matters arising

Similarities between "cholinergic proteolipid" and detergent-extracted cholinergic proteins

A REPORT from Changeux's group[1] concludes that the cholinergic receptor proteolipid[2] is different from the detergent-extracted cholinergic protein[3]. This conclusion is based on the observation that there is no immunological cross reactivity between the proteins and their respective antisera. Furthermore, confirming some earlier observations of Karlin[4], they found that after affinity labelling of the receptor *in situ* with ^{3}H-MPTA, the radioactive ligand is not extracted by chloroform–methanol. They failed, however, to establish whether the cholinergic receptor proteolipid is still extractable after the affinity labelling and thus committed the same error as did Karlin previously.

In an attempt to clarify the problem we have repeated the experiments (Table 1). In membranes from *Electrophorus* we found that treatment with dithiothreitol (the preliminary step in the affinity labelling) followed by mercaptoethanol, produces a drastic reduction (61%) in the receptor proteolipid that can be extracted. By affinity labelling *Torpedo* membranes with ^{3}H-MPTA we confirmed the findings of Karlin[4] and Changeaux[1] in the sense that only 6% and 14% of the radioactivity was extracted with chloroform–methanol; however in the same experiments there was a reduction of 70% and 80% in the cholinergic receptor proteolipid (Table 1). Thus an alternative and more valid conclusion to draw from the experiments of both Changeux[1] and Karlin[4] may be that the cholinergic receptor proteolipid is labelled in the membrane but is no longer extractable by the organic solvents, although it can still be solubilised with strong detergents in aqueous solutions.

With respect to the immunological work, we can offer no explanation at present for the apparently negative results. The isolation procedure used by Changeux's group, however, is not that used by our group[2]. Thus the diethyl ether precipitate used by them contains only 10% of the total proteolipids and practically no cholinergic receptor proteolipid. Clearly therefore the question of the immunological comparison of the proteins must be investigated further before definite conclusions can be drawn.

It is convenient to summarise the many similarities between these two proteins. Both extraction procedures are designed to solubilise hydrophobic intrinsic membrane proteins and both proteins have been purified by affinity chromatography either in aqueous solutions[5] or in organic solvents[6].

The number of receptor sites that can be labelled with α-toxin in *Electrophorus*[1] and *Torpedo* corresponds closely to the amount of cholinergic receptor proteolipid that we extract[7]. Both isolated proteins show high affinity binding for a range of cholinergic nicotinic ligands, including acetylcholine, decamethonium, *d*-tubocurarine and α-bungarotoxin. We can transfer our proteolipid to aqueous solutions using Triton X-100 and in these conditions the binding of ^{3}H-α-bungarotoxin can be demonstrated. Incorporation of the proteolipid into lecithin liposomes has also been achieved and the binding of α-toxin in the aqueous solution has been observed. From this evidence we conclude that the cholinergic proteolipid of the electroplax is probably identical with the binding subunit of the detergent-extracted protein.

Table 1 Percentage reduction in the extraction of the cholinergic proteolipid from membranes of *Electrophorus* and *Torpedo* electroplax, after affinity labelling with ^{3}H-MPTA

	μg Receptor proteolipid per g fresh tissue Control	Treated	Reduction (%)
*Electrophorus**	5.2	2.0	61
Torpedo†	23.0	7.0	70
Torpedo†	24.9	5.0	80

The experiments were carried out as described in ref. 1. The amount of receptor proteolipid was determined after chromatography of the extract on Sephadex LH-20 in the presence of 10^{-6} M ^{14}C-acetylcholine[2].
*Treated with dithiothreitol and mercaptoethanol.
†Affinity labelled with ^{3}H-MPTA (4-(*N*-maleimido)-phenyltri-^{3}H-methylammonium).

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BARRANTES ET AL. REPLY—As De Robertis *et al.* point out[1], the extent to which chloroform–methanol (2 : 1) (C : M) extracts proteolipids after treatment of the receptor-rich membranes with dithiothreitol (DDT) and reaction with ^{3}H-MPTA was not mentioned in our report in *Nature*[2]. It was, however, a daily experience in the laboratory that such treatments did not modify, in any respect, the dissolution of the receptor protein by detergents such as Triton X-100 or sodium cholate; but as De Robertis claims, this might no longer be true in the case of the extraction of the proteolipids by chloroform–methanol.

To our surprise, when De Robertis carries out this control he assays the proteolipid by measuring ^{14}C-acetylcholine binding after affinity labelling with ^{3}H-MPTA. From Karlin's work and from our own experience it is well established that ^{3}H-MPTA attaches covalently to the cholinergic receptor site and therefore blocks cholinergic ligand binding. On the other hand, the fact that acetylcholine binds to the proteolipid after reaction with ^{3}H-MPTA suggests that, in the presence of organic solvents, ^{14}C-acetylcholine binding is largely nonspecific (and this might equally be true for α-bungarotoxin binding in such drastic conditions). Also C : M extracts about the same fraction (0.5–1%) of the total proteins from both fresh

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