A Novel Small Conductance Ca\(^{2+}\)-activated K\(^+\) Channel Blocker from *Oxyuranus scutellatus* Taipan Venom

RE-EVALUATION OF TAICATOXIN AS A SELECTIVE Ca\(^{2+}\) CHANNEL PROBE*

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Taicatoxin, isolated from the venom of the Australian taipan snake *Oxyuranus scutellatus*, has been previously regarded as a specific blocker of high threshold Ca\(^{2+}\) channels in heart. Here we show that taicatoxin (in contrast to a range of other Ca\(^{2+}\) channel blockers) interacts with apamin-sensitive, small conductance, Ca\(^{2+}\)-activated potassium channels on both chromaffin cells and in the brain. Taicatoxin displays high affinity recognition of \(^{125}\)I-apamin acceptor-binding sites, present on rat synaptosomal membranes (\(K_d = 1.45 \pm 0.22\) nm) and also specifically blocks affinity-labeling of a 33-kDa \(^{125}\)I-apamin-binding polypeptide on rat brain membranes. Taicatoxin (50 nm) completely blocks apamin-sensitive after-hyperpolarizing slow tail K\(^+\) currents generated in rat chromaffin cells (mean block 97 ± 3%, \(n = 12\) ) while only partially reducing total voltage-dependent Ca\(^{2+}\) currents (mean block 12 ± 4%, \(n = 6\) ). In view of these findings, the use of taicatoxin as a specific ligand for Ca\(^{2+}\) channels should now be reconsidered.

Neurotoxins, found in the venom of a wide variety of poisonous species (snakes, scorpions, spiders, and marine snails) have provided biologists with a formidable armory of molecular probes with which to study the structure and function of ion channels (1). In particular, identification of the wide range of subtypes of potassium channels that are now known to exist, originally owes much to the discovery of neurotoxins with highly selective pharmacological actions (2–5). Three types of potassium channel activated by intracellular Ca\(^{2+}\) (\(K_{Ca}\) channels)\(^{2}\) can be distinguished on a biophysical basis (2): large conductance, BK\(_{Ca}\) channels (typically 100–250 pS), intermediate conductance, IK\(_{Ca}\) channels (typically 20–100 pS), and small conductance, SK\(_{Ca}\) channels (typically 5–20 pS). Each channel subtype has a distinct and characteristic neurotoxin pharmacology. Many SK\(_{Ca}\) channels are specifically blocked by apamin, a peptide (~2000 Da) isolated from the venom of the European honey bee, *Apis mellifera*. Through the use of apamin, SK\(_{Ca}\) channels have been shown to be present in a wide variety of electrically excitable and non-excitable cells. In neurons, SK\(_{Ca}\) channels regulate repetitive firing by maintaining a slow after-hyperpolarizing potential following bursts of action potentials (6). In chromaffin cells, SK\(_{Ca}\) channels have been implicated in the control of adrenaline release (7) and in hepatocytes, they respond to increases in cytosolic [Ca\(^{2+}\)] which is in turn specifically regulated by inositol trisphosphate and cAMP (8).

High affinity binding sites for \(^{125}\)I-apamin have been characterized on plasma membranes prepared from numerous tissues (9–12). \(^{125}\)I-Apamin binding polypeptides (putative SK\(_{Ca}\) channel subunits) have been identified through cross-linking and photoaffinity labeling strategies (11–16). These studies indicate that hetero-oligomeric association of high (\(\alpha\)) and low (\(\beta\)) molecular mass polypeptide subunits may be a general structural feature of members belonging to this family of K\(^+\) channels. Most recently, members of a new ion channel gene family have been cloned (17) with functional and structural properties that implicate them as SK\(_{Ca}\) channel subunits.

Apamin is generally recognized as being highly specific for SK\(_{Ca}\) channels (5, 18), however, it has also been reported to have apparently anomalous effects on Ca\(^{2+}\) channels in heart. Apamin can block slow Ca\(^{2+}\) action potentials in cultured cells originating from the ventricles of 15-day-old chick embryos (19) and also L-type Ca\(^{2+}\) currents of embryonic chick and human fetal heart cells (20). These intriguing observations have prompted an examination of the complementary effects of Ca\(^{2+}\) channel blockers on SK\(_{Ca}\) channel function. To our surprise, these studies have led us to identify taicatoxin (a previously characterized Ca\(^{2+}\) channel blocker isolated from the venom of the Australian taipan, *Oxyuranus scutellatus* (21, 22)) as a potent inhibitor of SK\(_{Ca}\) channels, both in rat brain and chromaffin cells. To the best of our knowledge, taicatoxin is the first SK\(_{Ca}\) channel blocker to be found in snake venom.

MATERIALS AND METHODS

Toxins, Drugs, and Chemicals—Native apamin was purified from *A. mellifera* bee venom and radiodiosinated as described previously (23). Taicatoxin and other Ca\(^{2+}\) channel blockers were obtained from Alomone Labs. Cardiotoxin was purified from the venom of *Naja nigricollis* nigricollis and was a generous gift from Dr. A. Menez (Department d’Ingenierie et d’Etudes des Proteines, CEA, Saclay, Gil-sur-Yvette, France). Molecular weight markers were obtained from Pharmacia Biotech Inc. Protease inhibitors, bovine serum albumin (BSA, fraction V, protease free), and hyalurondase type I-S were obtained from Sigma. Fetal bovine serum was obtained from Life Technologies, Inc. and collagenase type I was obtained from Worthington. Disuccinimidyl suberate was obtained from Pierce Chemical Co. All other chemicals used were reagent grade.

\(^{125}\)I-Apamin Binding Assays and Affinity Labeling—\(^{125}\)I-Apamin binding to rat cerebrocortical synaptic plasma membranes and subsequent analysis of data was performed as described previously (12). The incubation medium (1 ml) consisted of 10 mM KCl, 1 mM EGTA, 25 mM Tris, pH 8.4, containing 0.1% (w/v) BSA. In saturation experiments,

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† The abbreviations used are: \(K_{Ca}\) channels, Ca\(^{2+}\)-activated K\(^+\) channels; BK\(_{Ca}\) channels, high conductance Ca\(^{2+}\)-activated K\(^+\) channels; IK\(_{Ca}\) channels, intermediate conductance Ca\(^{2+}\)-activated K\(^+\) channels; SK\(_{Ca}\) channels, small conductance Ca\(^{2+}\)-activated K\(^+\) channels; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophorisis; \(I_{SKCa}\), K\(^+\) current through small conductance Ca\(^{2+}\)-activated K\(^+\) channels; \(I_{Ca}\), calcium current through voltage-dependent calcium channels.

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aliquots (~40 μg of protein) of pure plasma membranes were incubated with increasing concentrations of 125I-apamin (0.2–150 pm) in the presence or absence of either 0.1 μM native apamin (to determine non-saturable binding) or 2 nm taicatoxin. Following equilibration on ice (1 h) the reaction was quenched by the addition of ice-cold incubation medium and rapid filtration through GF/B filters, and then presoaked (1 h at 4 °C) in 0.5% (v/v) polyethyleneimine. In displacement experiments, aliquots (~100 μg of protein) of plasma membranes were incubated with a single fixed concentration of 125I-apamin (10 pm) in the absence or presence of increasing concentrations of taicatoxin or single fixed concentrations of various ligands (as detailed in Table I). In both saturation and displacement experiments triplicate assays were run to be performed; the standard deviation of the means was typically between 3 and 5%. Affinity labeling of rat synaptic plasma membranes with 125I-apamin using the homobifunctional agent disuccinimidyldi- berate was performed as described elsewhere (12).

**SDS-PAGE**—Affinity labeled membrane pellets were solubilized by heating (95 °C for 5 min) in sample buffer (4% (v/v) SDS, 10% (v/v) glycerol, 20 mM Tris, pH 6.8) containing protease inhibitors (2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 25 μM MgCl2) in the presence of 5% (v/v) 2-mercaptoethanol. Aliquots (~150 μg of protein) were analyzed by discontinuous SDS-PAGE using 12% (w/v) acrylamide slab gels. Radioactive bands were identified by exposing the dried gel to x-ray film (Hyperfilm-MP; Amersham Corp.) for 2–5 days using an intensifying screen. Molecular masses were determined by comparison with migration of known standards. Taicatoxin samples were examined by SDS-PAGE using a high-molarity Tris buffer system adopted for analysis of small peptides (24). The resolving gel contained 0.75% Tris, pH 8.9, and the composition of the running buffer was 0.192 M glycine, 0.05 M Tris, pH 8.9, containing 0.1% (w/v) SDS. Samples were heated (95 °C for 5 min) in sample buffer (as above) containing 2 mM EGTA, 2 mM EDTA and then analyzed by discontinuous gradient pore (15%–30% (v/v) acrylamide) SDS-PAGE run under reducing or nonreducing conditions in the presence or absence, respectively, of 5% (v/v) 2-mercaptoethanol. Gels were silver stained for total protein using the method of Morrissey (25).

**Cell Culture**—Rat chromaffin cells were prepared by procedures based on the methods of Neely and Lingle (26) and Park (27). In brief, 2–3 rats (~200 g weight) were killed by CO2 asphyxiation, according to Home Office guidelines. The adrenal glands were removed and the medullas isolated by dissection. The medullas were incubated in Ham’s F-14 medium containing hyaluronidase (2.4 mg/ml), 0.1% (w/v) collagenase, and 10% (v/v) fetal bovine serum for 45–60 min at 37 °C in a 5% CO2 incubator. The medullas were then washed three times with F-14 medium containing 10% (v/v) fetal bovine serum and the cells dissociated by trituration through a fine-polished Pasteur pipette. Cells were plated on polyornithine-coated coverslips and maintained at 37 °C for 3–5 days in a 5% CO2 incubator prior to electrophysiological measurements.

**Electrophysiology**—Coverslips with attached chromaffin cells were placed in a recording chamber and visualized on the stage of a Nikon Diaphot microscope. The general electrophysiological procedures were as reported in Bevan and Yeats (28). Membrane currents were studied with whole cell voltage clamp methods (29) using fire polished patch pipettes. Series resistances were between 3 and 10 MΩ. The external solution contained (mM) 129 NaCl, 30 KCl, 10 CaCl2, 1 MgCl2, 10 HEPES, adjusted to pH 7.4 with NaOH. The internal solution contained (mM) 120 potassium aspartate, 20 KCl, 5 MgCl2, 0.1 EGTA, 3 NaATP, 0.1 leupeptin, and 20 HEPES, adjusted to pH 7.2 with KOH.

A variety of both peptide and non-peptide blockers of voltage-dependent Ca2+ channels were tested for their ability to interact with 125I-apamin acceptors present on rat synaptosomal plasma membranes. Potent and widely used 1,4-dihydropyridine Ca2+ channel antagonists, such as nifedipine, nitrendipine, nimodipine, nicardipine, and PN 200-110 caused no significant change in 125I-apamin binding when present at a 100,000-fold molar excess (relative to 125I-apamin) (Table I). Similarly, Ca2+ channel toxins ω-agatoxin IVA, calcispeptine, ω-conotoxin MVIIIC, and ω-conotoxin GVIA were without effect when included in the assay at a 5000-fold molar excess. However, under identical conditions, the high threshold Ca2+ channel blocker taicatoxin, a complex oligomeric protein isolated from the venom of the Australian taipan snake O. scutellatus scutellatus (21) caused a marked reduction of binding to 11% of control values (Table I).

Taicatoxin (Mw 52000) has been reported to be an oligomeric complex of three noncovalently linked polypeptides (21). Analysis of taicatoxin samples by SDS-PAGE under reducing conditions followed by silver staining identified only three polypeptides (Fig. 1). Although the broad nature of the venom of the Australian taipan snake O. scutellatus scutellatus (21) caused a marked reduction of binding to 11% of control values (Table I).

| Table I | Effect of a variety of calcium channel blockers on 125I-apamin binding to rat brain plasma membranes |
|---------|--------------------------------------------------------------------------------------------------|
| Concentration | Competing ligand | % of control ± S.D. |
| Drugs | | |
| 1 μM | Nifedipine | 105 ± 7 |
| | Nicardipine | 105 ± 4 |
| | Nimodipine | 102 ± 3 |
| | Nitrendipine | 106 ± 5 |
| | PN 200–110 | 104 ± 4 |
| Toxins | | |
| 50 nm | Taicatoxin | 11 ± 2 |
| | ω-Agatoxin IVA | 92 ± 9 |
| | Calcispeptine | 95 ± 5 |
| | ω-Conotoxin GVIA | 102 ± 9 |
| | ω-Conotoxin MVIIIC | 100 ± 5 |
| 1 μM | Cardiotoxin γ | 102 ± 1 |
| | Apamin | 7 ± 1 |

RESULTS
A variety of both peptide and non-peptide blockers of voltage-dependent Ca2+ channels were tested for their ability to interact with 125I-apamin acceptors present on rat synaptosomal plasma membranes. Potent and widely used 1,4-dihydropyridine Ca2+ channel antagonists, such as nifedipine, nitrendipine, nimodipine, nicardipine, and PN 200-110 caused no significant change in 125I-apamin binding when present at a 100,000-fold molar excess (relative to 125I-apamin) (Table I). Similarly, Ca2+ channel toxins ω-agatoxin IVA, calcispeptine, ω-conotoxin MVIIIC, and ω-conotoxin GVIA were without effect when included in the assay at a 5000-fold molar excess. However, under identical conditions, the high threshold Ca2+ channel blocker taicatoxin, a complex oligomeric protein isolated from the venom of the Australian taipan snake O. scutellatus scutellatus (21) caused a marked reduction of binding to 11% of control values (Table I).

Taicatoxin (Mw 52000) has been reported to be an oligomeric complex of three noncovalently linked polypeptides (21). Analysis of taicatoxin samples by SDS-PAGE under reducing conditions followed by silver staining identified only three polypeptides of ~16, 8, and 7 kDa (Fig. 1). Although the broad nature of the 8-kDa polypeptide band on SDS gels made it rather difficult to accurately assess the relative abundance of the three components, our results are consistent with the conclusions of Possani and colleagues (21) who reported a stoichiometry of 1:1:4 of the 16-, 8-, and 7-kDa polypeptides, respectively. As no other polypeptides were observed after prolonged silver staining of SDS gels (not shown), our results attest to the purity of the toxin sample.

Since the 16-kDa sunit displays phospholipase activity (21), it is possible that this is responsible for the observed antagonism of 125I-apamin binding. However, this seems unlikely, since the ability of taicatoxin to inhibit 125I-apamin binding was unaltered in the presence of either 1 mM EGTA or 2 mM strontium chloride (Sr2+) is a competitor for Ca2+ binding (determined in the absence of any competing ligand) and are expressed as the mean ± S.D., determined on three different preparations of plasma membrane.
similar manner (cardiotoxins are extremely basic, surface-active polypeptides that have a wide range of membrane perturbing activities, thought to be due to their highly charged character (30)). Cardiotoxin γ from *N. nigricollis nigricollis* (31) did not alter 125I-apamin binding (Table I), suggesting that random charge effects are unlikely to underlie taicatoxin’s inhibitory action.

Further examination of taicatoxin’s activity showed that inhibition of 125I-apamin binding was complete over two log units of taicatoxin concentration ($K_i = 1.45 \pm 0.22 \, \text{nM}$) (Fig. 2A). In agreement with this finding, saturation experiments (in which increasing concentrations of 125I-apamin were incubated with brain membranes in the presence or absence of 2 nM taicatoxin) demonstrated that the inhibitory effect of taicatoxin was due to a reduction in the affinity of 125I-apamin for its acceptor, rather than an alteration of acceptor binding site density. The $K_d$ determined for 125I-apamin in the absence and presence of taicatoxin was 5 and 13 nM, respectively; $B_{max}$ varied by <2% (Fig. 2B). Importantly, these data re-affirm our earlier conclusion that the effects of taicatoxin on 125I-apamin binding are not due to site depletion through phospholipase activity.

125I-Apamin selectively labels high and low molecular weight polypeptides associated with SK$_{Ca}$ channels on both brain and liver membranes (16). Such affinity labeling can be blocked by structurally diverse pharmacological agents known to inhibit apamin-sensitive SK$_{Ca}$ channel K$^+$ currents (12). Taicatoxin completely abolished the incorporation of 125I-apamin into a 33-kDa polypeptide (putative SK$_{Ca}$ channel β-subunit (16)) present on rat brain membranes (Fig. 3, lane 7). However, no significant difference in the labeling of this polypeptide was seen in the presence of a large molar excess of other Ca$^{2+}$ channel blockers (Fig. 3, lanes 3–6). (The small decrease in labeling apparent in the presence of ω-conotoxin (lane 3) was not reproducibly observed).

In many cells, an apamin-sensitive Ca$^{2+}$-activated slow tail K$^+$ current through SK$_{Ca}$ channels ($I_{SK_{Ca}}$) contributes to the

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**Fig. 1.** Polyacrylamide gel electrophoresis of taicatoxin. An aliquot (5 μg) of taicatoxin was analyzed by gradient pore (15–30% (w/v) acrylamide) SDS-PAGE run under reducing conditions. Silver staining of the gel for total protein revealed only the subunits described by Possani et al. (21): 16, 8, and 7 kDa. Molecular masses were determined by comparison with the migration position of known standards.

**Fig. 2.** 125I-Apamin binding to rat brain plasma membranes in the presence of taicatoxin. *A*, rat brain synaptic plasma membranes (∼100 μg of protein/ml) were incubated (on ice for 1 h in 10 mM KCl, 1 mM EGTA, 25 mM Tris, pH 8.4, containing 0.1% (w/v) BSA) in the presence of 10 pM 125I-apamin and increasing concentrations of taicatoxin (range 0.01–300 nM). Following the collection of membranes by filtration, the extent of 125I-apamin binding ($B$) was expressed as a percent of total binding determined in the absence of any competing ligand ($B_0$). Data points are the mean of triplicate determinations ± S.D. The figure is typical of results obtained with three preparations of membranes. *B*, rat brain synaptosomal plasma membranes (37 μg of protein/ml) were incubated (in binding medium, as above) with increasing concentrations of 125I-apamin (0.2–150 pM), in the absence or presence of either 0.1 μM native apamin or 2 nM taicatoxin. Membranes were collected as described under “Materials and Methods.” Saturable binding in the absence (○) and presence (●) of taicatoxin is presented in the form of Scatchard plots. The figure is representative of three experiments, performed with three preparations of membranes.
after-hyperpolarization that is observed following an action potential (6). The effects of taicatoxin on ISK(Ca) were examined in voltage-clamp studies using the slow after-hyperpolarizing tail currents found in cultured rat chromaffin cells. Fig. 4 (parts A-C) demonstrates the currents evoked by stepping the membrane potential from an initial holding potential of −80 mV to 0 mV (for 2 s) and then to −120 mV (for 7 s). Under normal recording conditions, a biphasic outward current was elicited by the depolarizing voltage step and a long-lasting inward tail current was seen when the membrane potential was stepped to −120 mV (e.g. Fig. 4A, trace 1). The current is inward at −120 mV, as this holding potential is negative to the K⁺ equilibrium (zero current) potential. An application of 2 μM apamin for 1 s during the slow tail current resulted in rapid and complete block (Fig. 4A, traces 2 and 3), confirming the nature of the current as an ISK(Ca)

Application of 50 nM taicatoxin for 1 s during the tail current had no immediate effect (Fig. 4B, trace 3) but abolished the slow ISK(Ca) current evoked by subsequent depolarizing-hyperpolarizing voltage steps (Fig. 4B, trace 4). This low concentration of taicatoxin, eventually reduced the tail current by 97 ± 3% (n = 12). In contrast, higher concentrations of taicatoxin (5 μM) produced an immediate block of the ISK(Ca) tail current (Fig. 4C, trace 3). The specificity of taicatoxin for apamin-sensitive currents was tested in 4 cells in which a low dose of apamin (2 nM) was first applied, followed by the combined application of taicatoxin (50 nM) and apamin (2 nM). Application of taicatoxin had no effect on either the outward currents evoked by voltage steps from −80 mV to +20 mV or the fast tail currents evoked when the potential was stepped back to −80 mV. For the outward current, the ratio (amplitude in the presence of both toxins/amplitude in the presence of apamin alone) was 1.02 ± 0.06, while for the tail currents the ratio was 0.97 ± 0.06. These results suggest that taicatoxin does not block any additional apamin-insensitive components, such as the Ca²⁺-activated K⁺ current through BKCa channels.

At the same concentration of taicatoxin (50 nM) that completely blocked SKCa currents in chromaffin cells, only a partial block of ICa was achieved in the same cells (mean block 12 ± 4%, n = 6; Fig. 5A). This is consistent with the observations of Possani et al. (21) who showed that taicatoxin blocked calcium currents in heart cells with IC₅₀ values ranging from 10 to 500
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Australian taipan \( O. \text{scutellatus scutellatus} \) (21), is to our knowledge the first snake venom toxin shown to recognize apamin-sensitive \( \text{SK}_{\text{Ca}} \) channels. This toxin is an oligomeric complex, consisting of a peptide bearing homology to \( \alpha \)-neurotoxins (8 kDa), a neurotoxic phospholipase (16 kDa), and four copies of a serine protease inhibitor (7 kDa) whose primary structure bears homology to protease inhibitors from other snake venoms (21). Taicatoxin binds with high affinity (\( K_i \approx 1 \text{nM} \)) to \( ^{125}\text{I}-\text{apamin} \) acceptors present on rat brain membranes and the inhibition of \( ^{125}\text{I}-\text{apamin} \) binding is both competitive and phospholipase-independent. Taicatoxin also specifically blocks affinity labeling of a 33-kDa \( ^{125}\text{I}-\text{apamin} \) binding polypeptide implicated in the structure of a hetero-oligomeric \( \text{SK}_{\text{Ca}} \) channel. These biochemical findings are supported by the demonstration that in chromaffin cells, taicatoxin (50 nM) completely blocks the slow \( I_{\text{SK(Ca)}} \) tail current. At the same concentration, taicatoxin reduces total \( I_{\text{Ca}} \) by only \( \sim 12\% \).

Although taicatoxin competes for \( ^{125}\text{I}-\text{apamin} \) acceptors with high affinity, it is considerably less potent than apamin itself (\( K_i \approx 3 \text{pt} \) (12)). One cannot therefore unequivocally rule out the possibility that activity may be due to the presence (<0.01% by mass, assuming a \( M_f \) of 2000) of an unidentified neurotoxin with affinity analogous to that of apamin. However, evidence suggesting that this is not the case stems from the observation that taicatoxin’s constituent \( \alpha \)-neurotoxin possesses a structural motif consistent with the recognition of \( \text{SK}_{\text{Ca}} \) channels. Apamin has two adjacent arginine residues (Arg-13 and Arg-14) that are essential for its biological activity (37–39) and it has been proposed that this motif (more specifically the approximately 11 Å separation of two positive charges) provides the basis for specificity of \( \text{SK}_{\text{Ca}} \) channel blocking activity. A similar spatial separation of two positive charges is also present in dequalinium and tubocurarine (as well as other bisquaternary neuromuscular blocking agents), and this structural feature is thought to underlie their ability to inhibit \( ^{125}\text{I}-\text{apamin} \) binding to liver and brain membranes and to block \( \text{Ca}^{2+} \)-activated \( K^+ \) currents in both hepatocytes and neurons (12, 40, 41). Structure-function studies have also demonstrated that the scorpion toxins PO5 and scyllatoxin possess two functionally critical arginine residues responsible for \( \text{SK}_{\text{Ca}} \) channel inhibition. These two arginine residues are adjacent in PO5 (34) while in the case of scyllatoxin, they are brought into close proximity through secondary folding (33, 42). The close apposition of two non-contiguous arginine residues within \( \text{Tsk} \) is similarly thought to underlie its recognition of \( ^{125}\text{I}-\text{apamin} \) acceptor-binding sites (36). Since the \( \alpha \)-neurotoxin of the taicatoxin complex contains two adjacent arginine residues at its N-terminus (21), this suggests a structural basis for the recognition of \( ^{125}\text{I}-\text{apamin} \) acceptor-binding sites. We have no insights as to a possible structural basis for taicatoxin’s recognition of high threshold \( \text{Ca}^{2+} \) channels, although given the large size and oligomeric composition of taicatoxin, one can speculate that this may involve a different region of the toxin. The involvement of a single toxin domain in recognizing two distinct classes of ion channel would mean that such a toxin motif must exploit similar regions of like-charge distribution (presumably within the pore regions) on the two channel types. However, since there is no primary sequence homology between \( \alpha \)-subunits of \( \text{SK}_{\text{Ca}} \) channels and high threshold \( \text{Ca}^{2+} \) channels (17, 43), any similarity would be purely fortuitous. Nevertheless, such a scenario may explain the anomalous observation of apamin-sensitive \( \text{Ca}^{2+} \) channels in immature heart cells (19, 20). If there is any similarity in electrostatic topography between \( \text{SK}_{\text{Ca}} \) channels and certain forms of \( \text{Ca}^{2+} \) channel, then it is severely restricted; the overwhelming consensus of data in the literature would indicate that \( \text{Ca}^{2+} \) chan-
nels and SK\textsubscript{Ca} channels have distinct and non-overlapping pharmacology.

In summary, our results indicate that taicatoxin, previously shown to block high threshold Ca\textsuperscript{2+} channels in heart, interacts with SK\textsubscript{Ca} channels in both chromaffin cells and the brain. The toxin blocks \textsuperscript{125}I-apamin acceptor sites on rat synaptosomal membranes with high affinity and is an effective inhibitor of I\textsubscript{SKCa} in rat chromaffin cells. In view of these findings the use of taicatoxin as a specific ligand for voltage-dependent Ca\textsuperscript{2+} channels should now be reconsidered.

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