Structural Conservation of the Pores of Calcium-activated and Voltage-gated Potassium Channels Determined by a Sea Anemone Toxin*

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The structurally defined sea anemone peptide toxins ShK and BgK potently block the intermediate conductance, Ca2+-activated potassium channel IKCa1, a well recognized therapeutic target present in erythrocytes, human T-lymphocytes, and the colon. The well characterized voltage-gated Kv1.3 channel in human T-lymphocytes is also blocked by both peptides, although ShK has a ~1,000-fold greater affinity for Kv1.3 than IKCa1. To gain insight into the architecture of the toxin receptor in IKCa1, we used alanine-scanning in combination with mutant cycle analyses to map the ShK-IKCa1 interface, and compared it with the ShK-Kv1.3 interaction surface. ShK uses the same five core residues, all clustered around the critical Lys22, to interact with ShK-IKCa1 and Kv1.3, although it relies on a larger number of contacts to stabilize its weaker interactions with IKCa1 than with Kv1.3. The toxin binds to IKCa1 in a region corresponding to the external vestibule of Kv1.3, and the turret and outer pore of the structurally defined bacterial potassium channel, KcsA. Based on the NMR structure of ShK, we deduce the toxin receptor in IKCa1 to have x-y dimensions of ~22 Å, a diameter of ~31 Å, and a depth of ~8 Å; we estimate that the ion selectivity lies ~13 Å below the outer lip of the toxin receptor. These dimensions are in good agreement with those of the KcsA channel determined from its crystal structure, and the inferred structure of Kv1.3 based on mapping with scorpion toxins. Thus, these distantly related channels exhibit architectural similarities in the outer pore region. This information could facilitate development of specific and potent modulators of the therapeutically important IKCa1 channel.

The intermediate conductance, calcium-activated potassium channel, IKCa1, plays a role in regulating membrane potential and in modulating the calcium signal in many different peripheral tissues (1–12), including human T-lymphocytes (3, 4), B-lymphocytes (EST accession no. AA937083), erythrocytes (AF042487, AF053403, AF072984), hemopoietic stem cells (AA558247), colonic epithelia (AA897997, T24528), pancreatic islets (AA076338, AA076337, AA122017), fibroblasts (AL034286), prostate (AA603035, AA65228), ovary (AA424836, AA443903, AA425636), testis (AI081834), and platelets (10). IKCa1 is activated by intracellular calcium via a calmodulin-dependent mechanism (13), and its amino acid sequence exhibits ~40% identity with the sub-family of small conductance calcium-activated potassium channels (1–6). Clotrimazole, a potent but nonselective inhibitor of this channel, is currently being evaluated in the therapy of sickle cell disease and secretory diarrheas. Although initial results have been encouraging (5, 14–16), there is clearly a need for more specific inhibitors of IKCa1, and architectural information on this channel may facilitate drug development.

The use of peptide toxins to obtain insight into the topology of the external vestibule of potassium channels has a long and successful history. Structurally defined peptides from scorpion venom and sea anemone have been used as molecular yardsticks to gauge the dimensions and shape of the external vestibules of the voltage-gated Shaker and Kv1.3 channels (17–23). The deduced dimensions are in good agreement with the recently published structure of the outer pore region of the bacterial potassium channel, KcsA, based on crystallographic data (24, 25). Therefore, the use of peptide toxins as mapping tools, set in the context of the known KcsA crystal structure, can facilitate the architectural mapping of the outer pore regions of pharmacologically important mammalian potassium channels in the absence of direct structural data for these channels.

Although IKCa1 is only distantly related to the Shaker and Kv1.3 voltage-gated channels, it is potently blocked by some of the same scorpion and sea anemone peptides that inhibit these channels. It might therefore be feasible to use the peptide-mapping approach to gain insight into the dimensions and shape of the toxin receptor on IKCa1 and to compare this topology with the external vestibules of Kv1.3 and KcsA. For this purpose, we have used the structurally defined 35-amino acid peptide toxin, ShK,1 from the sea anemone Stichodactyla helianthus. We used the alanine scanning method, coupled with mutant cycle analyses, to map the interactive surface between ShK and IKCa1 and compared this with the ShK:Kv1.3 interface. Our studies indicate that ShK binds to IKCa1 in an external vestibule that is architecturally similar to that of Shaker, Kv1.3, and KcsA, although ShK uses a significantly wider surface to interact with IKCa1 compared with its interaction with Kv1.3. Such structural differences might be exploited to guide the design of novel peptides that specifically target IKCa1.

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1 The abbreviations used are: ShK, Stichodactyla helianthus toxin; BgK, Bunodosoma granulifera toxin; Dap, diaminopropionic acid; Nle, norleucine; Fmoc, N-(9-fluorenylmethoxy carbonyl).
Peptide Synthesis—Fmoc-amino acid derivatives were obtained from Bachem A.G. (CH-4416 Bubendorf, Switzerland). Solid-phase assembly was initiated with an Fmoc-Cys(Ttr)-2-chlorotriyl resin to minimize potential racemization of the C-terminal Cys residue. Automated stepwise assembly was carried out entirely on an ABI-431A peptide synthesizer (Applied Biosystems, Foster City, CA). The ShK analogues were solubilized, oxidized, and purified by reverse phase-high pressure liquid chromatography using the method described previously (23, 26), and high pressure liquid chromatography-pure fractions were pooled and lyophilized. The structure and purity of the peptides were confirmed by reverse phase-high pressure liquid chromatography, amino acid analysis, and electrospray ionization-mass spectroscopy analysis. Samples were weighed and adjusted to account for peptide content before bioassy.

Expression and Electrophysiological Analysis—The human wild-type IKCa1 and IKCa1-Lys239 constructs were linearized with NotI, the Kv1.3-Val294 mutant with EcoRI, and these constructs were transcribed in vitro (20, 28). The cRNA along with a marker dye was injected into rat basophilic leukemia cells as described previously (13, 28). After 2–6 h, dye-containing cells with specific currents could be characterized using the patch-clamp method. Cell lines stably expressing Kv1.3-wild type (27) were trypsinized and plated onto glass coverslips at least 3 h before measurement. All cells were measured in the whole-cell configuration and bathed in mammalian Ringer solution with 0.1% bovine serum albumin (Sigma) containing (in mM): 160 NaCl, 4.5 KCl, 1 MgCl2, 10 HEPES, adjusted to pH 7.4 with NaOH, with an osmolarity of 290–320 mOsm. In the K+-Ringer solution, NaCl was replaced with KCl. A simple syringe-driven perfusion system was used to exchange the bath solutions in the recording chamber. The internal pipette solution for the Kv1.3 channel recordings contained (in mM): 134 potassium fluoride, 1 CaCl2, 10 HEPES, 10 EGTA, pH 7.2 (with KOH), 290–310 mOsm (free [Ca2+] = 1 μM). The holding potential in all experiments was −80 mV. IKCa1 currents were activated with 1 μM internal Ca2+ and 200-ms voltage ramps from −150 to 50 mV applied every 5 s. Kv1.3 currents were measured following 200-ms depolarizing pulses to 40 mV from the holding potential, applied every 30 s. Series resistance compensation (80%) was used if the current exceeded 2 nA. Capacitative and leak currents were subtracted using the P/8 procedure for Kv1.3 currents. K+ values were calculated using the equation $K_+$ = $\sqrt{(\text{Theory}(0)/10))} - 1$ with $y$-unblock fraction; as shown mean ± S.D. where $n$ ≥ 3 for all experiments.

Free Energy Difference of Binding ($\Delta F$) —The free energy difference of binding was calculated as $\Delta F = RT \ln K_i$, where $K_i$ = $\frac{[\text{Peptide}]_{\text{free}}}{[\text{Peptide}]_{\text{bound}}}$. The $\Delta F$ values were corrected for the $\Delta G$ of binding (see Materials and Methods). A greater change in free energy ($\Delta F$), the greater the influence of a particular ShK residue for channel binding.

In the representative example shown in Fig. 1B (left), an alanine substitution at ShK position 20 significantly reduces the affinity of the toxin for IKCa1 ($K_+ = 2.450$ nM) compared with that of the wild-type toxin ($K_+ = 30$ nM). The change in free energy of binding, caused by this substitution, is 2.5 kcal mol$^{-1}$. Using this approach, we determined $\Delta F$ values measured in this “Ala-scan” were used to calculate the free energy difference of binding, $\Delta F$ (see “Materials and Methods”). The greater the change in free energy ($\Delta F$), the greater the influence of a particular ShK residue for channel binding.

RESULTS

The Sea Anemone Toxins, ShK and BgK, Block IKCa1 and Kv1.3 Channels.—We compared the toxin sensitivities of the two K$^+$ channels, present in activated human T lymphocytes, IKCa1 and Kv1.3. Two potent peptide inhibitors, ShK and BgK, from the sea anemones S. helianthus and B. granulifera, were chosen for analysis (30, 31). These peptides share 31% sequence identity (Fig. 1A). Both contain six conserved cysteines that form three disulfide bonds, a feature common to many channel-blocking peptide inhibitors from scorpion venom as well as many defensins (32). However, the structures of ShK and BgK are significantly different from that of the scorpion toxins and defensins (23, 33, 34).

The cloned IKCa1 and Kv1.3 genes were expressed in mammalian cells, and representative currents are shown in Fig. 1B. IKCa1 currents were elicited by 1 μM calcium in the pipette following break-in, whereas depolarizing pulses were used to generate Kv1.3 currents. ShK and BgK, when applied externally, block the IKCa1 channel in the low nanomolar range with $K_+$ values of $30 \pm 7$ nM and $172 \pm 43$ nM, respectively (Fig. 1B, left top panel). BgK blocks Kv1.3 with comparable potency ($K_+ = 39 \pm 4$ nM; Fig. 1B, right top panel). In contrast, ShK exhibits a markedly greater affinity for Kv1.3 channels ($K_+ = 0.016 \pm 0.003$ nM) compared with IKCa1 (Fig. 1B, top panel), indicating that this peptide has an exquisite ability to discriminate between the two T lymphocyte K$^+$ channels.

Determining the IKCa1 Channel-Binding Surface of ShK—To elucidate the molecular basis for the ability of ShK to discriminate between IKCa1 and Kv1.3, we used alanine-scanning mutagenesis to identify ShK residues essential for binding to both these channels. A series of monosubstituted peptide analogues, in which each residue is substituted with alanine, was tested for their ability to block the IKCa1 and Kv1.3 channels. The only exception is His19, which was replaced by a lysine. Structurally critical ShK residues that were not substituted included the six half-cysteine residues, as well as Asp5, Ala14, and Gly33. $K_+$ values measured in this “ Ala-scan” were used to calculate the free energy difference of binding, $\Delta F$ (see “Materials and Methods”). The greater the change in free energy ($\Delta F$), the greater the influence of a particular ShK residue for channel binding.

In the representative example shown in Fig. 1B (left), an alanine substitution at ShK position 20 significantly reduces the affinity of the toxin for IKCa1 ($K_+ = 2.450$ nM) compared with that of the wild-type toxin ($K_+ = 30$ nM). The change in free energy of binding, caused by this substitution, is 2.5 kcal mol$^{-1}$. Using this approach, we determined $\Delta F$ values for each alanine substitution. The ShK residues most critical for IKCa1 binding, with $\Delta F$ values greater than or equal to 2.5, are shown in red in Fig. 2A and include Arg11, His19, Ser20, Lys22, Tyr23, Arg24, and Phe27. Alanyl substitution at ShK position 21 (Met21) also significantly disrupts the toxin-channel interaction ($\Delta F = 1.8$; orange). Arg3, Ile7, Thr13, Leu25, and Ser26 (shown in yellow, where $\Delta F = 0.75$–1.5), and Thr6, Phe15, Lys18, and Arg29 (shown in blue, $\Delta F = 0.5$–0.75), are only moderately important for binding, whereas Ala substitutions at the remaining ShK positions (shown in white, $\Delta F < 0.5$) have minimal effects (Fig. 2A).

Fig. 3 (left) shows the positions of the ShK residues (bottom and side view) that contribute to its interaction with IKCa1, color coordinated with the histograms in Fig. 2A. All the highly critical residues ($\Delta F \geq 2.5$) cluster together on one surface of the ShK peptide (red), and Met21 (orange) lies immediately adjacent. Residues with moderate influence (yellow and blue) form the margins of the ShK/IKCa1-binding surface. In general, nonessential residues cluster together on the opposite surface of the peptide (Fig. 3, left). Thus, the surface of ShK that binds IKCa1 extends in its greatest distance from Arg1 on one side to Phe15 on the other (Fig. 3, left, bottom view). Lys22 lies at the lowest point in the channel-binding surface (Fig. 3, left, side view).

Comparison of the ShK Toxin-binding Surfaces in IKCa1 and Kv1.3—All ShK analogues were tested on the Kv1.3 channel to
define the ShK-binding surface for this structurally well-defined channel (20, 21, 23). Fig. 1B, bottom panel, compares the effect of one ShK alanine substitution (at ShK 20) on currents through Kv1.3 and IKCa1, whereas Fig. 2A compares the $\Delta F$ values for all ShK substitutions on these channels. Replacements of His19, Ser 20, Lys 22, Tyr 23, and Arg 24 significantly disrupt the interaction of ShK with Kv1.3 ($\Delta F > 1.5$) (Fig. 2A, and see Fig. 1B), whereas to a lesser extent than with IKCa1 ($\Delta F > 2.5$; Fig. 2A, and see Fig. 1B, bottom panel). Alanine substitutions at ShK positions 11, 21, and 27 ($\Delta F = 0.75$–1.5) and positions 1, 6, 7, 15, and 26 ($\Delta F < 0.75$) are also substantially less disruptive on Kv1.3 compared with IKCa1 (Fig. 2B). The only exception is ShK-Arg 29, which seems to be slightly more important for binding to Kv1.3 than for IKCa1 ($\Delta F = 0.88$ and 0.56, respectively). Our results thus suggest that ShK uses a larger number of residues to stabilize its interaction with IKCa1 than with Kv1.3, although it uses the same core domain of five clustered residues for binding to both channels.

ShK-Lys 22 protrudes into the Kv1.3 pore and lies in close proximity to Tyr 400 and Asp 402 in the selectivity filter, and is critical for the interaction of the toxin with this channel (23). To determine the contribution of Lys 22 to the interaction of the toxin with Kv1.3, we compared the affinity of Kv1.3-Lys 22 analogues (Fig. 2B); we also examined the effect of these analogues on the Kv1.3-His 404 → Val 404 mutant, and these data are presented in a subsequent section. In two analogues, the positively charged lysine is replaced with the shorter, positively charged amino acids, ornithine (Orn) and diaminopropionic acid (Dap), whereas the other two analogues have the neutral residues norleucine (Nle) and alanine (Ala) at position 22. These residues differ in their side chain lengths (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å). Our results show that Ala 22 and Nle 22 substitutions significantly decrease the affinity of the toxin for both channels (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å). Our results show that Ala 22 and Nle 22 substitutions significantly decrease the affinity of the toxin for both channels (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å). Our results show that Ala 22 and Nle 22 substitutions significantly decrease the affinity of the toxin for both channels (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å). Our results show that Ala 22 and Nle 22 substitutions significantly decrease the affinity of the toxin for both channels (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å). Our results show that Ala 22 and Nle 22 substitutions significantly decrease the affinity of the toxin for both channels (Dap, 2.5 Å; Ala, 2 Å; Orn, 5.0 Å; Nle, 5.0 Å; lysine, 6.3 Å).
alter the affinity of ShK for Kv1.3 (Fig. 1B, bottom left and Fig. 2B). The longer ShK-Orn22 substitution also significantly disrupts the affinity of the toxin for IKCa1 (∆F = 1.7), although to a lesser extent than Dap22, possibly because it is better anchored in the channel pore, whereas this analogue blocks Kv1.3 with potency equivalent to wild-type ShK (Fig. 2B).

Fig. 3 highlights the residues that ShK uses to interact with IKCa1 (left) compared with those for Kv1.3 (right). The residues are color-coordinated with the histogram in Fig. 2, A and B. ShK residues required for binding to both channels are clustered together on one surface (colored), whereas white residues not required for the interaction are located on the opposite toxin surface. ShK uses eight essential residues, Arg11, His19, Ser20, Met21, Lys22, Tyr23, Arg24, and Phe27, to interact with IKCa1, whereas the toxin interaction with Kv1.3 relies on only five essential contacts (His19, Ser20, Lys22, Tyr23, and Arg24). Alanine substitutions at any of these five critical positions in the binding core domain severely disrupt the ShK-channel interaction in both channels (∆F > 1.5, Fig. 3, red and orange).

ShK residues that surround this critical core domain (ShK-positions 1, 6, 7, 11, 13, 15, 21, 26, and 27) exhibit a lower influence on binding to Kv1.3 compared with IKCa1. Thus, the overall binding surface of ShK for IKCa1 is larger and contains more essential interacting residues than its binding surface for
Kv1.3 (Fig. 3). Nevertheless, the affinity of the toxin for Kv1.3 is significantly greater than for IKCa1 (Fig. 1B), suggesting that the picomolar affinity of ShK for Kv1.3 is dependent on a few very tight toxin-channel contacts, whereas its ~1,000-fold lower nanomolar affinity for IKCa1 is because of a greater number of weaker interactions.

**Interactions of ShK with Residues in the External Vestibule of IKCa1**—Peptides from scorpion and snake venom, as well as from sea anemone, bind to residues in the external vestibule of the ion conduction pathway of eukaryotic voltage-gated potassium channels and occlude their pores (17–23, 34). This vestibule corresponds to the outer pore and turret region in the structurally defined bacterial K⁺ channel, KcsA (24), and mutations in the turret region of KcsA greatly enhance the ability of this channel to bind peptide inhibitors (25). We therefore undertook a series of mutational analyses to define the interactions of ShK with residues in the external vestibule of IKCa1.

**Charge-reversal Mutation at IKCa1-Asp239 Dramatically Reduces Sensitivity to ShK**—We first aligned the turret and pore regions of KcsA, Kv1.3, and IKCa1 (Fig. 4A). All three channels are remarkably similar in the pore region with absolute conservation of the GYGD motif (Fig. 4A). Interestingly, IKCa1 contains an aspartate (Asp²³⁹) at the position homologous to Asp³⁸⁶ in the turret of Kv1.3 that is essential for the interaction of Kv1.3 with ShK and various scorpion toxins (20, 23). A charge-reversal mutation involving Asp³⁸⁶ in Kv1.3 (Asp → Lys) substantially reduces the channel sensitivity to ShK (ΔF = 1.9), kaliotoxin (ΔF > 2.5), and charybdotoxin (ΔF > 2.5) (20, 23). The converse charge-reversal mutation at the homologous position in KcsA (Arg → Asp) enhances this channel sensitivity for agitoxin-2 (25). We therefore replaced IKCa1 Asp²³⁹ with the positively charged Lys, and examined the sensitivity of the mutant channel to ShK. The IKCa1-Lys²³⁹ mutant was expressed in rat basophilic leukemia cells, and representative currents, elicited by 1 mM calcium in the pipette solution, are shown in Fig. 4B. The mutant channel is ~18-fold less sensitive to ShK (Kₑ₅ = 548 ± 37 nM) than wild-type IKCa1, suggesting that the Asp → Lys charge-reversal mutation at position 239 in IKCa1 has the same consequences on ShK binding as the identical mutation at the homologous position in Kv1.3 (23). The change in free energy for this channel mutant (ΔF = 1.7 kcal mol⁻¹) is equivalent to severe Ala substitutions (ΔF > 1.5 kcal mol⁻¹) in ShK (compare with Fig. 2A), indicating

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**Fig. 3. Channel-binding surface of ShK.** Left panel, surface of ShK that interacts with IKCa1; right panel, ShK surface that interacts with Kv1.3. ShK-binding surface is shown above (bottom view), and side view of the toxin is shown below. The residues are color coordinated to the histograms (ΔF values) in Fig. 2 (for color code, see Fig. 2A). The models were generated with the RasMol program.
that this channel residue is a critical contact point at the toxin-channel interface.

**IKCa1-Asp**

**Interacts with ShK-Arg**

As expected, because of its position on the opposite side of the interactive toxin surface, ShK-Lys is not coupled with **IKCa1-Asp** in different IKCa1 subunits, Arg being closer to Asp than Arg. As expected, because of its position on the opposite side of the interactive toxin surface, ShK-Lys is not coupled with **IKCa1-Asp**. The selectivity filter motif (GYGD) is conserved with respect to all ShK-Lys substitutions (Fig. 4D). The ΔΔG value for this cycle (0.91 kcal mol⁻¹) indicates that ShK-Lys lies within 5 Å of a K⁺-binding site located in the IKCa1 pore. We compared the effect of changing the external K⁺ concentration from 4.5 to 164.5 mM on the affinity of the IKCa1 channel for ShK-Lys and ShK-Ala. Consistent with earlier reports, increasing external K⁺ reduces the selectivity for the ShK channel more sensitive to ShK-Lys than the ShK-Lys pore, especially those involving shorter positive charged residues (Orn and Dap). A comparison of the sequences of the outer pore regions of the two channels suggests an explanation (Fig. 4A). K⁺ occupies the selectivity filter of the IKCa1 pore. Might the difference in the nature of the residue at the pore entrance account for the differential sensitivity to Shk-Dap and Shk-Orn? To test this possibility, we replaced Arg with a valine, a mutation that makes the outer pore of K⁺ more closely resemble that of IKCa1 and tested the sensitivity of this channel to the ShK analogues. In keeping with our hypothesis, the K⁺-valine mutant behaves more like IKCa1 with respect to all ShK-Lys analogues (Fig. 4B). We were unable to determine the effect of the reverse mutation (Val → His) on the selectivity of IKCa1 to the Lys analogues, because this channel mutant is nonfunctional. These results are consistent with the notion that the differential responsiveness of the K⁺-selective IKCa1 pores to ShK substitutions is due, in part, to the residue at the channel mouth of K⁺ (His) and the homologous position in IKCa1. In summary, ShK binds residues in the external vestibule of IKCa1, with ShK-Arg and Arg interacting with Asp residues in adjacent subunits of IKCa1 and with ShK-Lys projecting into the pore.

**DISCUSSION**

Using alanine-scanning mutagenesis, we have compared the surface that ShK, a 35-amino acid peptide toxin from the sea
Core domain involving His 19, Ser 20, Lys 22, Tyr 23, and Arg 24 stabilized by a greater number of weaker interactions.

**FIG. 5. Topology of the ShK-binding site.** Dimensions of the external vestibule of IKCa1 were determined using the NMR-derived ShK structure as a molecular caliper. Left, schematic representation of the external vestibule with Asp$^{239}$ residues highlighted (derived from the KcsA structure) with ShK (gray) docked. ShK-Arg$^{11}$ and Arg$^{29}$ are ~21 Å apart, interacting with Asp$^{239}$ residues in adjacent channel subunits. ShK-Lys$^{22}$ is shown at the geometric center of the tetramer, protruding into the pore. In this geometry Arg$^{1}$ and Phe$^{15}$ (at opposing ends of the ShK/IKCa1-binding surface) are located at opposite channel subunits. The diameter of the tetramer based on the distance between ShK-Arg$^{1}$ to Phe$^{15}$ and the Asp$^{239}$ x-y triangulation is approximately 31 Å. Right, schematic side view of the ShK toxin (compare Fig. 3, bottom) with the channel-binding surface highlighted. The distances between key toxin residues are indicated and correspond approximately to dimensions of the IKCa1 external vestibule (see ‘Discussion’).

Anemone *S. helianthus*, uses to interact with two distinct potassium channels present in activated human T-lymphocytes: the intermediate conductance, Ca$^{2+}$-activated K$^+$ channel IKCa1, and the voltage-gated K$^+$ channel Kv1.3. Although using the same core domain, the IKCa1-binding surface of ShK is more extensive than its Kv1.3-binding surface, and yet ShK blocks Kv1.3 with ~1,000-fold greater potency than IKCa1. A few tight Kv1.3-ShK contacts appear to underlie the picomolar affinity of ShK for To 1.3, whereas the IKCa1-ShK interface is stabilized by a greater number of weaker interactions.

Because ShK interacts with IKCa1 and Ko 1.3 using the same core domain involving His$^{19}$, Ser$^{20}$, Lys$^{22}$, Tyr$^{23}$, and Arg$^{24}$ (Fig. 3), this toxin is likely to sit in both channels with a similar geometry. Several lines of evidence support this idea. First, charge-reversal mutations at homologous positions in IKCa1 (Asp$^{239}$ → Lys$^{239}$) and Ko 1.3 (Asp$^{386}$ → Lys$^{386}$) reduce the sensitivity of these channels to ShK block in a similar fashion. Second, ShK-Arg$^{29}$ shows energetic coupling with Asp$^{239}$ in IKCa1, as well as with the homologous Ko 1.3-Asp$^{386}$ (Fig. 4C) (23). Third, ShK-Arg$^{11}$ and ShK-Arg$^{29}$ appear to couple with residues in adjacent subunits of IKCa1 as has been reported for Ko 1.3 (23). Fourth, the critical ShK-Lys$^{22}$ lies close to a potassium-binding site in the selectivity filter of the Ko 1.3 pore (23), and mutant cycle analyses suggests the same is true for IKCa1 (Fig. 4D). Last, replacement of the critical ShK-Lys$^{22}$ with bulky neutral residues (Nle and Ala) substantially reduces the affinity of the toxin for both channels, possibly because such residues are not tolerated in the channel pore. These results indicate that ShK binds to IKCa1 in a region corresponding to the external vestibules of Ko 1.3 and uses a comparable docking geometry. Such a docking configuration would place Arg$^{1}$ and Phe$^{15}$, the two residues at opposite margins of the IKCa1-binding surface (Fig. 3A), in close proximity to channel residues in opposite subunits in the IKCa1 tetramer (Fig. 5).

Knowing the NMR structure of ShK (33) and its approximate docking configuration in IKCa1, we used this peptide as a structural template to estimate the dimensions of the toxin receptor in the external vestibule of IKCa1. We have obtained two independent estimates of the diameter of the toxin receptor in the IKCa1 external vestibule. First, we estimate the width of the IKCa1 toxin receptor to be ~31 Å (Fig. 5) based on the width of the IKCa1-binding surface of ShK (distance from Arg$^{1}$ to Phe$^{15}$). Second, the x-y dimension of the toxin receptor is estimated to be ~22 Å (Fig. 5, left), based on the distance between the two toxin residues, ShK-Arg$^{11}$ and ShK-Arg$^{29}$ (Fig. 5, right), that interact with Asp$^{239}$ residues in adjacent IKCa1 subunits. This value implies (by Pythagorean triangulation) a distance of ~31 Å between Asp$^{239}$ residues in opposite subunits (Fig. 5). We estimate that the toxin receptor is approximately ~8–9 Å deep, based on the vertical distance between one horizontal line joining Arg$^{1}$ and Phe$^{15}$, and a second horizontal line connecting the terminal amines of Arg$^{11}$ and Arg$^{29}$ (Fig. 5, right). The selectivity filter is estimated to lie ~12–13 Å below the outer edge of the toxin receptor in the vestibule based on the vertical distance between the terminal amine of Lys$^{22}$ and the horizontal line joining Arg$^{1}$ and Phe$^{15}$ (Fig. 5, right). Our deduced dimensions of the toxin receptor in the IKCa1 vestibule are in good agreement with those obtained by crystallography for the KcsA vestibule (24, 25), and by toxin-mapping for Ko 1.3 (20, 21, 23) and Shaker (17–19, 22).

Thus, the IKCa1 external vestibule appears to be topologically similar to those of the distantly related Ko 1.3, Shaker, and KcsA channels, a result that provides support for the development of homology models of the IKCa1 outer pore based on the KcsA crystal structure. The experimentally determined differences in the ShK-IKCa1 and ShK-Ko 1.3 binding interfaces raises the possibility of designing novel peptides and small molecule inhibitors that selectively target the IKCa1 channel.
channel. Such reagents might be useful in elucidating the role of the IKCa1 channel in diverse cell types and may also have therapeutic value.

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