Sea Anemone Peptides with a Specific Blocking Activity against the Fast Inactivating Potassium Channel Kv3.4*

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Sylvie Diochot‡, Hugues Schweitz‡, László Béress‡, and Michel Lazdunski††

From the 2Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France and the 3Klinikum der Christian-Albrechts-Universität, Abteilung Toxikologie, Brunswiker Strasse 10, 2300 Kiel, Federal Republic of Germany

Sea anemone venom is known to contain toxins that are active on voltage-sensitive Na⁺ channels, as well as on delayed rectifier K⁺ channels belonging to the Kv1 family. This report describes the properties of a new set of peptides from Anemonia sulcata that act as blockers of a specific member of the Kv3 potassium channel family. These toxins, blood depressing substance (BDS)-I and BDS-II, are 43 amino acids long and differ at only two positions. They share no sequence homologies with other K⁺ channel toxins from sea anemones, such as AsKS, AsKC, ShK, or BgK. In COS-transfected cells, the Kv3.4 current was inhibited in a reversible manner by BDS-I, with an IC₅₀ value of 47 nM. This inhibition is specific because BDS-I failed to block other K⁺ channels in the Kv1, Kv2, Kv3, and Kv4 subfamilies. Inward rectifier K⁺ channels are also insensitive to BDS-I. BDS-I and BDS-II share the same binding site on brain synaptosomes, with K₅₀ values of 12 and 19 nM, respectively. We observed that BDS-I and BDS-II have some sequence homologies with other sea anemone Na⁺ channel toxins, such as AsI, AsII, and AsX. However, they had a weak effect on tetrodotoxin-sensitive Na⁺ channels in neuroblastoma cells and no effect on Na⁺ channels in cardiac and skeletal muscle cells. BDS-I and BDS-II are the first specific blockers identified so far for the rapidly inactivating Kv3.4 channel.

**EXPERIMENTAL PROCEDURES**

**Purification of a Blocker for the Kv3.4 Channel**—The purification started with a previously described chromatographic procedure (8) that had been used by us for the purification of other peptides with K⁺ channel blocking activity from the crude toxic material extracted from the sea anemone A. sulcata. Fraction 28 (AsF28) (8) eluted at 500 mM ammonium acetate. We found that it displayed a blocking activity against the K⁺ current expressed by Xenopus oocytes injected with the Kv3.4 channel. This fraction was then chromatographed on a cation exchange column TSK/SP 5PW (7.5 × 75 mm) (Toyosoda, Tokyo, Japan) with 1% acetic acid as solvent A and 1 M ammonium acetate as solvent B. The AsF28 fraction (10 mg) was loaded onto the column equilibrated with 30% solvent B. An inactive material eluted for 50 min, and then three peaks were eluted in a gradient from 30 to 100% solvent B in 20 min. The last two peaks contained the Kv3.4 potassium channel blocking activity. They were pooled and loaded on a Hypersil BDS column (4.6 × 250 mm), C18, 5 µm particle diameter (Shandon). Elution was performed with a linear gradient from 10 min from 10 to 50% acetonitrile in water complemented with 0.1% trifluoroacetic acid. Activity was found in the third peak, which was both the sharpest and the highest.

**Peptide Sequencing**—The primary structure of the peptide was determined by Edman degradation of the complete peptide using an Applied Biosystems model 477 A microsequencer. The native peptide was reduced with 2-mercaptoethanol and pyridylethylated with 4-vinylpyridine before sequencing.

**Membrane Preparations**—Rat brain synaptosomal membranes were prepared as described by Jones and Matus (33).

**Iodination of BDS-I**—BDS-I was iodinated by the IODO-GEN method (Pierce). Three nanomoles were mixed with 0.5 nM of ¹²⁵I-

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† To whom correspondence should be addressed: Tel.: 33-0-4-93-95-77-02 or 33-0-4-93-95-77-03; Fax: 33 0 4 93 95 77 04; E-mail: ipmc@unice.fr.

The abbreviations used are: MCD, mast cell degranulating; BDS, blood depressing substance; DTX, dendrotoxin; ChTX, charybdotoxin; AsKS, kaliseptine; AsKC, kalichodicine; AsI, toxin I from A. sulcata; AsX, toxin I from A. xanthogrammica.
labeled sodium (2000 Ci/mmol; 1 Ci = 37 GBq; NEN Life Science Products) and buffered at pH 7.5 with 100 mM Tris-Cl in an Eppendorf tube coated with 1 nM of IODO-GEN (total volume, 40 μl). After a 15-min incubation, free iodine was quenched with 500 μl of bovine serum albumin in 0.1% TFA (solvent A). 125-I-BDS-I was separated from native BDS-I by high performance liquid chromatography on a Spheri- sorb ODS1 5 μm (250 × 4.1-mm) Intercolumn column (Interchrom, Asnières, France) at 1 ml/min with a gradient from 20 to 50% solvent B in 60 min, where solvent B was 0.1% TFA in acetonitrile. 125-I-BDS-I eluted between 50 and 55 min, after native BDS-I. Fractions (1 ml) were neutralized and stabilized by addition of 100 μl of a mixture containing bovine serum albumin (10 mg/ml) and water in 1 ml Tris, pH 7.4. They were aliquoted and kept frozen at −80 °C.

**Binding to Membranes**—Protein concentrations were determined by the Bio-Rad assay with bovine serum albumin used as a standard. The buffer used for incubations contained 50 mM Tris-HCl, pH 7.4, 140 mM NaCl, 5 mM KCl, and 1.3 mM MgSO4, 1 mg/ml of bovine serum albumin and 0.2 mg/ml of sodium azide as a bacteriostatic. For the dose-response curves, series of predilutions (10 times as concentrated as that in the incubation medium) were done with pure water in low protein absorption tubes.

Incubations were carried out for 3 h at room temperature, and then the samples were diluted with 2 ml of cold washing buffer (100 mM NaCl, 20 mM Tris-HCl, pH 7.4) and filtered on Whatman GF/C filters that were eluted with 0.5% ethyleneimine at pH 7.4 and washed with 5 ml of washing buffer before use. The samples were washed twice with 5 ml of washing buffer, and the radioactivity retained on the filters was counted.

**Contraction Measurements on Isolated Organs**—Cardiac muscle contraction measurements were carried out as described elsewhere (34). The following medium was used for atria: 127 mM NaCl, 4 mM KCl, 0.5 mM NaH2PO4, 1 mM MgSO4, 1.8 mM CaCl2, 12 mM NaHCO3, 5 mM glucose. Left atria were stimulated by an electric field of 12 V/cm. Stimulation duration and frequency were 2 ms and 1 Hz, respectively.

**Action Potential Measurements**—Action potentials were recorded from primary cultures of thigh muscle of newborn rats (myotubes and myosacs) using a single microelectrode technique as described in Ref. 21.

**Electrophysiological Measurement in Xenopus Oocytes**—Cloning of cDNA and synthesis of complementary RNA have been previously described (27).

**Xenopus laevis** specimens were purchased from Centre de Recherches en Biochimie Macromoléculaire (CRBM) (Montpellier, France). Pieces of the ovary were surgically removed, and individual oocytes were dissected away in a saline solution containing (in mM): 150 NaCl, 2 mM KCl, 1.8 mM CaCl2, and 2 mM HEPES at pH 7.4 with NaOH. Stage V and VI oocytes were treated for 2 h with collagenase (1 mg/ml, type Ia, Sigma) in ND96 to discard follicular cells. cRNA solutions were injected (50–100 ng/μl, 50 nl/oocyte) using a pressure microinjector. This laboratory has previously shown that a high level of expression of some cloned K+ channels could lead to high magnitude K+ currents with major variations in their kinetics, pharmacological sensitivities, and voltage-dependences as compared with currents of lower intensity (29, 31). Therefore, particular attention was given to comparisons of currents of similar and relatively low intensities (below 3 μA for a test pulse at ±30 mV).

The oocytes were kept at 19 °C in the ND96 saline solution supplemented with gentamycin (5 μg/ml). Oocytes were studied within 2–4 days following injection of cRNA. In a 0.3-ml perfusion chamber, a single oocyte was impaled with two standard glass microelectrodes (1–2.5 MΩ resistance) filled with 3 M KCl and maintained under voltage-clamp using a Dagan TEV 200 amplifier. Stimulation of the preparation, data acquisition and analysis were performed using pClamp software (Axon Instruments). All experiments were performed at room temperature (21–22 °C) in ND96 solutions. Drugs were systematically applied externally by gently puffing 100 μl of drug solution near the oocyte. Control puffs revealed no mechanical effects on Kv currents.

**Cell Cultures and Transfection**—COS M6 cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum and antibiotics (60 μg/ml penicillin and 50 μg/ml streptomycin). Two days before transfection, 10% cells were plated on coverslips coated with 35-mm Petri dishes. The cells were transfected by a modification of the DEAE-dextran/chloroquine method (35) using 0.01 μg of supercoiled pRc/CMV Kv3.4 plasmid DNA to have low density expression of Kv3.4 channel. Currents were recorded within 1–2 days following transfection. Rat dorsal root ganglion neurons were dissected from 2-day-old postnatal embryos. Cells were plated at a density of 20,000 cells per 35-mm-diameter Petri dish.

**Cell Solutions**—The pipette solution used in voltage-clamp experiments for K+ currents recorded in COS M6 cells was 150 mM KCl, 3 mM MgCl2, 5 mM EGTA, 10 mM HEPES-KOH, pH 7.4. For action potential measurements, the pipette solution contained 140 mM KCl, 3 mM MgCl2, 5 mM EGTA, 10 mM HEPES-KOH, pH 7.3. For Na+ currents in NIE-115 cells and Ca2+ currents in dorsal root ganglion cells, the composition was 150 mM CsCl, 1 mM MgCl2, 5 mM EGTA, 10 mM HEPES-CsOH, pH 7.4.

The extracellular solution composition for K+ currents recordings in COS M6 cells was 5 mM KCl, 150 mM NaCl, 1 mM CaCl2, 3 mM MgCl2, 10 mM HEPES-NaOH, pH 7.4. For action potential measurements, the composition was 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES-NaOH, pH 7.4. For Ca2+ currents, it was 124 mM TEACl, 10 mM BaCl2, 1 mM MgCl2, 5 mM KCl, 10 mM HEPES-TEAOH, pH 7.4. 0.1% bovine serum albumin was added in BDS-containing solutions to prevent its adsorption onto containers.

**RESULTS**

**Purification of a Kv3.4 Channel Blocker from A. sulcata**—The initial purpose of this work was to find new specific and sufficiently high affinity ligands for K+ channels that have no specific pharmacology as yet. As a first step, we tested more than 200 peptidic fractions from the venom of different snakes, scorpions, spiders, and sea anemones on K+ currents recorded in Xenopus oocytes injected with cRNA from different cloned K+ channels, and we found interesting activities in A. sulcata extracts. A. sulcata venom has previously been shown to contain two classes of K+ channel blockers for the Shaker subfamily, kaliseptine (AsKS) and kalicludines (AsKCs); the latter is structurally similar to DTXs from snake venoms (8). Other blockers of the DTX-sensitive Kv channels have been found in other sea anemone species (1, 36–38). We observed that a peptidic fraction (AsF28, 1 mg/ml) that we had previously purified from A. sulcata when searching for other K+ channel toxins (see Fig. 1A in Ref. 8) produced more than a 50% inhibition of the Kv3.4 current recorded in Xenopus oocytes. This was a preliminary indication that this venom contained a component (or components) that blocked a K+ channel belonging to a subfamily different from Shaker. The AsF28 fraction (10 mg) was then separated as described under “Experimental Procedures”; this led to the isolation of a pure peptide active on the Kv3.4 channel.

**Amino acid Sequence Determination**—The active component was reduced, pyridylethylated, and subjected to Edman degradation to determine its primary structure (Fig. 1A). This peptide is identical to BDS-II and very similar to BDS-I, two peptides that had been previously purified from A. sulcata extracts by Bérès et al. (39) and that had been described as blood-pressure-depressing substances in the cat but apparently not in other animal species (39, 40). BDS-I differs from BDS-II at only two positions (Ser-7 instead of Pro, and Gly-11 instead of Asp; Fig. 1A). Both peptides have 43 amino acids and are cross-linked by three disulfide bridges. Their molecular absor- bency at 280 nm is ε280 16910, and their molecular weights are 4715 for BDS-I and 4783 for BDS-II.

**Sequence Homology with Other Toxins and Activity on Different Types of Voltage-sensitive Channels**—The primary amino acid sequence of BDS-I and BDS-II is compared in Fig. 1A with those of other well known sea anemone toxins active on voltage-dependent Na+ channels. BDS-I displays only 24–26% identity with toxins AaI (ATX-I), AaII (ATX-II), and AaV (ATX-V) from A. sulcata and AaI (AP-A) from Anthopleura xanthogrammica. All four peptides behave as selective and high affinity effectors of voltage-sensitive Na+ channels and slow down Na+ current inactivation (41). Sequences of BDS-I and BDS-II and those of identified Na+ channel toxins have a total of six cysteines, with five cysteines at identical positions and one cysteine at a dif-
ferent position (Fig. 1A). These cysteine residues are known to form disulfide bridges (42–45). Due to their apparent overall structural similarities with toxins active on Na⁺ channels, BDS-I (the most abundant) (100 nM) was tested for its possible activity against these channels. First, it had only small effects on TTX-sensitive Na⁺ channels from neuroblastoma cells (Fig. 1B). Even at a high concentration of 500 nM BDS-I, the slowing down of Na⁺ channel inactivation was much smaller than that observed with AsII or Axl at the same concentration. Both AsII and Axl were active on cardiac Na⁺ channels. The toxin-induced slowing down of the inactivation of cardiac Na⁺ channels led to an inotropic action of the two toxins (46). BDS-I and BDS-II were also tested for their possible positive inotropic action on isolated cardiac organ. No modifications in amplitude and frequency of rat and guinea-pig atria contractions were observed with 1 μM BDS-I, but they were drastically prolonged by AsII (100 nM; data not shown). None of the three Na⁺ channel toxins, AsI, AsII, or Axl, at a concentration of 10 μM, was able to inhibit the Kv3.4 current (n = 3; data not shown).

The effect of BDS-I was also tested on voltage-sensitive calcium channels. Perfusion of 1 μM BDS-I failed to affect low or high voltage-activated Ca²⁺ currents recorded in rat dorsal root ganglion neurons (n = 6; data not shown).

BDS-I, in particular, was analyzed for its ability to block a variety of K⁺ channels expressed in Xenopus oocytes. The specificity of BDS-I for channels of the Shaker subfamily was first analyzed by assaying its possible action on different members of other families of K⁺ channels, such as the Shaker family (Kv1.1, Kv1.2, Kv1.3, Kv1.4, and Kv1.5), the Shab family (Kv2.1 and Kv2.2), and the Shal family (Kv4.2 and Kv4.3). The sensitivity to the toxin of inward rectifier K⁺ channels, such as IRK1, IRK2, and ROMK1, was also tested. None of these cloned channels expressed in Xenopus oocytes (n = 3–6 for each channel type) was significantly affected by BDS-I. In the Shal family, only one member, Kv3.4, was extensively inhibited by BDS-I (Fig. 2). These results clearly show that BDS-I has a particularly high specificity for the Kv3.4 channel as compared with other K⁺ channels.

The pharmacological effect of BDS-I and BDS-II was then analyzed in more detail in COS cell lines transiently transfected with a Kv3.4-expressing plasmid. Whole cell voltage-clamp recordings indicated a high level of expression of Kv3.4 channels, with current amplitudes ranging from 500 pA to 6 nA for depolarizing pulses from −80 to +50 mV. Kv3.4 currents recorded from high density transfected cells (>0.1 μg of Kv3.4 plasmid per 35-mm-diameter Petri dish) displayed nonactivating kinetics and a lower sensitivity to BDS-I and BDS-II. This phenomenon has already been demonstrated for delayed rectifier Kv1.2 and Kv1.3 channels, which displayed a much lower sensitivity to toxins when high cRNA concentrations were injected in Xenopus oocytes, resulting in high current levels (29, 31). Therefore, particular attention was paid to the analysis of the effect of the toxin in cells expressing Kv3.4 currents of relatively lower intensities (under 2 nA for a test pulse at +50 mV) and rapidly inactivating kinetics, which probably correspond to the normal function of these K⁺ channels (47). Under these conditions, currents induced by a series of depolarizing pulses at −20 to +50 mV in 10-mV steps from a holding potential of −80 mV rapidly evoked an inactivating outward Kv3.4 current (τ = 17 ± 8 ms; n = 19 at +50 mV; Fig. 3A) very similar to that recorded in Xenopus oocytes. Inhibition of the Kv3.4 current, induced by a perfusion with 50 nM BDS-I, was not voltage-dependent (Fig. 3A). The time course of the block and the reversibility is presented in Fig. 3B. Inhibition developed after 1–2 min of toxin application. Extensive recovery occurred during the 5–6-min period after completion of the toxin exposure. The concentration dependence of the inhibitory effect of BDS-I and BDS-II on Kv3.4 currents is presented in Fig. 3C. This curve indicates a 1:1 association of the two partners, with IC₅₀ values of 47 and 56 nM for BDS-I and BDS-II, respectively.

Voltage-clamp studies carried out in outside-out patches from COS-transfected cells showed that a rapid inhibition of
Kv3.4 current occurred when BDS-I or BDS-II was applied to the outside surface of the patch, indicating a direct action of toxins on an external binding site of the channel structure (Fig. 3D).

**Affinity of Different Toxins for \(^{125}\text{I}\)-BDS-I Receptors**—
BDS-I binding sites can be easily identified on synaptic membranes (Fig. 4). Unlabeled BDS-I and BDS-II compete with \(^{125}\text{I}\)-BDS-I for binding to brain receptors, with \(K_{0.5}\) values of 12 and 19 nM, respectively. As expected, none of the Kv1 potassium channel toxins tried (DTX-I, MCD peptide, and ChTX) had any significant effect on these binding properties. The Na\(^+\) channel toxin AsII can only partially inhibit the binding of \(^{125}\text{I}\)-BDS-I at a high concentration of 1 \(\mu\text{M}\) (Fig. 4). At this concentration, the AsII binding site on neuronal voltage-sensitive Na\(^+\) channel is completely saturated (\(K_{d} = 150\) nM) (48).

**DISCUSSION**

For more than 20 years, sea anemones have provided toxins that have been essential tools for studies of structure, function, and localization of voltage-dependent Na\(^+\) channels (41, 49–51). It is only recently that new sea anemone peptides that are active on K\(^+\) channels have been discovered (1, 8, 36–38, 52, 53).

The most classic toxins used to block voltage-sensitive K\(^+\) channels are DTX-I, MCD peptide, and ChTX (3, 9, 17). They potently inhibit delayed rectifier Kv channels but do not rapidly inactivating K\(^+\) currents (I\(_{A}\) currents) generated by Kv1.4, Kv3.3, Kv3.4, and the Kv4 channel subfamily. New toxins for new K\(^+\) channels have been recently discovered in spider venoms (19). For example, Kv4.2 was recently found to be inhibited by three short peptides, called heteropodatoxins, purified from *Heteropoda venatoria* venom (32). The results presented here describe the identification and characterization in *A. sulcata* extracts of the first specific peptide blockers of a rapidly inactivating K\(^+\) channel. BDS-I and BDS-II are two structurally very similar peptides that reversibly inhibit Kv3.4 channels. BDS-I failed to block significantly other voltage-gated potassium channels in the Kv1, Kv2, Kv3, and Kv4 subfamilies. BDS toxins have no sequence homologies with other potassium channel toxins from sea anemones, such as ShK from *Stichodactyla helianthus*, BgK from *Bunodosoma granulifera*, or AsKS and AsKC from *A. sulcata*, which are active on Kv1.2 and Kv1.3 channels (8, 36–38).

BDS-I and BDS-II have some overall structural homology with classical Na\(^+\) channel toxins from *A. sulcata* or *A. xanthogrammica*. Nuclear magnetic resonance studies of BDS-I showed that the core of the polypeptide is formed by a triple-stranded antiparallel \(\beta\)-sheet composed of residues 14–16 (strand 1), 30–34 (strand 2), and 37–41 (strand 3), with an additional mini-antiparallel \(\beta\)-sheet at the N terminus (residues 6–9). In addition, a long exposed loop (residues 17–30) connects the first and second strands of the antiparallel \(\beta\)-sheet (42). A comparison of the three-dimensional structure of BDS-I with that of Na\(^+\) channel modulatory toxins from *A. sulcata* and related anemones reveals a similar antiparallel \(\beta\)-sheet conformation (43). The major difference is the presence in BDS-I of the short antiparallel \(\beta\)-sheet encompassing residues 6–9 and a type II turn instead of the unstructured loop that is present in sea anemone peptides, such as AxI (44) and AsI (45).

BDS-I has only a small effect on TTX-sensitive Na\(^+\) channels in neuroblastoma cells; this effect is probably achieved by binding to the same binding site as the other sea anemone toxins specific for these Na\(^+\) channels (50). BDS-I slowed down inactivation but only very slightly as compared with highly Na\(^+\) channel-selective toxins, such as AsI, AsII, and AxI from *A. sulcata* and *A. xanthogrammica* (41, 51). BDS-I had no action at 1 \(\mu\text{M}\) on voltage-sensitive Na\(^+\) channels in cardiac cells or in skeletal muscle myotubes. It failed to produce the large prolongations of myotube action potentials, as well as the strong cardiotoxic effects that can be observed at low concentrations of AsII (100 nM). This relative lack of action of BDS-I on voltage-sensitive Na\(^+\) channels is not really surprising because, excluding the conservation of 5 of the 6 cysteine residues that are essential for the overall folding of the toxins, there is only a limited percentage of sequence homologies between the BDS toxins and other sea anemone toxins selective for the TTX-sensitive Na\(^+\) channel (Fig. 1A). The presence of basic amino

**FIG. 2.** Effect of BDS-I on different families of K\(^+\) channels expressed in *Xenopus* oocyte. Kv currents were elicited by voltage steps from –80 to +30 mV every 20 s before and during the application of 10 \(\mu\text{M}\) BDS-I and IRK1 current from 0 to –80 mV. Kv3.4 current was almost completely inhibited (94%) by application of BDS-I, and there was only a slight effect on Kv3.1 (22 \(\pm\) 10%; \(n = 5\)), Kv1.2 (19 \(\pm\) 0.5%; \(n = 3\)), and Kv1.3 (17 + 6%; \(n = 3\)).

Sea Anemone Peptides Active Against the Kv 3.4 Channel

6747

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*Kv1.2 (196)*

*A. sulcata* describe the identification and characterization in *Heteropoda venatoria* by three short peptides, called heteropodatoxins, purified from animal venoms. BDS-I failed to block significantly other voltage-gated potassium channels in the Kv1, Kv2, Kv3, and Kv4 subfamilies. BDS toxins have no sequence homologies with other potassium channel toxins from sea anemones, such as ShK from *Stichodactyla helianthus*, BgK from *Bunodosoma granulifera*, or AsKS and AsKC from *A. sulcata*, which are active on Kv1.2 and Kv1.3 channels (8, 36–38).

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Sea Anemone Peptides Active Against the Kv 3.4 Channel

Figure 3. Effect of BDS-I on the Kv3.4 channel activity expressed in transfected COS cells. Cells were transfected with 0.5 μg of plasmid to have small and rapidly inactivating Kv3.4 currents. Currents were recorded in whole cell configuration (A–C) and outside out configuration (D). A, I-V relationship for Kv3.4 peak current measured before (●) and during (○) application of 50 nM BDS-I. Currents were induced by a set of depolarizing pulses at −20 to +50 mV (in 10-mV steps); holding potential, −80 mV. The inset shows the averaged trace corresponding to control I-V; B, time course for peak current block with 100 nM BDS-I and reversibility during washing on Kv3.4 current evoked at +50 mV from a holding potential of −80 mV. The pulses were repeated every 20 s before and during application of the toxin. C, concentration-response relationship for BDS-I (●) and BDS-II (○) block of the Kv3.4 current in COS cells. Kv3.4 currents were measured at +50 mV from a holding potential of −80 mV. Each point is the mean ± S.E. of data from three to six cells. The averaged trace is a theoretical fit with a Hill coefficient of n_H = 2.3. The individual IC_{50} values are 47 nM for BDS-I and 56 nM for BDS-II. The inset shows the effect of 200 nM BDS-I (trace 2) on Kv3.4 current. Trace I, control. D, effect of 75 nM BDS-I on Kv3.4 current recorded on patches in the outside-out configuration at a test pulse of +50 mV. Holding potential, −80 mV.

Kv3.4 channels are insensitive to DTX and MCD peptide (57), but they are blocked by relatively high concentrations of TEA, 4AP, and quinine (IC_{50} = 0.1–0.5 mM). However, these blockers are nonselective K^+ channel inhibitors. BDS-I and BDS-II are the first peptides that have been found to be able to specifically block this rapidly-inactivating K^+ channel, Kv3.4, with a relatively high affinity (IC_{50} = 47 and 56 nM, respectively). This blocking effect is rapid, direct, and largely reversible. The IC_{50} values found for the blockade of the Kv3.4 channel by BDS-I and BDS-II are very similar to the K_{0.5} values (K_{0.5} = 12 and 19 nM) obtained with ^{125}_1-BDS-I binding experiments. Like DTX-I, MCD peptide, and ChTX, which discriminate among members of the Kv1 family (3, 4, 9, 10, 17), BDS-I and BDS-II can also discriminate among members of the Kv3 subfamily. Other members of the Kv3 subfamily, such as Kv3.1, Kv3.2, and Kv3.3 channels, are essentially insensitive to BDS-I. Other polypeptide toxins might be found in the future for these other channels.

An intriguing observation is that sea anemones use three different types of protein folds to create K^+ channel toxins. One type is the protein fold of toxins with 35–37 amino acids and 3 disulfide bridges comprising BgK, ShK, and AsKS. A second type, present in A. sulcata venom, is that of AsKC. It corresponds to the protein fold found for trypsin inhibitors of the Kunitz type, for dendrotoxins, which are K^+ channel toxins found in mamba venoms, and for calcicludine, the Ca^2+ channel toxin also found in mamba venoms. The third type of protein fold is that of BDS-I and BDS-II, and it is probably close to the one adopted by Na^+ channel toxins in the same venom.
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