Vol. 163, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1989 Pages 394-397

INTERACTIONS BETWEEN DENDROTOXIN, A BLOCKER OF VOLTAGE-DEPENDENT POTASSIUM CHANNELS, AND CHARYBDOTOXIN, A BLOCKER OF CALCIUM-ACTIVATED POTASSIUM CHANNELS, AT BINDING SITES ON NEURONAL MEMBRANES

A.L. Harvey, D.L. Marshall, F.A. De-Allie* and P.N. Strong*

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, U.K.
*Jerry Lewis Muscle Research Centre, Department of Paediatrics and Neonatal Medicine, Royal Postgraduate Medical School, London W12 0NN, U.K.

Received July 14, 1989

SUMMARY. Dendrotoxin I (DpI) from black mamba venom (*Dendroaspis polylepis*) has high affinity binding sites on rat brain synaptic membranes. Native DpI displaced [^{125}I]-DpI binding with a K_i of 1 x 10^{-10} M, and over 90% of specific binding was displaceable. Charybdotoxin isolated from the Israeli scorpion venom (*Leiurus quinquestriatus hebraeus*), also displaced [^{125}I]-DpI binding, with a K_i of approximately 3 x 10^{-9} M, although the displacement curve was shallower than with native DpI. Both toxins are thought to be high affinity blockers of specific K^+ currents. Charybdotoxin selectively blocks some types of Ca^{2+}-activated K^+ channels, whereas dendrotoxins only block certain voltage-dependent K^+ channels. The interaction between the two types of toxin at the DpI binding site is unexpected and may suggest the presence of related binding sites on different K^+ channel proteins.

INTRODUCTION. The dendrotoxins are a family of small proteins from mamba venoms that facilitate transmitter release and block some types of neuronal K^+ currents (1,2,3). High affinity binding sites for dendrotoxins have been found in mammalian and avian brain (4,5) and binding proteins, which are thought to be part of some voltage-dependent K^+ channels, have been isolated (6,7).

Charybdotoxin has been isolated from venom of the Israeli scorpion *Leiurus quinquestriatus hebraeus* (8). At low concentrations it blocks large conductance Ca^{2+}-activated K^+ channels (8,9,10), but does not appear to affect voltage-dependent K^+ channels in mammalian neurones. The distinction between the effects of dendrotoxin and charybdotoxin on transmitter release at mammalian peripheral nerve terminals has been demonstrated (11,12).

During studies on the binding of dendrotoxins to synaptosomal membranes, we found that charybdotoxin unexpectedly displaced the specific binding of a radiolabelled dendrotoxin homologue, *Dendroaspis polylepis* toxin I (DpI).

MATERIALS AND METHODS

Dendrotoxin I (DpI) was isolated from *Dendroaspis polylepis* venom (Sigma Chemical Co.), as described earlier (1,13). It was homogeneous on reverse phase HPLC. Charybdotoxin was isolated from *Leiurus quinquestriatus hebraeus* venom (Latoxan) (14,15).

0006-291X/89 $1.50

Preparation of [^{125}I]-DpI. [^{125}I]-DpI was prepared using 1 mCi Na[^{125}I] (Amersham), 20 μg DpI and Iodogen (Pierce) in a solid phase oxidation procedure, according to Rehm *et al.* (16). Toxin and free iodide were separated on an SP-Sephadex C-25 ion exchange column. The specific activity of the iodinated toxin was 36-250 Ci/mmole.

Binding of [^{125}I]-DpI to synaptosomal membranes. Synaptosomal membranes were prepared from rat brain. Each brain was homogenised in 10 ml 320 mM sucrose, 2 mM Tris. HCl (pH 7.4) at 4°C. The suspension was centrifuged at 900x g for 10 min, the supernatant was recovered and re-centrifuged at 15000x g for 12 min. The resulting pellet was lysed by addition of 10 ml 5 mM Tris. HCl (pH 8.1). The suspension was centrifuged at 48000x g for a further 20 min, and the pellet was finally resuspended in 25 ml synaptosomal buffer (NaCl 130, KCl 3, $CaCl_2$ 2, $MgCl_2$ 2, and Tris. HCl 20 mM, pH 7.4).

For competition binding experiments, 200 μl of membrane suspension were incubated at room temperature (19-21°C) with a standard concentration of [^{125}I]-DpI (0.72 - 1.5 nM) and a range of concentrations of the competing toxin. After 30 min, the membranes were collected by centrifugation and washed in 1 ml synaptosomal buffer containing bovine serum albumin (1 mg/ml). Membrane-bound radioactivity was measured in a calibrated gamma counter.

RESULTS AND DISCUSSION

[^{125}I]-DpI demonstrated saturable binding to rat brain synaptosomal membranes, as has been shown previously for both DpI and dendrotoxin (4,6,17). The K_D for [^{125}I]-DpI was estimated to be around 5 x 10^{-11}M. Non-specific binding was 20-30% of total binding at 1.5 nM [^{125}I]-DpI.

The binding of [^{125}I]-DpI was displaced by native DpI (Fig. 1). Using the formula, $K_i = IC_{50} / \{1 + ([^{125}I]\text{-DpI})/K_D\}$, the K_i was calculated to be 1.1 ± 0.5 x 10^{-10}M (mean ± sem of 7 separate determinations).

Binding of [^{125}I]-DpI was also reduced by the presence of charybdotoxin at concentrations of 2 nM and above (Fig. 1). The displacement curve was shallower than that with DpI and the maximum displacement appeared to be only about 60% of specific binding. From the IC_{50} values, the K_i for charybdotoxin was estimated to be 3 ± 2 x 10^{-9} M.

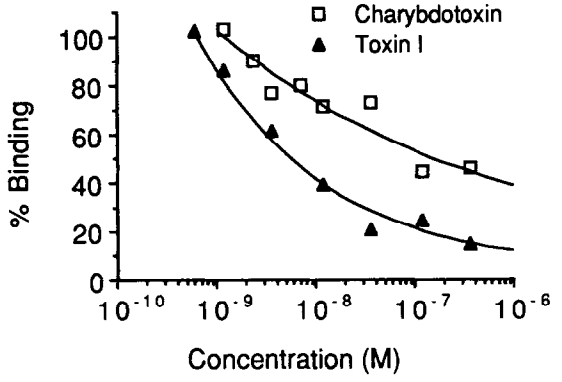

Fig. 1. Displacement of [^{125}I]-DpI binding to rat brain synaptic membranes by native DpI (▲) or by charybdotoxin (□). Each point represents the average of duplicate determinations. 200 μl of membrane suspension were incubated for 30 min with a standard amount of [^{125}I]-DpI (final concentration 1.5 nM) and with a range of concentrations of competing toxin.

There has been no previous evidence to suggest that dendrotoxins can affect Ca^{2+}-activated K^+ channels (2), and it has been tacitly assumed that these toxin binding sites on synaptosomal membranes are related to voltage-dependent K^+ channels. Charybdotoxin, on the other hand, appears to be selective for large conductance Ca^{2+}-activated channels, although there is a report of charybdotoxin blocking voltage-dependent K^+ channels in murine lymphocytes (18). Charybdotoxin does block the function of voltage-dependent K^+ currents resulting from the expression of *Drosophila Shaker* cDNA in oocytes, although the toxin does not affect ionic currents in the normal fruitfly (19). There are conflicting reports regarding the ability of charybdotoxin to block cloned rat brain K^+ channels (20,21). The displacement of radiolabelled DpI binding by charybdotoxin indicates that there might be similar binding sites on both voltage- and Ca^{2+}-dependent K^+ channels in the brain. Although this is unexpected from functional studies with these two toxins, there has been a previous report demonstrating that noxiustoxin, a specific blocker of voltage-dependent K^+ channels in neurones (22), also blocks (but with lower affinity) charybdotoxin-sensitive Ca^{2+}-activated K^+ channels in T tubules (23). Unfortunately, attempts to radiolabel charybdotoxin have so far resulted in a loss of specific binding, so we have not been able to perform the reverse interaction studies.

We think it is unlikely that the apparent cross-reactivity between dendrotoxin and charybdotoxin represents non-specific binding because of the high affinities observed. It is possible that some of the binding sites might be located on the inside of the membrane, and therefore not normally be accessible to toxin during functional experiments. More probably, this interaction has identified similar structural motifs on two types of physiologically distinct K^+ channels. Perhaps this is not so surprising considering that the primary function of any K^+ channel is to filter K^+ ions selectively, and that any further ability to modify ion flux, whether through a voltage sensor or by intracellular modulation, is an embellishment on the basic structure.

ACKNOWLEDGMENTS. The authors thank the Muscular Dystrophy Group of Great Britain, the Science and Engineering Research Council and the Wellcome Trust for support.

REFERENCES

1. Harvey, A.L., and Karlsson, E. (1980) Naunyn-Schmeidebergs Arch. Pharmacol. 312, 1-6.
2. Harvey, A.L., and Anderson, A.J. (1985) Pharmacol. Ther. 31, 35-55.
3. Stansfeld, C, and Feltz, A. (1989) Neurosci. Lett. 93, 49-55.
4. Black, A.R., Breeze, A.L., Othman, I.B., and Dolly, J.O. (1986) Biochem. J. 237, 397-404.
5. Black, A.R., and Dolly, J.O. (1986) Eur. J. Biochem. 156, 609-617.
6. Rehm, H., and Lazdunski, M. (1988) Proc. Natl. Acad. Sci. USA 85, 4919-4923.
7. Black, A.R., Donegan, C.M., Denny, B.J.,and Dolly, J.O. (1988) Biochemistry 27, 6814-6820.
8. Miller, C., Moczydlowski, E., Latorre, R., and Phillips, M. (1985) Nature 313, 316-318.
9. Smith, C., Phillips, M., and Miller, C. (1986) J. Biol. Chem. 261, 14607-14613.
10. Moczydlowski, E., Lucchesi, K., and Ravindran, A. (1988) J. Memb. Biol. 105, 95-111.
11. Anderson, A.J., and Harvey, A.L. (1988) Br. J. Pharmacol. 93, 215-221.
12. Anderson, A.J., Harvey, A.L., Rowan, E.G., and Strong, P.N. (1988) Br. J. Pharmacol. 95, 1329-1335.
13. Harvey, A.L., and Karlsson, E. (1982) Br. J. Pharmacol. 77, 153-161.

14. Castle, N.A., and Strong, P.N. (1986) FEBS Lett. 209, 117-121.
15. Strong, P.N., Weir, S., Beech, D.J., Hiestand, P., and Kocher, H. (1989) Br. J. Pharmacol., in press.
16. Rehm, H., Bidard, J.-N., Schweitz, H., and Lazdunski, M. (1988) Biochemistry 27, 1827-1832.
17. Harvey, A.L., and Karlsson, E. (1984) Trends Pharmac. Sci. 5, 71-72.
18. Lewis, R.S., and Cahalan, M.D. (1988) Science 239, 771-775.
19. MacKinnon, R., Reinhart, P.H., and White, M.M. (1988) Neuron 1, 997-1001.
20. Pongs, O. (1989) Pflügers Arch., in press.
21. Christie, M.J., Adelman, J.P., Douglass, J., and R.A. North (1989) Science 244, 221-224.
22. Carbone, E., Wanke, E., Prestipino, G., Possani, L.D., and Maelicke, A. (1982) Nature 296, 90-91.
23. Valdivia, H.H., Smith, J.S., Martin, B.M., Coronado, R., and Possani, L.D. (1988) FEBS Lett. 226, 280-284.