CHARACTER OF INTERACTION OF ENKEPHALIN WITH LIPID COMPONENTS OF CELL MEMBRANES

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Enkephalin is found in neurons in different parts of the nervous system but its concentration is particularly high in nerve endings [8]. Calcium ions have been shown to cause secretion of enkephalins [3].

The discovery of a highly effective and specific enzyme inactivating enkephalin [6] lay the foundations for advancement of the hypothesis of the mediator role of enkephalin in the CNS, exerting its effect through opiate receptors which, according to existing information, are composed of acid lipids and cerebrosides [5].

It was therefore of great interest to study the effect of enkephalin on different components of cell membranes. In the investigation described below the effect of enkephalin was studied on model membranes composed of ovolecithin and brain cerebroside.

EXPERIMENTAL METHOD

Bilayer lecithin membranes were formed by Mueller's method [7] and model membranes from cerebrosides by the method described previously [9]. The electrical measurements were undertaken by means of a high-ohmic "Vibron" electrometer, using a pair of Ag-AgCl electrodes [1] in 0.1 M solutions of KCl and $CaCl_2$ at 26°C. To determine the conductance of the model membranes at different ionic strengths, 0.1, 0.05, 0.01, and 0.001 M solutions of KCl and $CaCl_2$ were used. All points used to plot graphs represented mean values of at least six measurements on 2 or 3 different films. The investigations were carried out with enkephalin in a concentration of 10^{-5} to 10^{-8} M. The ovolecithin used in the work was purified beforehand by thin-layer chromatography; cerebrosides from bovine brain were isolated and generously provided by O. P. Sotskii, on the staff of the Department of Clinical Chemistry, Erevan Medical Institute, and the enkephalin was from Fluka (Switzerland). Liposomes from ovolecithin were obtained by the method [2] by sonication with the UZDN-1-1 ultrasonic disintegrator; the dimensions of the liposomes were determined by the light scattering method (diameter 30 nm).

Interaction of enkephalin with liposomes from ovolecithin was investigated by microcalorimetry. The measurements were made on a DAK1-1A microcalorimeter. Solutions of enkephalin and liposomes were made up in 0.1 M KCl and 0.1 M $CaCl_2$. The concentrations of enkephalin were 0.4×10^{-5} and 0.8×10^{-5} M, and of liposomes 6.7×10^{-7} of the molecular weight (mol. wt.), taking mol. wt. of liposomes to be 2.0×10^6 [4]. The working temperature was 25°C. Values of the thermal effect of interaction of enkephalin with liposomes were obtained with allowance for thermal effects of dilution of the reagents.

EXPERIMENTAL RESULTS

Model membranes formed from components of cell membranes (lecithin and cerebrosides) had low conductance for potassium and calcium ions (Fig. 1). The effect of enkephalin on these membranes was to increase their conductance. It will be clear from Fig. 1 that the greatest increase in conductance of the test membranes was observed with enkephalin in a concentration of 10^{-5} M, with a gradual decrease to 10^{-8} M. Enkephalin had

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TABLE 1. Enthalpy (ΔH) of Interaction of Enkephalin with Liposomes (concentration of liposomes 6.7×10^{-7} mol. wt.)

Solution	Enkephalin concentration, M	ΔH, kcal/mol. wt.
0,1 M KCl	$0{,}4 \cdot 10^{-5}$	12±0,5
	$0{,}8 \cdot 10^{-5}$	15,0±0,5
0,1 M $CaCl_2$	$0{,}4 \cdot 10^{-5}$	22,7±0,7
	$0{,}8 \cdot 10^{-5}$	120,0±1,5

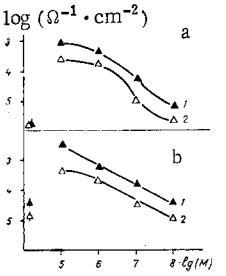

Fig. 1. Dependence of conductance of model membranes made of lecithin (a) and cerebroside (b) for potassium (2) and calcium (1) ions on enkephalin concentration. Abscissa, log of enkephalin concentration; ordinate, log of conductance. Figures on left indicate conductance of unmodified membranes for Ca^{++} and K^{+} respectively.

its strongest effect on lecithin membranes, whose permeability it increased by three orders of magnitude; it increased the permeability of cerebroside membranes by 2-2.5 orders of magnitude for potassium and calcium ions (membrane permeability was always higher for calcium ions). Measurement of the membrane potential in a tenfold KCl gradient with enkephalin in a concentration of 10^{-5} M gave the following results: 34.5 ± 1.8 mV for lecithin and 30.1 ± 0.9 mV for cerebroside membranes. These results are evidence that the test membranes, when modified by enkephalin, have high permeability for cations. Determination of conductance in solutions of KCl and $CaCl_2$ with different ionic strengths showed that enkephalin creates mainly calcium conductance in the membranes. These results suggest that the ability of enkephalin to create high permeability for certain basic lipid components of cell membranes on account either of disturbances in the structure of the lipid bilayer or of binding of enkephalin with cations may indicate that it possesses ionoform properties.

To study the possible mechanism of interaction of enkephalin with lipids, thermodynamic experiments were carried out. These showed that the thermal effect of interaction of enkephalin in different salt solutions (0.1 M KCl and 0.1 M $CaCl_2$) with liposomes is accompanied by absorption of heat. The sign and values of the endothermic reaction energy (ΔH) of interaction with liposomes (Table 1) suggest possible conformational changes in the structure of the bilayer during its interaction with enkephalin. The great difference in reaction energies between interaction of enkephalin with liposomes in the presence of different ions must be noted. The greater reaction energy for interaction of enkephalin with liposomes in the presence of Ca^{++} may perhaps be connected with more substantial changes in liposome conformation in calcium solution than in potassium, a view supported by the results showing changes in permeability of model membranes.

The results of these experiments thus suggest that enkephalin can increase the permeability of cell membranes through lipid fractions, probably on account of conformational changes in the structure of the test membranes as a result of their interaction with enkephalin.

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EFFECT OF D,L-DOPA ON PROTEIN ANTIGEN CONTENT IN SOME RAT BRAIN STRUCTURES

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The problem of the role of neurotransmitter systems in trans-synaptic regulation of protein synthesis in postsynaptic structures is a key problem in neurobiology. A decisive role has been ascribed to the possible selective effect of neurotransmitters on post-translation modification of proteins in the conversion of short-term changes in unit activity into stable, long-term changes. Induction of modifications of individual brain-specific proteins (antigens) by mediators undoubtedly has a direct bearing on the mechanisms of plasticity [9].

Data on the mechanisms of concrete interaction between neurotransmitters and protein-synthesizing brain systems are very scanty and are concerned chiefly with synthesis. Changes in RNA and protein synthesis in the brain have been demonstrated under the influence of noradrenalin (NA) and its analogs [2, 3, 14]. For example, stimulation of adrenoreceptors by amphetamine inhibited incorporation of radioactive label in RNA and proteins, but with an increase in the dose of the drug their synthesis was activated [2, 3]; D-amphetamine inhibited protein synthesis in the rat brain [14]; a decrease in the NA content in the brain by diethylthiocarbonate and reserpine reduced, whereas elevation of the NA level increased, the intensity of RNA synthesis [4].

Changes in nucleic acid and protein metabolism under the influence of NA may also be an important stage in the mechanism of regulation of synaptic efficiency [7]. Evidence of this is given by reorganization of the chemoreactive properties of cerebral neuron membranes by microiontophoretic application of NA and by stimulation of the locus coeruleus, the principal site of concentration of noradrenergic neurons [5, 13]. Clearly the role of individual proteins in this process may be very considerable. An electrophoretic study of water-soluble proteins of the cerebral and cerebellar cortex revealed divergent changes in individual protein fractions in response to electrical stimulation of the locus coeruleus [8]. However, the question of the functional role of different individual, including tissue-specific, proteins in the mechanisms of action of NA remains unsolved.

The aim of the present investigation was to study (by crossed immunoelectrophoresis) the character of the effect of the NA precursor D,L-dopa, on the content of protein antigens in the hypothalamus, cerebellum, and frontal cortex of the rat brain.

EXPERIMENTAL METHOD

An antiserum obtained by immunizing rabbits with water-soluble extract of rat brain by the scheme described previously [11] was used for the immunochemical investigations. To remove antibodies against serum protein the immune serum was exhausted with rat blood serum [6].

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