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The Adsorption of Adrenocorticotropin-(1-24)-tetracosapeptide to Lecithin Bilayer Membranes Formed from Liposomes

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Abstract. The interaction of the peptide hormone adrenocorticotropin ($ACTH_{1-24}$) with solvent-free planar lipid bilayers has been studied by use of the capacitance minimization method. The membranes were formed from artificial vesicles according to the method described by Schindler. In contrast to analogous studies with hexane-containing membranes, experiments with these vesicle-derived bilayers were completely reproducible and gave no indication that $ACTH_{1-24}$ spans such hexane-free bilayers.

Key words: Lipid-protein interaction – Capacitance minimization method – Solvent-effect on lipid bilayers

Introduction

Adrenocorticotrophic hormone (adrenocorticotropin, corticotropin, ACTH) carries vital biological information from the anterior lobe of the pituitary to the adrenal cortex and other parts of the mammalian body. It is a linear, flexible, amphiphilic nonatricontapeptide for which the structure-function relationship is known in some detail (e.g., Schwyzer 1977). Practical interest in ACTH has been stimulated by its unique and valuable therapeutic properties. The derivative of the hormone used in our studies, adrenocorticotropin-(1-24)-tetracosapeptide ($ACTH_{1-24}$), is a full agonist with a net positive charge of 6.

Using the recently developed method of capacitance minimization (Schoch et al. 1979a), evidence has been presented that $ACTH_{1-24}$ is rapidly adsorbed on and slowly transposes a fraction of its positive charges across lipid membranes containing hexane (Schoch et al. 1979b; Schoch 1980). As neither the mechanism of the incorporation nor the exact conditions for achieving it are known, we undertook further studies with solvent-free membranes formed from liposomes according to the method described by Schindler (Schindler 1980; Schindler and Quast 1980). Among many other advantages of lipid bilayer membranes formed by this technique, they are essentially free of organic

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solvents except for a very small amount of hexadecane originating from the pretreatment of the teflon septum over which the two monolayers are raised.

Materials and Methods

$ACTH_{1-24}$ was synthesized according to Schwyzer and Kappeler (1963). The lecithin used for the hexane-containing membranes was isolated from egg yolks by alumina column chromatography (for more details see Schoch and Sargent 1980). 1,2-Dioleoyl-sn-glycero-3-phosphorylcholine (DOPC) for the hexane-free bilayers was obtained from Berchtold (Biochemical Laboratory, Bern).

Black lipid membranes (BLM) were prepared by a modified Montal-Mueller technique (Montal and Mueller 1972) in which two surface films of lipid plus hexane are apposed across a hole in a thin teflon septum before all the hexane can evaporate. The aqueous phase was a 9 mM NaCl solution buffered by 2 mM imidazole (pH 7.4).

Hexane-free bilayers were formed from DOPC vesicles prepared by the method of Batzri and Korn (1973) in a buffer solution containing 10 mM KCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 10 mM HEPES, pH 7.4 (total ionic strength equals 33 mM: the concentrations of K^+, Ca^{2+}, and Mg^{2+} are about equal to the physiological values). The ethanol content of the vesicle solutions (usually 1 mg lipid/ml) was less than 0.2%. The equilibrium surface pressure at the air/vesicle solution interface was 42 ± 1 mN/m as determined with a ring tensiometer (Krüss, Hamburg). The vesicles could be stored for up to 3 days at 4° C. Hexane-free bilayers are formed by the apposition of the pure lipid monolayers which form spontaneously at the surface of the liposome suspension, as described by Schindler (1980). The aperture ($2 \cdot 10^{-4}$ cm^2) of the teflon septum on which the bilayers were formed was pretreated with hexadecane (Purum, Fluka, Buchs).

The interaction of $ACTH_{1-24}$ with the membranes was monitored using the capacitance minimization technique (Schoch et al. 1979a), which senses the intrinsic electric field (difference in surface potentials) accross the membrane. Surface charge and dipole components can be distinguished, as only the former depends on the ionic strength. In our experimental set-up the capacitance minimization potential ($V_{C\,\min}$), which is a measure of the amount of substance adsorbed, is monitored continuously and the time course following additions to the aqueous phase is plotted on a chart recorder. The aqueous solutions were stirred after each addition. Since determination of $V_{C\,\min}$ during stirring is accompanied by noise, the corresponding portions of the curves are indicated by dotted lines. All our experiments started with symmetrical conditions with respect to lipid and aqueous solutions, i.e., $V_{C\,\min} = 0$.

Results

The time course of the adsorption of $ACTH_{1-24}$ to a strongly hexane-containing membrane is shown in Fig. 1. At time A, upon addition of $ACTH_{1-24}$ (final

concentration $1.1 \cdot 10^{-4}$ M) to one side ("*cis*") of the bilayer, a rapid initial rise of $V_{C\,min}$ is followed by a slower decline of the signal to a steady state value of 24 mV. The polarity of the signal corresponds to a binding of positive charges to the *cis*-side of the membrane. At time B the salt concentration on the opposite side ("*trans*") was increased from 10–120 mM NaCl. [This is done to test for the

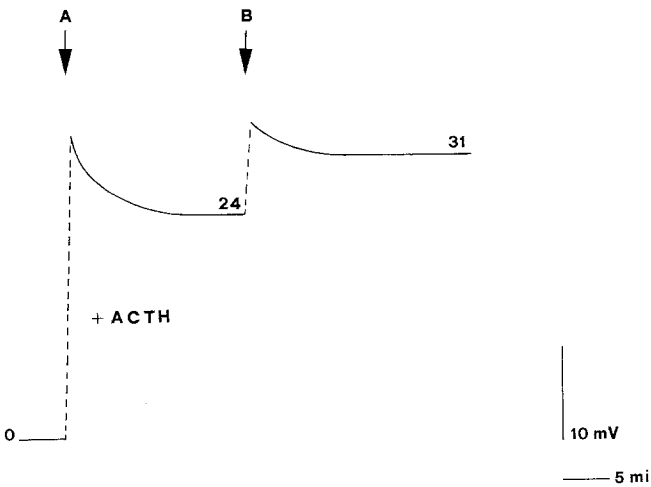

Fig. 1. Changes in capacitance minimization potential, $V_{C\,min}$, of BLM containing a considerable amount of hexane, upon addition of $ACTH_{1-24}$ to a final concentration of $1.1 \cdot 10^{-4}$ M (time A) and a subsequent increase of the ionic strength on the opposite side of the membrane from 10–120 mM (time B). The numbers above the curves give $V_{C\,min}$ in mV. Within experimental error, no change in $V_{C\,min}$ could be seen after increasing the ionic strength with unmodified bilayers (pure lecithin BLM)

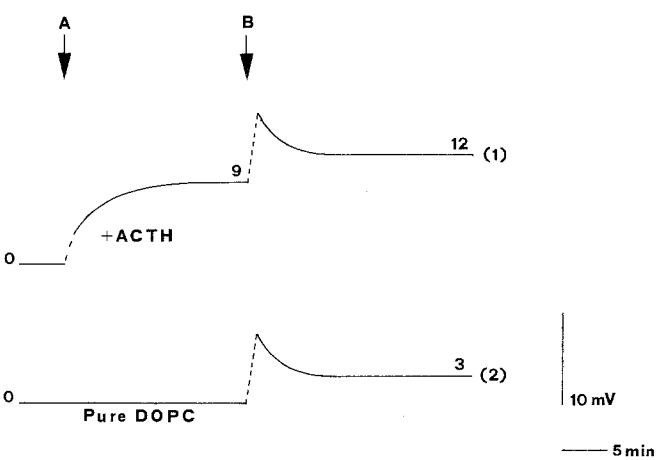

Fig. 2. Changes in capacitance minimization potential, $V_{C\,min}$, of hexane-free bilayers on addition of $ACTH_{1-24}$ to a final concentration of $1.4 \cdot 10^{-4}$ M [curve (1), time A], and a subsequent increase in the ionic strength on the opposite side from 33–300 mM by addition of NaCl (time B). The numbers above the curves give $V_{C\,min}$ in mV. Curve (2) shows the change seen after increasing the ionic strength (time B) with unmodified bilayers (pure DOPC), and represents the baseline for curve 1

appearance of charges on the *trans*-side of the membrane (shielding effects), as would be expected from the incorporation reaction.] An increase in $V_{C\,min}$ of several mV is observed. As no change of $V_{C\,min}$ was found for the pure lecithin BLM for the same change in ionic strength, the net "*trans*-effect" amounts to 7 mV. Such a *trans*-effect was by no means always reproducible, however.

Curve (1) of Fig. 2 shows the corresponding results for hexane-free bilayers. At time A, $1.4 \cdot 10^{-4}$ M $ACTH_{1\text{-}24}$ (final concentration) is added to the *cis*-side of the membrane and an increase of $V_{C\,min}$ is observed. Even after correcting the equilibrium value of 9 mV for the different ionic strengths used, this is still significantly lower than that for the hexane-containing BLM. At time B, after equilibration, the ionic strength on the *trans*-side of the membrane is increased from 33–300 mM by addition of NaCl. The time course of these changes has not been analyzed as it depends on stirring conditions.

In curve (2) of Fig. 2 the effect on the native DOPC bilayers of increasing the ionic strength on the *trans*-side from 33–300 mM is demonstrated. After equilibration, $V_{C\,min}$ reaches a value of + 3 mV. This change may be caused either by slight impurities in the lipid or be due to the pure lipid itself, e.g., a small head-group reorientation. In any case, comparison of curves (1) and (2) shows that the increase in ionic strength on the *trans*-side of the membrane results in the same change of the equilibrium value of $V_{C\,min}$ whether $ACTH_{1\text{-}24}$ is added to the *cis*-side or not.

Unfortunately, the useful range of concentrations for $ACTH_{1\text{-}24}$ turned out to be rather narrow. At a concentration of $1.4 \cdot 10^{-5}$ M the measured value of $V_{C\,min}$ was zero within experimental error. At a concentration of $7 \cdot 10^{-4}$ M $ACTH_{1\text{-}24}$ the lifetimes of the membranes became so short that no reliable value of $V_{C\,min}$ could be determined. Thus the search for a "*trans*-effect" could not be extended to higher levels of bound $ACTH_{1\text{-}24}$.

Discussion

The over-all time course for the interaction of $ACTH_{1\text{-}24}$ with strongly hexane-containing membranes (cf. Fig. 1) has been interpreted as follows (Schoch et al. 1979b). After addition of $ACTH_{1\text{-}24}$ a rapid adsorption (initial rise of $V_{C\,min}$) is followed by a slower incorporation of part of the adsorbed molecule to expose some of the positive charges to the opposite side of the membrane (subsequent decline of $V_{C\,min}$). Increasing the ionic strength on the *trans*-side increases the shielding of the transferred charges, resulting in an increase in $V_{C\,min}$ ("*trans*-effect"). Our own experiences with hexane-containg BLM revealed that this *trans*-effect occurred only with strongly hexane-containing BLM (Gremlich 1980) and has not always been reproducible.

In contrast to this, our experiments with hexane-free bilayers have been completely reproducible and the observed time course of the adsorption of $ACTH_{1\text{-}24}$ to the membrane always revealed the characteristic feature of Fig. 2: there is no indication that charges have appeared on the *trans*-side. [In this respect it resembles the adsorption to planar lipid bilayers of melittin (Schoch et al. 1979b; Schoch and Sargent 1980) which was not found to span the

membranes.] Furthermore, the level of binding is also significantly reduced compared with the membranes containing hexane.

The concentration of $ACTH^{6+}_{1-24}$ used in this study is clearly above the physiological hormone concentration. Biological membranes contain about 10% negatively charged lipids, however, and this causes the surface concentration of sixfold positively charged molecules to be increased over the bulk concentration by the Boltzmann distribution factor $[\exp(-zFV_G/RT)]$, which equals about 1,000 at physiological ionic strengths. That means that with biological membranes the observed interactions would occur in the sub-micromolar region. As this is already much nearer to physiological conditions, the observed effects are indeed of potential biological interest.

In summary, we can say that in contrast to strongly hexane-containing membranes, our investigations with hexane-free bilayers gave no evidence that $ACTH_{1-24}$ spans the membrane. The chemical mechanism of the interaction of $ACTH_{1-24}$ with planar lipid membranes remains to be established. To this aim further experiments with other techniques, e.g., infrared attenuated total reflection spectroscopy, are in progress. Details will be published later.

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