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Charybdotoxin blocks both Ca-activated K channels and Ca-independent voltage-gated K channels in rat brain synaptosomes

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Charybdotoxin (ChTX), a 4.3 kDa polypeptide toxin from the venom of the scorpion *Leiurus quinquestriatus*, blocks both a Ca-activated K channel ($IC_{50} \approx 15$ nM) and a Ca-independent voltage-gated K channel ($IC_{50} \approx 40$ nM) in rat brain synaptosomes. These results indicate that in this preparation ChTX is not specific for the Ca-activated K channel and suggest that there may be structural homology among the toxin-binding sites on various types of K channels.

Charybdotoxin; K^+ channel; Synaptosome

1. INTRODUCTION

Voltage-gated potassium (K) channels are found in virtually all excitable cells. Recently, polypeptide toxins have been isolated that block different classes of K channels with high potency. For example, α-dendrotoxin (α-DaTX, from the snake, *Dendroaspis angusticeps*) blocks inactivating K channels (associated with the A-current) [1], noxiustoxin (NTX, from the scorpion *Centruroides noxius* [2]) blocks non-inactivating (delayed rectifier) K channels [3,4], charybdotoxin (ChTX, from the scorpion *Leiurus quinquestriatus*) blocks large conductance ('maxi' or 'BK') Ca-activated K channels [5], and apamin (from bee venom) blocks small conductance Ca-activated K channels [6]. Although each of these toxins initially seemed to be highly selective for a single K channel type, it now appears that in some cases there is considerable overlap in their specificity. Thus, while NTX preferentially blocks Ca-insensitive, voltage-gated, non-inactivating K channels, it also blocks Ca-activated K channels at high concentrations [7]. The goal of this study was to carry out analogous experiments on ChTX: does this Ca-activated K channel blocker also block Ca-independent voltage-gated K channels, and if so, which ones? A preliminary report of some of these findings has appeared in abstract form [8].

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Abbreviations: ChTX, charybdotoxin; α-DaTX, α-dendrotoxin; NTX, noxiustoxin; 4-AP, 4-aminopyridine; PCP, phencyclidine (1-[1-cyclohexyl] piperazine); TEA, tetraethylammonium

2. MATERIALS AND METHODS

2.1. *Venom fractionation*

Venom from *L. quinquestriatus* var. *hebraeus* (Latoxan, Rosans, France) was acetone extracted [9], and fractionated on a CX-300 cation-exchange HPLC column (Brownlee Labs, Santa Clara, CA). Adsorbed material was eluted with an ammonium acetate gradient (0.02–0.7 M, pH 7.0); elution was monitored at 280 nm (Beckman, model 160). ChTX activity was assayed by block of synaptosome ^{86}Rb efflux as described below. The ChTX peak was lyophilized and resuspended in 100 mM NaCl, 20 mM Tris, pH 7.4. The ChTX protein concentration was determined using extinction coefficients of 15.05 and 1.52 $(mg/ml)^{-1} \cdot cm^{-1}$ at 215 and 280 nm, respectively [10].

Pure ChTX, a gift from Drs G. Kaczorowski and M. Garcia (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ), co-chromatographed with our ChTX on a C-18 reversed-phase HPLC column.

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2.2. *Synaptosome ^{86}Rb efflux*

Synaptosomes were prepared from rat forebrain as described [11]. A crude synaptosome preparation, the pellet (P_2) obtained from the second 10 000 × *g* spin, was used without further purification. The synaptosomes were loaded with ^{86}Rb [1] and preincubated for 16 min in 5K solution with or without ChTX prior to efflux measurement. ^{86}Rb efflux was then measured by a filtration assay [1]. Briefly, the percent efflux into 5K, 100K, or 100K + Ca solutions during a 1–5 s period was determined as the amount of ^{86}Rb released into the filtrate divided by the sum of the ^{86}Rb in the filtrate and that remaining in the synaptosomes. When used, 4-aminopyridine (4-AP) and/or phencyclidine (1-[1-cyclohexyl]piperazine, PCP) were present in both the wash and efflux solutions. The 5K solution contained (in mM) 145 NaCl, 5 KCl, 0.1 RbCl, 2 $MgCl_2$, 10 glucose, 0.5 NaH_2PO_4, and 10 Hepes adjusted to pH 7.4 with NaOH. In 100K solution, Na was reduced to 50 mM and K was increased to 100 mM. In 100K + Ca solution, $MgCl_2$ was reduced to 1 mM and replaced with 1 mM $CaCl_2$.

3. RESULTS AND DISCUSSION

3.1. *ChTX blocks Ca-activated K channels in synaptosomes*

Fig. 1 shows the effect of ChTX on Ca-activated K channels in synaptosomes as revealed by block of ^{86}Rb efflux. In this experiment we used a pharmacological maneuver to 'isolate' the Ca-activated component of ^{86}Rb efflux: previous studies had shown that 10 mM 4-AP completely blocked both non-inactivating and inactivating voltage-gated, Ca-independent components of efflux without affecting efflux through Ca-activated K channels [12]. Thus, in fig. 1, 100K-stimulated Rb efflux was measured in the presence of 10 mM 4-AP to block both of the voltage-gated, Ca-independent components of efflux. Ca-stimulated efflux (ΔCa) was measured in 100K + Ca solution. This Ca-dependent component was partially blocked by 10 nM ChTX and completely blocked by 100 nM (i.e. ΔCa = ΔChTX). ΔCa is most likely due to a maxi Ca-activated K channel, since this component of efflux is also blocked by 10 mM tetraethylammonium (TEA) [13]. In the presence of 4-AP, the ChTX-insensitive component of efflux (identical to the Ca-independent component) in 100K solution was due to depolarization-stimulated efflux of Rb through a voltage-independent (resting) K conductance [12].

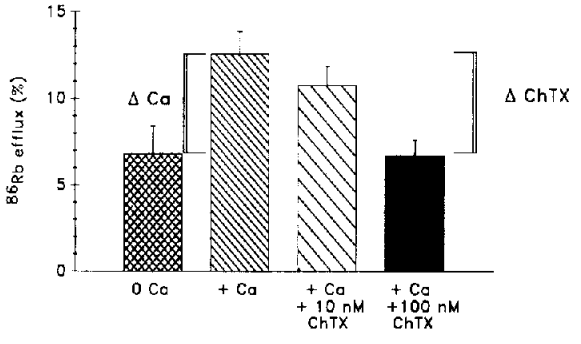

Fig.1. Effects of 10 and 100 nM ChTX on ^{86}Rb efflux through Ca-activated K channels in rat brain synaptosomes. ^{86}Rb efflux was stimulated by 100 mM K in the absence (left) or presence of 1 mM Ca. 10 nM ChTX blocked about 30% and 100 nM ChTX blocked 100% of the Ca-dependent component (Δ Ca). The 100K-stimulated Rb efflux is the percent efflux in 100K solution minus that in 5K solution. 4-AP (10 mM) was present to block Ca-independent voltage-gated K channels. The bars represent the average (± SE) of four or five determinations.

The dose response for ChTX block of the Ca-activated K channels is shown in fig. 2 (circles). Block was well-described by a monophasic inhibition curve with an IC_{50} of about 15 nM, close to that reported by Anderson et al. [9] for block of maxi Ca-activated K channels from skeletal muscle sarcolemma under similar ionic conditions.

3.2. *ChTX also blocks inactivating, Ca-independent K channels*

To determine whether ChTX also blocks Ca-independent voltage-gated K channels, its effects were tested on the K-stimulated Rb efflux in the

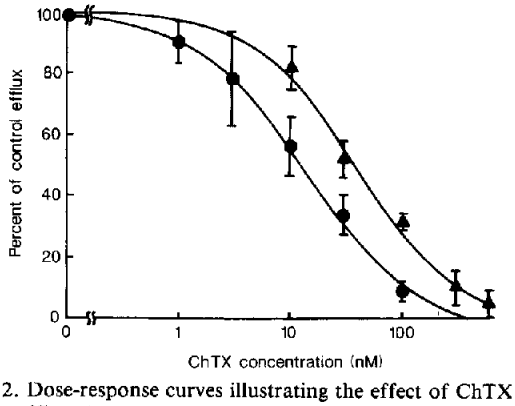

Fig.2. Dose-response curves illustrating the effect of ChTX on ^{86}Rb efflux through Ca-activated K channels (●) and the Ca-independent, rapidly inactivating voltage-gated K channels (▲) in synaptosomes. (●) 100% is the Ca-dependent component of depolarization-induced ^{86}Rb efflux measured in the presence of 4-AP; (▲) 100% is the Ca and PCP-independent, 4-AP-sensitive component of depolarization-induced efflux. Each symbol represents the mean (± SE) of quadruplicate determinations from each of three or four separate experiments. The curves show single-site block with IC_{50} values of 15 (●) and 40 (▲) nM.

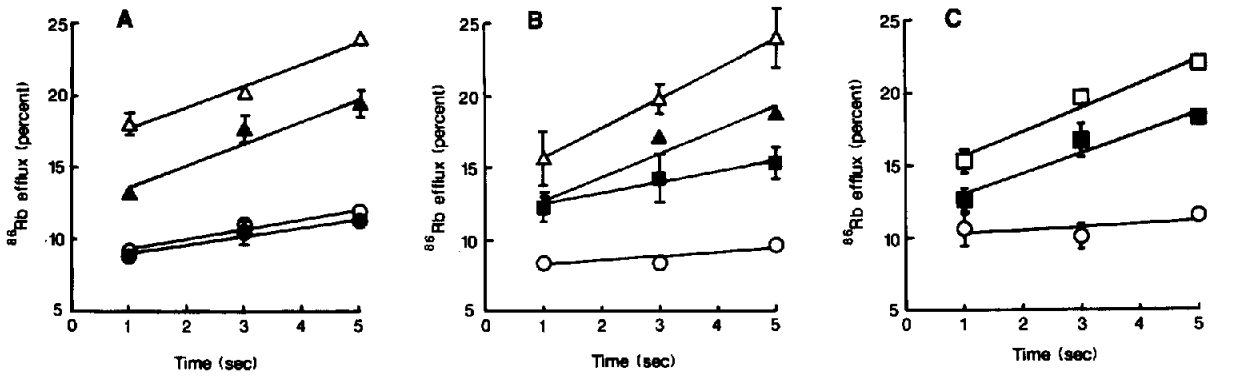

Fig. 3. Effect of ChTX on time course of Ca-independent ^{86}Rb efflux from rat brain synaptosomes. (A) Efflux into Ca-free 5K (circles) or 100K (triangles) solutions was measured in the absence (open symbols) and presence (filled symbols) of 100 nM ChTX. (B) Similar experimental protocol, except that the effect of 10 μM PCP (■) in the presence of 100 nM toxin was also determined. (C) Efflux into 100K + 10 μM PCP was measured in the absence (□) and presence of 100 nM (■) ChTX. Each symbol represents the mean (± SE) of four or five determinations; the lines were derived by least-squares regression analysis. In these three experiments the mean reduction in the ordinate intercept by 100 nM ChTX was 52%.

absence of Ca and 4-AP. As illustrated by the time-course experiments in fig. 3A,B raising K produced an increase in ^{86}Rb efflux that consisted of two components [12]: (i) an increase in the ordinate intercept due to efflux through α-DaTX-sensitive, rapidly-inactivating (A-type) voltage-gated K channels [1], and (ii) an increase in the rate of ^{86}Rb efflux from 1–5 s that corresponds, in part, to a PCP-sensitive non-inactivating ('delayed rectifier') voltage-gated K current [14]. 100 nM ChTX had a negligible effect on the Rb efflux into 5 mM K (fig. 3A) or on the rate of efflux from 1–5 s in 100 mM K (fig. 3A–C), indicating that it blocked neither the resting K conductance nor the non-inactivating Ca-independent voltage-gated K channels. However, 100 nm ChTX did reduce the K-stimulated increase in the ordinate intercept by about 50% (fig. 3A–C), due to block of the rapidly inactivating, voltage-gated K channels. As illustrated in fig. 3B, 10 μM PCP reduced the non-inactivating component of Rb efflux (slope) remaining in the presence of ChTX, with no further effect on the ordinate intercept. Fig. 3C shows that when the non-inactivating voltage-gated K channels were blocked by 10 μM PCP, 100 nM ChTX still blocked the rapidly inactivating voltage-gated K channels as indicated by the decrease in the ordinate intercept. This is further evidence that the two kinetically distinguished Ca-independent components of the Rb efflux correspond to different K channels.

The dose-response curve for the ChTX block of the K-stimulated, Ca-independent, PCP-insensitive (fig. 3B) ^{86}Rb efflux is also shown in fig. 2 (triangles). The IC_{50} for block of this flux by ChTX was about 40 nM.

An additional feature of this study is the demonstration that Rb efflux from synaptosomes can be used conveniently to screen the actions of toxins and other pharmacological agents on three different classes of K channels in the same preparation.

3.3. *ChTX is not a specific K channel blocker*

Our results demonstrate that ChTX blocks both Ca-activated K channels and Ca-independent, inactivating K channels in the same tissue (rat brain synaptosomes); however, we estimate block of the Ca-activated K channels to be about 2–3-fold more potent than that of the Ca-independent channels. Recently, MacKinnon et al. [15] demonstrated that ChTX also blocks inactivating K channels from *Drosophila* expressed in *Xenopus* oocytes after injection of mRNA transcripts from a cDNA (*Shaker*) clone coding for the A-current channels. The K_i for block of the *Drosophila* channels was about 4 nM, somewhat more potent than for block of mammalian maxi Ca-activated K channels and about 10-times more potent than for block of inactivating K channels in synaptosomes (fig. 2, triangles). Although they share the properties of inactivation and sensitivity to ChTX, the relationship

between the insect A-current channel (*Shaker*) and the inactivating K channels in mammelian brain synaptosomes, described here, remains unclear.

3.4. *Overlapping specificies among scorpion polypeptide toxins suggest structural similarities among K channels*

As described above, ChTX is not specific for one channel type. In addition NTX, originally reported to block non-inactivating (delayed rectifier) K channels in squid axon [3], also blocks maxi Ca-activated K channels from mammalian skeletal muscle but with about 10-fold lower potency than ChTX [7]. Since ChTX and NTX have considerable sequence homology [7,10] it might have been expected that ChTX would block the same two types of channels in synaptosomes, albeit with different affinities. Instead, although ChTX, like NTX, blocks maxi Ca-activated K channels, ChTX blocks the inactivating but not the non-inactivating voltage-gated K channels, in contrast to NTX which is a potent blocker of the latter. Our results raise the possibility that the different K channel types share some common structural features at the toxin-binding site. This site may be located at the extracellular mouth of the channel pore as suggested by the competitive interaction between ChTX and TEA in the maxi Ca-activated K channel [16]. This, together with the finding that all K channels have high selectivity for K and Rb over other cations, suggests that the pores of these different types of K channels may be quite similar, possibly having evolved from a common precursor gene. Different pharmacological properties, especially differential sensitivity to the various polypeptide toxins, may reflect relatively minor variations in this common pore structure. Resolution of this question will have to await sequence determination of one or more voltage-gated K channels in addition to the *Drosophila* channel. One or more of these polypeptide toxins may play an important role in this process by providing a probe for K channel isolation and characterization.

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REFERENCES

[1] Benishin, C.G., Sorensen, R.G., Brown, W.E., Krueger, B.K. and Blaustein, M.P. (1988) Mol. Pharmacol. 34, 152–159.
[2] Possani, L.D., Martin, B.M. and Svendsen, I. (1982) Carlsberg Res. Commun. 47, 285–289.
[3] Carbone, E., Wanke, E., Prestipino, G., Possani, L.D. and Maelicke, A. (1982) Nature 296, 90–91.
[4] Carbone, E., Prestipino, G., Spadavecchia, L., Franciolini, F. and Possani, L.D. (1987) Pflügers Arch. (Eur. J. Physiol.) 408, 423–431.
[5] Miller, C., Moczydlowski, E., Latorre, R. and Phillips, M. (1985) Nature 313, 316–318.
[6] Romey, G. and Lazdunski, M. (1984) Biochem. Biophys. Res. Commun. 118, 669–674.
[7] Valvidia, H.H., Smith, J.S., Martin, B.M., Coronado, R. and Possani, L.D. (1988) FEBS Lett. 226, 280–284.
[8] Schneider, M.J., Krueger, B.K. and Blaustein, M.P. (1989) Biophys. J. 55, 549a.
[9] Anderson, C.S., MacKinnon, R., Smith, C. and Miller, C. (1988) J. Gen. Physiol. 91, 317–333.
[10] Gimenez-Gallego, G., Navia, M.A., Reuben, J.P., Katz, G.M., Kaczorowski, G.J. and Garcia, M.L. (1988) Proc. Natl. Acad. Sci. USA 85, 3329–3333.
[11] Krueger, B.K., Ratzlaff, R.W., Strichartz, G.R. and Blaustein, M.P. (1979) J. Mem. Biol. 50, 287–310.
[12] Bartschat, D.K. and Blaustein, M.P. (1985) J. Physiol. 361, 419–440.
[13] Bartschat, D.K. and Blaustein, M.P. (1985) J. Physiol. 361, 441–457.
[14] Bartschat, D.K. and Blaustein, M.P. (1986) Proc. Natl. Acad. Sci. USA 83, 189–192.
[15] MacKinnon, R., Reinhart, P.H. and White, M.M. (1988) Neuron 1, 997–1001.
[16] Miller, C. (1988) Neuron 1, 1003–1006.