Biochimica et Biophysica Acta, 775 (1984) 246–254
Elsevier

BBA 72238

ADRENOCORTICOTROPIC HORMONE (ACTH)-LIPID INTERACTIONS

IMPLICATIONS FOR INVOLVEMENT OF AMPHIPATHIC HELIX FORMATION

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(Received March 12th, 1984)

Key words: ACTH-lipid interaction; Synaptic plasma membrane; Monolayer; Permeability; Fluorescence anisotropy; Protein-lipid interaction; Hormone-receptor interaction

ACTH-lipid interactions were investigated by: (1) lipid-monolayer studies using several zwitterionic and anionic phospholipids and gangliosides, (2) permeability experiments by following the swelling rate of liposomes in isotonic glycerol solutions by light scattering, using liposomes of synthetic lipids and liposomes made of lipids extracted from light synaptic plasma membranes, and (3) by steady-state fluorescence anisotropy measurements on liposomes derived from light synaptic plasma membranes employing 1,6-diphenyl-1,3,5-hexatriene as fluorescent probe. (1) The monolayer experiments demonstrated an interaction with gangliosides G_{T1}, G_{M1}, dioleoylphosphatidic acid and phosphatidylserine, but little or no interaction with phosphatidylcholine or sphingomyelin. The interaction with monolayers of G_{T1} or phosphatidic acid decreased for $ACTH_{1\text{-}13}\text{-}NH_2$ and $ACTH_{1\text{-}10}$. (2) The liposome experiments showed that $2 \cdot 10^{-5}$ M $ACTH_{1\text{-}24}$ increased the glycerol permeability by 20% and decreased the activation energy only when liposomes derived from light synaptic plasma membranes were used. Treatment of the liposomes with neuraminidase abolished the ACTH-induced permeability increase. (3) Steady-state fluorescence depolarization measurements revealed that $ACTH_{1\text{-}24}$, $ACTH_{1\text{-}16}\text{-}NH_2$ and $ACTH_{1\text{-}10}$ did not change the fluidity of liposomes derived from light synaptic plasma membranes as sensed by diphenylhexatriene. It is concluded that $ACTH_{1\text{-}24}$ can bind to negatively charged lipids and can form an amphipathic helix aligned parallel to the membrane surface involving the N-terminal residues 1 to 12, possibly to 16. Polysialogangliosides will favorably meet the condition of a high local surface charge density under physiological circumstances. It is suggested that ACTH-ganglioside interactions will participate in ACTH-receptor interactions.

Introduction

Corticotropin (ACTH) and its N-terminal fragments have shown to influence specific animal and human behavior [1–3]. In studying membrane processes associated with the central action of ACTH peptides, it was found that synaptosomal protein phosphorylation [4,5], polyphosphoinositide (polyPI) metabolism [6,7] and adenylate cyclase activity [8] are affected by $ACTH_{1\text{-}24}$. (For reviews see Refs. 10–13.)

The ACTH-induced excessive grooming behavior and the ACTH-induced inhibition of B-50 protein phosphorylation showed a similar structure-activity relationship [5,14]. It appeared that the effect of ACTH on the phosphorylation of the

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B-50 protein, a presumably presynaptically localized brain-specific membrane protein [15–17], is mediated by the inhibition of the B-50 protein kinase [9]. The B-50 protein kinase is a Ca^{2+}-activated phospholipid-dependent protein kinase similar to kinase C [13,20]. Recently, a crucial role has been attributed to B-50 protein phosphorylation in modulating synaptosomal polyPI metabolism [13,21,22].

Despite the various well-documented specific neurochemical actions of $ACTH_{1-24}$, attempts to demonstrate putative ACTH receptors have as yet been unsuccessful [21]. It is suggested, however, that ACTH affects directly the membrane properties and/or the B-50 protein kinase [13]. Considering that the effect of $ACTH_{1-24}$ on lipid fluidity of light synaptic plasma membranes [22] shows a similar structure-activity relationship as observed for the inhibition of B-50 protein phosphorylation (unpublished results), and that lipid fluidity can modulate protein phosphorylation and receptor binding in synaptic plasma membranes [23], the possibility arises that $ACTH_{1-24}$ indeed exerts its synaptic effects by directly affecting membrane properties. Therefore it was of interest to investigate ACTH-lipid interactions in more detail.

Materials and Methods

Chemicals. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycerol-3-phosphoserine (DOPS) were synthesized by standard methods [24]. 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-*sn*-glycero-3-phosphate were prepared from DOPC according to Cullis and De Kruijff [25].

Egg phosphatidylcholine (egg PC) was isolated by the method of Pangborn [26] and purified by high-pressure liquid chromatography [27].

Phosphatidylinositol (PI) was isolated from rat liver microsomes [28]. Sphingomyelin from total bovine brain and neuraminidase ex *Vibrio cholerae* (EC 3.2.1.18) were purchased from Koch-Light Laboratories (Colnbrook, UK).

The gangliosides G_{T1} and G_{M1} (nomenclature according to Svennerholm [29]) were obtained from Supelco (Houston, TX, U.S.A.). Gangliosides from total bovine brain were purchased from Sigma (St. Louis, MO, U.S.A.). All lipids were pure as evidenced by thin-layer chromatography, except the gangliosides from total bovine brain, which contained small impurities of PS and PI. All lipid manipulations were carried out under a N_2 atmosphere and all lipids were stored in chloroform/methanol (1 : 1, by vol.) at −20°C.

The following synthetic fragments of adrenocorticotrophic hormone (ACTH) were used: $ACTH_{1-24}$, $ACTH_{1-16}$-NH_2, $ACTH_{1-13}$-NH_2 and $ACTH_{1-10}$. These peptides were a gift from Dr. H.M. Greven (Organon Int. BV, Oss, The Netherlands).

All solvents and reagents used were of analytical grade.

Isolation of synaptic plasma membranes. Light synaptic plasma membranes were prepared from female Wistar rats (approx. 140 g body weight), using whole brain without cerebellum as described by Kristjansson et al. [17]. All manipulations were carried out at 4°C.

Lipid extraction. Extraction of lipids from the light synaptic plasma membrane pellet was performed at room temperature basically according to Bligh and Dyer [30], repeating the following cycle three times: chloroform/methanol added to form a monophasic composition in an air-tight tube; 5 min sonication in a waterbath (Philips, type 2100/10); chloroform/methanol added to form a biphasic composition; 5 min sonication in a waterbath; centrifugation for 10 min at $4000 \times g$ (Heraeus-Christ, type UJ-3); careful collection of lower phase with a blunt syringe. In the first cycle chloroform and methanol were added to the in water resuspended light synaptic plasma membrane pellet to obtain a monophasic composition of chloroform/methanol/water (2 : 2 : 1, by vol.). In the second and third cycle, 0.5 vol. methanol and 0.5 vol. chloroform were added (monophasic) and 1 vol. chloroform (biphasic).

The three lower phases were combined, evaporated at 37°C under reduced pressure, dried with absolute ethanol, dissolved in chloroform/methanol (1 : 1, by vol.) and filtered through a G-5 glass filter, evaporated to dryness and finally dissolved in chloroform/methanol (1 : 1, by vol.) to a concentration of approx. 10 mg/ml. Any precipitate was removed by centrifugation at $20000 \times g$ for 15 min.

Light synaptic plasma membrane lipids con-

tained 57.4 mol% phospholipid, 36.8 mol% cholesterol and 5.7 mol% ganglioside. The phospholipids contained 35 mol% phosphatidylethanolamine, 40 mol% phosphatidylcholine, 10 mol% phosphatidylserine, 5 mol% phosphatidylinositol, 5 mol% phosphatidic acid and 5 mol% sphingomyelin.

Monolayer experiments. Pressure increase measurements of lipid monolayers at the air/water interface [31] were performed in a Teflon trough (3 cm in diameter and 0.5 cm deep). The trough was filled with 150 mM KCl buffer (10 mM Tris-HCl, pH 7.4). The subphase (5.5 ml) was stirred with a magnetic bar placed in an additional saving of 1 cm diameter and 0.5 cm deep in the middle of the trough.

Mono-molecular films were spread from a chloroform/methanol (9:1, v/v) solution with a capillary pipette until the desired interfacial pressure was reached. After the initial surface pressure had stabilized, peptides were injected underneath the monolayer (275 μl, via a small bypass connection) and the increase in surface pressure was determined with a recording Beckman LM-500 electrobalance by the Wilhelmy-plate method, using a sandblasted platinum plate (1.96 by 1 cm). Measurements were performed at 25 ± 1°C in a thermostated box.

Permeability measurements. The permeability of liposomes towards glycerol was expressed as the relative initial swelling rate, % $(dl/A)/(dt)$, of liposomes in isotonic solution as observed by light scattering according to Bangham et al. [32] and De gier et al. [33].

Liposomes were prepared by dispersing an aliquot of the light synaptic plasma membrane lipid extract or other lipid mixtures (approx. 10 mg lipid/ml liposomes) in 150 KCl buffered with 10 mM Tris-HCl, pH 7.4 (KCl buffer). Prior to the determination of glycerol permeability, 30 μl liposomes were incubated at room temperature with 30 μl KCl buffer, with or without ACTH fragments for 10 min. The initial swelling rate was recorded at 438 nm (Vitatron, type MPS) and at 25°C, after adding 55 μl of incubation mixture to 5 ml of 300 mM glycerol in 10 mM Tris-HCl (pH 7.4), vigorously stirred in a thermostatically controlled cuvette. The activation energies for the permeation were calculated by linear regression from Arrhenius plots in which $\ln[\% (dl/A)/(dt)]$ was plotted against $1/T$, T being the absolute temperature.

Steady-state fluorescence depolarization. Steady-state fluorescence depolarization measurements were performed with diphenylhexatriene as fluorescent probe using a self-constructed instrument described previously [34].

Labeling of the liposomes was performed by incubating 400 μl liposomes with 500 μl of a 2 μM dispersion of diphenylhexatriene in KCl buffer for 30 min at 35°C. The diphenylhexatriene dispersion was prepared by mixing a 2 mM diphenylhexatriene solution in tetrahydrofuran with KCl buffer for 30 min at room temperature under a constant flow of N_2. After the labeled liposomes were incubated with 100 μl ACTH fragments for 5 min at 35°C, $I_\perp$ and $I_\parallel$ were measured independently at about 20 temperatures between 20°C and 37°C. The final liposome concentration was 0.1 mg/ml.

The liposome concentration and the incubation time of the liposome with diphenylhexatriene were chosen at their P-plateau. The structural order parameter S was estimated using a semi-empirical method [35,36]. After linear regression S at 25°C was calculated by interpolation.

Miscellaneous. Phosphorus was determined as described by Rauser et al. [37] and cholesterol as outlined by Broekhuyse [38]. *N*-Acetylneuraminic acid was determined by the method of Svennerholm [39] as modified by Miettinen and Takki-Luukkainen [40].

Neuraminidase treatment was based on Ada et al. [41] and Haksar et al. [42]. A liposomal suspension in KCl buffer (approx. 10 mg lipid per ml) was incubated with 1 mM $CaCl_2$ and 10 mU/ml neuraminidase at 37°C for 30 min. After cooling to room temperature the whole incubation mixture was processed as described under permeability measurements.

Light synaptic plasma membrane gangliosides were isolated from light synaptic plasma membrane lipids by preparative thin-layer chromatography on silica (Kieselgel 60, Merck, Darmstadt, F.R.G.). After elution with chloroform/methanol/ammonia/water (55:45:5:5, by vol.) and localization with iodine-vapor (on the side) the corresponding track was scraped from the

glass support, transferred into a glass column and eluted with chloroform/ methanol/ water (30 : 60 : 20, by vol.) [43].

Results and Discussion

Monolayer experiment

A significant increase in surface pressure ($\Delta\pi$) was observed after the injection of $ACTH_{1-24}$ underneath mono-molecular layers of negatively charged lipids at different initial pressures (π_i) (Fig. 1A, B). At initial pressures of 30–35 m · Nm^{-1}, which are considered to be most relevant for biological membranes [44,45], the highest pressure increase is observed for dioleoylphosphatidic acid and gangliosides G_{T1} and G_{M1}. A smaller effect is measured for dioleoylphosphatidylserine and phosphatidylinositol. Neutrally charged lipids as dioleoylphosphatidylcholine, dioleoylphosphatidylethanolamine and sphingomyelin, had hardly any effect. While interaction of $ACTH_{1-24}$ with phosphatidic acid and phosphatidylserine monolayers ($\Delta\pi$) decreases progressively at increasing π_i, with G_{T1} and G_{M1} monolayers this interaction levels off at $\pi_i > 25$ m · Nm^{-1}. This different behavior can possibly be explained by the large lateral compressibility of gangliosides, relative to phosphatidic acid and phosphatidylserine [46].

The concentration dependency of the $ACTH_{1-24}$ monolayer interaction (Fig. 2) shows that already at 0.3 μM $ACTH_{1-24}$ an increase in surface pressure can be measured, dependent on the type of lipid. At an initial pressure of 35 m · Nm^{-1}, 20–30 μM $ACTH_{1-24}$ increases the surface pressure similarly for phosphatidic acid, gangliosides G_{T1} and G_{M1}, demonstrating that a negative charge is important for the $ACTH_{1-24}$ monolayer interactions. It was found that the rate of $ACTH_{1-24}$ monolayer interaction was significantly different for the various lipids. At 5 μM $ACTH_{1-24}$ the maximal pressure increase was obtained after 1 min for phosphatidic acid, 3 min for ganglioside G_{T1}, 7 min for phosphatidylserine and 25 min for ganglioside G_{M1}. Structure-activity studies of ACTH fragment-monolayer interactions using phosphatidic acid and ganglioside G_{T1} monolayers, showed a decreasing activity with a decrease in the chain length of the N-terminal fragment of ACTH (Fig. 3). The interaction with mono-molecular layers decreased in the order $ACTH_{1-24}$, $ACTH_{1-16}$-NH_2, $ACTH_{1-13}$-NH_2 and $ACTH_{1-10}$. A similar decrease in activity with shorter chain length has been found for a variety of biological effects of the peptide [3,5,6,8,47–49].

It can be concluded that both the negative charge of the monolayer lipids and the positive charge of the ACTH peptide are of critical importance for the interaction of the peptide with a membrane interface. The pressure increase indicates that not only polar interactions but also hydrophobic interactions occur which could be reflected in a permeability change of membranes.

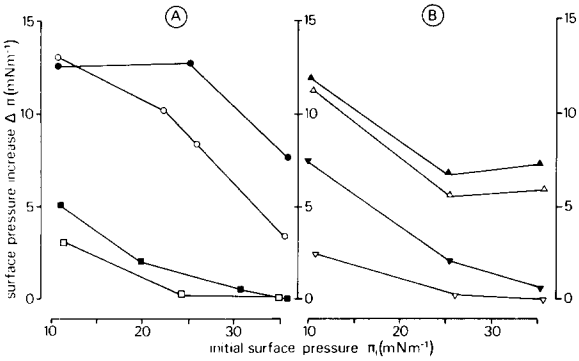

Fig. 1. Increase in surface pressure ($\Delta\pi$) after injection of $ACTH_{1-24}$ (final concentration 5 μM, in 150 mM KCl, 10 mM Tris-HCl, pH 7.4) underneath monolayers at different initial surface pressure (π_i). (A) Dioleoylphosphatidic acid (●), dioleoylphosphatidylserine (○), dioleoylphosphatidylcholine (■), dioleoylphosphatidylethanolamine (□). (B) Monosialoganglioside G_{M1} (△), trisialoganglioside G_{T1} (▲), phosphatidylinositol (▼), sphingomyelin (▽).

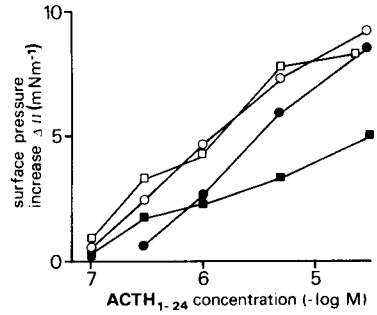

Fig. 2. Increase in surface pressure ($\Delta\pi$) after the injection of $ACTH_{1-24}$ underneath mono-molecular films of trisialoganglioside G_{T1} (○), dioleoylphosphatidic acid (□), monosialoganglioside G_{M1} (●) and dioleoylphosphatidylserine (■). The initial surface pressure was 35 m · Nm^{-1}.

TABLE I

EFFECT OF ACTH ON THE PERMEABILITY OF LIPOSOMES, MADE FROM LIGHT SYNAPTIC PLASMA MEMBRANE LIPIDS, TOWARDS GLYCEROL

Data (relative initial swelling rate) are expressed as mean ± S.E. ($n = 5$) as percentage of control. Control values: mean ± S.E. ($n = 15$). A: concentration of $ACTH_{1-24}$ was 30 μM, concentration of other fragments was 50 μM. Liposomes made from light synaptic plasma membrane lipids were found to behave as ideal osmometers according to Boyle-Van 't Hoff. Statistically significant differences from control values were obtained: * 2 $P < 0.05$; ** 2 $P < 0.01$ (Student's t-test).

A. Effect of ACTH fragments	
Control	100 ± 2
$ACTH_{1-10}$	105 ± 1
$ACTH_{1-13}$-NH_2	113 ± 2 *
$ACTH_{1-16}$-NH_2	119 ± 3 *
$ACTH_{1-24}$	117 ± 3 **
B. Effect of $ACTH_{1-24}$ concentration (M)	
Control	100 ± 2
$ACTH_{1-24}$, 10^{-7}	99 ± 4
10^{-6}	105 ± 3
$4 \cdot 10^{-6}$	103 ± 2
$7 \cdot 10^{-6}$	111 ± 4 *
10^{-5}	110 ± 3 *
$3 \cdot 10^{-5}$	120 ± 3 **

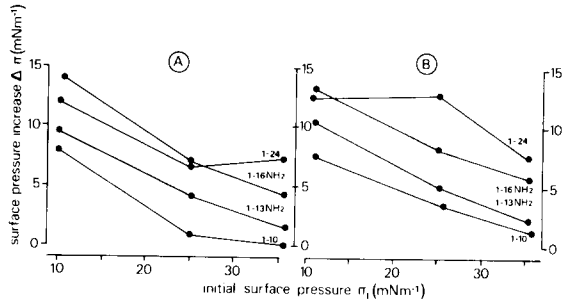

Fig. 3. Increase in surface pressure ($\Delta\pi$) after the injection of ACTH fragments underneath monolayers of trisialoganglioside G_{T1} (A), and dioleoylphosphatidic acid (B) at different initial surface pressures (π_i). The peptide concentration in the subphase was 5 μM.

Permeability experiment

The permeability of liposomes derived from light synaptic plasma membrane lipids, for small non-electrolytes was found to be affected by the interaction with $ACTH_{1-24}$. $ACTH_{1-24}$ increases the permeability of these liposomes towards glycerol by about 20% (Table I). A similar effect was found for $ACTH_{1-16}$-NH_2. The effect decreased in the presence of $ACTH_{1-13}$-NH_2 and $ACTH_{1-10}$ in this

TABLE II

THE INVOLVEMENT OF LIGHT SYNAPTIC PLASMA MEMBRANE GANGLIOSIDES IN THE INCREASE OF LIPOSOMAL GLYCEROL PERMEABILITY INDUCED BY $ACTH_{1-24}$

DOPC/Dioleoylphosphatidic acid liposomes contained 15 μM DOPC + 0.6 μM dioleoylphosphatidic acid per ml KCl buffer (150 mM KCl, 10 mM Tris-HCl, pH 7.4). DOPC/dioleoylphosphatidic acid + bovine gangliosides: 0.5 mg of total bovine brain gangliosides added to DOPC/dioleoylphosphatidic acid, per ml. Egg-PC + light synaptic plasma membrane gangliosides: 12 mg egg-PC + 1 mg light synaptic plasma membrane gangliosides (isolated by preparative TLC), per ml. Light synaptic plasma membrane liposomes contained approx. 10 mg lipid per ml. Data are expressed as mean ± S.E. ($n = 5$) as percentage of control. Neuraminidase data are expressed as mean ± S.E. ($n = 5$) as percentage of control before neuraminidase treatment.

Liposomal composition	Control	30 μM $ACTH_{1-24}$
DOPC/dioleoylphosphatidic acid	100 ± 2	97 ± 3
DOPC/dioleoylphosphatidic acid + bovine brain gangliosides	100 ± 3	106 ± 6
Egg PC + light synpatic plasma membrane gangliosides	100 ± 1	113 ± 4 *
Light synaptic plasma membrane lipid liposomes before neuraminidase treatment	100 ± 1	113 ± 1 *
Light synaptic plasma membrane lipid liposomes after neuraminidase treatment	97 ± 2	95 ± 2

* Statistically different from control values: 2 $P < 0.05$ (Student's t-test).

order. The dose-response relationship for the ACTH-induced permeability increase shows that the effect becomes evident at concentrations of 10^{-6} M (Table I). At a concentration of $3 \cdot 10^{-5}$ M a permeability increase for glycerol was measured of about 20%. This ACTH concentration and structure dependency as measured for the glycerol permeability is very similar to that of the inhibition of the B-50 protein phosphorylation [14]. Since monolayer studies showed an interaction of $ACTH_{1-24}$ with negatively charged lipids, it is well possible that the presence of specific anionic lipids in light synaptic plasma membranes can cause the increase in liposome permeability. The presence of about 3.8 mol% bovine brain gangliosides in dioleoylphosphatidylcholine liposomes enhanced the permeability by about 6%, and the presence of about 7 mol% light synaptic plasma membrane gangliosides in egg-PC liposomes with about 13% (Table II). Although $ACTH_{1-24}$ showed a strong interaction with phosphatidic acid in monolayers, the presence of this negatively charged lipid in liposomes did not enhance the glycerol permeability (Table II). That gangliosides might be required for the enhanced glycerol permeability as induced by $ACTH_{1-24}$, is supported by the observation that neuraminidase treatment of the liposomes abolished this effect.

It might be well possible that polysialogangliosides are involved, since they provide the highest charge density. In mono-molecular layers the interaction was more rapid with G_{T1} than with G_{M1}. In addition, neuraminidase preferentially hydrolyzes polysialogangliosides to monosialogangliosides [50,51], whereas light synaptic plasma membrane lipids are enriched in polysialogangliosides compared to total brain [52]. From the temperature dependency the activation energy for the glycerol permeation of light synaptic plasma membrane liposomes was calculated [53]. In the absence and presence of 30 μM $ACTH_{1-24}$ the activation energy for the glycerol permeation through these liposomes was 20.0 ± 0.2 and 18.3 ± 0.1 kcal $\cdot mol^{-1}$, respectively. The activation energy is the sum of the dehydration energy, and the diffusion energy. The dehydration energy is a constant and is for glycerol 11 kcal $\cdot mol^{-1}$ [54]. It can be calculated that the diffusion energy is decreased by 19% in the presence of ACTH.

Steady-state fluorescence depolarization

The glycerol permeability experiments indicated a perturbation of the hydrophobic core of the lipid bilayer by $ACTH_{1-24}$. It has been reported that light synaptic plasma membrane fluidity increases after $ACTH_{1-24}$ addition [22]. Therefore, the effect of $ACTH_{1-24}$ on the order parameter of light synaptic plasma membrane lipid liposomes was investigated as determined by diphenylhexatriene (*S*) [35]. It was found that $ACTH_{1-10}$, $ACTH_{1-16}$-NH_2 and $ACTH_{1-24}$ did not perturb the hydrophobic core in a way that could be sensed by diphenylhexatriene. This effect of $ACTH_{1-24}$ on diphenylhexatriene depolarization in light synaptic plasma membrane lipid liposomes deviates from the effect of $ACTH_{1-24}$ on diphenylhexatriene depolarization in light synaptic plasma membranes. It is well possible that the ACTH-membrane interaction is not identical with light synaptic plasma membranes and light synaptic plasma membrane lipid liposomes and that diphenylhexatriene senses fluidity changes in the central part of the membrane [55,56], rather than closer to the polar headgroup. Considering the affinity of diphenylhexatriene for apolar regions of proteins [56], it is also possible that diphenylhexatriene depolarization monitors structural changes of proteins as a consequence of the ACTH-membrane interaction. It has been suggested that the increased fluidity in adrenal cell membranes after ACTH addition originates from conformational changes in receptor proteins [57]. It is most likely that the positively charged amino acids at position 15–18 are involved in the ionic interaction with negatively charged lipids. The monolayer and permeability experiments suggest that ACTH penetrates into the hydrophobic core. A penetration of the N-terminal part of $ACTH_{1-24}$ deep into the hydrophobic core of the membrane is opposed by the presence of charged amino acids at positions 1 (Ser), 5 (Glu) and 8 (Arg) and is not supported by fluorescent depolarization experiments. An undeep penetration of $ACTH_{1-24}$ into the membrane lipid layer could be visualized by an amphipathic helix [58].

In order to determine the amphipathic properties of $ACTH_{1-24}$ in an α-helical conformation, the approach of Eisenberg et al. [59] was used. They showed that amphipathic peptide sequences (ap-

prox. 15–20 residues) in an α-helical conformation are characterized by a high hydrophobic moment ($\langle \mu_H \rangle$), which is a measure for the degree of amphipathic character, combined with a negative mean hydrophobicity ($\langle H \rangle$), indicating the general hydrophilic character. For the most amphipathic 5–22 segment of mellitin they calculated a hydrophobic moment ($\langle \mu_H \rangle$) of 0.40 and a mean hydrophobicity ($\langle H \rangle$) can be calculated of -0.02. Applying this approach to $ACTH_{1-24}$ as illustrated in Fig. 4, it is found that the most amphipathic N-terminal segment $ACTH_{1-16}$ gives a $\langle \mu_H \rangle = 0.34$ and a $\langle H \rangle = -0.38$. In general helices with a high amphipathic character and a negative mean hydrophobicity are surface-seeking helices [59].

This possibility becomes increasingly plausible recognizing the well-documented behavior of melittin [60], a small peptide sharing structural similarities with $ACTH_{1-24}$ and other peptide hormones [61]. Also Schwyzer et al. [62] assumes that the N-terminal part of ACTH ($ACTH_{1-10}$) has an α-helical structure when being part of $ACTH_{1-24}$. The fragment $ACTH_{1-10}$ itself, when studied without the sequence (11–24) seems to prefer a α-pleated sheet structure. If indeed the α-helical structure of the fragment $ACTH_{1-10}$ is dependent on its presence in $ACTH_{1-24}$, then the difference in effectiveness between $ACTH_{1-10}$ and $ACTH_{1-24}$ (Table I) may originate from these conformational differences. In view of the similarity in effectiveness between $ACTH_{1-10}$ and $ACTH_{1-24}$ in several behavioral test situations, Schwyzer et al. [62] assume that the, as yet unidentified, brain receptors differ in their recognition properties from those of peripheral target cells to ACTH. In contrast with the β-pleated sheet structure of the (1–10) fragment, Van Nispen and Greven [63] suggest that the fragment $ACTH_{4-10}$ itself may have an α-helical conformation at the brain receptor sites. The present data are clearly in line with the notion that the biological important N-terminal part of ACTH assumes an α-helix.

It can be visualized that an amphipathic helix aligned parallel to the membrane surface and penetrating only a short distance to it, may explain the observed effects of $ACTH_{1-24}$ in the monolayer, permeability and fluorescence experiments. It is well possible that proline at position 12 might interrupt the amphipathic helix structure.

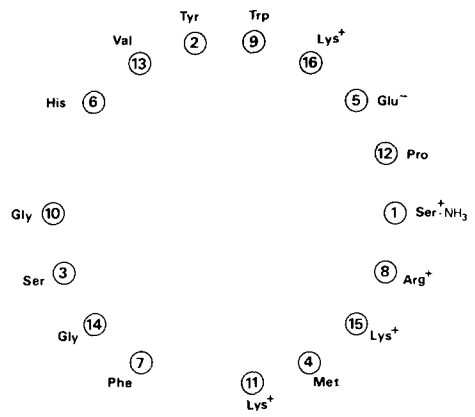

Fig. 4. Projection of $ACTH_{1-16}$-NH_2 in an α-helical conformation along the helical axis.

Biological significance

As described already before, the interaction of an amphipathic helix of $ACTH_{1-12}$, possibly to 16, with membranes is favored by negatively charged lipids giving a high charge density. The involvement of these localized charges can be understood applying the amphipathic helix model. The importance of a negative charge near the hydrophobic core is well in agreement with the localization of Lys^{11} and Lys^{16}, bordering the hydrophilic side of the amphipathic helix in $ACTH_{1-16}$ almost opposite to each other (Fig. 4).

A high local surface charge density may well be needed to induce and stabilize the C-terminal part of the amphipathic helix in ACTH considering that Lys^{11}, Lys^{15} and Lys^{16} will become located close to each other. The condition of a high local surface charge density can be met in biological membranes by gangliosides due to their clustering properties [64–68].

After early suggestions that gangliosides may participate in the internalization of environmental signals [69], it has been shown that gangliosides participate in the actions of glycoprotein hormones [70–72] and bacterial toxins [73,74] with considerable specificity. The monolayer and permeability experiments do not allow a definite conclusion with respect to ganglioside headgroup specificity since the permeability experiments suggest specific interactions between ACTH and gangliosides while the monolayer experiments point to general

ACTH-anionic lipid interactions. In addition, an absolute headgroup specificity in ganglioside-ACTH interactions seems very unlikely considering the metabolic constraints, especially when is recognized that gangliosides are located over the entire neuronal surface [75,76] and are found in brain microsomal subfractions with a species composition similar to that of synaptic plasma membranes [77]. The specificity in ACTH-receptor interactions does not necessarily involve specific ganglioside-ACTH interactions as is implicated by the observation that ACTH binds with high affinity to adrenal proteinaceous receptors [78].

Judging the importance of the lipid-induced amphipathic helix in ACTH in ACTH-receptor interactions, we share the conviction of Epand [79] and Gysin and Schwyzer [80,81] that ACTH-lipid interaction may be part of ACTH-receptor interaction by capturing ACTH from the surrounding fluid and by inducing conformations and topological arrangements that are favorable for receptor interaction.

Though ACTH-lipid interaction may provide some of the specificity of the ACTH-receptor interaction by means of the conditional properties of the anionic lipid and possibly by headgroup specificity of the anionic lipid, it seems appropriate that ACTH-lipid interactions may at best not be more than part of the ACTH-receptor interactions, as judged by the specific binding of ACTH to adrenal [78] and pituitary proteins [82] and by the complex pleiotropic actions of ACTH in several tissues.

The participation in ACTH-receptor interactions of the amphipathic helix in ACTH along the membrane surface introduces a few feature, which may explain some of the peculiarities observed in detailed structure-activity studies: the direction of the hydrophobic moment, which will differ among different fragments, resulting in different exposure of the residues relative to the membrane surface. Obviously, besides these differences between ACTH fragments, also differences in surface concentration (due to different charges) and differences in conformation will complicate the interpretation of structure-activity studies.

Acknowledgement

We wish to thank Dr. Siegfried de Laat (Hubrecht Laboratory, Utrecht) for his help with the fluorescence depolarization experiments.

References

1 De Wied, D. (1977) Ann. N.Y. Acad. Sci. 297, 263–274
2 De Wied, D. and Gispen, W.H. (1977) in Peptides in Neurobiology (Gainer, H., ed.), pp. 397–448, Plenum Press, New York
3 De Wied, D. and Jollès, J. (1982) Physiol. Rev. 62, 976–1059
4 Zwiers, H., Veldhuis, H.D., Schotman, P. and Gispen, W.H. (1976) Neurochem. Res. 1, 669–677
5 Zwiers, H., Wiegant, V.M., Schotman, P. and Gispen, W.H. (1978) Neurochem. Res. 3, 455–463
6 Jollès, J., Bär, P.R. and Gispen, W.H. (1981) Brain Res. 224, 315–326
7 Jollès, J., Zwiers, H., Dekker, A., Wirtz, K.W.A. and Gispen, W.H. (1981) Biochem. J. 194, 283–291
8 Wiegant, V.M., Dunn, A.J., Schotman, P. and Gispen, W.H. (1979) Brain Res. 168, 565–584
9 Zwiers, H., Schotman, P. and Gispen, W.H. (1980) J. Neurochem. 34, 1689–1699
10 Gispen, W.H. (1980) Progr. Brain Res. 53, 193–206
11 Gispen, W.H., Van Someren, H. and Schotman, P. (1981) Adv. Physiol. Sci. 13, 223–232
12 Wiegant, V.M., Zwiers, H. and Gispen, W.H. (1981) Pharmac. Ther. 12, 463–490
13 Zwiers, H., Jollès, J., Aloyo, V.J., Oestreicher, A.B. and Gispen, W.H. (1982) Progr. Brain Res. 56, 405–417
14 Gispen, W.H., Zwiers, H., Wiegant, V.M., Schotman, P. and Wilson, J.E. (1979) Adv. Exp. Med. Biol. 116, 199–224
15 Oestreicher, A.B., Zwiers, H., Schotman, P. and Gispen, W.H. (1981) Brain Res. Bull. 6, 145–153
16 Sorensen, R.G., Kleine, L.P. and Mahler, H.R. (1981) Brain Res. Bull. 7, 57–61
17 Kristjansson, G.I., Zwiers, H., Oestreicher, A.B. and Gispen, W.H. (1982) J. Neurochem. 39, 371–378
18 Aloyo, V.J., Zwiers, H. and Gispen, W.H. (1983) J. Neurochem. 41, 649–653
19 Jollès, J., Zwiers, H., Van Dongen, C., Schotman, P., Wirtz, K.W.A. and Gispen, W.H. (1980) Nature 286, 623–625
20 Oestreicher, A.B., Van Dongen, C.J., Zwiers, H. and Gispen, W.H. (1983) J. Neurochem. 41, 331–340
21 Witter, A. (1980) Adv. Biochem. Psychopharmacol. 21, 407–414
22 Hershkowitz, M., Zwiers, H. and Gispen, W.H. (1982) Biochim. Biophys. Acta 692, 495–497
23 Hershkowitz, M., Heron, D., Samuel, D. and Shinitzky, M. (1982) Progr. Brain Res. 56, 419–434
24 Van Deenen, L.L.M. and De Haas, G.H. (1964) Adv. Lipid Res. 2, 167–234
25 Cullis, P.R. and De Kruijff, B. (1976) Biochim. Biophys. Acta 436, 523–540
26 Pangborn, M.C. (1951) J. Biol. Chem. 188, 471–476

27 Geurts van Kessel, W.S.M., Tieman, M. and Demel, R.A. (1981) Lipids 16, 58–63
28 Demel, R.A., Kalsbeek, R., Wirtz, K.W.A. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 466, 10–22
29 Svennerholm, L. (1964) J. Lipid Res. 5, 145–153
30 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917
31 Demel, R.A. (1974) Methods Enzymol. 32, 539–545
32 Bangham, A.D., De Gier, J. and Greville, G.D. (1967) Chem. Phys. Lipids 1, 225–246
33 De Gier, J., Mandersloot, J.G. and Van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 666–675
34 Teichberg, V.I. and Shinitzky, M. (1973) J. Mol. Biol. 74, 519–531
35 Van Blitterswijk, V.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332
36 Pottel, H., Van der Meer, W. and Herreman, W. (1983) Biochim. Biophys. Acta 730, 181–186
37 Rauser, J., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496
38 Broekhuyse, R.M. (1976) Clin. Chim. Acta 66, 88
39 Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611
40 Miettinen, T. and Takki-Luukkainen, I.-T. (1959) Acta Chem. Scand. 13, 856
41 Aga, G.L., French, E.L. and Lind, P.E. (1961) J. Gen. Microbiol. 24, 409–421
42 Haksar, A., Baniukiewicz, S. and Péron, F.G. (1973) Biochem. Biophys. Res. Commun. 52, 959–966
43 Nilsson, O. and Svennerholm, L. (1982) J. Lipid Res. 23, 327–334
44 Demel, R.A., Geurts van Kessel, W.S.M., Zwaal, R.F.A., Roelofsen, B. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 97–107
45 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44
46 Maggio, B., Cumar, F.A. and Caputto, R. (1978) Biochem. J. 171, 559–565
47 Schwyzer, R. (1977) Ann. N.Y. Acad. Sci. 297, 3–26
48 Opmeer, F.A., Van Ree, J.M. and De Wied, D. (1978) Naunyn-Schmiedelbergh's Arch. Pharmacol. 302, 31–36
49 Terenius, L., Gispen, W.H. and De Wied, D. (1975) Eur. J. Pharmacol. 33, 395–399
50 Miller-Prodaza, H., Bradley, R.M. and Fishman, P.H. (1982) Biochemistry 21, 3260–3264
51 Massarelli, R., Wong, T.Y., Harth, S., Louis, J.C., Freysz, L. and Dreyfus, H. (1982) Neurochem. Res. 7, 301–316
52 Leskawa, K.C. and Rosenberg, A. (1981) Cell. Mol. Neurobiol. 1, 373–388
53 De Gier, J., Blok, M.C., Van Dijck, P.W.M., Mombers, C., Verkleij, A.J., Van der Neut-Kok, E.C.M. and Van Deenen, L.L.M. (1978) Ann. N.Y. Acad. Sci. 308, 85–100
54 Cohen, B.E. (1975) J. Membrane Biol. 20, 205–234
55 Stubbs, G.W., Litman, B.J. and Barenholz, Y. (1976) Biochemistry 15, 2766–2772
56 Mély-Goubert, B. and Freedman, M.H. (1980) Biochim. Biophys. Acta 601, 315–327
57 Rowlands, J.R. and Allen-Rowlands, C.F. (1978) Mol. Cel. Endocrinol. 10, 63–80
58 Segrest, J.P. and Jackson, R.L. (1977) Membr. Proteins 1, 21–45
59 Eisenberg, D., Weiss, R.M. and Terwilliger, T.C. (1982) Nature 299, 371–374
60 Vogel, H., Jähnig, F., Hoffmann, V. and Stümpel, J. (1983) Biochim. Biophys. Acta 733, 201–209
61 Kaiser, E.T. and Kézdy, F.J. (1984) Science 223, 249–255
62 Schwyzer, A., Gremlieu, H., Gysin, B. and Sargent, D.E. (1983) in Peptides Structure and Function, Proc. 8th Am. Peptide Symp. (Hruby, V.J. and Rich, D.H., eds.), pp. 657–664, Pierce Chem. Co., Rockford, IL
63 Van Nispen, J.W. and Greven, H.M. (1982) Pharmac. Ther. 16, 67–102
64 Sharom, F.J. and Grant, C.W.M. (1978) Biochim. Biophys. Acta 507, 280–293
65 Delmelle, M., Dufrane, S.P., Brasseur, R. and Ruysschaert, J.M. (1980) FEBS Lett. 121, 11–14
66 Maggio, B., Cumar, F.A. and Caputto, R. (1981) Biochim. Biophys. Acta 650, 69–87
67 Bertoli, E., Masserini, M., Sonnino, S., Ghidoni, R., Cestaro, B. and Tettamanti, G. (1981) Biochim. Biophys. Acta 467, 196–202
68 Endo, T., Nojima, S. and Inoue, K. (1982) J. Biochem. 92, 1883–1890
69 Fishman, P.H. and Brady, R.O. (1976) Science 194, 906–915
70 Kohn, L.D. (1978) in Receptors and Recognition, Series A (Cuatrecasas, P. and Creaves, M.F., eds.), Vol. 5, pp. 134–212, Chapman and Hall, London
71 Gardas, A. and Nauman, J. (1981) Acta Endocrinol. 98, 549–555
72 Laccetti, P., Grollman, E.F., Aloj, S.M. and Kohn, L.D. (1983) Biochem. Biophys. Res. Commun. 110, 772–778
73 Fishman, P.H. (1982) J. Membrane Biol. 69, 85–98
74 Van Heyningen, S. (1983) Curr. Topics Membranes Transp. 18, 446–471
75 Ledeen, R.W. (1978) J. Supramol. Struct. 8, 1–17
76 Rösner, H. and Merz, G. (1982) Brain Res. 236, 63–76
77 Skrivanek, J.A., Ledeen, R.W., Margolis, R.U. and Margolis, R.K. (1982) J. Neurobiol. 13, 95–106
78 Ramachandran, J., Muramoto, K., Kenez-Keri, M., Keri, G. and Buckley, D.I. (1980) Proc. Natl. Acad. Sci. USA 77, 3967–3970
79 Epand, R.M. (1983) Trends Biochem. Sci. 8, 205–206
80 Gysin, B. and Schwyzer, R. (1983) Arch. Biochem. Biophys. 225, 467–474
81 Gysin, B. and Schwyzer, R. (1983) FEBS Lett. 158, 12–16
82 Muramoto, K. and Ramachandran, J. (1980) Biochemistry 19, 3280–3286