Volume 250, number 2, 519–522 FEB 07323 July 1989

Charybdotoxin blocks dendrotoxin-sensitive voltage-activated K^+ channels

Hugues Schweitz, Catherine E. Stansfeld*+, Jean-Noël Bidard, Laurent Fagni*, Pierrette Maes° and Michel Lazdunski

*Centre de Biochimie du Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, *Laboratoire d'Etude des Régulations Physiologiques, 23 rue Becquerel, 67087 Strasbourg* and °*Institut Pasteur de Lille, 1 rue du Professeur Calmette, 59019 Lille, France*

Received 14 April 1989

Charybdotoxin, a short scorpion venom neurotoxin, which was thought to be specific for the blockade of Ca^{2+}-activated K^+ channels also blocks a class of voltage-sensitive K^+ channels that are known to be the target of other peptide neurotoxins from snake and bee venoms such as dendrotoxin and MCD peptide. Charybdotoxin also inhibits ^{125}I-dendrotoxin and ^{125}I-MCD peptide binding to their receptors. All these effects are observed with an IC_{50} of about 30 nM.

K^+ channel; Charybdotoxin; Dendrotoxin; Mast cell degranulating peptide; (Dorsal root ganglia, Rat brain)

1. INTRODUCTION

Charybdotoxin (ChTX) is a small neurotoxin from the venom of the scorpion *Leiurus quinquestriatus hebraeus* [1–4]. It has been described as a potent blocker of high conductance K^+-specific channels which are activated by both intracellular Ca^{2+} and membrane potential variations [1]. The affinity of ChTX for the large conductance (200 pS) channel is in the nanomolar to 10 nM range [2,3]. ChTX has also been shown to block a lower conductance (30 pS) Ca^{2+}-activated K^+ channel in molluscan neurons [4]. In this preparation it is without effect on other types of K^+ channels. Other K^+ channels are blocked by other types of polypeptide toxins [5–7]. Dendrotoxins from snake venoms, and particularly dendrotoxin I, the most potent representative in this series, are high-affinity blockers of voltage-sensitive K^+ channels [8–11]. Dendrotoxin-sensitive voltage-activated K^+ channels have a conductance of 5–10 pS and they appear to be insensitive to intracellular Ca^{2+} [11]. This type of channel is also blocked by the bee venom peptide toxin, the mast cell degranulating peptide (MCD) [12].

This report shows that ChTX is not selective for Ca^{2+}-dependent K^+ channels. It also blocks dendrotoxin-sensitive voltage-activated K^+ channels.

Correspondence address: H. Schweitz, Centre de Biochimie du Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, France

+ *Present address:* MRC Neuropharmacology Research Group, Department of Pharmacology, University College, Gower Street, London WC1E 6BT, England

2. MATERIALS AND METHODS

2.1. *Purification of ChTX*

The venom of the scorpion *Leiurus quinquestriatus hebraeus* was obtained from Latoxan (France). The purification procedure included the following steps: a filtration on Sephadex G50, a succession of HPLC steps on the ion-exchange column TSK SP 5PW, reverse-phase C_8, a second chromatography on TSK SP 5PW, another reverse-phase C_{18}, and finally desalting on Sephadex G25. The purity of the toxin was over 99%, estimated from the last HPLC on reverse-phase C_{18}. Recovery was 0.17% of the dry venom. The purity of the toxin was also checked by the sequence determination which was found to be

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

identical to that previously reported [3]. The toxin was fully active on large Ca^{2+}-activated K^+ channels.

2.2. *Short-term culture of the rat dorsal root ganglion (DRG) cells*

DRG cells from 4-day-old rats were mechanically dissociated following enzyme treatment, collagenase (Sigma, 1 mg/ml) and dispase (Sigma, 5 mg/ml), and allowed to attach to collagen-coated culture dishes for 6 h in an L15 medium containing 15% horse serum, as described by Bossu et al. [13]. Recordings were made between 6 and 14 h in culture.

2.3. *Electrophysiological measurements*

Electrophysiological studies have been performed on rat DRG cells using conventional patch-clamp recording methods with the use of a List EPC7 amplifier (Darmstadt, FRG). Currents were recorded on a Racal taperecorder and subsequently filtered and digitized for analysis. The pipette filling solution contained 110 mM KCl, 20 mM KF, 10 μM $AlCl_3$, 1 mM EGTA, 2 mM $MgCl_2$, 10 mM Hepes-Tris, pH 7.2. External 'physiological' K^+ solution 5 mM Hepes-Tris, 10 mM glucose. For 'symmetrical' K^+ conditions the choline Cl was replaced by KCl.

2.4. *Binding assays*

Synaptosomal P_3 membranes from rat brain were prepared as described [14]. Protein concentrations were determined according to Bradford [15] using bovine serum albumin as a standard. The conditions of ^{125}I-MCD and ^{125}I-DTX_I binding to membranes was carried out as previously described [16], free and bound labelled toxins were separated by a rapid centrifugation method as previously described [14]. Incubations were carried out at 4°C for 30 min. The concentration of ^{125}I-labelled toxins was 20 pM, that of unlabelled toxins and membranes in the binding experiments are indicated in the legends of the figures. Membrane pellets were counted in a γ-counter. All binding data were corrected for binding to tubes measured in the absence of membranes and for non-specific binding measured in the presence of 100 nM of the unlabelled toxin. Each data represented the mean value of duplicate determinations.

3. RESULTS AND DISCUSSION

Electrophysiological studies of the action of charybdotoxin on a known DTX-sensitive K^+ current have been performed, using conventional patch-clamp recording methods [17] on rat dorsal root ganglion cells (DRG) in short-term culture. These cells have a large voltage-activated, non-Ca^{2+}-activated K^+ current which is blocked by low nanomolar concentrations of DTX [8,11]. The current can be evoked by stepping the membrane voltage up to between −60 and 0 mV from a holding potential of around −90 mV. Further, with appropriate ionic conditions given in section 2, channel activity can be restricted almost exclusively to this particular current. The current is equally well expressed in either the whole cell or in isolated outside-out patches of cell membrane containing multiple channels. Using both of these conditions, ChTX (10 nM–1 μM) applied to five preparations (two whole cell, three outside out patches), induced a rapid (a few seconds) concentration-dependent reduction in current. An example of this current and its blockade by ChTX is seen in fig.1. Recovery was slow: 20 min for the partial recovery shown in fig.1.

A dose-response curve of the ChTX effect on the DTX-sensitive K^+ current is presented in fig.2. The IC_{50} of the blockade was around 30 nM.

Both DTX_I and MCD receptors have been previously identified using their ^{125}I-labelled derivatives [12,14]. ^{125}I-DTX_I and ^{125}I-MCD associate with high affinity (K_d = 41 pM and 158 pM, respectively) to their binding sites in brain synaptic membranes [16]. DTX_I and MCD receptors are distinct and allosterically related [12]. However they are both situated within the same protein component [18–20] of 77 kDa.

Fig.2 shows that ChTX inhibits, in exactly the same way, both ^{125}I-DTX_I and ^{125}I-MCD binding to their respective receptors. The IC_{50} for the inhibitory effect of ChTX was 30 nM. Binding data and electrophysiological data are nearly superimposable.

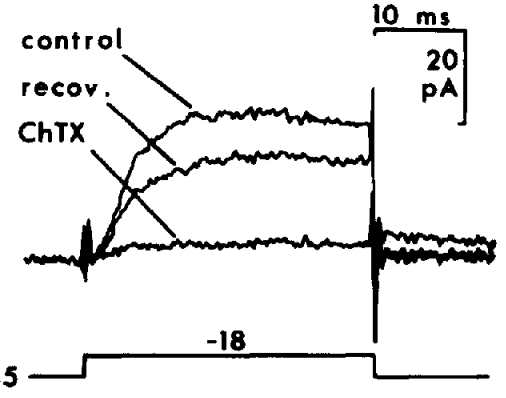

Fig.1. Blockade of K^+ currents by ChTX in cultured DRG cells recorded by patch-clamp methods. Records were made at 22°C. Toxin was applied by close perfusion. Averaged currents (n = 14) recorded from a large outside-out patch containing approximately 60 channels, in response to depolarizing steps according to the protocol shown below the traces. The K^+ gradient is 'physiological' (E_K −100 mV). ChTX (600 nM) produced almost total blockade. Partial recovery at 20 min is shown.

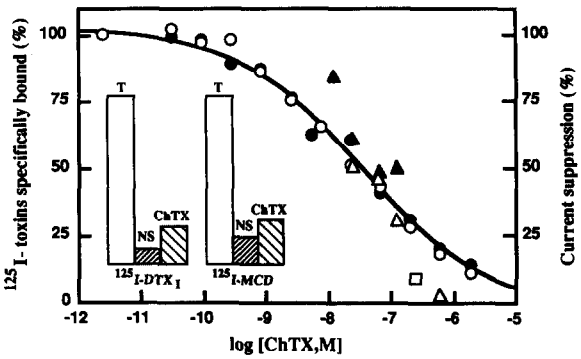

Fig.2. Comparison of the inhibition by ChTX of ^{125}I-DTX$_I$ (●) and ^{125}I-MCD (○) binding to synaptosomal membranes from rat brain with the dose-response curve showing proportion of evoked current blockade by ChTX (▲, △, □). Synaptic membranes (180 μg of protein/ml or 40 μg of protein/ml) were incubated with ^{125}I-MCD or with ^{125}I-DTX$_I$, respectively. Diagram (in arbitrary unit) shows for both ^{125}I-labelled toxins the total binding (T), the non-specific binding (NS) obtained in the presence of 100 nM of the respective unlabelled toxin and the binding obtained in the presence of 1 μM ChTX. For the dose-response curve showing proportion of evoked current blockade by ChTX three symbols apply to three different types of preparations: whole-cell recording (▲), isolated patches with either 60–80 channels approximately in 'physiological' K^+ gradient (△), or with fewer channels in 'symmetrical' K^+ gradient (□).

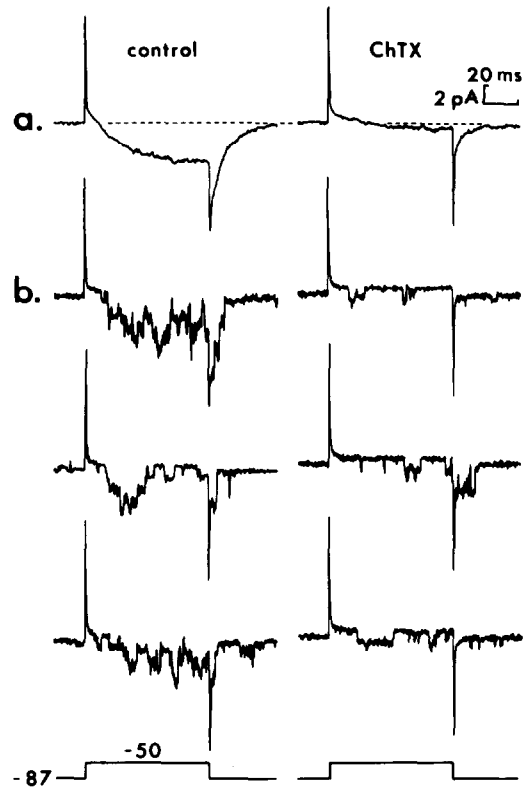

Fig.3. The effect of ChTX (200 nM) on inward current evoked from an isolated patch in 'symmetrical' K^+. Sampled at 8 kHz, filter: low pass, 2 kHz, 8 pole Bessel. No leak subtraction. Voltage protocol shown below traces. (a) Averaged currents, n = 62 for control and n = 30 in the presence of toxin. (b) Examples of individual current traces showing unitary current activity in some cases.

In one relatively small patch (fig.3) the channel activity was studied using a 'symmetrical' (130 mM) K^+ distribution (see section 2) allowing clearer resolution of individual channel openings (fig.3). Channel conductance for an inward current between −85 and −40 mV was around 35 pS, in agreement with that reported for DTX-sensitive channels under the same conditions [8,11]. In the presence of the toxin the general characteristics of individual channel openings was not obviously different from those in the control period. This is consistent with a reduction in the numbers of active channels and reminiscent also, using synaptosomal membranes, of the earlier reported manner of blockade by DTX.

Charybdotoxin is very widely used with the belief that it blocks Ca^{2+}-activated K^+ channels of large conductance (200 pS) [4]. This paper shows that the pure toxin is also a potent blocker of one well-identified class of voltage-sensitive K^+ channels: those sensitive to dendrotoxin. However it may have a better affinity (about 1 order of magnitude) for Ca^{2+}-activated K^+ channels [2,3].

The fact that a single toxin blocks both a class of voltage-sensitive K^+ channels and a class of Ca^{2+}-activated K^+ channels strongly suggests that there are homologies between these 2 types of channels. This conclusion is supported by the observation that another, much less potent scorpion toxin, noxiustoxin, which blocks voltage-sensitive K^+ channels in squid giant axons with an IC_{50} above 1.5 μM [21] also blocks large conductance Ca^{2+}-activated K^+ channels in skeletal muscle with an IC_{50} of 450 nM [22].

Acknowledgements: This work was supported by the Institute National de la Santé et de la Recherche Médicale (grant CRE 88.20.07), the Royal Society (London) Overseas Fellowship and the Fondation pour la Recherche Médicale. We thank Dr A.

Feltz, Dr A. Tartar and H. Rehm for their help and support and Martine Valetti for skilful technical assistance. We are grateful to Dr Georges Romey and Catherine Van Renterghem for assaying the classical activity of purified ChTX on Ca^{2+}-activated K^+ channel.

REFERENCES

[1] Miller, C., Moczydlowski, E., Latorre, R. and Phillips, M. (1985) Nature 313, 316–318.
[2] Smith, C., Phillips, M. and Miller, C. (1986) J. Biol. Chem. 261, 14607–14613.
[3] Gimenez-Gallego, M., Navia, A., Reuben, J.P., Katz, G.M., Kaczorowski, G.J. and Garcia, M.L. (1988) Proc. Natl. Acad. Sci. USA 85, 3329–3333.
[4] Hermann, A. and Erxleben, C. (1987) J. Gen. Physiol. 90, 27–47.
[5] Moczydlowski, E., Lucchesi, K. and Ravindran, A. (1988) J. Membr. Biol. 105, 95–111.
[6] Bernardi, H., Bidard, J.N., Fosset, M., Hugues, M., Mourre, C., Rehm, H., Romey, G., Schmid, H., Schweitz, H., De Weille, J. and Lazdunski, M. (1988) in: Proceedings of the Unesco Symposium on Molecular Basis of Membrane Associated Disease, Springer-Verlag, Prague, in press.
[7] Castle, N.A., Haylett, D.G. and Jenkinson, D.H. (1989) Trends Neurosci. 12, 59–65.
[8] Penner, R., Petersen, M., Pierau, F.K. and Dreyer, F. (1986) Pflügers Arch./Eur. J. Physiol. 407, 365–369.
[9] Harvey, A.L. and Anderson, A.J. (1985) Pharmacol. Ther. 31, 33–55.
[10] Stansfeld, C.E., Marsh, S.J., Parcej, D.N., Dolly, J.O. and Brown, D.A. (1987) Neuroscience 23, 893–902.
[11] Stansfeld, C.I. and Feltz, A. (1988) Neurosci. Lett. 93, 49–55.
[12] Bidard, J.N., Mourre, C. and Lazdunski, M. (1987) Biochem. Biophys. Res. Commun. 143, 383–389.
[13] Bossu, J.L., Feltz, A. and Thomann, J.M. (1985) Pflügers Arch./Eur. J. Physiol. 403, 360–368.
[14] Taylor, J.W., Bidard, J.N. and Lazdunski, M. (1984) J. Biol. Chem. 259, 13957–13967.
[15] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[16] Bidard, J.N., Mourre, C., Gandolfo, G., Schweitz, H., Widmann, C., Gottesmann, C. and Lazdunski, M. (1989) Brain Res., in press.
[17] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch./Eur. J. Physiol. 391, 85–100.
[18] Rehm, H., Bidard, J.N., Schweitz, H. and Lazdunski, M. (1988) Biochemistry 27, 1827–1832.
[19] Rehm, H. and Lazdunski, M. (1988) Proc. Natl. Acad. Sci. USA 85, 4919–4923.
[20] Stühmer, W., Stocker, H., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A. and Pongs, O. (1988) FEBS Lett. 242, 199–206.
[21] Carbone, E., Prestipino, G., Spadavecchia, L., Franciolini, F. and Possani, L.D. (1987) Pflügers Arch./Eur. J. Physiol. 408, 423–431.
[22] Valdivia, H.H., Smith, J.S., Martin, B.M., Coronado, R. and Possani, L.D. (1988) FEBS Lett. 2, 280–284.