Biochemical Aspects of Cholinergic Transmission in Insect Central Nervous System

JOHN F. DONNELLAN* and ROY HARRIS†

*Shell Biosciences Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, U.K., and †Department of Biological Sciences, The Polytechnic, Wolverhampton WV1 1LY, West Midlands, U.K.

Neuropharmacological studies have indicated that acetylcholine is probably the major excitatory transmitter at synapses in the insect central nervous system (Pitman, 1971). Further evidence for the cholinergic mode of transmission has been the demonstration that high concentrations of acetylcholine, acetylcholinesterase (EC 3.1.1.7) and choline acetyltransferase (EC 2.3.1.6) occur throughout the central nervous system of a number of insects (Pichon, 1974). In the present paper we describe recent progress on the characterization of a protein with the binding properties of the acetylcholine receptor, an integral component of insect cholinergic synapses. The isolation from insect central nervous system of a synaptosomal fraction with cholinergic properties is also discussed.

Cholinergic receptor

A convenient source of insect central nervous system is the brain (10–20 μg fresh wt.) of the housefly (Musca domestica), an insect which can be reared on a comparatively large scale. A particular property of frozen houseflies is that mechanical shaking and sieving techniques can be used to separate the heads as a discrete entity from the remaining body parts. Eldefrawi & O'Brien (1970) established that such fly heads contained protein(s) which bound a number of cholinergic ligands with high affinity.
Furthermore the binding profile exhibited by the insect material was of the broad spectrum indicated by electrophysiological studies with nicotinic and muscarinic ligands, having the same order of affinity for the receptor proteins (Pitman, 1971).

Clarke & Donnellan (1975) confirmed the subcellular localization and nature of the binding activity in a 100000g-supernatant fraction prepared from fly heads. They also showed that α-bungarotoxin, the essentially irreversible nicotinic antagonist, did not appear to bind to the fly head fraction, precluding the use of the toxin as a diagnostic label for the insect receptor. The binding activity could be purified by Sepharose 6B chromatography after solubilization of the membranous material present in the 100000g-supernatant fraction prepared from fly head homogenates (Clarke & Donnellan, 1975). Ligand binding to this purified fly head fraction, as measured by an ultrafiltration technique, also established that a variety of muscarinic and nicotinic ligands showed comparable affinities (Jewess et al., 1975).

Isoelectric focusing of the receptor fraction indicated that the binding material has a pI of 4.8, a value similar to that for the eel nicotinic receptor (Biesecker, 1973). The fly head receptor protein has a mol.wt. of 360000, as measured by gel-permeation chromatography; gel electrophoresis in the presence of sodium dodecyl sulphate shows the presence of two populations of subunits of 83000 and 90000 daltons. Cross-linkage studies of these subunits can produce a protein of molecular weight similar to that of the native receptor, suggesting an αβ configuration of four subunits for the receptor. The native receptor and subunits all stain as glycoproteins, and g.l.c. of the alditol acetate derivatives of the native receptor's neutral sugars (3%, w/w) has demonstrated that mannose, glucose and galactose are the major constituents.

**Insect central-nervous-system synaptosomes**

Synaptosomes have been extensively used in the studies of the vertebrate cholinergic synapses (Jones, 1975). Preliminary attempts to isolate such a nerve-ending fraction from insect central nervous system were unsuccessful when discontinuous sucrose gradients were used to resolve a brain-mitochondrial fraction prepared from fleshflies (*Sarcophaga barbata*). Substitution of iso-osmotic Ficoll gradients has allowed the isolation of a fraction with some of the morphological properties of synaptosomes (Donnellan et al., 1976). The insect synaptosomal fraction is enriched in occluded choline acetyltransferase and also in acetylcholine (0.7 nmol per mg of protein).

Osmotic lysis of the insect synaptosomes releases choline acetyltransferase in the soluble form predicted if the enzyme is a constituent of the terminal cytoplasm of cholinergic synapses (Fonnum, 1973). The fleshfly head synaptosomes also possess a Na+-dependent uptake system for choline ($K_m = 2 \mu M$) with properties similar to those of the system present in vertebrate cholinergic synaptosomes (Yamamura & Snyder, 1972). Choline uptake, for example, is inhibited by hemicholinium and low temperatures, and is abolished by low concentrations of detergents.