Fig. 1. Effect of KCl (0.6 M) and DTT (10 mM) on the dissociation of ^{125}I -dendrotoxin₁ from bovine brain membranes

Bovine brain membranes (50 μg of protein) were incubated with ^{125}I -dendrotoxin₁ (0.4 pM) for 20 min at room temperature in a total volume of 200 μl of membrane buffer: 20 mM-sodium Hepes (pH 7.2)/120 mM-KCl/2 mM- CaCl_2 /2 mM- MgCl_2 /0.01 % (w/v) Triton X-100. To remove unbound toxin, 1 ml of cold membrane buffer was added and the mixture was centrifuged for 10 min at 15000 rev./min and 4 $^\circ\text{C}$. The pelleted membranes with the bound ^{125}I -dendrotoxin₁ were resuspended in 1 ml of cold membrane buffer (control) or in 1 ml of cold membrane buffer containing 0.6 M-KCl or 10 mM-DTT respectively. The membranes were incubated for the times indicated, then pelleted by centrifugation (10 min, 15000 rev./min, 4 $^\circ\text{C}$) and the pellets counted for ^{125}I . The figure shows the amount of ^{125}I -dendrotoxin₁ still bound to the membranes as a percentage of control. The data are the means of triplicate experiments.

channel from the dendrotoxin₁ affinity column by 0.6 M-KCl is due to an ionic strength effect: the binding of dendrotoxin₁ to the K^+ channel is also inhibited by high concentrations of NaCl or MgCl_2 (H. Rehm, unpublished work).

H. R. is a stipendiat of the Deutsche Forschungsgemeinschaft.

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Elegance persists in the purification of K^+ channels

To clarify the reference by Rehm [1] to a putative K^+ channel protein, it must be noted that a family of such proteins [2] has been detected in mammalian brain, using dendrotoxin (DTX) or toxin I. These snake polypeptides were first shown [3] to block a fast-activating, voltage-dependent K^+ current ('A-type') in hippocampal CA1 neurons [4]; variants of this K^+ conductance have been recorded electrophysiologically in a variety of peripheral nerve cells (reviewed in [5]). The acceptor subtypes for DTX, which have characteristic locations in the central nervous system [3,4,6], can also be distinguished by their differing affinities for β -bungarotoxin [3,7]. In fact, the acceptor species in synaptic membranes that displays high affinities for both these toxins was identified using ^3H -labelled β -bungarotoxin [8]; accordingly, the latter has been found to inhibit certain voltage-activated, K^+ conductances in peripheral neurons [9,10]. Mast cell degranulating peptide blocks a DTX-sensitive K^+ current in nodose ganglion A cells and antagonizes the binding of DTX and β -bungarotoxin [11] but with efficacies that are much lower than its K_D for the purified protein [12]. Although an attempt has been made to construct a simplified model for the various findings [7], future purification of the individual subtypes is a prerequisite for gaining insight into the molecular basis of heterogeneity in this group of K^+ channels.

Complete purification of DTX-binding sites from rat and bovine cortex was achieved independently by two groups and yielded preparations with the same subunit composition (polypeptides of M_r ~ 75000 and ~ 37000) [12,13]. Although both used affinity chromatography on immobilized toxin I, the methods employed for gel preparation and acceptor elution were very different; moreover, the lengthier procedure [12] involved additional steps. The merits of our protocol must be judged on data derived from experiments carried out as recommended [13]. Unfortunately, the affinity resin tested in [1] was not prepared according to this protocol since chemical activation (glutaraldehyde versus CNBr) and density (10-fold higher) of conjugated ligand were used. It is not surprising, therefore, that under these conditions performance was far from optimal, thereby confirming the importance that we placed on these parameters. The specified procedure routinely gives a highly enriched preparation of acceptor with a recovery as high as 25%. In contrast, elution with high K^+ concentration (~ 0.6 M) gave impure material, an observation also made by Rehm & Lazdunski [12]. Despite subjecting the crude extract to anion-exchange chromatography prior to the affinity chromatographic step, these investigators obtained a preparation containing eight or more major contaminants (Fig. 1 in [12]), additional procedures being required to yield maximal purity. We developed a sophisticated elution for the affinity column using dithiothreitol; this yielded acceptor of much greater purity (Fig. 1) in a single step. Complete purification was achievable after final chromatography on a lectin resin, with an overall recovery that was somewhat higher than obtained by the other group.

The unsatisfactory elution of the affinity resins with K^+ was explained by our demonstration that inhibition of toxin-acceptor interaction is not restricted to K^+ [13],

Received 8 May 1989

Fig. 1. Purity of K⁺ channel proteins isolated by a single affinity chromatography step

A crude detergent extract of bovine brain membranes was loaded onto a toxin I-Sepharose 4B column (the gel was CNBr-activated to $\sim 0.5 \mu\text{mol}$ of cyanate esters/ml before conjugation with toxin at a density of 0.3 mg/ml), washed with 25 mM-imidazole/HCl buffer, pH 8.2, containing 100 mM-KCl and then eluted with 10 mM-dithiothreitol in the latter. SDS/polyacrylamide-gel electrophoresis of the eluate was performed; a silver stained track (F2₁) from Fig. 2(a) of Parcej & Dolly [13] is reproduced here. Arrows show the mobilities of the two major subunits [M_r of 75000 (upper) and 37000] of the putative K⁺ channels.

as now confirmed by Rehm [1]. Hence, it is to be expected that such a non-specific effect of high ionic strength would occur rapidly. The dithiothreitol elution performed by Rehm [1], again, does not simulate conditions of the affinity column. He studied dissociation of the complex between membrane-bound acceptor and ¹²⁵I-labelled toxin I instead of monitoring elution of detergent-solubilized acceptor from toxin I immobilized on CNBr-activated Sepharose 4B (prepared according to published specifications [13]); the latter is likely to dissociate faster than the native toxin. Also, in the

affinity chromatography dithiothreitol could inhibit the reformation of complex favoured by the high toxin concentration on the gel. Finally, due to the high acceptor capacity of our affinity resin ($\sim 100 \text{ pmol/mg}$ of toxin I), re-use of the gel has not been evaluated. However, reoxidation of toxin I in solution can be accomplished readily [14], raising the possibility of re-using the affinity gel.

In conclusion, our optimized affinity chromatography procedure remains a fast and effective method for routine isolation of these novel K⁺ channel proteins.

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Received 21 July 1989