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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_i = 1.45 ± 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)[1] 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 neurones, 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 (α) and low (β) 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 α 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 radioiodinated 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, Gif-sur-Yvette, France). Molecular weight markers were obtained from Pharmacia Biotech Inc. Protease inhibitors, bovine serum albumin (BSA, fraction V, protease free), and hyaluronidase 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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[1] 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 electrophoresis; $I_{SK(Ca)}$, K^+ current through small conductance Ca^{2+}-activated K^+ channels; I_{Ca}, calcium current through voltage-dependent calcium channels.

aliquots (~40 μg of protein) of purified plasma membranes were incubated with increasing concentrations of ^{125}I-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 Whatman GF/B FP-100 filters 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 ^{125}I-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 routinely performed; the standard deviation of the means was typically between 3 and 5%. Affinity labeling of rat synaptic plasma membranes with ^{125}I-apamin using the homobifunctional agent disuccinimidyl suberate was performed as detailed elsewhere (12).

SDS-PAGE—Affinity labeled membrane pellets were solubilized by heating (95 °C for 5 min) in sample buffer (4% (w/v) SDS, 10% (v/v) glycerol, 20 mM Tris, pH 6.8) containing protease inhibitors (2 mM EDTA, 2 mM EGTA, 10 μg/ml soybean trypsin inhibitor, 0.2 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, and 25 μg/ml bacitracin) 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 M 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% (w/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 CO_2 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% CO_2 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 fire polished Pasteur pipette. Cells were plated on polyornithine-coated coverslips and maintained at 37 °C for 3–5 days in a 5% CO_2 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 and an 80–85% series resistance compensation was applied. Potassium currents through small conductance Ca^{2+}-activated channels ($I_{SK(Ca)}$) were studied by examination of the tail currents, evoked when the membrane potential was stepped from an initial holding potential of −80 mV to more depolarized potentials (to evoke an inward Ca^{2+} current), and then subsequently stepped back to −120 mV for 7 s. The external solution contained (mM) 122.5 NaCl, 30 KCl, 10 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES, adjusted to pH 7.4 with NaOH. The internal solution contained (mM) 120 potassium aspartate, 20 KCl, 5 $MgCl_2$, 0.1 EGTA, 3 Na_2ATP, 0.1 leupeptin, and 20 HEPES, adjusted to pH 7.2 with KOH. The same solutions were also used to study any effects of taicatoxin on potassium currents through BK_{Ca} channels. Delayed rectifier currents were evoked by 100 ms voltage steps from −80 mV to more depolarized potentials in "Ca^{2+}-free" solution. In most experiments drugs were applied by pressure ejection from a puffer pipette although a U-tube application system was employed in some cases. Data are presented as mean ± S.E. of *n* observations. For Ca^{2+} current (I_{Ca}) experiments the external solution contained (mM) 150 choline chloride, 10 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, buffered to pH 7.4 with Tris-OH, and the internal solution contained (mM) 130 CsCl, 1 $CaCl_2$, 10 EGTA, 1 $MgCl_2$ and 10 HEPES, adjusted to pH 7.2 with CsOH.

TABLE I

Effect of a variety of calcium channel blockers on ^{125}I-apamin binding to rat brain plasma membranes

Displacement experiments using ^{125}I-apamin were performed on purified plasma membranes as described under "Materials and Methods." Values shown are a percent of control 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.

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
	Calciseptine	95 ± 5
	ω-Conotoxin GVIA	102 ± 9
	ω-Conotoxin MVIIC	100 ± 5
1 μM	Cardiotoxin γ	102 ± 1
1 nM	Apamin	7 ± 1

RESULTS

A variety of both peptide and non-peptide blockers of voltage-dependent Ca^{2+} channels were tested for their ability to interact with ^{125}I-apamin acceptors present on rat synaptosomal plasma membranes. Potent and widely used 1,4-dihydropyridine Ca^{2+} channel antagonists, such as nifedipine, nitrendipine, nimodipine, nicardipine, and PN 200-110 caused no significant change in ^{125}I-apamin binding when present at a 100,000-fold molar excess (relative to ^{125}I-apamin) (Table I). Similarly, Ca^{2+} channel toxins ω-agatoxin IVA, calciseptine, ω-conotoxin MVIIC, 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 Ca^{2+} 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 (M_r 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 subunit displays phospholipase activity (21), it is possible that this is responsible for the observed antagonism of ^{125}I-apamin binding. However, this seems unlikely, since the ability of taicatoxin to inhibit ^{125}I-apamin binding was unaltered in the presence of either 1 mM EGTA or 2 mM strontium chloride (Sr^{2+} is a competitor for Ca^{2+} binding, which is essential for phospholipase activity) (data not shown). Nevertheless, since EGTA alone (1 mM) produced no discernible inhibition of ^{125}I-apamin binding, it was routinely included in the buffer used in all further binding experiments. Another possible explanation for taicatoxin's block of ^{125}I-apamin binding could be nonspecific charge effects, owing to the high density of positive charge on the toxin. To test this possibility, we examined the ability of a snake venom cardiotoxin to act in a

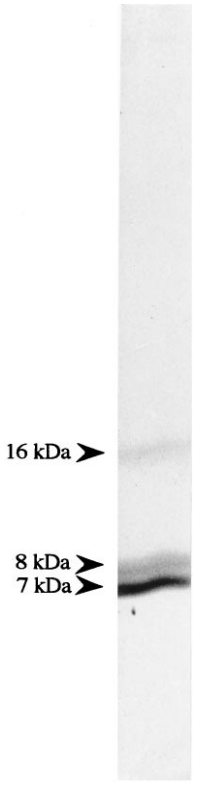

FIG. 1. **Polyacrylamide gel electrophoresis of taicatoxin.** An aliquot (5 μg) of taicatoxin was analyzed by gradient pore (15–30% (w/v) acrylamide) SDS-PAGE run under reducing conditions. Silver staining of the gel for total protein revealed only the subunits described by Possani *et al.* (21): 16,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

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 ^{125}I-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 ^{125}I-apamin binding was complete over two log units of taicatoxin concentration (K_i = 1.45 ± 0.22 nM) (Fig. 2*A*). In agreement with this finding, saturation experiments (in which increasing concentrations of ^{125}I-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 ^{125}I-apamin for its acceptor, rather than an alteration of acceptor binding site density. The K_d determined for ^{125}I-apamin in the absence and presence of taicatoxin was 5 and 13 pM, respectively; B_{max} varied by <2% (Fig. 2*B*). Importantly, these data re-affirm our earlier conclusion that the effects of taicatoxin on ^{125}I-apamin binding are not due to site depletion through phospholipase activity.

^{125}I-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 ^{125}I-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).

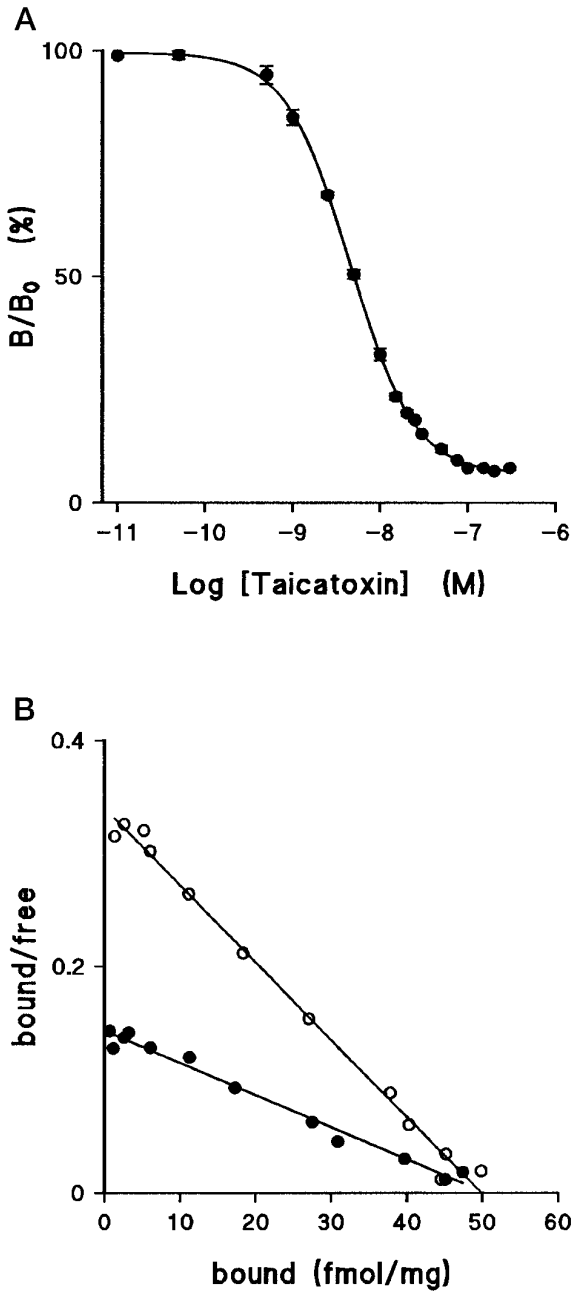

FIG. 2. **^{125}I-Apamin binding to rat brain plasma membranes in the presence of taicatoxin.** *A*, rat brain synaptic plasma membranes (~100 μg of protein/ml) were incubated (on ice for 1 h in 10 mM KCl, 1 mM EGTA, 25 mM Tris, pH 8.4, containing 0.1% (w/v) BSA) in the presence of 10 pM ^{125}I-apamin and increasing concentrations of taicatoxin (range 0.01–300 nM). Following the collection of membranes by filtration, the extent of ^{125}I-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 ^{125}I-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.

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

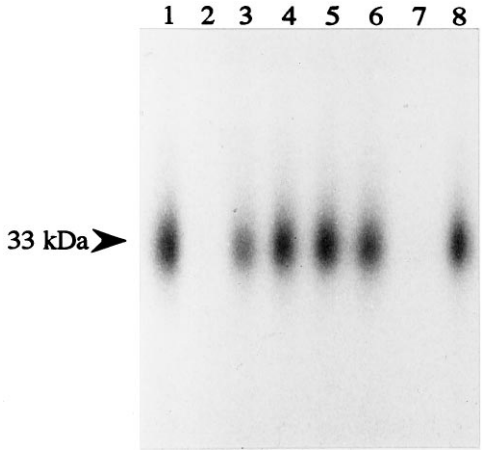

FIG. 3. **Cross-linking of ^{125}I-apamin to rat brain synaptosomal plasma membranes in the presence of a variety of toxins and drugs.** Rat brain plasma membranes were equilibrated (on ice for 1 h in 10 mM KCl, 1 mM EGTA, 25 mM borax, pH 9.0, containing 0.1% (w/v) BSA) with 100 pM ^{125}I-apamin in the absence (*lane 1*) or presence (*lanes 2–8*) of various competing ligands and subsequently reacted with 5 mM disuccinimidyl suberate (in buffer lacking BSA) as described under "Materials and Methods." Competing ligands were 10 nM native apamin (*lane 2*), 1 μM ω-conotoxin GVIA (*lane 3*), 1 μM nicardipine (*lane 4*), 1 μM nitrendipine (*lane 5*), 1 μM nimodipine (*lane 6*), 500 nM taicatoxin (*lane 7*), and 1 μM cardiotoxin γ (*lane 8*). Aliquots (~150 μg) of protein were analyzed by discontinuous SDS-PAGE on 12% (w/v) acrylamide slab gels run under reducing conditions in the presence of 5% (v/v) 2-mercaptoethanol. Radioactive bands were identified by exposing the dried gel to x-ray film. Molecular masses were determined by comparison with the migration position of known standards.

after-hyperpolarization that is observed following an action potential (6). The effects of taicatoxin on $I_{SK(Ca)}$ 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. 4*A, 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. 4*A, traces 2* and *3*), confirming the nature of the current as an $I_{SK(Ca)}$. Application of 50 nM taicatoxin for 1 s during the tail current had no immediate effect (Fig. 4*B, trace 3*) but abolished the slow $I_{SK(Ca)}$ current evoked by subsequent depolarizing-hyperpolarizing voltage steps (Fig. 4*B, 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 $I_{SK(Ca)}$ tail current (Fig. 4*C, 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 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 Ca^{2+}-activated K^+ current through BK_{Ca} channels.

At the same concentration of taicatoxin (50 nM) that completely blocked SK_{Ca} currents in chromaffin cells, only a partial block of I_{Ca} was achieved in the same cells (mean block 12 ± 4%, n = 6; Fig. 5*A*). This is consistent with the observations of Possani *et al.* (21) who showed that taicatoxin blocked calcium currents in heart cells with IC_{50} values ranging from 10 to 500

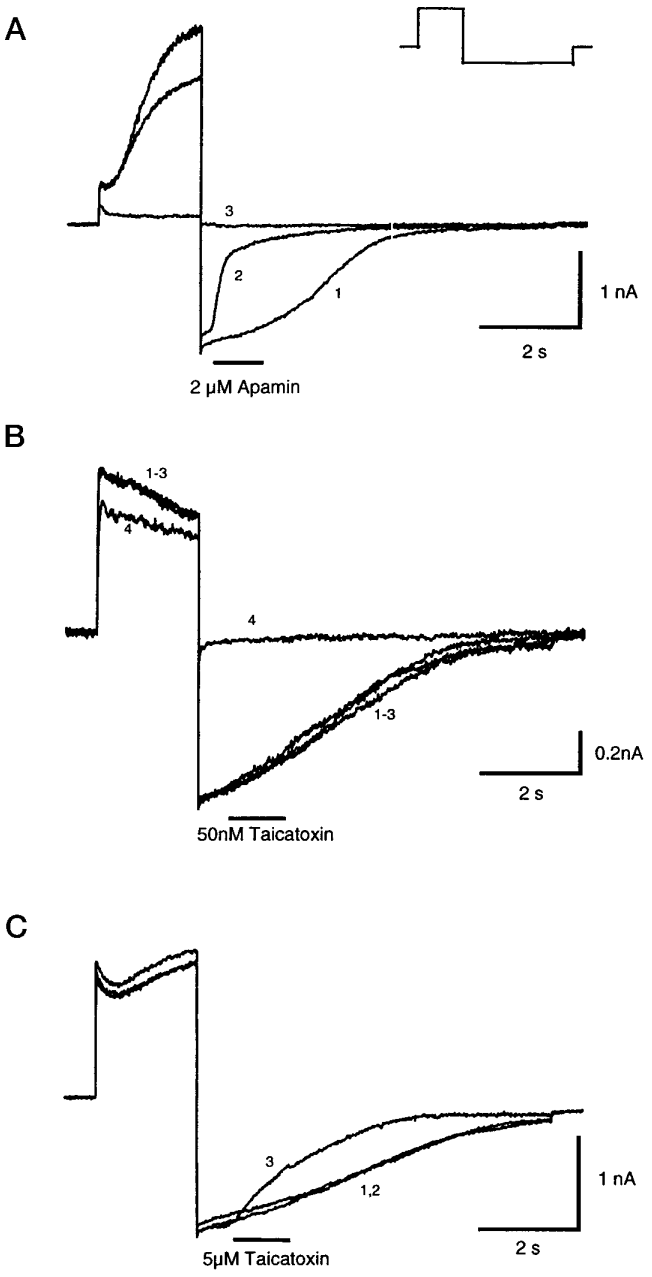

FIG. 4. **Blockade of rat chromaffin cell $I_{SK(Ca)}$ slow tail potassium currents by apamin and taicatoxin.** Experimental procedures were as outlined under "Materials and Methods." In *panels A-C*, slow tail potassium currents were evoked by a step in membrane potential from a holding potential of −80 to 0 mV (for 2 s) (evoking an influx of calcium) and subsequently back to −120 mV for 7 s. *Inset* shows the voltage step protocol. *A*, effect of apamin on slow tail potassium currents. The interval between the traces was 5 s. *Trace 1*, control slow tail current. *Trace 2*, apamin (2 μM) applied for 1 s (*horizontal bar*) during the tail current. *Trace 3*, reduction of the outward current (leaving residual delayed rectifier and calcium currents) and abolition of the tail current, in response to apamin. *B*, effect of taicatoxin on $I_{SK(Ca)}$ tail currents. *Traces 1* and *2*, control slow tail currents. *Trace 3*, taicatoxin (50 nM) applied for 1 s during the tail current showed little or no immediate block; however, the tail current was completely abolished 5 s later, *trace 4. C*, immediate blockade of $I_{SK(Ca)}$ tail current induced by a high concentration of taicatoxin. *Traces 1* and *2*, control currents. *Trace 3*, taicatoxin (5 μM) applied for 1 s during the tail current.

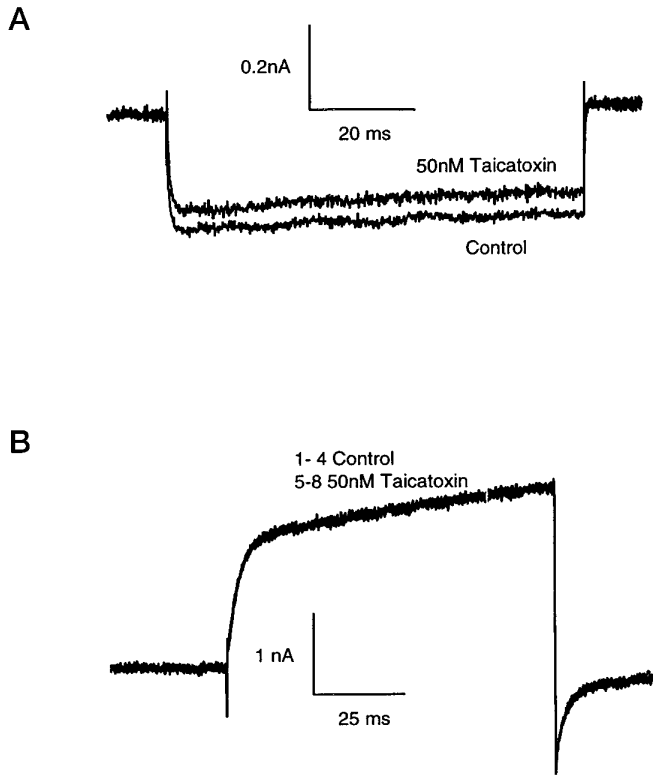

FIG. 5. **Effect of taicatoxin on calcium and delayed rectifier potassium currents in rat chromaffin cells.** Experimental procedures were as outlined under "Materials and Methods." *A*, effect of taicatoxin on calcium currents. Taicatoxin was applied between traces. I_{Ca} evoked by a depolarizing step from −80 to 0 mV for 1 s. Traces show the control current and a current recorded 30 s after continuous application of taicatoxin (50 nM). *B*, effect of taicatoxin on delayed rectifier potassium currents recorded in "calcium-free" external solution. The interval between traces was 10 s. Potassium currents were evoked by depolarizing steps (100 ms duration) to +20 mV from a prepulse potential of −120 mV, after which the potential was stepped to −80 mV. *Traces 1–4*, control currents. *Traces 5–8*, currents recorded in the presence of taicatoxin (50 nM).

nM, dependent on holding potential (−30 to −80 mV). The relatively small effect on total I_{Ca} observed here is consistent with the notion that the reduction of $I_{SK(Ca)}$ represents a direct effect of taicatoxin on SK_{Ca} channels. The specificity of taicatoxin for $I_{SK(Ca)}$ was also tested by examining its effect on delayed rectifier currents which are present in chromaffin cells (26). In Ca^{2+}-free media, taicatoxin (50 nM) had no significant effect on the amplitude of the delayed rectifier K^+ currents (Fig. 5*B*, mean amplitude 96 ± 3%, $n = 5$, of control value after toxin application).

DISCUSSION

The ability of neurotoxins to selectively recognize different ion channels with high affinity renders them extremely useful probes for determining the distribution, biophysics, pharmacology, and structure of individual channel subtypes. As the number of recognized channel subtypes increases, so too does the demand for novel, highly selective toxins.

Apamin, an octadecapeptide from the venom of the European honeybee *A. mellifera* is an established blocker of SK_{Ca} channels in both the central nervous system and in diverse peripheral tissues (3, 5, 18). The properties of SK_{Ca} channels have been largely deduced through the use of apamin in both biochemical and physiological studies. Although three other toxins, scyllatoxin (leiurotoxin I), PO5, and Tsκ, possessing apamin-like activity have been characterized from scorpion venoms (32–36), taicatoxin, purified from the venom of the Australian taipan *O. scutellatus scutellatus* (21), is to our knowledge the first snake venom toxin shown to recognize apamin-sensitive SK_{Ca} channels. This toxin is an oligomeric complex, consisting of a peptide bearing homology to α-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 ~1 nM) to ^{125}I-apamin acceptors present on rat brain membranes and the inhibition of ^{125}I-apamin binding is both competitive and phospholipase-independent. Taicatoxin also specifically blocks affinity labeling of a 33-kDa ^{125}I-apamin binding polypeptide implicated in the structure of a hetero-oligomeric SK_{Ca} channel. These biochemical findings are supported by the demonstration that in chromaffin cells, taicatoxin (50 nM) completely blocks the slow $I_{SK(Ca)}$ tail current. At the same concentration, taicatoxin reduces total I_{Ca} by only ~12%.

Although taicatoxin competes for ^{125}I-apamin acceptors with high affinity, it is considerably less potent than apamin itself (K_d ~3 pM (12)). One cannot therefore unequivocally rule out the possibility that activity may be due to the presence (<0.01% by mass, assuming a M_r 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 α-neurotoxin possesses a structural motif consistent with the recognition of SK_{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 SK_{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}I-apamin binding to liver and brain membranes and to block Ca^{2+}-activated K^+ currents in both hepatocytes and neurones (12, 40, 41). Structure-function studies have also demonstrated that the scorpion toxins PO5 and scyllatoxin possess two functionally critical arginine residues responsible for SK_{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 Tsκ is similarly thought to underlie its recognition of ^{125}I-apamin acceptor-binding sites (36). Since the α-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}I-apamin acceptor-binding sites. We have no insights as to a possible structural basis for taicatoxin's recognition of high threshold 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 α-subunits of SK_{Ca} channels and high threshold Ca^{2+} channels (17, 43), any similarity would be purely fortuitous. Nevertheless, such a scenario may explain the anomalous observation of apamin-sensitive Ca^{2+} channels in immature heart cells (19, 20). If there is any similarity in electrostatic topography between SK_{Ca} channels and certain forms of Ca^{2+} channel, then it is severely restricted; the overwhelming consensus of data in the literature would indicate that Ca^{2+} chan-

nels and SK_{Ca} channels have distinct and non-overlapping pharmacology.

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

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