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THE EFFECT OF ACTH ON RAT BRAIN SYNAPTIC PLASMA MEMBRANE LIPID FLUIDITY

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The effect of ACTH on the lipid fluidity was examined in synaptic plasma membranes from rat forebrain. $ACTH_{1-24}$ increased the fluidity of the synaptic plasma membranes in a dose-dependent way, the lowest effective dose being 10^{-5} M. The shorter N-terminal fragment $ACTH_{1-10}$ was not effective. The significance of this finding is discussed in relation to the known effects of ACTH on synaptic membrane phosphorylation.

ACTH has a broad spectrum of biological effects. One of the more important extra-adrenal influences of the peptide seems its action on both brain metabolism and behavior [1,2]. At the level of the synaptic plasma membrane, $ACTH_{1-24}$ affects protein phosphorylation [3], adenylcyclase activity [4] and polyphosphoinositide metabolism [5]. None of these membrane-related processes was affected by the shorter N-terminal fragment $ACTH_{1-10}$. Until now, using radioligand assays, no high affinity receptors for ACTH could be demonstrated [6]. This opens the possibility that at least part of the neurochemical effects of ACTH are mediated by a more general mechanism, conceivably involving a change in the membrane lipid fluidity. Indeed, there are several reports describing the effect of various hormones and neurotransmitters on the fluidity of membranes [7,8]. Furthermore, the lipid fluidity of membranes seems to affect the activity of the various proteins in the membrane, such as carriers [9], receptors [10,11], phosphoproteins [12] and enzymes [13]. In this paper, we report the effect of $ACTH_{1-24}$ on the lipid fluidity of synaptic plasma membranes from rat forebrain.

Synaptic plasma membranes were prepared from Wistar rat forebrain as was described previously [3,15]. In short, the tissue was homogenized in 0.32 M sucrose (1 : 10, w/v) and centrifuged for 10 min at $1000 \times g$ to remove nuclei and tissue debris. The supernatant was spun for 10 min at $10\,000 \times g$ and the resulting pellet was subjected to osmotic shock and again centrifuged at $10\,000 \times g$ for 20 min. The supernatant was layered on a discontinuous sucrose gradient (16 ml 1.0 M; 8 ml 0.4 M) and spun for 80 min at $100\,000 \times g_{max}$ in the Beckman SW 27.1 rotor. The material floating on the 1.0 M layer was collected, washed and the membraneous pellet was resuspended in buffer (A), containing 10 mM sodium acetate, 10 mM Mg^{2+}, pH 6.5, 70 mg wet weight membrane per ml. The membrane fraction was characterized by electron microscopy [14] and presence of marker proteins specific for a number of subcellular fractions [15]. It appeared that the fraction was free of myelin and highly enriched in synaptic plasma membranes. For fluidity measurements, 1.0 mg membranes was incubated in 2 ml of a buffered salt solution (1.4 mM NaCl, 0.09 mM Na_2HPO_4, 0.013 mM Na_2PO_4, pH 7.4), containing the fluo-

rescence lipid probe 1,6-diphenyl-1,3,5-hexatriene (final concentration 0.5 μM) (for more experimental details, see Ref. 10). After 30 min incubation at 25°C, the fluoresence polarization was measured at 25°C with a selfconstructed instrument which was described previously [16]. $I_{\parallel}$ and $I_{\perp}$, the fluorescence intensities parallel and perpendicular to the direction of polarization of the excitation beam, were measured independently. Membrane lipid microviscosity was estimated from the $I_{\parallel}/I_{\perp}$ ratio using the approximate derivation method described previously [17].

Following estimation of control membrane lipid microviscosity, the peptide was added and mixed with the suspension of synaptic plasma membranes and diphenylhexatriene. The effect of the peptide on the degree of fluorescence depolarization was determined at least five times during 1 min beginning 10 s after the addition of the peptide to the incubation mixture.

$ACTH_{1-24}$ has a dose-dependent effect on the microviscosity of synaptic plasma membranes (Table I). It reduced the microviscosity of the membranes at a concentration of 10^{-6} M and at 10^{-5} M $ACTH_{1-24}$ the reduction is statistically significant ($P < 0.01$, Student's t-test, two-tailed). The N-terminal sequence $ACTH_{1-10}$ even at a concentration of 10^{-4} M had no effect. Thus, using this fluorescence depolarization method as an indicator of membrane fluidity, we were able to show that $ACTH_{1-24}$ rapidly can fluidize synaptic plasma membranes. The dose relationship and structure-activity requirements are in agreement with those observed for the ACTH-induced inhibition of the phosphorylation of the brain-specific protein B-50 [15,18] and the enhancement of the synthesis of phospho*myo*inositol 4,5-diphosphate [5]. More detailed structure-activity analysis is required as to whether there is a possible link to the ACTH-induced release of a 41 kDa protein from synaptic plasma membranes [19]. As no ATP was added to the incubation mixture it is highly unlikely that phosphorylation of proteins and/or lipids is required for the peptide-induced change in microviscosity of the synaptic plasma membranes. Conversely, previously it was shown that changes in fluidity may affect membrane phosphorylation and receptor characteristics [12].

TABLE I

THE EFFECT OF ACTH ON THE MICROVISCOSITY OF SYNAPTIC PLASMA MEMBRANES

Synaptic plasma membranes were prepared from the forebrain of four rats as described in the text. Values are the mean of 3–6 experiments ± S.D. The $\bar{\eta}$-values of the microviscosity were determined by the fluorescence depolarization method using the diphenylhexatriene probe as described in the text.

	$\bar{\eta}$ at 25°C (poise)
Control	6.45 ± 0.18
$ACTH_{1-24}$ (10^{-6} M)	6.25 ± 0.20
$ACTH_{1-24}$ (10^{-5} M)	5.70 ± 0.17 [a]
$ACTH_{1-24}$ ($5 \cdot 10^{-5}$ M)	5.50 ± 0.14 [a]
$ACTH_{1-24}$ (10^{-4} M)	5.40 ± 0.15 [a]
$ACTH_{1-10}$ (10^{-4} M)	6.25 ± 0.15

[a] Statistically different from the control value, $P < 0.01$ by Student's t-test, two-tailed.

Certainly not all enzymes of the synaptic plasma membranes studied thus far are affected by $ACTH_{1-24}$, for instance phospho*myo*inositol kinase, $(K^+ + Na^+)$-ATPase, B-50 protein phosphatase, etc. [20]. Presently, experiments are carried out addressing in detail what if any is the relationship between the known effects of ACTH-like peptides on the activities of the enzymes of synaptic plasma membranes and its effect on membrane fluidity.

In summary, the present study shows an immediate increase of synaptic membrane fluidity by $ACTH_{1-24}$, using the fluorescence depolarization method.

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