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SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID LEVELS IN THE BRAIN AND IMMUNOCOMPETENT ORGANS AFTER IMMUNIZATION

O. V. Galkina, E. L. Al'perina, O. V. Suslyakova, and L. V. Devoino

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One aspect of the study of mechanisms of neuroimmunomodulation is the detection of neurochemical changes in specialized brain structures and changes in concentrations of those monoamines which perform the function of neurotransmitters in the brain in immunocompetent organs during formation of the immune response.

The serotoninergic system of the brain is known to be responsible for the inhibitory mechanism of immunomodulation [3, 5, 8]. Sporadic studies have demonstrated a fall in the serotonin concentration 20 min after immunization in the ventromedial part of the anterior hypothalamus [6] and an increase in the serotonin concentration in the hypothalamus of rats immunized with sheep's red blood cells (SRBC) on the 2nd and 4th days of the immune response, followed by a fall of the serotonin level on the 11th and 20th days [1]. So far as the immunocompetent organs are concerned, a fall of the serotonin concentration has been found in the thymus after immunization [2, 6, 7], and a very small increase in the serotonin concentration in the spleen on the 3rd day of the immune response [7].

The aim of this investigation was to analyze changes in the concentrations of serotonin (5-HT) and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in brain structures related to the serotoninergic system of the mesencephalic nuclei raphe, the immunocompetent organs, and adrenals, which constitute the peripheral component in regulation of the immunosuppressive action of the serotoninergic system [5] in the early period after injection of the antigen.

Laboratory of Mechanisms of Memory Regulation and Laboratory of Mechanisms of Neurochemical Modulation, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. P. Nikitin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 178-180, August, 1990. Original article submitted August 30, 1989.

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TABLE 1. Concentrations of 5-HT (I) and 5-HIAA (II) (in ng/g tissue) in Brain Structures and Immunocompetent Organs during Immunization of Rats with SRBC ($5 \cdot 10^8$ M)

Structure	IN	Group of animals				
		1	2	3	4	5
		Serotoninergic system				
		Nuclei raphe				
B7+B8	I	8 274	760**	76 337**	3 756*	297**
	II	N.D.	N.D.	76 197**	329 324**	5 136**
		Nigrostriatal system				
A9	I	7 745	—	21 429**	2 318*	267**
	II	N.D.	—	20 607**	17 314**	1 520**
Caudate nucleus	I	5 684	426**	13 188**	1 602*	200**
	II	N.D.	N.D.	13 939**	104 700**	1 688**
Hippocampus	I	787	—	18 732	1 426	126**
	II	314	—	13 433	12 458**	1 410**
		Mesolimbic system				
A10	I	5 246	—	20 812**	2 756*	247**
	II	N.D.	—	20 787**	331 580**	1 723**
Nucleus accumbens	I	4 070	200**	13 259**	2 460**	200**
	II	N.D.	N.D.	26 730**	49 052**	1 696**
Basal amygdala	I	2 980	—	34 300**	2 022	2 843
	II	N.D.	—	N.D.	34 173**	N.D.
Corticomedial	I	3 336	—	35 803**	2 186*	2 420*
amygdala	II	N.D.	—	N.D.	8 962**	N.D.
		Periaqueductal system				
A11	I	4 130	—	20 683**	2 550*	278**
	II	N.D.	—	23 177**	108 324**	2 060**
		Hypothalamus				
Anterior	I	4560	—	21 430**	1 776*	1 289*
	II	N.D.	—	N.D.	31 890**	N.D.
Mediobasal	I	2198	—	42 478**	2 042	2 320
	II	N.D.	—	N.D.	1 020**	N.D.
Posterior	I	4859	—	12 695**	2 374*	1 561*
	II	N.D.	—	5 375**	65 050**	36 450**
		Immunocompetent organs and adrenals				
Bone marrow	I	7896	—	7 153	133 442**	240**
Spleen	I	5700	—	8 020	14 190*	200**
Thymus	I	5300	—	5 480	1 688*	210**
Adrenals	I	7090	—	5 786	3 248*	230**

Legend. IN) Indoles, N.D.) not detected or not determined. In view of the restrictions imposed on the size of the table, the mean error is not indicated, but only the values of the mean (M) and the significance of differences from control values. $*p < 0.05$, $**p < 0.01$.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male Wistar rats weighing 160-180 g. The animals were divided into five groups: group 1 (control, intact rats) received physiological saline by intraperitoneal injection in the same volume as the antigen; the remaining groups received an intraperitoneal injection of a suspension of SRBC in a dose of $5 \cdot 10^8$ and the rats were decapitated 2, 20, and 60 min or 24 h after immunization (groups 2, 3, 4, and 5 respectively). The brain was quickly removed from the skull and frozen in liquid nitrogen, and the spleen, thymus, bone marrow, and adrenals also were quickly removed and frozen. The brain structures were identified by the use of stereotaxic coordinates of the atlas [10]. Glass capillary tubes were used to extract the nuclear formations.

The isolated brain tissues were weighed and homogenized during cooling in glass homogenizers for 2 min in 0.3-0.5 ml (for samples weighing under 10 mg) and in 0.5-0.7 ml (for samples weighing 10-40 mg) of 0.2 N CH_3COOH in CH_3OH [9]. The homogenates were centrifuged at 4°C and 12,000*g* for 20 min. The supernatants were transferred into transparent plastic test tubes (height 0.5-1 cm) and held in a current of warm air for a few minutes until the liquid had evaporated, after which 50-100 μl of 0.1 N HCl was added to the contents of the tubes and a concentrated solution containing, besides catecholamines, 5-HT and 5-HIAA, was obtained. Indoles were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (using an instrument from LKB, Sweden). The conditions of chromatography were as follows: stainless steel column

measuring 250 × 4 mm, packed with a sorbant for reverse-phase chromatography (Lichrosorb RP-18, LKB), with a particle size of 5 μ.

The mobile phase, made up in bidistilled water, contained 0.1 M NaH_2PO_4, 1 mM EDTA, 10 mM NaCl, 5 mg/liter of sodium octylsulfonate (SOS), and 10% (by volume) methyl alcohol, pH 4.0. The rate of elution was 1 ml/min. The potential of the glass-carbon electrode was set at +0.65 V. The solution containing indoles was introduced through an injector (Rheodyne Inc., USA) with 20-μl loops into a column for HPLC.

To calculate the concentrations of 5-HT and 5-HIAA, 60-80 ng (20 μl) of 5,7-dihydroxytryptamine (5,7-DHT) was added during homogenization.

To determine indoles in the immunocompetent organs and adrenals, tissues were prepared by the method described above, with a corresponding increase in the volume of homogenizing mixture and in the duration of homogenization to 4-5 min.

The 5-HT, 5-HIAA, 5,7-DHT, EDTA, and SOS used in the work were obtained from "Sigma" (USA). All other reagents used in the work were of Soviet origin, of high purity or recrystallized.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Analysis of the data showed that 2 min after injection of antigen, the concentration of 5-HT fell in the mesencephalic nuclei raphe, which give rise to the ascending pathways of the serotoninergic system, and whose involvement in the regulation of formation of the immune response has been established [4, 5]. A marked decrease (more than tenfold) in the 5-HT concentration at this time also was observed in the caudate nucleus and nucleus accumbens; meanwhile the 5-HIAA concentrations in these structures were below the level of detection (Table 1).

By the 20th minute of the immune response activity of the serotoninergic system of the brain was sharply increased, especially in the region of localization of serotoninergic neurons, namely the mesencephalic nuclei raphe B7 + B8, in which a simultaneous increase in the concentrations of 5-HT and 5-HIAA was observed compared with the control values. Metabolism of 5-HT was intensified (the concentrations of 5-HT and 5-HIAA were increased) in the hippocampus and dopaminergic structures of the brain, containing serotonin terminals, such as the caudate nucleus, nuclei A9, A10, and A11, and the nucleus accumbens. In the amygdala and hypothalamus (anterior and mediobasal) only the 5-HT level rose (Table 1).

After the 60th minute of the immune response the 5-HT concentration in the mesencephalic nuclei raphe fell below the level detected in intact animals, and became very low after 24 h; however, the 5-HIAA concentration remained significantly higher than in the control until the end of the 1st day, evidence of a high level of 5-HT metabolism during this period. Similar changes were observed in the dopaminergic structures of the brain (nuclei A9, A10, All, caudate nucleus, nucleus accumbens) and in the posterior hypothalamus. Toward the end of the day the 5-HT concentration fell in the hippocampus while the 5-HIAA level in this region still remained quite high compared with the control, although lower than 60 min after immunization; the 5-HT and 5-HIAA concentrations in the amygdala (basal amygdala) and mediobasal hypothalamus also returned to the level determined in intact animals.

It will be clear from Table 1 that 20 min after immunization the 5-HT concentration in the immunocompetent organs and adrenals was unchanged compared with the control, although in the early stages of the immune response the serotonin concentration in the thymus could fall a little [2, 6].

As regards the adrenals, the level of biogenic amines in this organ underwent very considerable fluctuations after immunization, possibly connected with their initial functional state. By the 60th minute of the immune response a considerable fall of the 5-HT level was observed in the thymus and adrenals, but in the bone marrow and spleen the 5-HT level rose; however, 24 h after immunization the 5-HT concentration showed a sharp fall in all the immunocompetent organs and in the adrenals.

It can thus be concluded from these results that starting with the 20th minute after injection of an antigen, activation of the serotoninergic system of the nuclei raphe of the mesencephalon and brain structures containing terminals of neurons of the nuclei raphe became activated. A high level of mediator exchange in most of the brain structures tested was maintained 24 h after immunization also. Changes in the 5-HT level in the immunocompetent organs and adrenals occurred much later after immunization than in the brain.

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REGULATION OF IMMUNOGLOBULIN PRODUCTION BY MYELOPIDE IN PERIPHERAL BLOOD LYMPHOCYTE CULTURE FROM NORMAL INDIVIDUALS AND PATIENTS WITH A SECONDARY IMMUNODEFICIENCY STATE

R. Ya. Vlasenko, R. N. Stepanenko, and L. V. Koval'chuk

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Myelopide (MP) is an immunocorrective agent based on myelopeptides — a group of low-molecular-weight peptides secreted by bone marrow cells, and discovered because of their ability to intensify antibody production in the productive phase of the immune response [2]. Addition of MP to a culture of immune mouse lymph node cells or injection of MP into animals at the peak of the secondary immune response leads to an increase in the number of antibody-forming cells and in the titers of antibodies both to soluble and to corpuscular antigens in the blood [3, 7]. Moreover, the antibody-stimulating effect of MP is more marked against the background of immunodeficiency states. For instance, in mice of the MRL/1 pr line with genetically determined disturbance of the immune system, injection of MP restored the secondary immune response to sheep's red blood cells (SRBC) [4]. The study of antibody production by peripheral blood lymphocytes (PBL) from patients with agammaglobulinemia [6] showed that MP increases the IgG and IgM levels in cultures stimulated by pokeweed mitogen.

In this investigation we studied the effect of MP on secretion of immunoglobulins (Ig) in vitro in PBL of healthy individuals and of patients for heart surgery. It was shown previously that operations for rheumatic diseases of the heart valve in these patients lead to the development of an immunodeficiency state [5].

It has been shown that MP has a stimulating effect on mitogen-induced IgA and IgM production.

Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 180-182, August, 1990. Original article submitted October 8, 1989.

