Equilibrium Binding of Fluorescent Cholinergic Ligands to the Purified Acetylcholine Receptor from Electrophorus electricus

H. Prinz

Max-Planck-Institut für Ernährungsphysiologie, Rheinlanddamm 201, 4600 Dortmund, West-Germany

The cholinergic ligands, NBD-5-acylcholine (N-5-C), a pure agonist (1), and N-n-propyl-NBD-5-acylcholine (N-5-CP), a depolarizing antagonist, (2) lost more than 90% of their fluorescence upon binding to the purified acetylcholine receptor from Electrophorus electricus. This effect was reversible and specifically cholinergic. It was used to monitor the binding equilibria of N-5-C and N-5-CP with the receptor. The binding of the representative cholinergic ligands acetylcholine, carbamylcholine, tubocurarine, gallamine, decamethonium and hexamethonium was measured in competition binding studies.

Data analysis was based on a simple scheme of two non-interacting binding sites to which both the fluorescent and non-fluorescent ligand could bind randomly. Initially only the titration curves of receptor and fluorescent ligands were fitted. The same titration curves were then performed in the presence of competing ligand. From these experiments the dissociation constants for the non-fluorescent cholinergic ligands could be deduced.

The basic results were as follows (2):

1. Two classes of binding sites in a 1:1 ratio were detected on the nicotinic acetylcholine receptor.
2. All cholinergic ligands competed for the same binding sites.
3. Agonists and antagonists showed different binding patterns upon interaction with the solubilized receptor.
4. The disulfide reducing agent dithiothreitol (DTT) changed both binding patterns and affinities in accordance with electrophysiological observations.

(1) Jürss, R., Prinz, H., and Maelicke, A. (1979) Proc. Natl. Acad. Sci. USA 76, 1064-1068
(2) Prinz, H. and Maelicke, A. (1980) submitted