of the same protein being regulated by different control and leader sequences in different tissues.

If *reg* and PSP-S genes are alleles, the *reg*/PSP-S gene is expressed both in exocrine cells and in regenerating endocrine cells, since transcripts were quantified in purified regenerating islets apparently free of exocrine parenchyma, and were absent from normal islets (Terazono *et al.*, 1988). A possible explanation would be that during regeneration islet cells dedifferentiate to a stage equivalent to the embryonic 'protodifferentiated state' (11–14 days of gestation in the rat) described by Pictet & Rutter (1972), in which exocrine proteins and insulin have been observed in the same cells. If, on the contrary, *reg* and PSP-S genes are similar but non-allelic, *reg* might be a foetal gene temporarily expressed in dedifferentiated islets.

The identity of PSP-S and *reg* protein primary sequences does not exclude that tissue-specific post-translational maturation might confer to the *reg* protein other properties than CaCO_3 crystal growth inhibition. The physiological role of *reg* protein secretion by regenerating islets remains therefore unknown.

Finally, the recent demonstration by Ozturk *et al.* (1989) that a protein very similar if not identical to PSP-S was present in significant amounts in Alzheimer disease brain raises the possibility that members of the *reg*/PSP-S gene family might be expressed in several tissues upon perturbation of their normal phenotypic expression.

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Purification methods for neuronal K^+ channels

In brain a protein exists which binds with high affinity the snake venom neurotoxins dendrotoxin and β -bungarotoxin and the bee venom peptide mast cell-

degranulating peptide [1–3]. This dendrotoxin-binding protein is a neuronal voltage-dependent K^+ channel [4]. It was first purified by Rehm & Lazdunski [5] in a three-step procedure: ion exchange chromatography, affinity chromatography on a dendrotoxin₁ affinity column (dendrotoxin₁-AcA 22) and chromatography on wheat germ agglutinin-Affigel 10. The key method in the purification scheme is the affinity chromatography on dendrotoxin₁-AcA 22. The channel was eluted from this column by a high concentration of KCl (0.6 M). Recently, Parcej & Dolly repeated and confirmed these experiments [6]. However, for K^+ channel elution from the affinity column Parcej & Dolly used 10 mM-dithiothreitol (DTT) instead of 0.6 M-KCl. The rationale for the elution with DTT is that the binding activity of dendrotoxin₁ depends on its three internal disulphide bridges. Splitting them should destroy the binding activity of the column-immobilized dendrotoxin₁ and thus lead to the elution of the K^+ channel. Here I want to point out that this apparently elegant method has two serious drawbacks.

1. Reoxidation of dendrotoxin₁ to its native state is not feasible: elution of the dendrotoxin₁ affinity column with DTT destroys the column, i.e. it can be used only once. In contrast, elution with 0.6 M-KCl leaves the column intact; it can be reused several dozens of times and is stable for at least 1 year (H. Rehm, unpublished work).

2. The recovery of K^+ channel from the dendrotoxin₁ affinity column using DTT elution is several times lower than by elution with 0.6 M-KCl. Dendrotoxin-binding K^+ channel (130 fmol) was solubilized from bovine brain membranes and bound to dendrotoxin₁-AcA 22 (100–150 μl of gel). After washing the dendrotoxin₁-AcA 22 three times with 1 ml of buffer A the K^+ channel was batch-eluted by incubation with 1 ml of buffer A + 0.6 M-KCl or with 1 ml of buffer A + 13 mM-DTT (30 min, 4 °C). The eluates were dialysed overnight against buffer A and the amount of recovered K^+ channel determined. Under these conditions 0.6 M-KCl recovered 39 fmol (30%) whereas 13 mM-DTT recovered only 6.5 fmol (5%). Buffer A was 20 mM-sodium Hepes (pH 7.2)/120 mM-KCl/1 mM-EDTA/10% (v/v) glycerol/0.05% (w/v) Triton X-100/0.01% (w/v) phosphatidylcholine; the methods for solubilization of the K^+ channel and preparation of the affinity column are described in [3] and [5].

What is the reason for the suboptimal recovery of K^+ channel by DTT? Fig. 1 demonstrates that DTT removes dendrotoxin₁ from the K^+ channel only slowly and inefficiently: a 2 min incubation with buffer containing 0.6 M-KCl is sufficient to remove more than 90% of dendrotoxin₁ bound to bovine brain membranes. In contrast, even after 40 min of incubation with buffer containing 10 mM-DTT, less than 50% of the bound dendrotoxin₁ is removed. The splitting of the dendrotoxin₁ disulphide bridges by DTT seems to be a slow and incomplete reaction. Therefore the three-step method with KCl elution [5] is superior to the two-step method with DTT elution [6], as the former gives both a several-fold higher recovery in the affinity chromatography and a purer product (compare the gel in [6] with the gels and scan in [5] and [7]). Unfortunately, the overall recovery of the two methods cannot be compared as Parcej & Dolly did not show a purification table in [6].

Finally, it is true that both elution methods are not biospecific. This was indicated by Rehm & Lazdunski ([5], p. 4921, last sentence). Clearly, the elution of K^+

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).

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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 $M_r \sim 75000$ 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 ($\sim 0.6 \text{ M}$) 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],