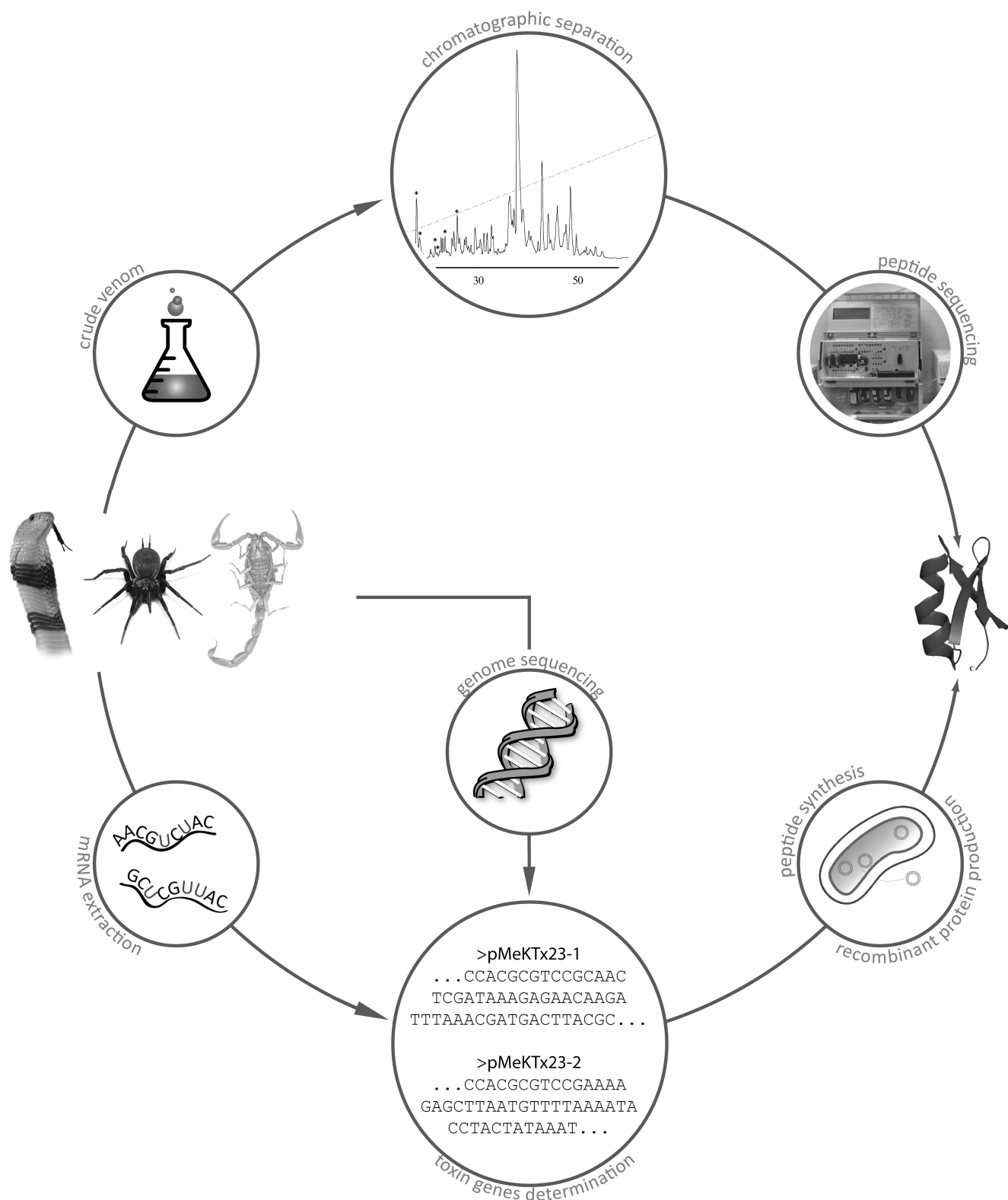
Similarly to snake venoms, crude venoms of sea anemones are complex mixtures of numerous compounds: actinoporins (~20 kDa) that can form pores in lipid bilayer [93], ligands of voltage-gated Na<sup>+</sup> channels [94], K<sup>+</sup> channel blockers [95], and polypeptide ligands of acid-sensing ion channels [96] and thermoreceptors [97].

More than 25 polypeptide ligands of K<sup>+</sup> channels have been identified in sea anemone venom [98]. All of

these can be subdivided into three structurally different groups [99]. The first group includes AeK, BgK, HmK, and ShK polypeptides. These polypeptides are composed of 34–37 amino acid residues. Six of the residues are cysteines that form C<sup>1</sup>-C<sup>6</sup>, C<sup>2</sup>-C<sup>4</sup>, and C<sup>3</sup>-C<sup>5</sup> disulfide bonds [95, 100–102], promoting formation of the toxin spatial structure as a combination of  $\alpha$ - and/or 3<sub>10</sub>-helices (Fig. 9b) [85]. The second group includes AsKC1 and APEKTx1 molecules (58–65 amino acid residues) with Kunitz-type fold [99]. Their six cysteine residues form C<sup>1</sup>-C<sup>6</sup>, C<sup>2</sup>-C<sup>4</sup>, and C<sup>3</sup>-C<sup>5</sup> disulfide bonds [103, 104]. Toxins of the third group (BDS-I, Am II, and APETx1) consist of 42–48 residues and display different disulfide bond formation pattern: C<sup>1</sup>-C<sup>5</sup>, C<sup>2</sup>-C<sup>4</sup>, and C<sup>3</sup>-C<sup>6</sup> [105–107]. The spatial structure of these toxins is represented by several  $\beta$ -strands [85]. Amino acid sequences of some K<sup>+</sup> channel blockers from sea anemones are shown in Table 3. Some active forms of the polypeptides undergo posttranslational modifications, such as conversion of proline into 4-hydroxyproline or amidation of the *C*-terminal amino acid residue [106]. Polypeptides from sea anemones mostly modulate K<sub>v</sub> channels (including K<sub>v</sub>11 and K<sub>v</sub>3.4) [105, 107] and IK<sub>Ca</sub> channels [108] in pico- and nanomolar concentrations.

**Cone snails.** Cone snails are predatory sea mollusks from the Conidae family that includes about 600 species [109]. The major weapons of these snails are short disulfide bond-containing peptides named conopeptides [110].

Conopeptides interacting with K<sup>+</sup> channels ( $\kappa$ -conotoxins) are extremely diverse and can be classified



**Fig. 8.** Main strategies for identification of active peptides in animal venoms. The first strategy is purification of active components from crude venom; the second strategy is identification of venom components by construction of cDNA libraries from mRNA of the venom glands with subsequent production of compounds of interest by chemical synthesis or by biosynthesis in recombinant systems.

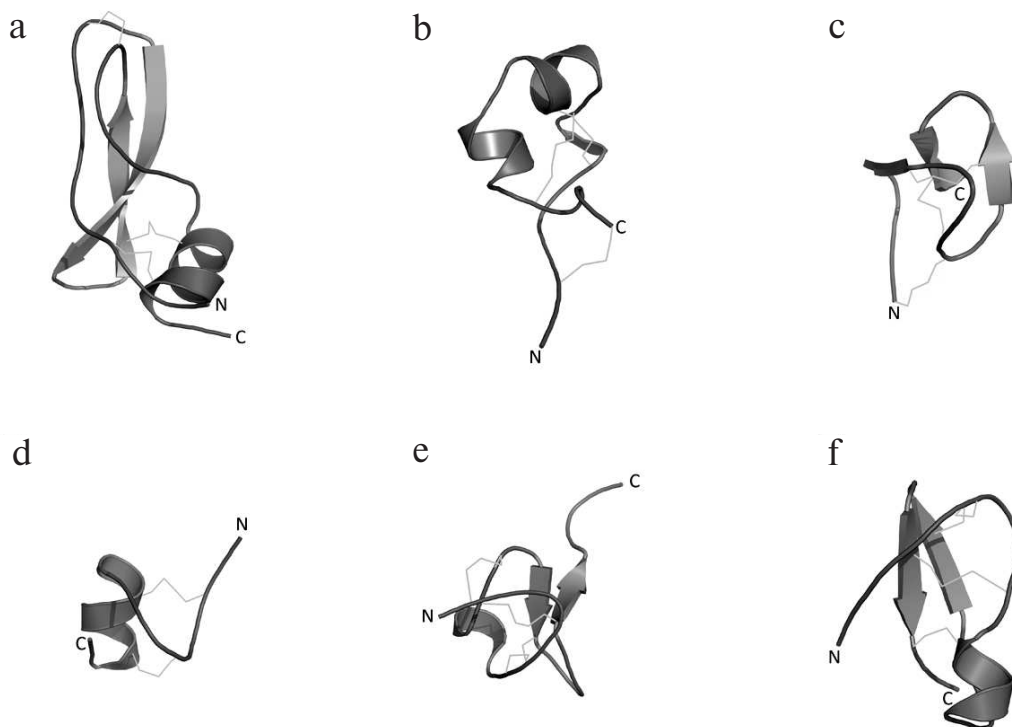
**Table 2.** K<sup>+</sup> channel ligands from snake venom

Toxin	ID	Amino acid sequence	Activity
<i>Dendroaspis angusticeps</i>			
$\alpha$ -DTX	P00980	ZPRRKL <b>C</b> ILHRNPGR <b>C</b> YDKIPAFYYNQKKK <b>Q</b> CE <sup>R</sup> FDWSGCGGNSNRFKTIEE <b>CRRT</b> <b>C</b> IG	1
$\delta$ -DTX	P00982	AAKY <b>C</b> KLPVRYG <b>P</b> CKKKIPSFYYKWKAK <b>Q</b> CLPFDYSGCGGNANRFKTIIEE <b>CRRT</b> <b>C</b> VG	1
E-DTX	P00984	LQHR <b>T</b> F <b>C</b> KLPAEPGP <b>C</b> KASIPAFYYNWA <b>A</b> AK <b>C</b> QLFHYGG <b>C</b> KGNANRFSTIEK <b>CR</b> H <b>A</b> <b>C</b> VG	1
<i>Dendroaspis polylepis polylepis</i>			
I-DTX	P00979	ZLRK <b>L</b> CILHRNPGR <b>C</b> YQKIPAFYYNQKK <b>Q</b> CEGFTWSGCGGNSNRFKTIEE <b>CRRT</b> <b>C</b> IRK	1
B-DTX	P00983	RPYA <b>C</b> ELIVAAG <b>P</b> CMFFISAFYYKSGANK <b>C</b> YPFTYSGCRGNANRFKTIIEE <b>CRRT</b> <b>C</b> VV	1
K-DTX	P00981	AAKY <b>C</b> KLPLRIG <b>P</b> CKRKRIPSFYYKWKAK <b>Q</b> CLPFDYSGCGGNANRFKTIIEE <b>CRRT</b> <b>C</b> VG	1

Notes: ID, accession number in the UniProt database; Z, pyroglutamate residue; 1, active toward K<sub>v</sub> channels (here and below, figures indicate activity toward corresponding channels). Cysteine residues are shown in bold.

into several groups according to their primary structure [111]. Amino acid sequences of some  $\kappa$ -conotoxins are shown in Table 4.  $\kappa$ A-Conotoxins (Ac4.2, MIVA, PIVF, SmIVA, SIVA, and other) are peptides of 24-37 amino

acid residues. Six of these residues are cysteines that comprise the CC-C-C-C-C motif. The pattern of formed disulfide bonds can be either C<sup>1</sup>-C<sup>5</sup>, C<sup>2</sup>-C<sup>3</sup>, C<sup>4</sup>-C<sup>6</sup> or C<sup>1</sup>-C<sup>3</sup>, C<sup>2</sup>-C<sup>5</sup>, C<sup>4</sup>-C<sup>6</sup> [112-114].  $\kappa$ O-Conotoxins (e.g.



**Fig. 9.** Spatial structures of K<sup>+</sup> channel ligands. a) I-DTX (PDB ID: 1DEM), a K<sub>v</sub> channel blocker from *Dendroaspis polylepis polylepis* snake venom; b) ShK (PDB ID: 1ROO), a high-affinity K<sup>+</sup> channel blocker from *Stichodactyla helianthus* sea anemone venom; c) PVIIA (PDB ID: 1AV3), a K<sub>v</sub> channel blocker from *Conus purpurascens* mollusk venom; d) tertiapin (PDB ID: 1TER), a K<sup>+</sup> channel peptide ligand from *Apis mellifera* bee venom; e) hanatoxin (PDB ID: 1D1H) from *Grammostola rosea* Chilean bird-eating spider venom; f) noxiustoxin (PDB ID: 1SXM) from *Centruroides noxius* scorpion venom.

**Table 3.** K<sup>+</sup> channel ligands from sea anemone venoms

Toxin	ID	Amino acid sequence	Activity
<i>Actinia equine</i>			
AeK	P81897	G <b>C</b> KDNFSANT <b>C</b> KHVKANNN <b>C</b> GSQKYATN <b>C</b> AKT <b>C</b> G <b>K</b> C	1
<i>Anemonia erythraea</i>			
AETX K	Q0EAE5	A <b>C</b> KDYLPKSE <b>C</b> TQFR <b>C</b> RTSMKYKYTN <b>C</b> KKT <b>C</b> G <b>T</b> C	1
<i>Anemonia sulcata</i>			
BDS-I	P11494	AAP <b>C</b> F <b>C</b> SGKPGRGDLWILRGT <b>C</b> PGGYGYTSN <b>C</b> YKWPNI <b>C</b> CYPH	1
AsKS	Q9TWG1	A <b>C</b> KDNFAAAT <b>C</b> KHVKENKN <b>C</b> GSQKYATN <b>C</b> AKT <b>C</b> G <b>K</b> C	1
AsKC1	Q9TWG0	INKD <b>C</b> LLPMDVGR <b>C</b> RASHPRYYN <b>S</b> SSKR <b>C</b> EKFYGG <b>C</b> RGNANNFHTLEE <b>C</b> EKV <b>C</b> GVR	1
<i>Antheopsis maculata</i>			
Am II	P69930	ALL <b>S</b> <b>C</b> R <b>C</b> EGKTEYGDKWL <b>F</b> HGG <b>C</b> ONNYGYNYK <b>C</b> FMKPGAV <b>C</b> CYPQNGR-NH <sub>2</sub>	5
<i>Anthopleura elegantissima</i>			
APETx1	P61541	GTT <b>C</b> Y <b>C</b> GKTIGIYWFGTKT <b>C</b> PSNRGYTGS <b>C</b> GYFLGI <b>C</b> CYPVD	5
<i>Bunodosoma caissarum</i>			
BcsTx1	C0HJC2	A <b>C</b> IDRFPTGT <b>C</b> KHVKKGG <b>S</b> C <b>K</b> NSQKYRIN <b>C</b> AKT <b>C</b> GL <b>C</b> H	1
<i>Bunodosoma granuliferum</i>			
BgK	P29186	VCRDWFKET <b>A</b> CRHAKSLGN <b>C</b> RTSQKYRAN <b>C</b> AKT <b>C</b> EL <b>C</b>	1, 4
<i>Cryptodendrum adhaesivum</i>			
κ-TATX	E2S064	RT <b>C</b> IDTIPKSR <b>C</b> TAFQ <b>C</b> KNSMKYRLSF <b>C</b> RK <b>T</b> C <b>G</b> T <b>C</b>	1
<i>Heteractis magnifica</i>			
HmK	O16846	RT <b>C</b> KDLIPVSE <b>C</b> TDIR <b>C</b> RTSMKYRLNL <b>C</b> RK <b>T</b> C <b>G</b> S <b>C</b>	1
<i>Stoichactis helianthus</i>			
ShK	P29187	RS <b>C</b> IDTIPKSR <b>C</b> TAFQ <b>C</b> KHSMKYRLSF <b>C</b> RK <b>T</b> C <b>G</b> T <b>C</b>	1, 4

Notes: 1, 4, and 5, active toward K<sub>v</sub> (except K<sub>v</sub>11), IK<sub>Ca</sub>, and K<sub>v</sub>11 channels, respectively; O, 4-hydroxyproline residue; -NH<sub>2</sub>, amidation. Cysteine residues are shown in bold.

PVIA) are short (~27 amino acid residues) peptides with six cysteine residues, whose distribution in the amino acid sequence (C-C-CC-C-C) differs from that in κA-conotoxins. The disulfide bond formation pattern is C<sup>1</sup>-C<sup>4</sup>, C<sup>2</sup>-C<sup>5</sup>, and C<sup>3</sup>-C<sup>6</sup> (Fig. 9c) [115, 116]. κM-Conotoxins

(RIIIJ and RIIIK) are composed of 24-25 amino acids; the number of cysteine residues and the pattern of disulfide bond formation in these polypeptides are the same as in κO-conotoxins; however, the distribution of six cysteine residues in the amino acid sequence is different:

**Table 4.** Conotoxins, ligands of K<sup>+</sup> channels

Toxin	ID	Amino acid sequence	Activity
<i>Conus achatinus</i>			
κ-Ac4.2	A3DT44	AOWMVVT <u>T</u> AT <u>T</u> N <b>CC</b> GYTGPA <b>CH</b> O <b>CL</b> CTQ <b>SC</b> -NH <sub>2</sub>	1
<i>Conus betulinus</i>			
BeTx	Q9U3Z3	<b>C</b> RAEGTY <b>C</b> ENDSQ <b>CC</b> LNE <b>CC</b> WGGCGHO <b>C</b> RHP-NH <sub>2</sub>	2
<i>Conus magus</i>			
κ-MIVA	P0C1X1	AOELVVT <u>T</u> AT <u>T</u> N <b>CC</b> GYNOMTICOO <b>CM</b> CTYS <b>CO</b> OKRKO-NH <sub>2</sub>	1
<i>Conus monile</i>			
κ-Mo1659	P84713	FHGGSWYRFPWGY-NH <sub>2</sub>	1
<i>Conus planorbis</i>			
α/κ-pl14a	Q0N4U8	FPRPRI <b>C</b> NL <b>AC</b> RAGIGHKY <b>P</b> F <b>CH</b> CR-NH <sub>2</sub>	1
<i>Conus purpurascens</i>			
κ-PIVF	P0C2C6	D <b>CC</b> GVKLEM <b>CH</b> P <b>CL</b> CDNS <b>CK</b> KSGK-NH <sub>2</sub>	1
κ-PVIA	P56633	<b>C</b> RIONQ <b>K</b> CFQHLDD <b>CC</b> SR <b>K</b> CNRF <b>NK</b> CV	1
<i>Conus radiatus</i>			
κ-RIIIJ	P0CG45	LO <b>CC</b> T <b>OO</b> KK <b>HC</b> OA <b>OA</b> CKY <b>KO</b> CC <b>KS</b>	1
κ-RIIK	P69769	LO <b>CC</b> SLN <b>LR</b> LC <b>OV</b> OA <b>CK</b> R <b>NO</b> CC <b>T</b> -NH <sub>2</sub>	1
<i>Conus spurius</i>			
κ-SrXIA	P0C615	<b>C</b> RTEGMS <b>C</b> EEN <b>Q</b> CC <b>WR</b> S <b>CC</b> R <b>GE</b> CEAP <b>C</b> RFGP-NH <sub>2</sub>	1
<i>Conus stercusmuscarum</i>			
κ-SmIVA	P0CE75	ZT <b>WL</b> V <b>P</b> ST <u>I</u> T <b>TC</b> CGYDOGT <b>MC</b> OT <b>CM</b> CDNT <b>CK</b> OKOK <b>KS</b> -NH <sub>2</sub>	1
<i>Conus striatus</i>			
κ-SIVA	P0C828	ZKSLV <b>P</b> SV <u>I</u> T <b>TC</b> CGYDOGT <b>MC</b> OO <b>CR</b> CTNS <b>C</b> -NH <sub>2</sub>	1
Conk-S1	P0C1X2	KDR <b>PS</b> L <b>CD</b> L <b>P</b> ADSGSGT <b>KA</b> E <b>K</b> RIY <b>NS</b> ARK <b>Q</b> CL <b>R</b> FDYTG <b>Q</b> GGN <b>EN</b> FR <b>RT</b> Y <b>D</b> C <b>Q</b> RT <b>CL</b> YT-NH <sub>2</sub>	1
<i>Conus ventricosus</i>			
Con-Vn	P83047	GDC <b>P</b> W <sup>D</sup> <b>K</b> P <b>WC</b> -NH <sub>2</sub>	1, 2

**Table 4.** (Contd.)

Toxin	ID	Amino acid sequence	Activity
<i>Conus villepinii</i>			
κ-vil14a	P84704	GGLGR <b>C</b> IY <b>N</b> CMNSGGGLSFIQ <b>C</b> KTMCY	1
<i>Conus virgo</i>			
κ-ViT <sub>x</sub>	Q7YZS9	SR <b>C</b> FP <b>P</b> GIY <b>C</b> TPYLP <b>C</b> WG <b>I</b> CCGT <b>C</b> RNV <b>C</b> HLRI-NH <sub>2</sub>	1

Notes: 1 and 2, active toward K<sub>v</sub> (except K<sub>v</sub>11) and BK<sub>Ca</sub> channels, respectively; O, 4-hydroxyproline residue; Z, pyroglutamate residue; E, 4-carboxyglutamate residue; T and S, O-glycosylated threonine and serine residues, respectively; W<sup>D</sup>, D-tryptophan residue; -NH<sub>2</sub>, amidation. Cysteine residues are shown in bold.

CC-C-C-CC [117, 118]. Another group, κI-conotoxins (BeTx, SrXIA, and ViTx), includes molecules composed of ~30 amino acid residues with eight cysteine residues (C-C-CC-CC-C-C) and varying patterns of disulfide bond formation [119-122]. Cone snail venom also contains κL-conotoxins (vil14a) and κJ-conotoxins (α/κ-pl14a) – small (~25 amino acid residues) peptides with two disulfide bonds. They have similar cysteine residue distribution (C-C-C-C), but different patterns of disulfide bond formation: C<sup>1</sup>-C<sup>4</sup>, C<sup>2</sup>-C<sup>3</sup> in κL-conotoxins and C<sup>1</sup>-C<sup>3</sup>, C<sup>2</sup>-C<sup>4</sup> in κJ-conotoxins [123, 124]. Two toxins should also be mentioned that have an unusual structure: conkunitzin-S1 (Conk-S1), a 60 amino acid peptide with the Kunitz-type fold and only two disulfide bonds (C<sup>1</sup>-C<sup>4</sup>, C<sup>2</sup>-C<sup>3</sup>) [125], and contryphan-Vn (Con-Vn), a short peptide of nine amino acids with one disulfide bond [126]. These variations in the cysteine residue distribution and disulfide bonds patterns give origin to a wide variety of the κ-conotoxin spatial structures, such as inhibitor cystine knot (ICK, see below), as in PVIIA [116], Kunitz-type fold [127], or even helix-loop-helix motif [123].

Molecules of κ-conotoxins and other peptides from the cone snail venom are often subjected to posttranslational modifications that are rarely seen in other organisms [128]. In addition to typical modifications (cyclization of N-terminal glutamine, hydroxyproline formation, C-terminal amidation), other types of the polypeptide chain modification are observed, such as 4-carboxygluta-

mate formation, serine and threonine O-glycosylation [129], and incorporation of D-amino acids (e.g. D-tryptophan) in the polypeptide chain [130]. The major targets of κ-conotoxins are K<sub>v</sub> channels; however, some can affect the function of BK<sub>Ca</sub> channels [111].

**Bees.** Bee venom has been extensively characterized, and its components, such as melittin, secapin, apamin, and tertiapin, have become classical instruments in biochemical and physiological studies (Fig. 9d) [131]. Apamin and tertiapin, short peptides of 18 and 21 amino acid residues, respectively, affect various K<sup>+</sup> channels (Table 5) [132, 133]. Their molecules undergo posttranslational C-terminal amidation. Four cysteine residues form two disulfide bonds (C<sup>1</sup>-C<sup>3</sup> and C<sup>2</sup>-C<sup>4</sup>), and together with an amidated C-terminal residue, generate a spatial structure with a short β-turn and an α-helix [134-136]. Apamin preferentially binds to SK<sub>Ca</sub> channels [137, 138], while tertiapin mostly affects BK<sub>Ca</sub> [139] and K<sub>ir</sub> channels [140, 141].

**Spiders.** More than 45,000 species of spiders are known today [142]. However, venoms of only about 100 species have been studied [143], and just a few of these have been characterized in details [144]. Spider venoms are complex mixtures of biologically active compounds that can be divided into three groups: low molecular mass (<1 kDa) components, peptides (1-10 kDa), and high molecular mass (>10 kDa) compounds (enzymes and neurotoxins) [143]. K<sup>+</sup> channel ligands in spider venoms are peptides that inhibit mostly channel activation via

**Table 5.** Short polypeptide ligands of K<sup>+</sup> channels from bee venom

Toxin	ID	Amino acid sequence	Activity
<i>Apis mellifera</i>			
Apamin	P01500	<b>C</b> NCKAP <b>E</b> TAL <b>C</b> ARR <b>C</b> Q <b>Q</b> H-NH <sub>2</sub>	3
Tertiapin	P56587	AL <b>C</b> N <b>C</b> N <b>R</b> I I I P <b>H</b> M <b>C</b> W <b>K</b> K <b>C</b> G <b>K</b> K-NH <sub>2</sub>	2, 6

Notes: 2, 3, and 6, active toward BK<sub>Ca</sub>, SK<sub>Ca</sub>, and K<sub>ir</sub> channels, respectively; -NH<sub>2</sub>, amidation. Cysteine residues are shown in bold.



**Table 6.** K<sup>+</sup> channel ligands from spider venom

Toxin	ID	Amino acid sequence	Activity
<i>Chilobrachys guangxiensis</i>			
JZTX-I	P83974	ACGQFVWKC GEGKPPCCANFACKIGLYLCIWS P	1
JZTX-III	P62520	DGECGGFVWKC GRGKPPCCCKGYACSKTWGWC AVEAP	1
JZTX-V	Q2PAY4	YCQKWMWTC DSKRACCEGLRCKLWCRKII-NH <sub>2</sub>	1
<i>Grammostola rosea</i>			
HaTx1	P56852	ECRYLFGGCKTTS DCCKHLGCKFRDKYCAWDFTF S	1
VsTx1	P60980	ECGKFMWKC KNSNDCCCKDLVCS SRWKWCVLASF P	5
GsMTx4	Q7YT39	GCLFVWKC CNPNDKCCRPKLC SKLFLKLCNFSF-NH <sub>2</sub>	5
GsAFII	P61409	YCQKWMWTC DEERKCC EGLVCR LWCKKKIEW	5
<i>Hadronyche versuta</i>			
κ-HXTX	P82227	TICTGADRPC AACCPCCPGTSC QGPESNGVVYCRNF	2
<i>Haplopelma schmidti</i>			
HwTx-XI	P68425	IDTCRLP SDRGRCKASFERWYFN GR TCAKFIYGGCGGNGNKFP TQEACMKRCAKA	1
<i>Heteropoda venatoria</i>			
HpTX1	P58425	DCGTIWHYCGTDQSECC EGWKCSRQLCKYVIDW-NH <sub>2</sub>	1
<i>Heteroscodra maculata</i>			
HmTx1	P60992	ECRYLFGGCSSTS DCCKHLSCRS DWKYCAWDGTF S	1
<i>Paraphysa scrofa</i>			
PaTX1	P61230	YCQKWMWTC DSARKCC EGLVCR LWCKKII-NH <sub>2</sub>	1
<i>Phoneutria nigriventer</i>			
PNTx3-1	O76200	AECAAVYERCGKGYKRC CEERPCKCNIVMDNCTCKKFISE	1
<i>Psalmopoeus cambridgei</i>			
VaTx1	P0C244	SECRWFMGGCDSTLDCC KHLSCKMGLYYCAWDGTF-NH <sub>2</sub>	1
<i>Stromatopelma calceatum griseipes</i>			
SGTx1	P56855	TCRYLFGGCKTTADCC KHLACRS DGKYCAWDGTF	1
ScTx1	P60991	DCTRMFGACRRDS DCCPHLGCKPTSKYCAWDGTI-NH <sub>2</sub>	1

Table 6. (Contd.)

Toxin	ID	Amino acid sequence	Activity
<i>Theraphosa blondi</i>			
TITx1	P83745	AACLGMFES <b>C</b> DPNNDK <b>CC</b> PNRE <b>C</b> NRKHKW <b>C</b> KYKLW	1
<i>Thrixopelma pruriens</i>			
ProTx1	P83480	ECRYWLGG <b>C</b> SAGQT <b>CC</b> KHLV <b>C</b> SRRHGW <b>C</b> VWDGTF <b>S</b>	1

Notes: 1, 2, and 5, active toward K<sub>v</sub>, BK<sub>Ca</sub>, and K<sub>v</sub>11 channels, respectively; -NH<sub>2</sub>, amidation. Cysteine residues are shown in bold.

interaction with the voltage sensor [145]. This interaction is preceded by the binding of the toxin to the membrane due to the presence of hydrophobic amino acid residues on the molecule surface [146]. Spider venom toxins consist of ~30-40 amino acid residues (Table 6) with the C<sup>1</sup>-C<sup>4</sup>, C<sup>2</sup>-C<sup>5</sup>, C<sup>3</sup>-C<sup>6</sup> pattern of disulfide bond formation [147-150]. Their spatial structure is represented by the inhibitor cystine knot (ICK) fold characterized by the presence of β-hairpin and characteristic “knot”-like (hence the name) structure in which the third disulfide bond (C<sup>3</sup>-C<sup>6</sup>) penetrates the ring formed by the two other disulfide bonds and amino acid residues of the polypeptide chain between them (Fig. 9e).

Some spider venom polypeptides (κ-HXTX and PNTx3-1) have an additional fourth disulfide bond, but it does not noticeably affect the spatial structure of the molecule [151]. Spider venoms also contain K<sup>+</sup> ligands with

the Kunitz-type fold, which act as weak pore blockers [152].

Spider toxins mostly interact with K<sub>v</sub> channels: hanatoxin (HaTx1) is specific against K<sub>v</sub>2.1 [147]; HpTx1 and PaTx1 bind to the K<sub>v</sub>4 channels [149, 153]; ScTx1 and HmTx1 bind to the K<sub>v</sub>2 and K<sub>v</sub>4 channels [154]. Some ligands of voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channels, e.g. JZTX-I and VsTx1, can also inhibit K<sup>+</sup> channels [155-157].

**Scorpions.** An extremely important role in understanding the mechanism of action and physiological role of K<sup>+</sup> channels belongs to polypeptide channel blockers from scorpion venom (KTx). The application of these molecules in K<sup>+</sup> channel studies started over 30 years ago, when the first short peptide, noxiustoxin (Fig. 9f), was purified from the venom of the scorpion *Centruroides noxius* [158] and found to affect potassium flux in the squid

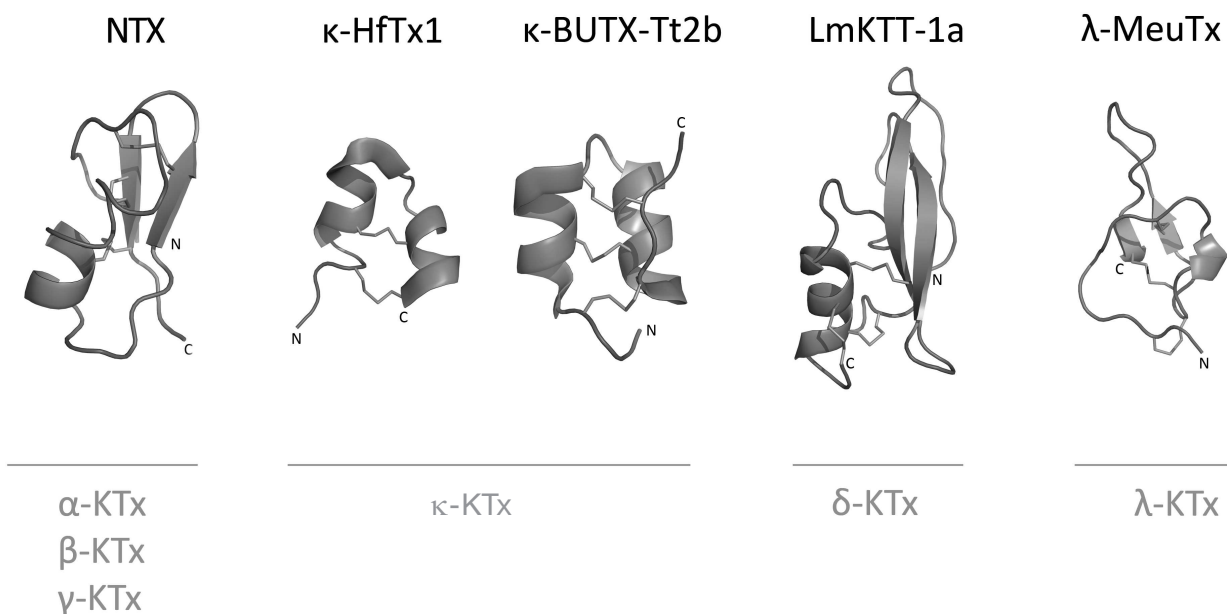


Fig. 10. Examples of structural folds in different groups of KTx: CS $\alpha$ / $\beta$  in NTX; CS $\alpha$ / $\alpha$  with two disulfide bonds in  $\kappa$ -HfTx1; CS $\alpha$ / $\alpha$  with three disulfide bonds in  $\kappa$ -BUTX-Tt2b; Kunitz-type fold in LmKTT-1a; ICK in  $\lambda$ -MeuTx.

**Table 7.** Occurrence of different structural folds in KTx (total number of toxins analyzed, 247)

CS $\alpha$ / $\beta$	CS $\alpha$ / $\alpha$ 2(C-C)	CS $\alpha$ / $\alpha$ 3(C-C)	Kunitz- type fold	ICK
220	16	2	7	2
89.2%	6.4%	0.8%	2.8%	0.8%

giant axon [159]. Isolation of charybdotoxin (ChTx) from the venom of the scorpion *Leiurus quinquestriatus hebraeus* [160] and its use for modeling ligand–receptor interactions (toxin binding to channels) helped to achieve a deeper understanding of the structure–functional properties of K<sup>+</sup> channels [161, 162]. Later, an array of isoform-specific selective blockers of K<sup>+</sup> channels were identified in the venoms of various scorpion species. Such peptides as iberiotoxin (IbTX) [163], margatoxin (MgTX) [164], kaliotoxin [165], and agitoxin-2 (AgTx2) [166] have become classical molecular instruments for studying K<sup>+</sup> channels [167].

**Structural properties of K<sup>+</sup> channel blockers from scorpion venom.** The UniProt database includes ~250 K<sup>+</sup> channel blockers from scorpion venoms (KTx). All of them are small polypeptides of ~20–75 amino acid residues (Mw ~ 2400–8500 Da) with two to four S–S bridges [168, 169]. These toxins are synthesized as precursors with the *N*-terminal signal peptide that is cleaved off in the process of toxin maturation [170]. Sometimes, KTx precursors contain a propeptide fragment of unknown function immediately after the signal peptide that is also cleaved off during processing [171]. Some peptides undergo posttranslational modification, such as

conversion of the *N*-terminal glutamine into pyroglutamate [172], *C*-terminal amidation of the *C*-terminal glycine, or removal of *C*-terminal positively charged residues (lysine, arginine) [173].

K<sup>+</sup> channel blockers from scorpion venoms can be classified into five groups according to their structure (see Fig. 10 for representatives of different structural classes).

1. Peptides with CS $\alpha$ / $\beta$  (cysteine-stabilized  $\alpha$ -helix– $\beta$ -sheet) fold. This type of peptide structure is the most represented fold in KTx; it is also present in Na<sup>+</sup> channel ligands and a group of chlorotoxin-like peptides [174–176].

2. KTx with two parallel  $\alpha$ -helices connected with two disulfide bonds: CS $\alpha$ / $\alpha$  2(C-C) [168].

3. Peptides with amino acid sequences similar to those of peptides from the first group, but with different spatial structure, CS $\alpha$ / $\alpha$  (cysteine-stabilized helix-loop-helix), due to an alternative pattern of disulfide bond formation [177].

4. KTx with the Kunitz-type fold characteristic of serine protease inhibitors [178].

5. KTx with the inhibitor cystine knot (ICK) fold that is present in polypeptides from various groups of living organisms (plants, fungi, and invertebrates) [179].

Table 7 shows the relative occurrence of different structural folds in known KTx (according to the UniProt database). It is evident that the CS $\alpha$ / $\beta$  fold dominates among the KTx structures.

*KTx with CS $\alpha$ / $\beta$  fold.* As mentioned above, CS $\alpha$ / $\beta$  is the most widely represented type of fold in scorpion venom toxins. It is characterized by the presence of two conserved motifs, CXXXC and CXC (where C is cysteine and X is any amino acid residue), in the amino acid sequence. Based on structural and functional properties,

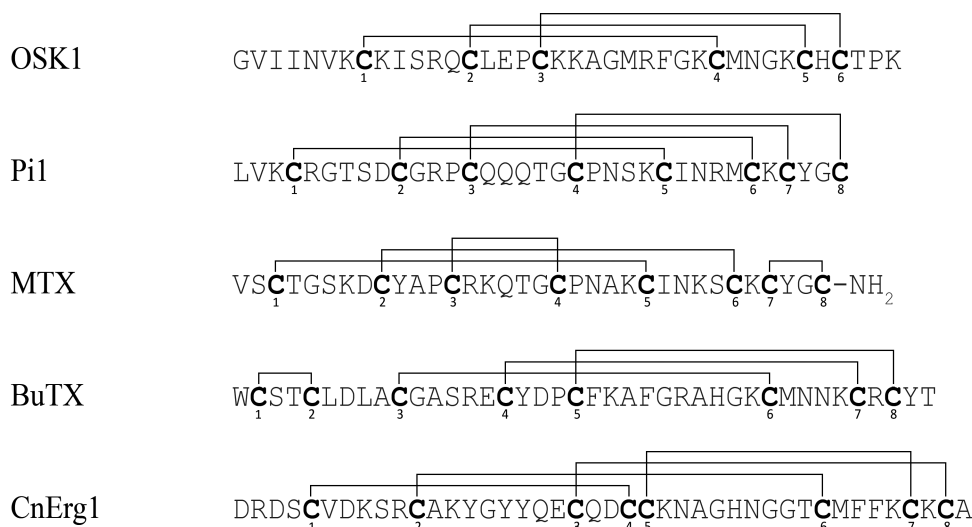
**Fig. 11.** Disulfide bond formation pattern in KTx with CS $\alpha$ / $\beta$  fold.



Fig. 12. Disulfide bond formation pattern in κ-KTx (κ-hefutoxin).



Fig. 13. Disulfide bond formation pattern in CS $\alpha$ / $\alpha$ -fold peptides with cysteine residue distribution characteristic of CS $\alpha$ / $\beta$ -fold toxins.

KTx with CS $\alpha$ / $\beta$  fold can be classified into three families:  $\alpha$ -KTx,  $\beta$ -KTx, and  $\gamma$ -KTx [180].

The  $\alpha$ -KTx family includes ~27 subfamilies (based on similarity of their primary structures) [181]. More than 165 polypeptides are now known to belong to this family, and this number continues to grow.  $\alpha$ -KTx consist of ~20-40 amino acid residues, six or eight being cysteines that form three or four disulfide bonds. The majority of toxins in this family have been isolated from the venoms of scorpions of the Buthidae family.  $\alpha$ -KTx have also been identified in the venoms of scorpions from other families (Chactidae, Euscorpidae, Vaejovidae, Caraboctonidae, Hemiscorpiidae, and Scorpionidae) [182-184].

$\beta$ -KTx are larger than  $\alpha$ -KTx (~45-75 amino acid residues) due to the presence of an additional linear *N*-terminal fragment. The *C*-terminal fragments of these toxins contain six cysteines that form three disulfide bonds. Most of  $\beta$ -KTx (22 peptides) have been found in scorpions from the Buthidae family; two  $\beta$ -KTx were discovered in the transcriptome and venom of *Hadrurus gertschi* from the family Caraboctonidae [169, 185]. It is believed that, in addition to the blocking effect on K<sup>+</sup> channels,  $\beta$ -KTx also possess cytolytic activity that is determined by the presence of the *N*-terminal linear fragment [186, 187].

The structure of  $\gamma$ -KTx is similar to that of  $\alpha$ -KTx. They are composed of ~35-45 amino acid residues and have three or four disulfide bonds. This group is distinguished by its selective activity toward the only type of K<sub>v</sub> channels – K<sub>v</sub>11. At present, 29  $\gamma$ -KTx are known; all of them have been identified in the venom or the venom gland transcriptome of scorpions from the Buthidae family [188, 189].

As mentioned above, the number of disulfide bonds in KTx with CS $\alpha$ / $\beta$  fold varies from three to four. The pattern of the disulfide bond formation can differ in different molecules (Fig. 11), but it does not affect the fold of the toxins [190-194].

*KTx with CS $\alpha$ / $\alpha$  fold and two disulfide bonds.* Peptides of this group are also called κ-KTx (similarly to the group 3 toxins that have CS $\alpha$ / $\alpha$  fold and three disulfide bonds). They are weak inhibitors of K<sup>+</sup> channels and have been found only in the venom of scorpions of the Scorpionidae

and Hemiscorpiidae families. κ-KTx consist of ~20-25 amino acid residues, four of which are cysteine residues that form two intramolecular disulfide bonds [168]. Cysteine residues are located within the conserved motifs C<sup>1</sup>XXXC<sup>2</sup> and C<sup>3</sup>XXXC<sup>4</sup> (where C is cysteine residue, X is any amino acid residue), and form the C<sup>1</sup>-C<sup>4</sup> and C<sup>2</sup>-C<sup>3</sup> disulfide bonds (Fig. 12). The spatial structure of these toxins is represented by two parallel  $\alpha$ -helices.

*KTx with CS $\alpha$ / $\alpha$  fold and three disulfide bonds.* Recently, a new type of fold was found in a group of scorpion venom toxins. Even if these peptides have the traditional positioning of cysteine residues typically found in the majority of CS $\alpha$ / $\beta$  toxins, the pattern of disulfide bond formation is C<sup>1</sup>-C<sup>5</sup>, C<sup>2</sup>-C<sup>4</sup>, C<sup>3</sup>-C<sup>6</sup> (Fig. 13) instead of the classical C<sup>1</sup>-C<sup>4</sup>, C<sup>2</sup>-C<sup>5</sup>, C<sup>3</sup>-C<sup>6</sup>. The spatial structure of these toxins is represented by two  $\alpha$ -helices connected by a loop. Peptides of this group are conventionally called κ-KTx (similarly to KTx with the CS $\alpha$ / $\alpha$  fold and two disulfide bonds) [195].

Only two short peptides (28-29 amino acid residues) with this type of structural fold have been found so far. They were isolated from the venoms of related scorpions *Tityus serrulatus* and *Tityus trivittatus* of the Buthidae family [177].

*KTx with Kunitz-type fold.* KTx with this type of fold can possess a dual activity. Almost all of them inhibit proteolytic enzymes (e.g. trypsin) in nanomolar concentration, and some of the toxins block K<sub>v</sub> channels. Seven different toxins with such properties have been identified so far: six from the venom of Buthidae family scorpions and one from the venom of Caraboctonidae scorpions [178]. KTx with the Kunitz-type fold consist of ~60-70 residues, six or eight of them are cysteines that form three or four intramolecular disulfide bonds with the C<sup>1</sup>-C<sup>5</sup>, C<sup>2</sup>-C<sup>3</sup>, C<sup>4</sup>-C<sup>6</sup> or C<sup>1</sup>-C<sup>7</sup>, C<sup>2</sup>-C<sup>4</sup>, C<sup>3</sup>-C<sup>5</sup>, C<sup>6</sup>-C<sup>8</sup> patterns (Fig. 14). The Kunitz-type fold is represented by two antiparallel  $\beta$ -strands and two or, more often, one helical regions [196]. Toxins of this group are named  $\delta$ -KTx.

*KTx with the inhibitor cystine knot fold.* Ten peptides with the inhibitor cystine knot (ICK) fold have been found in scorpion venom; only two of them are active toward K<sup>+</sup> channels. The structure of these peptides (37 amino acid residues) is represented by a  $\beta$ -sheet formed

**Table 8.** KTx subfamilies

Toxin	Subfamily	ID	Amino acid sequence	A.a.	Mm	Activity
<i>Androctonus australis</i>						
Aa1	$\alpha$ 15.1	P60233	ZNETNKK <b>C</b> QGGSCASV <b>C</b> RRRVIGVAAGK <b>C</b> INGR <b>C</b> V <b>C</b> YP	37	3851	1, 5
AaTXK $\beta$	$\beta$ 1	P69939	KLVKYAVPVGTLRLTILQTVVHKV <b>G</b> K <b>T</b> Q <b>F</b> <b>G</b> <b>C</b> PAYQGY <b>C</b> DDH <b>C</b> QDIKKEEG <b>F</b> <b>C</b> HG <b>F</b> K <b>C</b> K <b>G</b> I <b>P</b> M <b>G</b> F	64	7117	1
<i>Androctonus mauretanicus mauretanicus</i>						
KTX-1	$\alpha$ 3.1	P24662	GVEINVK <b>C</b> SGSPQ <b>C</b> LPK <b>C</b> KDAGMR <b>F</b> GG <b>K</b> CMNR <b>K</b> CH <b>C</b> TPK-NH <sub>2</sub>	38	4149	1, 2
AmP01	$\alpha$ 8.1	P56215	V <b>S</b> <b>C</b> ED <b>C</b> PEH <b>C</b> STQKAQAK <b>C</b> DNDK <b>C</b> V <b>C</b> EPI	29	3179	3
<i>Centruroides limpidus limpidus</i>						
CIIERG2	$\gamma$ 4.1	Q86QU9	DRDS <b>C</b> VDKSK <b>S</b> SKYGY <b>Y</b> Q <b>Q</b> DE <b>C</b> CKKAGDRAGN <b>C</b> VY <b>F</b> K <b>C</b> K <b>N</b> P	43	4832	5
<i>Centruroides noxius (Mexican scorpion)</i>						
NTX	$\alpha$ 2.1	P08815	TIINVK <b>T</b> SPK <b>Q</b> CSK <b>P</b> CKELYGSSAGAK <b>C</b> MNGK <b>C</b> K <b>C</b> YNN-NH <sub>2</sub>	39	4195	1, 2
CoTx1	$\alpha$ 10.1	O46028	AV <b>C</b> VYRT <b>C</b> DKD <b>C</b> KRRRGYRS <b>G</b> K <b>C</b> INNACK <b>C</b> YPY-NH <sub>2</sub>	32	3731	1, 3, 4
CnERG1	$\gamma$ 1.1	Q86QT3	DRDS <b>C</b> VDKSR <b>C</b> AKYGY <b>Y</b> Q <b>Q</b> DE <b>C</b> CKNAGHNGGT <b>C</b> MFF <b>K</b> C <b>K</b> CA	42	4730	5
CnERG2	$\gamma$ 3.1	P59939	GRDS <b>C</b> VNKSR <b>C</b> AKYGY <b>Y</b> S <b>Q</b> CEV <b>C</b> CKKAGHKGGT <b>C</b> DF <b>F</b> K <b>C</b> K <b>V</b>	43	4783	5
<i>Centruroides sculpturatus</i>						
CsERG5	$\gamma$ 5.1	Q86QU2	DRDS <b>C</b> VDKSR <b>C</b> AKYGY <b>Y</b> Q <b>Q</b> CEV <b>C</b> CKKAGHNGGT <b>C</b> MFF <b>K</b> CM <b>V</b> NS <b>K</b> M <b>N</b>	47	5263	*
<i>Hadrurus gertschi</i>						
Hge- $\beta$ -KTx	$\beta$ 2	Q0GY41	KSTVGQKLKKLNQAVDKVKEVLNKSEY <b>M</b> CPVSS <b>F</b> CK <b>Q</b> H <b>C</b> ARLGKSG <b>Q</b> CDLLE <b>C</b> IC <b>S</b>	58	6427	1
HgeScpl1	$\beta$ 3	Q0GY40	GWMSEKKVQ <b>G</b> ILD <b>K</b> KL <b>P</b> E <b>G</b> II <b>R</b> NA <b>A</b> K <b>A</b> IV <b>H</b> K <b>M</b> AK <b>N</b> Q <b>F</b> <b>G</b> <b>C</b> FANVDVK <b>G</b> D <b>C</b> K <b>R</b> H <b>C</b> K <b>A</b> ED <b>K</b> E <b>G</b> I <b>C</b> H <b>G</b> T <b>K</b> <b>C</b> K <b>C</b> G <b>V</b> P <b>I</b> S <b>Y</b> L	76	8369	1
Hg1	$\delta$ 1.1	P0C8W3	GHHNRV <b>N</b> CL <b>L</b> PP <b>K</b> T <b>G</b> P <b>C</b> K <b>G</b> S <b>F</b> AR <b>Y</b> Y <b>F</b> DI <b>E</b> T <b>G</b> S <b>C</b> K <b>A</b> F <b>I</b> Y <b>G</b> <b>C</b> EGNS <b>N</b> N <b>F</b> SE <b>K</b> H <b>H</b> CE <b>K</b> R <b>C</b> R <b>G</b> F <b>R</b> K <b>F</b> G <b>G</b> K	67	7539	1
<i>Heterometrus fulvipes</i>						
$\kappa$ -HfTx1	$\kappa$ 1.1	P82850	GH <b>A</b> C <b>Y</b> R <b>N</b> C <b>W</b> REGNDEET <b>C</b> K <b>E</b> R <b>C</b> -NH <sub>2</sub>	22	2655	1
<i>Heterometrus laoticus</i>						
HelaTx1	$\kappa$ 5.1	P0DJ41	S <b>C</b> K <b>K</b> E <b>C</b> SGSRRT <b>K</b> K <b>C</b> M <b>Q</b> K <b>C</b> NREHGH-NH <sub>2</sub>	25	2917	1
<i>Heterometrus petersii</i>						
HSP040C.1	$\kappa$ 3.1	P0DJ36	Q <b>W</b> I <b>N</b> A <b>C</b> F <b>N</b> V <b>C</b> M <b>K</b> I <b>S</b> SD <b>K</b> K <b>Y</b> <b>C</b> K <b>Y</b> L <b>C</b> G <b>K</b> N	27	3185	1

Table 8. (Contd.)

Toxin	Subfamily	ID	Amino acid sequence	A.a.	Mm	Activity
HSP040C.2	κ4.1	P0DJ40	DIPCFETCMKLYHIPKLCYIKCRKH	25	3080	1
<i>Leiurus quinquestriatus hebraeus</i>						
ChTX	α1.1	P13487	ZFTNVSC <sup>T</sup> TTSKEC <sup>W</sup> SVQC <sup>R</sup> RLHNTSRGK <sup>C</sup> MNKK <sup>C</sup> RCYS	37	4296	1
SeyTx	α5.1	P16341	AFCNLRMC <sup>Q</sup> LS <sup>C</sup> RSLGLLGK <sup>C</sup> IGDK <sup>C</sup> EVKH-NH <sub>2</sub>	31	3423	3
<i>Lychas mucronatus</i>						
LmKTx2	α29.1	D9U2A6	EGDC <sup>P</sup> ISEA <sup>I</sup> K <sup>C</sup> VEK <sup>C</sup> KEK <sup>V</sup> VEV <sup>C</sup> EPGV <sup>C</sup> K <sup>C</sup> SG	32	3395	1
LmKTT-la	δ2.1	P0DJ46	KKK <sup>C</sup> QLPSDVGK <sup>G</sup> KAS <sup>F</sup> TRY <sup>Y</sup> YNEES <sup>G</sup> K <sup>C</sup> ET <sup>F</sup> IYGGV <sup>G</sup> NSN <sup>N</sup> FLTKED <sup>C</sup> CRE <sup>C</sup> AQ <sup>G</sup> SC	59	6495	1
<i>Mesobuthus eupeus</i>						
BeKm-1	γ2.1	Q9BKB7	RPTDIK <sup>C</sup> SESY <sup>Q</sup> CF <sup>P</sup> V <sup>C</sup> KSR <sup>F</sup> GK <sup>T</sup> NGR <sup>C</sup> VNG <sup>F</sup> CD <sup>C</sup> F	36	4092	5
λ-MeuTx	λ	P86399	G <sup>C</sup> NRLN <sup>K</sup> K <sup>C</sup> NSDAD <sup>C</sup> CR <sup>Y</sup> GER <sup>C</sup> I <sup>S</sup> TGV <sup>N</sup> YY <sup>C</sup> RPDF <sup>G</sup> P	37	4174	—
<i>Mesobuthus martensii</i>						
BmKK6	α9.1	Q9NJP7	VG <sup>C</sup> EE <sup>C</sup> PM <sup>H</sup> CK <sup>G</sup> KNAP <sup>T</sup> CD <sup>D</sup> DG <sup>V</sup> C <sup>N</sup> C <sup>N</sup> V	28	2949	1, 3
BmKK1	α14.1	Q967F9	TP <sup>F</sup> AI <sup>K</sup> CAT <sup>D</sup> DAD <sup>C</sup> SR <sup>K</sup> C <sup>P</sup> GN <sup>P</sup> SCR <sup>N</sup> GF <sup>C</sup> ACT	31	3233	*
BmKK4	α17.1	Q95NJ8	ZT <sup>Q</sup> C <sup>Q</sup> S <sup>V</sup> RD <sup>C</sup> Q <sup>Q</sup> Y <sup>C</sup> L <sup>T</sup> P <sup>D</sup> R <sup>C</sup> S <sup>Y</sup> GT <sup>C</sup> Y <sup>C</sup> K <sup>T</sup> T-NH <sub>2</sub>	30	3460	—
BmBKTx1	α19.1	P83407	AAC <sup>Y</sup> SSD <sup>C</sup> RV <sup>K</sup> CVAM <sup>G</sup> FSS <sup>G</sup> GK <sup>C</sup> INS <sup>K</sup> CK <sup>C</sup> YK	31	3336	2
Kcugx	α22.1	Q8MUB1	EVD <sup>G</sup> RTAT <sup>F</sup> CT <sup>Q</sup> SI <sup>C</sup> EE <sup>S</sup> CK <sup>R</sup> Q <sup>N</sup> KNGR <sup>C</sup> VIEA <sup>E</sup> GS <sup>L</sup> IYHL <sup>C</sup> K <sup>C</sup> Y	44	4982	*
BmK86	α26.1	A7KJJ7	NFK <sup>V</sup> EGAC <sup>S</sup> K <sup>P</sup> CR <sup>K</sup> Y <sup>C</sup> ID <sup>K</sup> GAR <sup>N</sup> GK <sup>C</sup> INGR <sup>C</sup> H <sup>C</sup> Y <sup>Y</sup>	35	3981	1
BmKTT-2	δ3.1	P0DJ50	VD <sup>C</sup> TLP <sup>S</sup> DTGR <sup>C</sup> KAY <sup>F</sup> IR <sup>Y</sup> F <sup>Y</sup> NQ <sup>K</sup> AGE <sup>C</sup> Q <sup>K</sup> FV <sup>Y</sup> GG <sup>C</sup> EG <sup>N</sup> SN <sup>N</sup> FLTKSD <sup>C</sup> CK <sup>Q</sup> CSP <sup>G</sup> K <sup>C</sup>	58	6470	1
<i>Mesobuthus tamulus</i>						
TmTX	α16.1	P0C173	DLIDV <sup>K</sup> CISS <sup>Q</sup> EC <sup>W</sup> IA <sup>C</sup> CK <sup>K</sup> V <sup>T</sup> GR <sup>F</sup> E <sup>G</sup> K <sup>Q</sup> NR <sup>Q</sup> CR <sup>C</sup> Y	36	4207	1
<i>Opisthacanthus madagascariensis</i>						
OmTx1	κ2.1	P0C1Z3	DPCYEV <sup>C</sup> LQ <sup>Q</sup> HGN <sup>V</sup> KE <sup>C</sup> E <sup>E</sup> ACK <sup>H</sup> P <sup>V</sup> E	26	2984	1
<i>Pandinus imperator</i>						
Pi1	α6.1	Q10726	LVK <sup>C</sup> RG <sup>T</sup> SD <sup>C</sup> GR <sup>P</sup> C <sup>Q</sup> Q <sup>T</sup> GT <sup>G</sup> CP <sup>N</sup> SK <sup>C</sup> IN <sup>R</sup> M <sup>C</sup> K <sup>C</sup> Y <sup>G</sup> C	35	3835	1, 3
Pi2	α7.1	P55927	TIS <sup>C</sup> TNP <sup>K</sup> Q <sup>C</sup> YP <sup>H</sup> CK <sup>K</sup> ET <sup>G</sup> YP <sup>N</sup> AK <sup>C</sup> M <sup>N</sup> R <sup>K</sup> CK <sup>C</sup> CF <sup>G</sup> R	35	4034	1
<i>Parabuthus villosus</i>						
PBTx1	α11.1	P60164	DEEP <sup>K</sup> ES <sup>C</sup> SDEM <sup>C</sup> VI <sup>Y</sup> CK <sup>G</sup> E <sup>E</sup> Y <sup>S</sup> TG <sup>V</sup> CD <sup>G</sup> PQ <sup>K</sup> CK <sup>C</sup> SD	37	4090	1

Table 8. (Contd.)

Toxin	Subfamily	ID	Amino acid sequence	A.a.	Mm	Activity
<i>Tityus obscurus</i>						
To1	$\alpha$ 13.1	P83243	ACG <b>S</b> CR <b>KK</b> CKG <b>S</b> GK <b>C</b> INGR <b>C</b> K <b>C</b> Y	23	2448	1
To32	$\alpha$ 18.1	P60211	TGPQ <b>T</b> T <b>C</b> QAAM <b>C</b> EAG <b>C</b> KGLGK <b>S</b> ME <b>S</b> CQGD <b>T</b> C <b>K</b> KA	35	3522	1
<i>Tityus serrulatus</i>						
Tityustoxin	$\alpha$ 4.1	P46114	VFINAKCRGS <b>P</b> E <b>C</b> LPK <b>C</b> KEAIGKAAG <b>K</b> CMNG <b>K</b> C <b>K</b> C <b>P</b> Y	37	3942	1, 2
BuTX	$\alpha$ 12.1	P59936	WCST <b>C</b> LDLACGASRE <b>C</b> YD <b>P</b> CFKAFGRAHG <b>K</b> CMNN <b>K</b> CR <b>C</b> Y <b>T</b>	40	4506	1
Ts15	$\alpha$ 21.1	P86270	GKFG <b>K</b> CP <b>N</b> I <b>C</b> AK <b>T</b> C <b>Q</b> TEKGK <b>G</b> MG <b>Y</b> C <b>N</b> K <b>T</b> E <b>C</b> V <b>C</b> SE <b>W</b>	36	3956	1
<i>Tityus trivittatus</i>						
Tt28	$\alpha$ 20.1	P0C183	GCT <b>P</b> E <b>Y</b> CS <b>M</b> W <b>C</b> KV <b>K</b> VSQ <b>N</b> Y <b>C</b> V <b>K</b> N <b>C</b> K <b>C</b> P <b>G</b> R	29	3310	1
$\kappa$ -BUTX-Tt2b	$\kappa$	B3A0L5	G <b>C</b> M <b>P</b> E <b>Y</b> CAG <b>Q</b> CRG <b>K</b> VS <b>Q</b> D <b>Y</b> C <b>L</b> K <b>N</b> C <b>R</b> C <b>I</b> R	28	3180	1
<i>Vaejovis mexicanus smithi</i>						
Vm24	$\alpha$ 23.1	P0DJ31	AAA <b>I</b> S <b>C</b> VGS <b>P</b> E <b>C</b> PP <b>K</b> CR <b>A</b> Q <b>G</b> C <b>K</b> NG <b>K</b> CMNR <b>K</b> C <b>K</b> Y <b>C</b> -NH <sub>2</sub>	36	3864	1, 3

Notes: The table shows one representative of each subfamily.  $\alpha$ ,  $\beta$ , and  $\gamma$ , toxins with CS $\alpha$ / $\beta$ -fold;  $\kappa$ , toxins with CS $\alpha$ / $\alpha$  fold with two or three disulfide bonds;  $\delta$ , peptides with Kunitz-type fold;  $\lambda$ , peptides with ICK fold. Activity toward: 1, K<sub>v</sub> channels (except K<sub>v</sub>11 channels); 2, BK<sub>Ca</sub> channels; 3, SK<sub>Ca</sub> channels; 4, IK<sub>Ca</sub> channels; 5, K<sub>v</sub>11 channels; —, no data available; \*, amino acid sequence was determined from cDNA sequencing, no data on the activity available. Posttranslational modifications: Z, pyroglutamate residue, -NH<sub>2</sub>, amidation; a.a., amino acids; Mm, molecular mass. Cysteine residues are shown in bold.

by three antiparallel  $\beta$ -strands, and a  $3_{10}$ -helix [197]. Two disulfide bonds (C<sup>1</sup>-C<sup>4</sup> and C<sup>2</sup>-C<sup>5</sup>) and atoms of the polypeptide chain form a ring that is penetrated by the third disulfide bond C<sup>3</sup>-C<sup>6</sup> (Fig. 15). In addition to weak K<sub>v</sub> channel-blocking properties, toxins of this group also affect the activity of Ca<sup>2+</sup> channels [198].

#### Variety of K<sup>+</sup> channel blockers from scorpion venom.

As mentioned above, ~250 KTx have been identified in scorpions. Table 8 shows one representative of each KTx subfamily (the toxins are placed under the Latin names of the corresponding scorpion species, and the species names are arranged alphabetically). All K<sup>+</sup> channels ligands from scorpion venom are pore blockers that act at concentrations ranging from pM to  $\mu$ M and above. The most active K<sub>v</sub>1.3 channel blocker Vm24 from scorpion *Vaejovis mexicanus smithi* is characterized by complex dissociation constant ( $K_d$ ) of 2.9 pM [199]. Some of the toxins selectively inhibit specific isoforms of K<sub>v</sub> (e.g. BeKm-1 and OSK2) [188, 200] or BK<sub>Ca</sub> channels (IbTX) [163]. No selective blockers for the K<sub>ir</sub> and K<sub>2P</sub> channels have been identified in scorpion venom so far [53].

**KTx interaction with K<sup>+</sup> channels.** K<sup>+</sup> channel ligands can be pore blockers or modulators (see above). Interestingly, unlike in spiders, K<sup>+</sup> channel modulators are not typical for scorpion venoms; all KTx are pore blockers.

Since the known KTx predominantly affect K<sub>v</sub> channels and, to a lesser extent, BK<sub>Ca</sub> and SK<sub>Ca</sub> channels, and the most active peptides of this group have CS $\alpha$ / $\beta$  fold, the model for the interaction of the toxin with the channel (ligand–receptor binding) was based on data obtained for this type of toxins [201–203].

Early studies of  $\alpha$ -KTx suggested that the channel pore region is highly conserved and has a similar structure in all groups of K<sup>+</sup> channels [204]. Later, this hypothesis received robust experimental confirmation: first, spatial structures of several channels were resolved [22, 205, 206]; second, a hybrid channel based on the K<sup>+</sup> channel KscA from the bacterium *Streptomyces lividans* was obtained, and it was blocked by some  $\alpha$ -KTx (the wild-type channels are insensitive to the toxins) [207, 208].

Some of the toxins were found to interact with the selectivity filter in the pore region of K<sub>v</sub> and BK<sub>Ca</sub> chan-

nels via the  $\beta$ -hairpin amino acid residues [209-211]. Other blockers interact with the lower part of the SK<sub>Ca</sub> channel vestibule via amino acid residues of the  $\alpha$ -helix, but not of the  $\beta$ -hairpin [212-214]. Moreover, some  $\gamma$ -KTx interact with K<sub>v</sub>11 channels by binding to a site located at a considerable distance from the selectivity filter due to the presence of elongated extracellular loops in the channel molecule [215, 216].

Such broad structural diversity of peptides capable of interacting with K<sup>+</sup> channels suggests existence of key molecular determinants responsible for the ligand binding to the receptor. Several studies demonstrated that mutations of certain amino acid residues in both the channels and the toxins could considerably change KTx affinity and selectivity.

Many KTx contain the so-called “functional dyad” [217] that is involved in ligand–receptor interaction and is responsible for K<sup>+</sup> flux blockade. This dyad is composed of two highly conserved amino acid residues, the first being lysine, and the second being tyrosine, phenylalanine [213, 217], or leucine [218]. According to the functional dyad model, the lysine side chain enters the pore, where a ring of negatively charged aspartate or glutamate residues surrounds it (each aspartate or glutamate residue belonging to one of the four  $\alpha$ -subunits of the K<sup>+</sup> channel tetramer). Substitution of this lysine residue with another amino acid considerably decreases toxin affinity [219, 220]. The aromatic or aliphatic residue of the dyad might interact with a tyrosine or tryptophan residue of one of the channel  $\alpha$ -subunits [221-223].

It should be noted that this model is far from a comprehensive description of the ligand–receptor interactions. Thus, some of the toxins lack the dyad but display high affinity toward specific K<sup>+</sup> channel isoforms [224]. Moreover, a new model has been proposed that describes the importance of the “functional triad” (earlier identified Lys residue and two additional hydrophobic residues) for the toxin binding with the pore [225].

An alternative model of the “ring of basic residues” was suggested based on the results of molecular docking studies. Such ring is present in many K<sub>v</sub> channel blockers, and its positively charged residues form salt bridges with

the negatively charged residues of the channel  $\alpha$ -subunit. In some toxins, the ring is formed by four lysine or arginine residues, but this number may vary in other toxins [221, 222].

The observed differences in the selectivity of various KTx toward K<sup>+</sup> channel isoforms imply that neither the “functional dyad” nor the “ring of basic residues” model can comprehensively describe unique toxin–channel interactions. More and more data have been published that show that the determining role in the toxin binding to the channel does not belong exclusively to the residues of the dyad or the ring. For example, asparagine residue Asn30 of AgTx2 was found essential for toxin–channel complex stabilization [167]. Therefore, a third model was proposed that emphasizes the necessity for creation of functional maps of “complementary” surfaces of toxins and channels involved in close contact. In this model, each residue is given its “coefficient of importance”. Obviously, the dyad residues will have the maximum coefficient values [226].

The three-dimensional structure of ChTX complex with a chimeric K<sub>v</sub>1.2-K<sub>v</sub>2.1 channel has been resolved. It demonstrated that the interaction of the toxin with the channel does not considerably change the structure of the channel selectivity filter (Fig. 16), and it confirmed the important role of functional dyad lysine residue (Lys27) in the interaction. Lys27 is surrounded by four carbonyl groups of tyrosine residues of four channel  $\alpha$ -subunits. The  $\alpha$ -helical part of the toxin molecule faces away, while the part of the toxin formed by a fragment of the  $\beta$ -hairpin faces toward the pore. A tyrosine residue (Tyr36) is positioned to pack simultaneously against Asp375 and Val377 of one subunit, and Met29 is able to pack against Asp375 of an adjacent subunit. The side chain of Arg25 is within close proximity of the channel Gln353, and the peptide backbone near Thr8-Thr9 is held against Gln353 of another subunit. There are also residues that might be involved in long-range electrostatic interactions: Arg34 and Arg25 of the toxin electrostatically interact with Asp375 and Asp359 of the channel, respectively. Asn30 is another residue that appears to be close enough to Asp375 to enable an H-bonding interaction.



Fig. 14. Disulfide bond formation pattern in K<sup>+</sup> channel blockers with the Kunitz-type fold.



Fig. 15. Disulfide bond formation pattern in KTx with the ICK fold ( $\lambda$ -MeuTx).



The resolved structure of the ChTX complex with  $K_v1.2$ - $K_v2.1$  confirmed the earlier proposed models and explained the importance of certain residues (e.g. Lys27) in the interaction of the toxin with the channel. However, some questions remain that require further study. Thus, Tyr36 seems to participate in the complex formation due to the presence of its hydroxyl group and to the large size of its residue, but not because of its hydrophobic nature, as had been previously believed. To understand the molecular mechanisms of toxin selectivity, future studies should aim at resolving crystal structures of toxin complexes with channels from different families ( $K_v$ ,  $BK_{Ca}$ , and  $SK_{Ca}$ ).

#### PRACTICAL APPLICATIONS OF POLYPEPTIDE LIGANDS OF $K^+$ CHANNELS

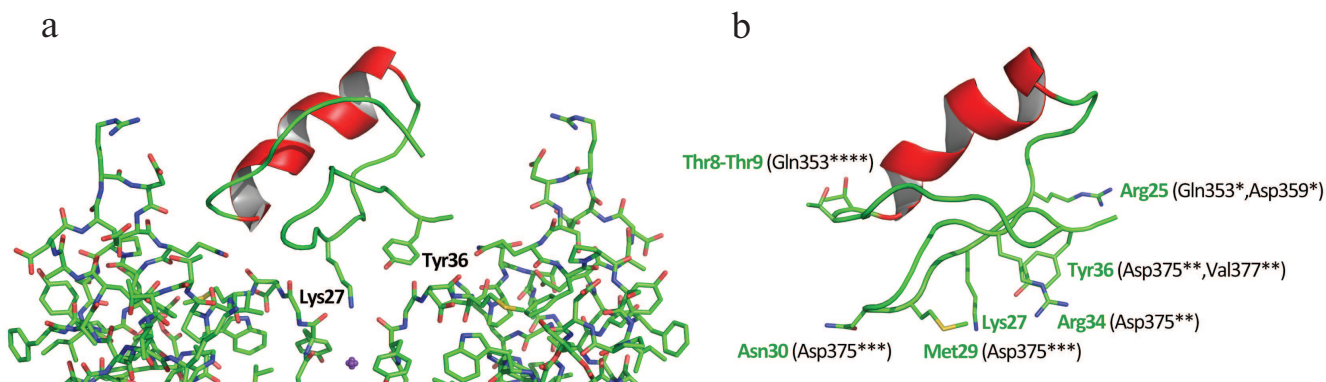
$K^+$  channel polypeptide ligands from living organisms have always played an important role in the investigations of  $K^+$  channels. Early studies used toxins to purify the channels. Later, radioactive and fluorescently

labeled toxin analogs were widely applied as molecular tools for investigating pharmacological properties of  $K^+$  channels and their localization [227, 228].

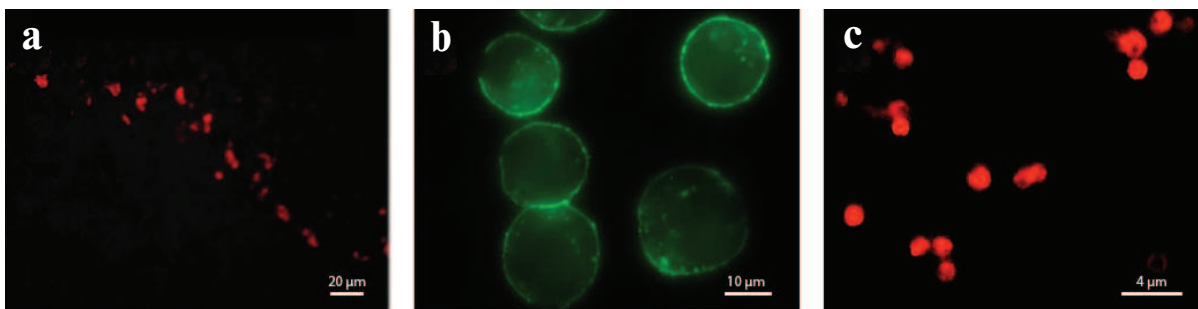
The role of  $K^+$  channels in cell excitability has been extensively studied; however, the importance of these channels in cell migration, proliferation, and angiogenesis has been demonstrated relatively recently [229]. The functional diversity and wide occurrence of  $K^+$  channels make this superfamily of membrane proteins an important pharmacological target in the development of new drugs (including polypeptides) [230].

#### Radioactive and fluorescently labeled toxin analogs.

Toxins labeled with radioactive iodine  $^{125}I$  on Tyr/His residues [231, 232] or with tritium  $^3H$  [233] have been widely applied for pharmacological profiling of  $K^+$  channels, channel purification from tissues, and investigation of channel distribution and subunit composition [227, 234–236]. However, the use of radioactive labeled toxins poses a number of serious problems, such as difficulties in the utilization of radioactive waste, specific working conditions, and high cost of experiments [237].



**Fig. 16.** a) Spatial structure of chimeric  $K_v1.2$ - $K_v2.1$  channel complex with ChTX (PDB ID: 4JTA). The key lysine residue Lys27 and the aromatic residue (Tyr36) of the dyad are shown. The side chain of Lys27 enters the channel pore. b) Amino acid residues of ChTX that interact with the channel residues (in parentheses). Asterisks indicate to which one of the four  $\alpha$ -subunits the residues belong.



**Fig. 17.**  $K^+$  channel staining with fluorescently labeled toxins and their analogs. a) Rat cerebellum  $K_v$  channels stained with  $HgTx_1$ -A19C-Alexa546 (from [239] with kind permission from Springer). b) HEK293 cells expressing  $BK_{Ca}$  channels treated with biotinylated IbTX and visualized with streptavidin-Alexa488 (from [240] with permission from Springer). c) Hybrid  $KcsA$ - $K_v1.3$  channels on the surface of spheroplasts [241] (with kind permission from K. S. Kudryashova).

In recent years, fluorescently labeled ligands have been increasingly used in K<sup>+</sup> channel studies (Fig. 17). Most of them are synthesized by covalent attachment of fluorescent organic molecules to the amino groups of toxins (*N*-terminal or lysine side chain) [238] or to a cysteine residue introduced into the toxin sequence [239]. In addition, biotinylated derivatives of toxins are used in combination with fluorescently labeled streptavidin [240]. The most common fluorescent labels for toxins are Cy3, Cy5, Alexa488, Alexa546, and TAMRA [237].

Fluorescent toxin derivatives have been used to demonstrate the ligand–receptor interactions at the monomolecular level [83] and to identify subpopulations of cells expressing specific K<sub>v</sub> isoforms by flow cytometry [82].

**K<sup>+</sup> channels as targets in treatment of various pathologies.** Ion channels form the third largest group (after G protein-coupled receptors and kinases) of proteins involved in signal transduction in human cells [51]. At present, ~13% of all medicinal compounds sold on the pharmaceutical market affect ion channels [242], which puts these membrane proteins in second place of the most widely used pharmacological targets after G protein-coupled receptors [243]. Since K<sup>+</sup> channels (78 genes in humans) are the largest superfamily of ion channels, it is not surprising that they are involved in pathogenesis of various diseases and, therefore, can be promising pharmacological targets [244].

The involvement of K<sup>+</sup> channels in autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, psoriasis, and type I diabetes, has been extensively studied [245–247]. One of the major roles in the development of these disorders belongs to effector T-lymphocytes. T-lymphocyte proliferation and activation, as well as interleukin production by these cells, require high Ca<sup>2+</sup> concentrations in the cytoplasm, which are achieved through the functioning of Ca<sup>2+</sup> channels [248]. Low membrane potential is maintained by release of K<sup>+</sup> via homotetrameric K<sub>v</sub>1.3 and IK<sub>Ca</sub> channels [249–251]. During the development of an autoimmune disorder, the biochemical profile of T-lymphocytes undergoes drastic changes that include hyperexpression of K<sub>v</sub>1.3 channels [245]. Inhibition of K<sup>+</sup> channels prevents K<sup>+</sup> efflux from the cells, which, in turn, abolishes Ca<sup>2+</sup> entry into the cytoplasm and suppresses proliferation of the effector T-lymphocytes and interleukin biosynthesis [252]. The functional activity of these cells is predominantly supported by K<sub>v</sub>1.3 channels; therefore, inhibition of this isoform may be an efficient approach to autoimmune disease therapy. The membrane potential of other types of lymphocytes is maintained mostly by IK<sub>Ca</sub> channels, and the functioning of those cells will not be affected [253].

It has long been observed that scorpion bites inhibit the development of multiple sclerosis [254]. Elucidation of molecular mechanism of this disease allowed proposing the use of K<sup>+</sup> channel blockers from animal venoms as

therapeutic agents in multiple sclerosis [230]. The most progress has been achieved in the development of a K<sub>v</sub>1.3 channel blocker based on the ShK toxin from the venom of the sea anemone *Stichodactyla helianthus* (Fig. 9b) [95, 255]. A number of improved toxin derivatives have been synthesized [256], and one of them, ShK-186, has already passed stage I of clinical trials [257]. Scorpion toxins MgTX, OSK1, ChTX, and their derivatives were found to be promising candidates for the treatment of autoimmune disease in model systems [258–260].

Recently, an important role of K<sub>v</sub>1.3 in asthma pathogenesis has been demonstrated [261]. Channel inhibition with ShK-186 provided an efficient treatment of the disease in model experiments [262]. In addition, combined inhibition of K<sub>v</sub>1.3 and IK<sub>Ca</sub> could be an approach to preventing tissue rejection in organ transplantation [263].

About 10 different types of K<sup>+</sup> channels are known to be involved in the development of cardiovascular disorders. K<sub>ir</sub>2.1, K<sub>ir</sub>3.4, K<sub>2p</sub>17.1, K<sub>v</sub>1.5, K<sub>v</sub>4.3, K<sub>v</sub>7.1, and K<sub>v</sub>11.1 channels [264] were found to be major players in some types of arrhythmias (atrial fibrillation, Andersen syndrome and long or short QT syndromes) [264]. In most cases, the dysfunction of a particular channel (channelopathy) results from mutations in the corresponding gene [265]. Therefore, selective modulators of these channels (e.g. BeKm-1) may be promising therapeutic agents in cardiovascular diseases [188].

As mentioned above, K<sup>+</sup> channels are the major molecules responsible for the generation of the action potential and nerve signal transmission (see introductory part). Disturbances in the normal functioning of these membrane proteins are related to numerous neurophysiological disorders, such as seizures, epilepsy, and autism [266–268]. Similarly to arrhythmia, the reasons for abnormal channel activity can be mutations in the channel-encoding genes (e.g. neuronal K<sub>v</sub>1.1, K<sub>v</sub>1.2, K<sub>v</sub>4.2, K<sub>v</sub>7.2, and K<sub>v</sub>7.3 channels). Therapy of neurophysiological disorders uses mostly low molecular mass K<sup>+</sup> channels ligands [51, 53].

Abnormal expression of K<sup>+</sup> channels has been described in many types of tumor cells [269]. Some isoforms (K<sub>ir</sub>1.1, K<sub>ir</sub>3.4, K<sub>2p</sub>5.1, K<sub>2p</sub>9.1, K<sub>v</sub>1.1, K<sub>v</sub>1.3, K<sub>v</sub>1.5, K<sub>v</sub>10.1, K<sub>v</sub>10.2, K<sub>v</sub>11.1, K<sub>Ca</sub>1.1, K<sub>Ca</sub>2.3, and K<sub>Ca</sub>3.1) play an important role in cancer pathogenesis and, therefore, can be considered as targets for treatment of oncological diseases [229]. An even broader array of K<sup>+</sup> channels is involved the chronic pain-associated processes [270], which make K<sup>+</sup> channel activators promising candidates as analgesics [271].

## CONCLUSION

K<sup>+</sup> channels are a superfamily of integral membrane proteins responsible for passive transport of K<sup>+</sup> across the cell membrane. Similarly to other ion channels, the

activity of K<sup>+</sup> channels can be modulated by various ligands. All ligands of K<sup>+</sup> channels can be classified into several groups, the three major groups being metal ions, small organic molecules, and polypeptide toxins. In addition, specific antibodies have been obtained against many of K<sup>+</sup> channel isoforms. K<sup>+</sup> channel ligands can be pore blockers or modulators.

About 400 polypeptide ligands of K<sup>+</sup> channels have been identified in animal venoms; ~250 of them are scorpion venom toxins (KTx). All KTx are pore blockers that can be subdivided into five groups according to their structural and functional properties. Some of the toxins (such as ChTX, BeKm-1, OSK1, and apamin) have become classical molecular tools in studies of K<sup>+</sup> channels. In recent years, K<sup>+</sup> channels have been considered promising pharmacological targets in the therapy of autoimmune, respiratory, and neurodegenerative diseases, and K<sup>+</sup> channel ligands have emerged as potential agents for treating these disorders.

Every year, new polypeptide ligands of K<sup>+</sup> channels are added to the UniProt database because of high scientific interest in this type of compounds. However, further studies should focus not only on identification and purification of these molecules, but on their detailed structural and physiological characterization.

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