0026-895X/90/020164-06$02.00/0

MOLECULAR PHARMACOLOGY, 38:164-169

Potassium Channel Blockade by the B Subunit of β-Bungarotoxin

CHRISTINA G. BENISHIN

Department of Physiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2H7 Canada

Received June 26, 1989; Accepted April 30, 1990

SUMMARY

β-Bungarotoxin (β-BTX) isolated from the venom of *Bungarus multicinctus* has previously been reported to be a neurotoxic protein. The toxin is composed of two subunit chains, denoted A and B. The intact toxin was first examined in this study for its ability to block central nerve ending K channels using the ^{86}Rb efflux technique. β-BTX, when preincubated with synaptosomes for 30 min in the absence of extracellular Ca^{2+}, selectively inhibited the slowly inactivating voltage-dependent (S) component of ^{86}Rb efflux. The EC_{50} for inhibition is 1 to 3 nM. The two subunit chains were separated and isolated by reduction and carboxymethylation of the parent toxin. The K channel-blocking activity was associated only with the reduced and carboxymethylated B-subunit of β-BTX (RCM-B). The dose-dependent inhibition by RCM-B exhibited an apparent biphasic response, with the noninactivating voltage-gated K channel being more susceptible to RCM-B inhibition (EC_{50} = 0.1 to 0.3 nM) than to inhibition by the parent compound. Additionally, the inactivating voltage-gated K channel (T) was sensitive to inhibition by higher concentrations of RCM-B (EC_{50} = 1 to 3 nM). These results suggest that the B-chain of β-BTX may be responsible for blockade of certain voltage-gated K channels in a manner that is not directly related to the known phospholipase activity of the intact molecule.

Recent studies have indicated that venoms from a family of snakes, Elapidae, contain certain peptide toxins that may be potent and selective antagonists of potassium channels (1, 2). Careful study of the venom of the Eastern green mamba (*Dendroaspis angusticeps*) resulted in identification of four distinct peptide toxins that block presynaptic K channels in nerve endings (1). Partial sequence analysis indicated that these four peptides share structural homology with known protease inhibitor components of a number of snake venoms. One peptide that also has structural homology with the protease inhibitors is the B-chain of β-BTX (3–6).

β-BTX intoxication has been previously described both *in vivo* (7) and *in vitro* (8–11). In particular, intoxication in the isolated neuromuscular junction preparation is characterized by an initial reduction in acetylcholine release, followed by a transient rise in the release of neurotransmitter and then by a gradual total cessation of nerve activity (12). *In vitro*, the action of β-BTX is associated with a depolarization of nerve ending plasma membranes (13, 14) and an increase in acetylcholine release from isolated nerve endings of brain (synaptosomes) (15–17).

Chemical analysis of β-BTX has shown that the toxin is composed of two polypeptide chains, denoted A and B (3, 4). Phospholipase A_2 activity has been ascribed to the A-chain (18, 19). However, this activity cannot explain the neurotoxic action of this toxin, because previous studies have shown that phospholipase A_2 enzyme from other sources does not produce the same pattern of toxicity (20, 21). Several attempts have been made to determine whether β-BTX acts on ion channels. Several groups have suggested that β-BTX may block neuronal K channels in the mammalian nerve-muscle preparation (22, 23) and in guinea pig dorsal root ganglion neurons (24). The effect of β-BTX on central nervous system K channels has not yet been examined, but several studies have reported the existence of β-BTX binding sites in rat (25–27) and chicken (26, 28, 29) brain. The identity of the β-BTX binding site with a K channel has not been conclusively demonstrated. Therefore, these studies were carried out to determine whether β-BTX might be capable of blocking central nerve ending K channels independently of the phospholipase activity. A preliminary report has been previously published as an abstract (30).

Materials and Methods

Synaptosomal K channel quantitation. Brain synaptosomes were prepared according to the procedure of Hajos (31), as described by Krueger *et al.* (32). Whole rat forebrains were homogenized in 0.32

This work was supported by an Alberta Heritage Foundation for Medical Research scholarship/establishment grant and a University of Alberta Central Research Fund grant to the author.

ABBREVIATIONS: β-BTX, β-bungarotoxin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; RCM-B, β-bungarotoxin reduced and carboxymethylated B subunit.

M sucrose, buffered with 10 mM HEPES at pH 7.4. The crude synaptosomal (P_2) pellet was prepared by differential centrifugation. Synaptosomes were purified from the P_2 fraction by sucrose gradient centrifugation. The fraction obtained from the sucrose gradient is reported to be 80–90% pure synaptosomes in the whole brain preparation (32).

Synaptosomal K channel activity was quantitated using the ^{86}Rb efflux technique, as described previously (33, 34). Synaptosomes collected from the preparative gradient were equilibrated in a resting physiological salt solution (composition in mM: NaCl, 145; KCl, 5; RbCl, 0.1; $MgCl_2$, 2; glucose, 10; NaH_2PO_4, 0.5; HEPES, 10; adjusted to pH 7.4 with NaOH; 5 K). Aliquots of synaptosomes were then incubated for 30 min at 30° in 5 K (approximately 40 mg/ml synaptosomal protein) with ^{86}Rb (10 to 20 μCi/ml) in order to load synaptosomes. In most experiments, during ^{86}Rb loading, some synaptosomes were also exposed to 500 μM EGTA (control) or EGTA plus the final concentration of the peptide toxin, where indicated. Aliquots of the loaded synaptosomes were washed (5 K plus 0.1% bovine serum albumin) to remove extracellular ^{86}Rb and then incubated in the presence of an efflux solution containing normal KCl (5 mM; 5 K) or elevated KCl (50 or 100 mM KCl, NaCl reduced to maintain osmolarity; 50 K or 100 K) with 0.5 mM EGTA, with or without the peptide toxins. At the approximate times (1 to 5 sec), the efflux was terminated with a stop solution (composition in mM: tetraethylammonium chloride, 145; tetrabutylammonium chloride, 5; RbCl, 0.1; $NiCl_2$, 10; $MgCl_2$, 10; HEPES, 10; adjusted to pH 7.4 with NaOH). Synaptosomes were separated from the effluent by rapid filtration on glass fiber filters (Schleicher & Schuell no. 25). Radioactivity was measured in both the effluent solution and the filter, and results are expressed as the percentage of the synaptosomal content released [[(counts in effluent)/(counts in effluent + counts on filter)] × 100]. Efflux into 5 K solution corresponds to conductances through channels that are active under nondepolarizing conditions, as well as other nonspecific effluxes. This flux is composed of the basal (*y*-intercept; 'B') and resting (slope; 'R') flux components. Efflux stimulated in the presence of elevated (depolarizing) KCl corresponds to efflux through voltage-gated K channels and is composed of two components. The fast (inactivating) component corresponds to the magnitude of the stimulated increase in the *y*-intercept and is designated T. This component has previously been demonstrated to be sensitive to inhibition by 4-aminopyridine (33) and α-dendrotoxin (1) and is thought to correspond to flux through the A-current channels. The slow (noninactivating) component corresponds to the magnitude of the stimulated increase in the slope and is designated S. Approximately one third of this component has previously been demonstrated to be sensitive to inhibition by phencyclidine (35) and β-dendrotoxin (1). The remainder of S is thought to be ^{86}Rb flux through resting K channels, which is stimulated by the increased driving force upon depolarization.

Because the experiments described herein were designed to inhibit phospholipase A_2 activity by omission of extracellular Ca, it was not possible to quantitate effects on Ca-activated K channels. However, an effect of β-BTX on Ca-activated K channels has been previously eliminated in the nerve-muscle preparation (23).

Synaptosomal integrity. In order to ensure that phospholipase A_2 activity of β-BTX was adequately inhibited and was not increasing the membrane permeability by lipolysis, synaptosomes were prepared as described above and incubated in the absence or presence of β-BTX. RCM-B, and extracellular Ca for 30 min at 30°. Following incubation, the synaptosomal suspensions were filtered (Schleicher & Schuell no. 25) and the supernatants were analyzed for release of the cytosolic enzyme lactate dehydrogenase according to standard procedures (36), monitoring oxidation of NADH by the change in extinction at 365 nm.

Reduction and carboxymethylation of β-BTX. The procedure of Crestfield *et al.* (37) was adapted for preparation of the β-BTX subunits. Aliquots of 0.5 to 1.0 mg of β-BTX were dissolved in 10 ml of Tris·HCl buffer (360 mM, pH 8.6) containing 6 M urea. Mercaptoethanol (1 μl) was added, and then the tube was flushed with N_2 and tightly capped. The solution was incubated for 4 hr at room temperature. After incubation, 13 μl of a solution of iodoacetic acid (134 mg/1.0 ml in 1.0 N NaOH) were added to the incubation medium. The tube was wrapped in foil (to protect it from light) and incubated further for 15 min. The mixture was then applied to a column of Sephadex G-25 (2.0 × 15 cm, also wrapped in foil), and RCM-B was eluted in 0.1 M formic acid. The insoluble A-chain (3) was then solubilized and eluted by washing with water, followed by 0.01 M NH_4OH. Protein was assayed according to the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL).

Electrophoresis. Purity of the peptide was ensured with discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoretic gels, which were run according to a modification of the procedure described by Anderson *et al.* (38). The composition of gels was: stacking gel, 3.125% T (total concentraion of monomer), 20% C (percentage of total monomer which is crosslinker); separating gel, 5% T, 5% C. Gels were run at 100 V, for approximately 100 min. Samples were run under reducing conditions except where it is indicated that nonreducing conditions were used. In this case, the Anderson sample buffer was simply modified to omit 2-mercaptoethanol.

Chemicals. β-BTX, obtained from Sigma Chemical Co. (St. Louis, MO), was isolated according to the procedure of Lee *et al.* (39). All other chemicals were from Sigma Chemical Co. as well.

Statistics. Data were analyzed by paired *t* test, Newman-Keuls test, or Duncan's multiple range test where appropriate, with the computer program PCS/PHARM. Significance was set at $p < 0.05$.

Results

Because early reports have demonstrated phospholipase A_2 activity associated with a portion of the β-BTX molecule (18, 19), initial experiments examined the effect of native β-BTX on ^{86}Rb efflux from synaptosomes in the presence of EGTA (phospholipase A_2 is Ca^{2+} dependent). β-BTX was also included in the loading preincubation in order to expose the synaptosomes to toxin for 30 min before the flux. This procedure slightly reduced the loading of synaptosomal ^{86}Rb in the toxin-treated samples versus the controls (data not shown). However, because there has never been any evidence to suggest intrasynaptosomal compartmentalization of K and results are expressed as a percentage of the total content, this should not influence the results. Fig. 1 presents the averaged results from five flux experiments. The data indicate that 10 nM β-BTX profoundly reduced the slope of the high KCl-stimulated efflux, corresponding to the nonactivating K channel (S), without altering the other components of flux. Table 1 summarizes the results from six experiments and supports the same conclusion, that β-BTX affects only S significantly. The effect of β-BTX on the voltage-dependent ^{86}Rb efflux was dose dependent (Fig. 2), with an IC_{50} of 1 to 3 nM. Results in Table 2 indicate that the conditions of incubation (omission of Ca, addition of EGTA) were adequate to abolish phospholipase activity of β-BTX, because incubation with the toxin with EGTA did not increase release of the cytosolic enzyme lactate dehydrogenase above control.

In order to determine whether the K channel-blocking activity can be separated from the phospholipase activity, the two substituent chains, A and B, which are held together by one disulfide bond, were separated by reduction of the disulfide bond and carboxymethylation of the resulting sulfhydryl groups. A sodium dodecyl sulfate-polyacrylamide gel of the native β-BTX (reducing and nonreducing conditions) and the reaction product RCM-B (under reducing conditions) is shown in Fig. 3. Native β-BTX run under nonreducing conditions

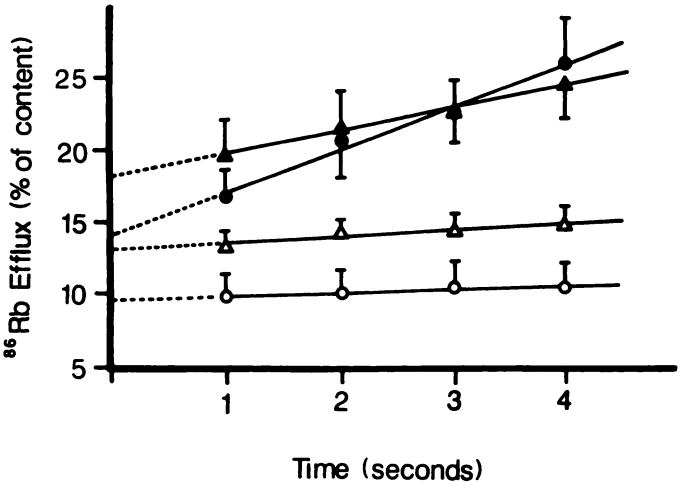

Fig. 1. Effect of 10 nM β-BTX on ^{86}Rb efflux. The efflux of ^{86}Rb from synaptosomes was measured, as described in Materials and Methods, in the presence of 5 K (*open symbols*) or 50 K (*closed symbols*) solutions and in the absence (*circles*) or presence (*triangles*) of β-BTX, for 1 to 4 sec. Each *point* represents the mean ± standard error for five experiments.

TABLE 1

Summary of the effects of β-BTX on synaptosomal ^{86}Rb efflux

Fluxes were quantitated as described in Materials and Methods. Flux was stimulated by 50 K. Results are expressed as percentage of content (B and T) or percentage of content/sec (R and S). Values are mean ± standard error for six experiments.

Component	^{86}Rb efflux			Statistics
	Control	10 nM β-BTX		
			% of control	
B	11.5 ± 2.6	14.8 ± 1.4	108	NS[a]
R	0.326 ± 0.136	0.447 ± 0.132	137	NS
T	4.78 ± 1.51	5.86 ± 2.38	122	NS
S	2.59 ± 0.29	1.19 ± 0.13	46	[b]

[a] NS, not significant.
[b] Toxin treatment alters flux component significantly from control, paired *t* test.

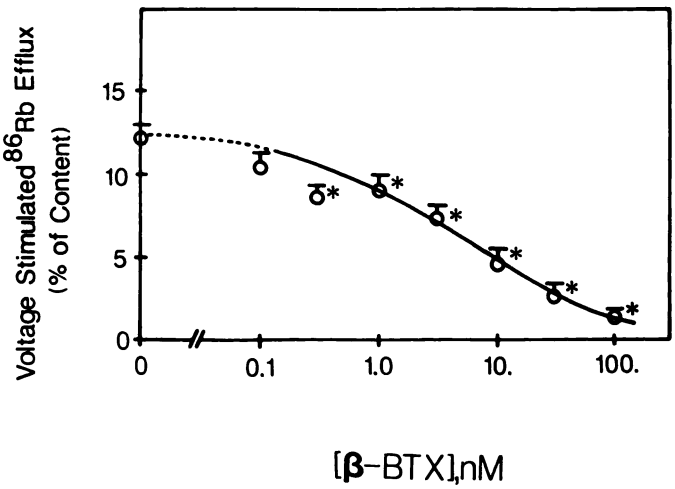

Fig. 2. Dose response of β-BTX on ^{86}Rb efflux in synaptosomes. Efflux was measured as described in Materials and Methods, in the presence of 5 K or 100 K, for 4 sec. The difference, which is plotted, represents the voltage-stimulated efflux. The EC_{50} is 1 to 3 nM. Each *point* is the mean ± standard error of six separate determinations, and each determination was made in quadruplicate. *Differs significantly from control, Newman-Keuls test.

TABLE 2

Analysis of synaptosomal integrity by release of cytosolic lactate dehydrogenase

Synaptosomes were prepared as described in Materials and Methods. An aliquot of synaptosome suspension (containing 0.6 mg of synaptosomal protein) was incubated in 5 K solution with added Ca (1 mM) or EGTA (10 mM) and in the presence of no toxin (control), β-BTX (100 nM), or RCM-B (10 nM). Lactate dehydrogenase (LDH) activity released from synaptosomes was measured in the supernatants as described in Materials and Methods. Results are expressed as mean ± standard error for three experiments.

Incubation	LDH activity released
	units/ml
Control + Ca	0.2066 ± 0.0213
Control + EGTA	0.2576 ± 0.0222
β-BTX + Ca	0.3928 ± 0.0259[a]
β-BTX + EGTA	0.2427 ± 0.0176[b]
RCM-B + Ca	0.2427 ± 0.0074
RCM-B + EGTA	0.2381 ± 0.0241

[a] Significantly different from control plus Ca group.
[b] Significantly different from β-BTX plus Ca group; Duncan's multiple range test.

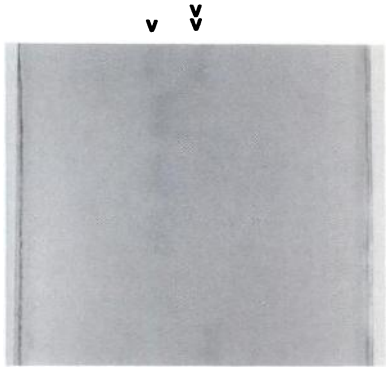

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of RCM-B and β-BTX. Gels were run according to the procedure of Anderson *et al.* (38), as described in Materials and Methods. Peptide bands were stained with Coomassie blue. Samples run are (*top* to *bottom*): *lane 1*, β-BTX, run in reducing sample buffer; *lanes 2–5*, RCM-B in decreasing amounts, run in reducing buffer; *lane 6*, β-BTX, run in nonreducing sample buffer. >, position of 6,500-dalton standard; ≫, position of 12, 500-dalton standard.

exhibited a protein band at approximately 20,000 daltons. Preparation of β-BTX in the reducing buffer results in the occurrence of two peptide bands, one at approximately 7,000 daltons and another at 12,000–13,000 daltons. This gel also verifies that the RCM-B fraction contains only a single band corresponding to the smaller peptide component of β-BTX. Results in Table 2 also indicate that phospholipase A_2 activity has been adequately removed from the RCM-B preparation.

Results presented in Fig. 4 indicate that the fraction of RCM-B prepared from the native toxin retained its K channel-blocking activity. However, at a concentration of 10 nM, RCM-B reduced both the slope and the *y*-intercept components of the voltage-stimulated ^{86}Rb efflux. This suggests that, at this concentration, RCM-B blocks both the nonactivating (S) and the inactivating (T) voltage-gated K channels in nerve endings. Pooled results from five experiments support this conclusion (Table 3). Further experiments using a lower concentration of RCM-B (1 nM; also summarized in Table 3) indicate that this lower concentration exhibits more selectivity. At 1 nM, only component S is significantly reduced. The inhibition of the voltage-stimulated flux of ^{86}Rb is also concentration dependent. The dose-response curve shown in Fig. 5 suggests a biphasic inhibition, with a lower EC_{50} of approximately 0.1 to 0.3 nM

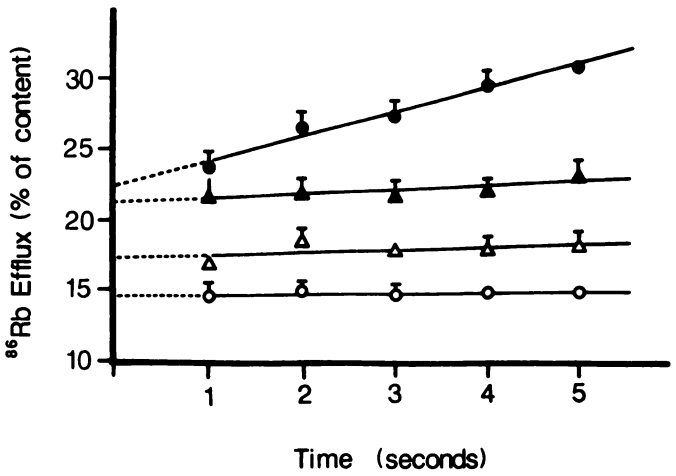

Fig. 4. Effects of 10 nM RCM-B on ^{86}Rb efflux in synaptosomes. Efflux was quantitated, as described in Materials and Methods, in the presence of 5 K (*open symbols*) or 100 K (*closed symbols*) solution and in the absence (*circles*) or presence (*triangles*) of RCM-B, for periods of 1 to 5 sec. Each *point* represents the mean ± standard error for five experiments.

TABLE 3

Summary of effects of RCM-B on synaptosomal ^{86}Rb efflux

Fluxes were quantitated as described in Materials and Methods. Flux was stimulated by 100 K. Results are expressed as percentage of content (B and T) or percentage of content/sec (R and S). Values are mean ± standard error for five experiments each.

Component	^{86}Rb efflux			Statistics
	Control	1 nM RCM-B		
			% of control	
B	13.5 ± 1.13	14.2 ± 1.5	105	NS[a]
R	0.176 ± 0.047	0.140 ± 0.061	79	NS
T	13.6 ± 2.2	14.7 ± 1.7	108	NS
S	2.04 ± 0.28	0.98 ± 0.16	48	[b]
Component	^{86}Rb efflux			Statistics
	Control	10 nM RCM-B		
			% of control	
B	14.3 ± 0.9	17.3 ± 0.9	121	[b]
R	00.246 ± 0.98	00.319 ± 0.131	129	NS
T	8.1 ± 1.4	3.0 ± 0.4	37	[b]
S	1.52 ± 0.16	0.27 ± 0.12	18	[b]

[a] NS, not significant.
[b] Toxin treatment alters flux component significantly from control, paired *t* test.

and a higher EC_{50} of 3 to 10 nM (corresponding to inhibition of the two voltage-stimulated components).

Reduced and carboxymethylated A-chain, also isolated in the reduction and carboxymethylation reaction, produced no effect on any of the components of ^{86}Rb efflux (data not shown).

Discussion

Results presented here demonstrate that the neurotoxin β-BTX is capable of blocking nerve ending K channels. Inhibition was dose dependent. The broad concentration range over which inhibition was observed may be due to the fact that the preparation is a heterogeneous mixture of K channels, which may vary slightly in their sensitivity to β-BTX inhibition. These results further support the hypothesis that selective K channel blockade may be a crucial part of the toxic action of this peptide. Additionally, the present results further verify the use of the

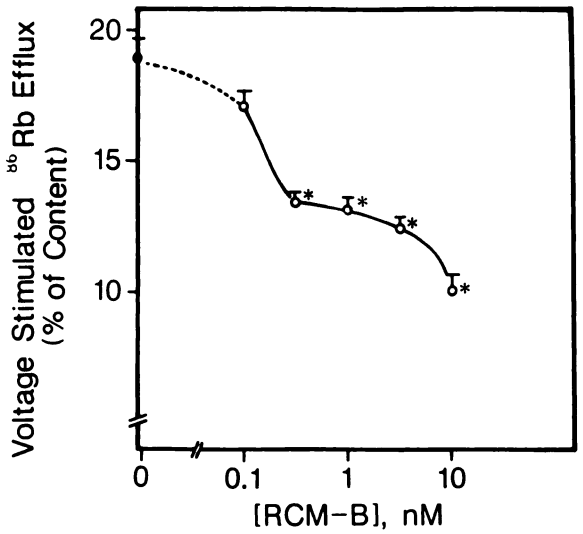

Fig. 5. Dose response of RCM-B on ^{86}Rb efflux in synaptosomes. Efflux was measured, as described in Materials and Methods, in the presence of 5 K or 100 K for 4 sec. The difference, which is plotted, represents the voltage-stimulated efflux. Two components of ^{86}Rb efflux may be discriminated on the basis of RCM-B sensitivity. The EC_{50} is 0.1 to 0.3 nM for the more sensitive component and 1 to 3 nM for the less sensitive component. Each *point* is the mean ± standard error of five separate determinations, and each determination was made in quadruplicate. *Differs significantly from control, Duncan multiple range test.

^{86}Rb efflux technique to quantitate nerve ending K channel activity.

The mechanism of the neurotoxic action of β-BTX has been extensively studied previously, as mentioned earlier. Until recently, the possible role of ion channel blockade in the neurotoxic action had been ruled out (17), although a mechanism involving an increase in Ca^{2+}_i in nerve terminals has been proposed (40). However, with the identification of the heterogeneity of ion channels in different cells and in different regions of the same cell (41), it is possible that the earlier studies failed to use a suitable model of ion channels to see an effect.

The recent observations of certain sequence homologies with other K channel blockers (5, 6, 24) have renewed interest in the study of this toxin. Several recent studies have, in fact, suggested that β-BTX may inhibit K channels in several peripheral preparations (22–24). A previous study (24) has reported that β-BTX partially blocks a noninactivating K channel in dorsal root ganglion neurons that is also sensitive to α-dendrotoxin. Dreyer and Penner (23) have also suggested that, in motor nerve terminals, β-BTX may block K channels with slow activation kinetics, which are sensitive to α-dendrotoxin. These results raise the question of the exact identity of the sensitive K channel, relative to other peripheral and central K channels. Other studies report that only the rapidly inactivating K channels are sensitive to inhibition by α-dendrotoxin (1, 42). Results of experiments described here indicate that β-BTX can block noninactivating nerve ending K channels in the central nervous system, which are only weakly sensitive to inhibition by α-dendrotoxin (1).

Separation of the larger A-chain by reduction and carboxymethylation yielded an active fragment, RCM-B, which also blocks nerve ending K channels. It is interesting to note that isolation of RCM-B produced two pronounced effects. 1) The ability of the toxin to block noninactivating K channels (S) was increased approximately 10 times (IC_{50} = 0.1 to 0.3 nM).

This is the same conductance that was previously demonstrated to be sensitive to inhibition by phencyclidine (35) and β-dendrotoxin (1) but is only weakly sensitive to α-dendrotoxin (1). 2) The ability of the toxin to also block inactivating K channels (T) was unmasked. The two types of voltage-gated nerve ending K channels in this preparation can be distinguished by their sensitivity to RCM-B. This may explain the ability of β-BTX to inconsistently interfere with α-dendrotoxin binding, only at higher concentrations (43) or not at all (44). The fragment RCM-B was about 10 times less potent at inhibiting the inactivating voltage-gated K channel (T) (IC_{50} = 1 to 3 nM). This channel was previously reported to be sensitive to 4-aminopyridine (33) and α-dendrotoxin (1). The effect of RCM-B on the T component was affected at concentrations similar to those required to block the dorsal root ganglion K current by β-BTX (24). It may be that peripheral fast and slow kinetics K channels have more structural features in common, such that they are not distinguished by α-dendrotoxin and β-BTX, whereas the central fast and noninactivating K channels are more dissimilar and can be distinguished. Inhibition of nerve ending K channels is consistent with a mechanism of neurotoxicity involving membrane depolarization (13, 14) and elevation of $Ca^{2+}{}_i$ (41). This action may account for the transient facilitation in neurotransmitter release associated with toxicity (9, 12).

Several recent reports have characterized the binding of radiolabeled β-BTX to brain membranes (24, 26) and synaptosomes (25, 28, 29, 45). β-BTX can also compete with binding of other toxins such as dendrotoxin (29, 46), toxin I (47), and mast cell-degranulating protein (29), all of which may be K channel ligands (26). The binding of β-BTX has been reported to be facilitated by Ca^{2+}, which increases the affinity of the ligand for its receptor (26, 45). In the present studies, the IC_{50} for inhibition of ^{86}Rb efflux by β-BTX of 1 to 3 nM correlates well with, or is only slightly higher than, the reported K_d values for toxin binding of 0.47 to 2.1 nM (25, 27, 45), even though these studies were conducted in the absence of extracellular Ca^{2+}. Therefore, it would appear that the omission of Ca^{2+} in the current experiments did not drastically affect the activity of this toxin on K channels. It may be that the Ca^{2+} requirement for binding involves the action of the phospholipase A_2-containing chain. The EC_{50} for inhibition of the more sensitive component (S, the noninactivating component) by RCM-B is slightly lower than the lowest K_d values reported by others (28). This indicates that the A-chain may hinder the most efficient binding of the B-chain to K channels.

In summary, the significant points of these findings are as follows: 1) native β-BTX can inhibit noninactivating K channels in the central nervous system, 2) the B-chain subunit of β-BTX will block noninactivating as well as inactivating K channels, and 3) the study of toxins such as β-BTX can help to define the relationship between K channels from different sources such as the peripheral and central nervous systems.

Acknowledgments

The author wishes to acknowledge the technical assistance of Christine Laverdure and Teresa Labedz and the assistance of Mrs. Ortella M. Findlay in manuscript preparation.

References

1. Benishin, C. G., R. G. Sorensen, W. E. Brown, B. K. Krueger, and M. P. Blaustein. Four polypeptide components of green mamba venom selectively block certain potassium channels in rat brain synaptosomes. *Mol. Pharmacol.* **34**:152–159 (1988).
2. Blaustein, M. P., D. K. Bartschat, C. G. Benishin, W. E. Brown, K. A. Colby, B. K. Krueger, M. J. Schneider, and R. G. Sorensen. Potassium channels in rat brain synaptosomes: pharmacology and toxicology. *NATO Adv. Study Inst. Ser.* **H32**:241–261 (1988).
3. Kondo, K., K. Narita, and C.-Y. Lee. Chemical properties and amino acid composition of β-bungarotoxin from the venom of *Bungarus multicinctus* (Formosan banded krait). *J. Biochem.* **83**:91–99 (1978).
4. Kondo, K., K. Narita, and C.-Y. Lee. Amino acid sequences of the two polypeptide chains in β-bungarotoxin from the venom of *Bungarus multicinctus*. *J. Biochem.* **83**:101–115 (1978).
5. Harvey, A., and E. Karlsson. Proteinase inhibitor homologues for mamba venoms: facilitation of acetylcholine release and interactions with prejunctional blocking toxins. *Br. J. Pharmacol.* **77**:153–161 (1982).
6. Dufton, M. J. Protease inhibitors and dendrotoxins: sequence classification, structural prediction and structure/activity. *Eur. J. Biochem.* **153**:647–654 (1985).
7. Chang, C. C. The action of snake venoms on nerve and muscle, in *Snake Venoms* (C.-Y. Lee, ed.). Springer-Verlag, New York, 309–376 (1979).
8. Chang, C. C., and C.-Y. Lee. Isolation of neurotoxins from the venom of *Bungarus multicinctus* and their modes of neuromuscular blocking action. *Arch. Int. Pharmacol. Ther.* **144**:241–246 (1963).
9. Abe, T., S. Alema, and R. Miledi. Isolation and characterization of presynaptically acting neurotoxins from the venom of *Bungarus* snakes. *Eur. J. Biochem.* **80**:1–12 (1977).
10. Caratsch, C. G., B. Maranda, R. Miledi, and P. N. Strong. A further study of the phospholipase-independent action of β-bungarotoxin at frog end plates. *J. Physiol. (Lond.).* **319**:179–191 (1981).
11. Tse, C. K., D. Wray, J. Melling, and J. O. Dolly. Actions of β-bungarotoxin on spontaneous release of transmitter at muscle end-plates treated with botulinum toxin. *Toxicon* **24**:123–130 (1986).
12. Chang, C. C., T. F. Chen, and C. Y. Lee. Studies of the presynaptic effect of β-bungarotoxin on neuromuscular transmission. *J. Pharmacol. Exp. Ther.* **184**:339–345 (1973).
13. Ng, R. H., and B. D. Howard. De-energization of nerve terminals by β-bungarotoxin. *Biochemistry* **23**:4976–4986 (1978).
14. Sen, I., and J. R. Cooper. Similarities of β-bungarotoxin and phospholipase A_2 and their mechanisms of action. *J. Neurochem.* **30**:1369–1375 (1978).
15. Sen, I., and J. R. Cooper. The effect of β-bungarotoxin on the release of acetylcholine from brain synaptosomes. *Biochem. Pharmacol.* **24**:2107–2109 (1975).
16. Sen, I., P. A. Grantham, and J. R. Cooper. Mechanism of action of β-bungarotoxin on synaptosomal preparations. *Proc. Natl. Acad. Sci. USA* **73**:2664–2668 (1976).
17. Spokes, J. W., and J. O. Dolly. Complete purification of β-bungarotoxin: characterization of its action and that of tityus toxin on synaptosomal accumulation and release of acetylcholine. *Biochim. Biophys. Acta* **596**:81–93 (1980).
18. Kondo, K., H. Toda, and K. Narita. Characterization of phospholipase A activity of β-bungarotoxin from *Bungarus multicinctus* venom. I. Its enzymatic properties and modification with *p*-bromophenacyl bromide. *J. Biochem.* **84**:1291–1300 (1978).
19. Kondo, K., H. Toda, and K. Narita. Characterization of phospholipase A activity of β-bungarotoxin from *Bungarus multicinctus* venom. II. Identification of the histidine residue of β-bungarotoxin modified by *p*-bromophenacyl bromide. *J. Biochem.* **84**:1301–1308 (1978).
20. Su, M. J., and C. C. Chang. Presynaptic effects of snake venom toxins which have phospholipase A_2 activity (β-bungarotoxin, taipoxin, crotoxin). *Toxicon* **22**:631–640 (1984).
21. Ho, C. L., I. H. Tsai, and C.-Y. Lee. The role of enzyme activity and charge properties on the presynaptic neurotoxicity and contracture-inducing activity of snake venom phospholipase A_2. *Toxicon* **24**:337–345 (1986).
22. Rowan, E. G., and A. L. Harvey. Potassium channel blocking actions of β-bungarotoxin and related toxins on mouse and frog motor nerve terminals. *Br. J. Pharmacol.* **94**:839–847 (1988).
23. Dreyer, F., and R. Penner. The actions of presynaptic snake toxins on membrane currents on mouse motor nerve terminals. *J. Physiol. (Lond.)* **386**:455–463 (1987).
24. Petersen, M., R. Penner, F.-K. Pierau, and F. Dreyer. β-Bungarotoxin inhibits a non-inactivating potassium current in guinea pig dorsal root ganglion neurones. *Neurosci. Lett.* **68**:141–145 (1986).
25. Othman, S. B., J. W. Spokes, and J. O. Dolly. Preparation of neurotoxin ^3H-β-bungarotoxin: demonstration of saturable binding to brain synapses and its inhibition by toxin I. *Eur. J. Biochem.* **128**:267–276 (1982).
26. Schmidt, R. R., H. Betz, and H. Rehm. Inhibition of β-bungarotoxin binding to brain membranes by mast cell degranulating peptide, toxin I, and ethylene glycol bis(β-amino ethyl ether)-*N,N,N',N'*-tetraacetic acid. *Biochemistry* **27**:963–967 (1988).
27. Pelchen-Matthews, A., and J. O. Dolly. Distribution of acceptors for β-bungarotoxin in the central nervous system of the rat. *Brain Res.* **441**:127–138 (1988).
28. Rehm, H., and H. Betz. Binding of β-bungarotoxin to synaptic membrane fractions of chick brain. *J. Biol. Chem.* **257**:10015–10022 (1982).
29. Breeze, A. L., and J. O. Dolly. Interactions between discrete neuronal membrane binding sites for the putative K^+-channel ligands, β-bungarotoxin,

dendrotoxin and mast cell degranulating peptide. *Eur. J. Biochem.* **178**:771–778 (1989).
30. Benishin, C. G. Neurotoxic action of β-bungarotoxin may involve K channel blockade. *Soc. Neurosci. Abstr.* **14**:456 (1988).
31. Hajos, F. An improved method for the preparation of synaptosomal fractions in high purity. *Brain Res.* **93**:485–489 (1975).
32. Krueger, B. K., R. W. Ratzlaff, G. R. Strichartz, and M. P. Blaustein. Saxitoxin binding to synaptosomes, membranes, and solubilized binding sites from rat brain. *J. Membr. Biol.* **50**:287–310 (1979).
33. Bartschat, D. K., and M. P. Blaustein. Potassium channels in isolated presynaptic nerve terminals from rat brain. *J. Physiol. (Lond.)* **361**:419–440 (1985).
34. Bartschat, D. K., and M. P. Blaustein. Calcium-activated channels in presynaptic nerve terminals from rat brain. *J. Physiol. (Lond.)* **361**:441–456 (1985).
35. Bartschat, D. K., and M. P. Blaustein. Phencyclidine in low doses selectively blocks a presynaptic voltage-regulated potassium channel in rat brain. *Proc. Natl. Acad. Sci. USA* **83**:189–192 (1986).
36. Bergmeyer, H. U., and E. Bernt. UV-assay with pyruvate and NADH, in *Methods of Enzymatic Analysis* (H. U. Bergmeyer, ed.), Vol. 2. Academic Press, New York, 574–579 (1974).
37. Crestfield, A. M., S. Moore, and W. H. Stein. The preparation and enzymatic hydrolysis of reduced and *S*-carboxymethylated proteins. *J. Biol. Chem.* **238**:622–627 (1963).
38. Anderson, B. L., R. W. Berry, and A. Telser. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis system that separates peptides and proteins in the molecular weight range of 2,500 to 90,000. *Anal. Biochem.* **132**:365–375 (1983).
39. Lee, C.-Y., S. L. Chang, S. T. Kau, and S.-H. Luh. Chromatographic separation of the venom of *Bungarus multicinctus* and characterization of its components. *J. Chromatogr.* **72**:71–82 (1972).
40. Gundersen, C. B., D. J. Jenden, and M. W. Newton. β-Bungarotoxin stimulates the synthesis and accumulation of acetylcholine in rat phrenic nerve diaphragm preparations. *J. Physiol. (Lond.)* **310**:13–35 (1988).
41. Hille, B. *Ionic Channels of Excitable Membranes.* Sinauer Associates, Inc., Sunderland, MA (1984).
42. Halliwell, J. V., I. B. Othman, A. Pelchen-Matthews, and J. O. Dolly. Central action of dendrotoxin: selective reduction of a transient K conductance in hippocampus and binding to localized acceptors. *Proc. Natl. Acad. Sci. USA* **83**:493–497 (1986).
43. Black, A. R., A. L. Breeze, I. B. Othman, and J. O. Dolly. Involvement of neuronal acceptors for dendrotoxin in its convulsive action in rat brain. *Biochem. J.* **237**:397–404 (1986).
44. Mehraban, F., A. L. Breeze, and J. O. Dolly. Identification by cross-linking of a neuronal acceptor protein for dendrotoxin, a convulsant polypeptide. *FEBS Lett.* **174**:116–122 (1984).
45. Rehm, H., and H. Betz. Solubilization and characterization of the β-bungarotoxin-binding protein of chick brain membranes. *J. Biol. Chem.* **259**:6865–6869 (1984).
46. Black, A. R., and J. O. Dolly. Two acceptor sub-types for dendrotoxin in chick synaptic membranes distinguishable by β-bungarotoxin. *Eur. J. Biochem.* **156**:609–617 (1986).
47. Rehm, H., and M. Lazdunski. Existence of different populations of the dendrotoxin I binding protein associated with neuronal K channels. *Biochem. Biophys. Res. Commun.* **153**:231–240 (1988).

Send reprint requests to: Christina G. Benishin, Department of Physiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2H7 Canada.