Further characterization of the nicotinic acetylcholine receptor from locust ganglia

DAVID R. E. MACALLAN and GEORGE G. LUNT
Department of Biochemistry, University of Bath, Bath BA2 7AY, U.K.

The nicotinic acetylcholine receptor is the most extensively characterized neurotransmitter receptor protein. The receptor has been isolated from a variety of peripheral tissues ranging from the electric organ of electric fish to human skeletal muscle. In all cases the receptor is seen to have a common structure comprising four subunits in the ratio α2βδ (Stevens, 1985). It has recently been shown that in chick optic tectus a similar receptor protein is present that is coded by a distinct but closely related set of genes to that coding for the muscle receptor (Conti-Tronconi et al., 1985). The status of the putative nicotinic receptor in insect ganglia is less well defined than that of its vertebrate counterparts. We have previously described its presence in locust ganglia (Filbin et al., 1981). We report here an improved purification of this receptor and a determination of its subunit composition. The nicotinic affinity ligand 4-(N-maleimide)-[3H]benzyltrimethylammonium (MBTA) has been used to label the protein.

The α-bungarotoxin-binding site in the supra-oesophageal ganglion of the locust Schistocerca gregaria, which we tentatively indentified as a nicotinic receptor (Filbin et al., 1983). We report here an improved purification of this receptor and a determination of its subunit composition. The nicotinic affinity ligand 4-(N-maleimide)-[3H]benzyltrimethylammonium (MBTA) has been used to label the protein.

The α-bungarotoxin-binding component from a membrane fraction (Filbin et al., 1983) from locust ganglia was purified by affinity chromatography of a Lubrol PX extract on an α-bungarotoxin-Sepharose 4B affinity column. Elution of the bound protein onto a DE52 ion-exchange column was achieved by recycling with 4 mM-benzoquinonium chloride (Lindstrom et al., 1981). The toxin-binding component was recovered from the DE52 column by salt elution. The purified protein was precipitated by acetone and subjected to polyacrylamide-gel electrophoresis under denaturing conditions in the presence of sodium dodecyl sulphate (Laemmli, 1970). Gels were stained according to Morrissey et al. (1981). The membrane fraction was labelled with the affinity ligand [3H]MBTA by the method of Barrantes et al. (1975). The labelled membranes were subjected to gel electrophoresis as described above. After electrophoresis the gels were sliced 2 mm, solubilized and their radioactivity determined. Parallel experiments were done in which the membranes were preincubated in the presence of 5 mM-nicotine for 1 h before exposure to the [3H]MBTA. Such experiments provided a measure of the non-specific labelling of the membranes with MBTA.

Four bands were observed on the polyacrylamide gels

![Gel profile of specific labelling of polypeptides of locust ganglia](image)

Abbreviation used: MBTA, 4-(N-maleimide)-[3H]benzyltrimethylammonium.
Glutamate binding in bovine retina

PAUL-FRANCIS URBAN, MARLYSE ZAEFFEL and PAUL REICHERT
Centre de Neurochimie-CNRS, 5 Rue Blaise Pascal, 67084 Strasbourg Cedex, France

L-Glutamate has been proposed as a retinal neurotransmitter on the evidence that uptake and release causing L-glutamate binding with our observations of binding of L-glutamate to washed retinal P2 (crude mitochondrial fraction) membranes is linear up to 90 min and proportional to the amount of protein up to 150 pg. As a function of increasing ligand concentration, binding representation shows one main population of sites with a KD of 390 nM and a mean Bmax of 75 pmol/mg. This binding can be displaced by various amino acids with the decreasing potency: L-glutamate > L-aspartate > L-cysteine sulphinate and L-cysteate are more powerful than L-glutamate and L-aspartate during perfusions of chick brain has four subunits of M, 48 000, 56 000, 69 000 and 72 000, and Norman et al. (1982) have previously shown specific affinity labelling of the M, 56 000 subunit. Our findings would support the view expressed by Contin-Tronconi et al. (1985) that the neuronal nicotinic receptors have the same general subunit composition as the vertebrate peripheral receptor.

We are grateful to the S.E.R.C. for support. D.R.E.M. is in receipt of an S.E.R.C. postgraduate training award.

Stevens, C. F. (1985) Trends Neurosci. 8, 1–2


Vol. 14