Changes in the Content of Met-Enkephalin in Different Brain Structures During the Development of Immune Response

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Changes in the content of the opiate peptide Met-enkephalin at the early stages of immune response are studied in different structures of rats brain 20 min and 24 h after immunization with sheep erythrocytes.

Key Words: *Met-enkephalins; immune response; dopaminergic and serotoninergic systems of the brain; high-performance liquid chromatography*

Met-enkephalin, a short neuropeptide with opiate activity, belongs to the enkephalin family and is a product of proenkephalin A [5]. Met-enkephalins (ME) produce various biological effects through interaction with a certain type of opiate receptors. It is known that neuropeptides, in particular, enkephalins act as neurotransmitters in the central and peripheral nervous systems. They have been considered as functional, physiological, and humoral transmitters between the central nervous system and the immune system. It has been shown that ME modulate the activity of natural killer cells in the spleen and peripheral blood [6], boost T cell functioning [11], in certain doses suppress the generation of antibody-forming cells and hemagglutinins, and inhibit skin allograft rejection [9]. In 1979 J. Wybran *et al.* [13] identified ME-like receptors on human blood lymphocytes. Moreover, cells of the immune system express genes of an enkephalin precursor and are able to synthesize and secrete enkephalins [8]. It was suggested that neuromodulatory effects of enkephalins are realized via interaction with central neurotransmitter systems [7,12] whose involvement into neuroimmunomodulation has been established [3]. Based onf these findings and the observation that the content of monoamines and their major metabolites in specialized brain structures changes within an early period after injection of antigen [1,2], it seems interesting to study the content of ME in different brain structures at the early stages of the immune response.

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MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats weighing 160-180 g. Intact rats intraperitoneally injected with physiological saline (0.4 ml) served as the control. Other rats received intraperitoneal injection of 0.4 ml sheep erythrocyte suspension (5×10^8/rat). The animals were decapitated 20 min and 24 h after immunization. The brain was removed and frozen at -20°C. The brain structures were identified according to stereotaxic coordinates. Nuclear structures were extracted with glass capillary tubes. Isolated brain specimens were weighted and homogenized with cooling in glass homogenizers for 1 min in a volume of 0.5-0.8 ml (for 20-50-mg specimens) in a buffer for high-performance liquid chromatography (HPLC, 0.5% trifluoroacetic acid in 25% acetonitrile, pH 2.3). The homogenate was centrifuged at 1800*g* for 5 min and transferred into HPLC tubes. ME were

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TABLE 1. Distribution of ME (ng/g Tissue) in Different Structures of Rat Brain After Immunization with Sheep Erythrocytes in a dose of 5×10^8 ($M\pm m$, n=10)

Structure	Intact rats	Time after immunization	
		20 min	24 h
A9	4.56±0.068	4.59±0.09	6.44±0.1**
Caudate nucleus	6.1±0.09	8.68±0.29**	8.95±0.07**
Hippocampus	6.89±0.15	7.13±0.15*	9.94±0.14**
A10	3.11±0.09	3.88±0.05	5.4±0.04**
Nucleus accumbens	5.99±0.14	9.21±0.04**	12.5±0.07**
Basal amygdala	3.8±0.08	4.01±0.03	4.12±0.05**
Corticobasal amygdala	5.06±0.13	5.02±0.13	8.29±0.03**
A11	2.53±0.04	2.5±0.06	3.16±0.03*
Anterior hypothalamus	4.89±0.06	5.6±0.097**	7.19±0.03**
Mediobasal hypothalamus	5.66±0.1	5.6±0.09	6.15±0.04**
Posterior hypothalamus	4.78±0.08	6.2±0.2**	7.97±0.07**
Nuclei raphe	1.34±0.045	3.29±0.1**	7.1±0.029**

Note. *p<0.05, **p<0.01 compared with intact rats.

measured by HPLC with spectrophotometrical detection in a Milikhrom-2 microcolumn chromatograph. Chromatography conditions were as follows: stainless steel column (64×3 mm) and a reverse-phase sorbent (Separon-C18, 5 μ, Chemapol). The mobile phase was prepared on bidistilled water and contained acetonitrile and trifluoroacetic acid (20: 48% linear acetonitrile gradient over 20 min). The elution rate was 100 μl/min. The measurements were performed at 206 nm. The retention time for [D-Ala^2]-ME standard was 11.5 min. [D-Ala^2]-ME (40 ng, 10 μl) was added to the homogenate for determination of the tissue ME content. Acetonitrile and [D-Ala^2]-ME were purchased from Sigma, other reagents of the extrapure grade were manufactured in Russia. The data were processed statistically using the Student test.

RESULTS

Changes in the content of ME in the nuclear and terminal zones of the nigrostriatal and mesolimbic dopaminergic systems, nuclei A11, and in the hypothalamus and the serotoninergic nucleus raphe system are presented in Table 1. In the anterior and posterior hypothalamus and hippocampus, a rise in the content of ME was noted as soon as 20 min after immunization. Simultaneously, we observed an increased content of ME in the terminal zones of the nigrostriatal (caudate nucleus) and mesolimbic (nucleus accumbens) dopaminergic systems and in the midbrain nuclei raphe giving rise to the ascending serotoninergic projections. Twenty-four hours after immunization, the content of ME was almost equally elevated in all analyzed structures, attaining the highest values in the accumbens, caudate nucleus, and hippocampus.

It should be noted that the content of ME constituted few ng/g tissue, which by several orders of magnitude exceeds the receptor density. Therefore, it was very difficult to detect changes in the ME content after binding to the opiate receptors, and consequently, its validity as a transmitter. More likely, ME in this concentration range acts as a hormone coupled, according to the published data, to the dopaminergic system.

In our previous studies, we determined the sequence of physicochemical transformations of dopamine (DA) accompanying DA-induced synaptic activation upon stimulation of the DA-system in rat brain. It was found that a necessary condition of these transformation is an interaction of DA molecule with pre- and postsynaptic membrane [4]. This interaction is effected through either oriented adsorption of DA molecule involving two (3,4)-hydroxyls or affinity interaction with the active center of the receptor mediated by one (3-OH) hydroxyl. However, DA is one of the most rapidly oxidized transmitters which under physiological pH loses the nativity of its hydroxyl groups, yielding, first, o-quinone and, finely, the insoluble product aminochrome [10]. In 1991 and 1992, we showed that ascorbic acid and opiate peptides (ME) prevent DA degradation in the synaptic gap. Ascorbic acid acts as a competitor in the reaction of oxidation, thus protecting the hydroxyl groups of DA molecule,

while ME interacts with DA, structurally preserving this molecule, and with synaptic membranes, increasing the number of peptide bonds and thus stabilizing the membrane transporting processes. In this aspect, ME is more effective, since ascorbic acid in large amounts may damage the synaptic membrane by hydrolyzing its phospholipids.

Previously, we found a considerable rise of DA and serotonin at the early stages of the immune response (20 min after immunization) in certain brain areas [1] that correspond to the areas with elevated content of ME observed in the present study. In view of this, it can be hypothesized that ME is directly involved into the synaptic membrane mechanisms, facilitating either re-uptake of DA and serotonin or their interaction with the postsynaptic membrane. It should be taken into account that ME rapidly crosses the synaptic membrane in comparison with other opiate peptides, therefore, we assume that ME plays a special role in the interaction between the central and peripheral synaptic mechanisms at the early stages of the immune response.

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