MEMBRANE PROTEIN RECEPTORS IN SUPPORTED LIPID BILAYERS AS BIOSENSORS

J. Ramsden (1), F. Hucho (2) and H. Vogel (3)

(1) Biozentrum der Universität Basel, Switzerland
(2) Institut für Biochemie, FU Berlin, FRG
(3) ETH Lausanne, CH 1015 Lausanne, Switzerland

SUMMARY

Acetylcholine receptors have been incorporated into planar lipid bilayers and coupled to the surface of a silicon wafer. The binding of ligands to these receptors have been quantified by measuring the concomitant changes of certain electrical properties of the membrane such as impedance and capacitance. The results show that there is principle feasibility to use membrane protein receptors as biosensors.

1. INTRODUCTION

In biotechnology and clinical medicine/chemistry, improved measurement devices are required for purposes of analysis, monitoring and control. Recently, the application of biosensors in this field has been rapidly evolving (1). The present communication discusses the design and the development of a new class of biosensor based on lipid bilayers and membrane protein receptors. The concept is to exploit the principle of self organization of the participating biomolecules. This has been accomplished by using an electronic signal transducer with planar lipid membranes directly coupled to the surface of a semiconducting electrode such as silicon, covered with a thin electrically nonconducting film of SiO_2. The lipid membrane which contain certain receptor molecules is surrounded by the aqueous solution of interest. Upon selective binding of ligands to the receptor molecules, structural changes in the membrane may occur which can be detected by measuring concomitant changes of both the impedance and the capacitance of the membrane using standard methods (2).

In order to show the principle selforganization working in such a device, we have used the nicotinic acetylcholine receptor (nAChR), one of the best characterized membrane receptor to date. nAchR functions as an ion channel in excitable membranes thereby lowering the energy barrier for the permeation of charged particles through the lipid bilayer. The receptor is located primarily in the postsynaptic membranes of the neuromuscular junction of skeletal muscle, called the endplate. When the neuron is electrically excited, the neurotransmitter acetylcholine (ACh) is secreted in a burst at this junction, where it diffuses from the presynaptic neuronal membrane to the skeletal muscle membrane. The nAChR is localized in densely packed clusters in the endplate structure in the cell plasma membrane. Upon interaction with Ach, the channels open which allows the selctive passage of cations. As the transmembrane potential changes with the flow of cations, the muscle cell is excited electrically, which eventually results in muscle contraction. The chemical signal, Ach, is used for excitatory synaptic transmission between nerve and muscle (3).

Large quantities of the nAChR are found in the electrical organ of electric eels such as Torpedo and Electrophorus. Because it is available in bulk the nAChR channel is much better characterized than any other channel proteins. The "fish" channels have been shown to be closely related to the human endplate channel and can be considered to be identical in most properties.

The relative molecular mass of the purified receptor is about 290'000, including surface carbohydrates of about 20'000 molecular mass. The receptor molecule consists of five polypeptide subunits of four different types with a stoichometry of $\alpha_2\beta\gamma\delta$. The four subunits have very homologous sequences. Based on the distribution of polar and apolar amino acids, models have been published describing the manner in which the protein subunits may be folded in the lipid membrane. However, the problem of folding the nAChR is still unclear and remains a matter of controversy. From a variety of biochemical, elctrophysiological and electron microscopic experiments the following picture of the three-dimensional structure of the AChR arises (Fig. 1). The five receptor subunits are arranged to traverse the membrane in a funnel-type form. The inner wall of the membrane

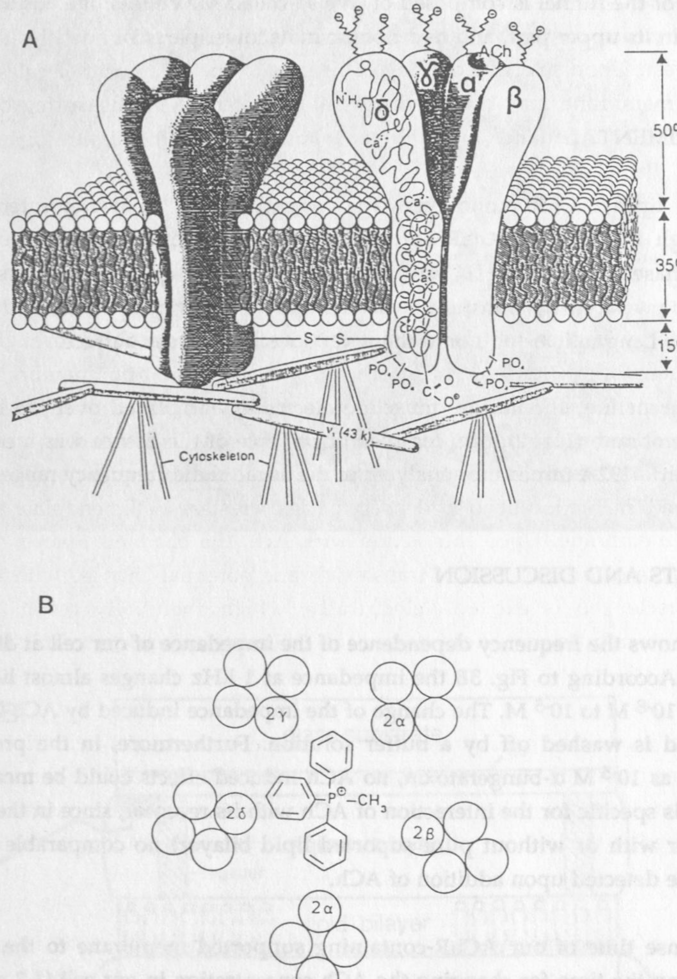

Fig. 1: Model of the ion channel of AChR. (A) Longitudinal section, (B) Cross-section in the bilayer plane (3).

embedded part of the funnel is composed of five so-called M2 helices; the postulated funnel is hydrophobic in its upper part, and hydrophilic in its lower part (3).

2. EXPERIMENTAL

A mixed lipid/AChR monolayer was formed at the air/water interphase of a Langmuir trough by injecting AchR enriched membranes from Torpedo californica into the buffer subphase (50 mM Tris-HCl pH 7.4, 1 mM EDTA). Supported bilayers containing the AChR were prepared by transfering sequentially two monolayers from the air-water interphase of the Langmuir trough onto the n <100 > silicon wafer surface.

A transparent metallic indium-tin oxide electrode was placed over the buffer layer covering the membrane (Fig. 2). The complex impedance of the device was measured with a Hewlett Packard 4192A impedance analyzer in the audio radio frequency range.

3. RESULTS AND DISCUSSION

Fig. 3A shows the frequency dependence of the impedance of our cell at different Ach concentrations. According to Fig. 3B the impedance at 1 kHz changes almost linearly with log (ACh) from 10^{-8} M to 10^{-5} M. The change of the impedance induced by ACh is reversible when the ligand is washed off by a buffer solution. Furthermore, in the presence of a antagonist such as 10^{-5} M α-bungeratoxin, no ACh induced effects could be measured. The observed effect is specific for the interaction of ACh with its receptor, since in the absence of AChR (Si waver with or without pure suported lipid bilayer) no comparable impedance changes could be detected upon addition of ACh.

The reponse time of our AChR-containing supported membrane to the binding of ACh is faster than the time for changing the ACh concentration in our cell (1-2 minutes). In our experiments we therefore cannot observe the opening and closing of the receptor channnel which ocurs in the msec time range upon binding of ACh.

Besides opening of the receptor channel, agonists such as ACh have a further effect on the AChR; prolonged ligand receptor interaction leads to receptor desensitization, accompanied by a channel closing and an increase of receptor affinity for agonists by two orders of magnitude.

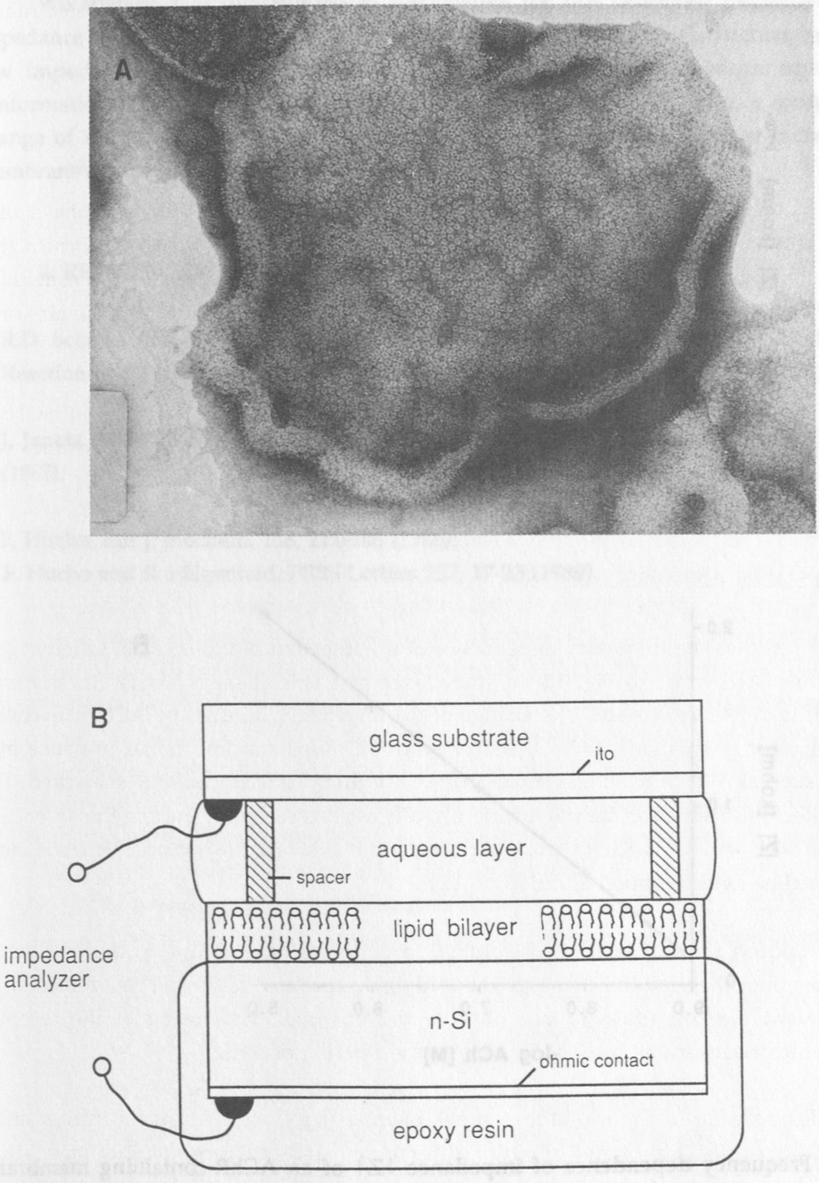

Fig. 2: (A) Electron microscopic image of negatively stained AChR-rich membranes. (B) Electrode-membrane-semiconductor capacitor.

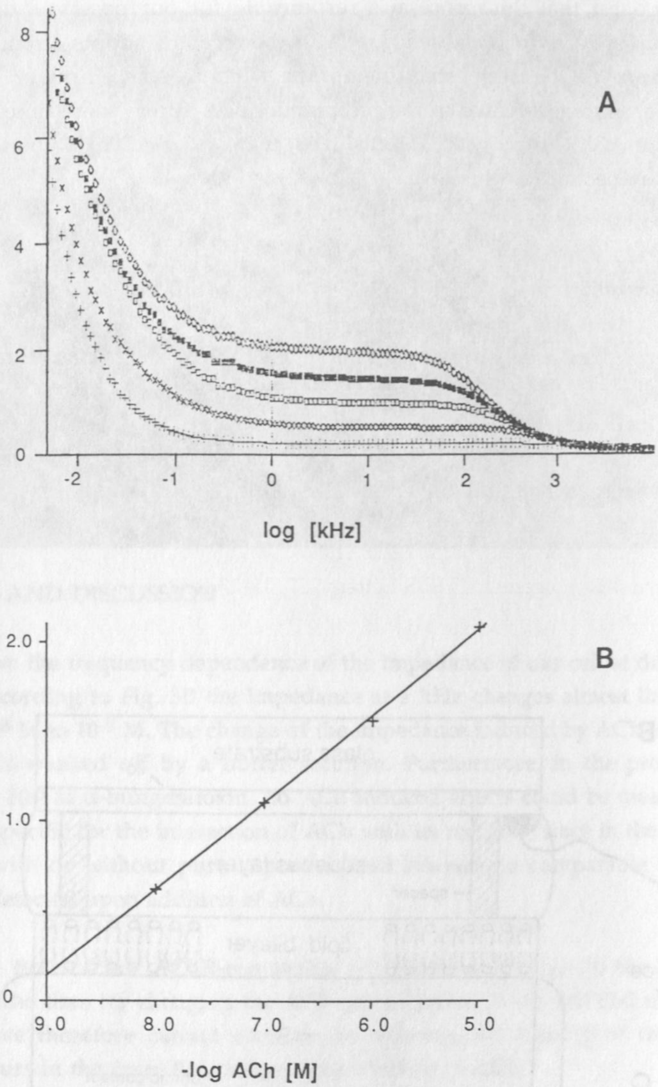

Fig. 3: (A) Frequency dependence of impedance |Z| of an AChR-containing membrane capacitor. Curves from bottom to top correspond to buffer with 0, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M acetylcholine, respectively. (B) Dependence of |Z| at 1 kHz on concentration of acetylcholine.

We suggest that this process is responsible for our observed dependence of the impedance upon ACh. In the absence of ACh the receptor adopts a structure leading to a low impedance of the supported membrane such as a preform of an open channel conformation. Binding of ACh to the receptor induces, after some time, a conformational change of the AChR to a closed, desensitized channel, which explains the increase of the membrane's impedance in this case.

4. REFERENCES

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