# Reconstitution of the Acetylcholine Receptor-Channel System in Planar Bilayer Membranes Below Lipid Phase Transition

G. Boheim and W. Hanke

Lehrstuhl Zellphysiologie, Ruhr-Univ. Bochum, D-4630 Bochum

F.J. Barrantes, H. Eibl and B. Sakmann

MPI Biophysikalische Chemie, D-3400 Göttingen

G. Fels and A. Maelicke

MPI Ernährungsphysiologie, D-4600 Dortmund

Reconstitution experiments of the acetylcholine receptor channel proteins were carried out using solvent-free planar lipid membranes. Bilayers were formed from a $\geqslant$ 99%-pure mixed-chain lipid (1-stearoyl-3-myristoyl-glycero-2-phosphocholine $\equiv$ 1,3-SMPC) at 21°C, i.e. below its phase transition temperature of $\approx$ 29°C. Fusion of vesicles with planar bilayers were observed under these conditions.

Three different vesicle preparations were used: acetylcholine receptor-rich membrane fragments from Torpedo marmorata, the same membrane fragments, however, after alkaline treatment, and Torpedo receptor protein purified by affinity chromatography and incorporated into 1,3-SMPC vesicles. Successful reconstitution was followed by electrochemical measurements. In each case single channel events could be observed which were qualitatively identical to those induced by acetylcholine in sarcolemmal muscle membranes and measured by the patch-clamp technique. The following properties specific for this protein channel were seen with all preparations, i.e. biological cells, membrane fragments, alkaline treated fragments, and purified receptor proteins: 1. a peculiar change in channel lifetime distribution with time after acetylcholine addition; 2. agonist specificity of mean lifetime but not of conductance amplitude; 3. complete channel blockade by α-bungarotoxin.