CHANGES IN LEVELS OF DOPAMINE AND ITS METABOLITES IN BRAIN STRUCTURES AND IMMUNOCOMPETENT ORGANS DURING FORMATION OF THE IMMUNE RESPONSE

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It has recently been shown that the central dopaminergic system is involved in activation mechanisms of neuroimmunomodulation. For example, it has been shown with the aid of substances acting selectively on the receptor mechanism of the dopaminergic system that activation of postsynaptic receptors by apomorphine (1 mg/kg) and by (+)-3-PPP (6.8 mg/kg), and also administration of blockers of dopamine reuptake, namely amantadine (50 mg/kg) and bupropione (20 mg/kg), stimulate immune reactions [3, 5, 6, 7], i.e., that pre- and postsynaptic dopamine receptors are involved in the mechanisms of neuroimmunomodulation.

Further evidence of the immunostimulating effect of the dopaminergic system also is given by results showing that electrolytic destruction of the nigrostriatal dopaminergic system inhibits the reaction of rosette formation in immunized rats [6], whereas injection of 6-hydroxydopamine into the region of the neostriatum reduces the number of E- and EAC-RFC in the peripheral blood of rats [1].

The problem of changes in the content of dopamine and its metabolites in the brain and in the immunocompetent organs during the formation of the immune response to an antigen remains unsolved.

Only elevation of the dopamine level 2 days or more after subcutaneous implantation of tumor cells into rats has been demonstrated in the hypothalamus [2].

Meanwhile, analysis of changes in concentrations of dopamine and its metabolites in the early period after immunization in dopamine-containing structures of the brain and in regions connected with it can reveal the significance of changes in the dopamine level and its turnover in the mechanisms of dopamine neuroimmunomodulation.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male Wistar rats weighing 160-180 g. The animals were divided into five groups. Intact rats of group 1 (control) received an intraperitoneal injection of physiological saline in the same volume as the antigen; rats of the four remaining groups were given intraperitoneal injections of sheep's red blood cells (SRBC) in a dose of 5×10^8, after which the rats of groups 2, 3, 4, and 5 were decapitated 2, 20, and 60 min and 24 h after immunization. The brain was quickly removed from the skull and frozen in liquid nitrogen; the spleen, thymus, bone marrow, and adrenals also were quickly removed and frozen. The brain structures were identified by stereotaxic coordinates in the atlas [10]. Glass capillary tubes were used to withdraw 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 0.02 N CH_3COOH in CH_3OH [9]. The homogenates were centrifuged at 4°C, 12,000*g*, for 20 min. The supernatants were transferred to transparent plastic test tubes (height 0.5-1 cm) and kept in a current of warm air for several minutes until the liquid had evaporated; 50-100 μl of 0.1 N

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TABLE 1. Concentrations of Dopamine and Its Metabolites (in mg/g tissue) in Brain Structures and Immunocompetent Organs and Adrenals of Rats on Immunization with SRBC (5×10^8)

Structure	CA	Group of animals				
		1	2	3	4	5
Nigrostriatal system						
A9	1	2365	—	8 420**	100**	3480*
	2	1378	—	1 853	3 004*	347**
	3	n.d.	—	5 760	n.d.	n.d.
Caudate nucleus	1	5345	7730*	92 406**	13 825**	8024*
	2	836	850	11 513**	2 322**	823
	3	n.d.	n.d.	4 133**	1 522**	n.d.
	4	n.d.	160**	n.d.	n.d.	1213**
Hippocampus	1	602	—	29 482**	115*	3618*
	2	n.d.	—	2 417	n.d.	1198*
	3	n.d.	—	1240	n.d.	n.d.
	4	n.d.	—	n.d.	n.d.	689
Mesolimbic system						
A10	1	2127	—	18 344**	96	3396*
	2	n.d.	—	1 492*	1 956**	343**
	3	n.d.	—	19 716**	n.d.	n.d.
Nucleus accumbens	1	9885	2310*	88 440**	6 171*	6514*
	2	844	630	20 516**	n.d.	322*
	3	n.d.	n.d.	9 363**	n.d.	n.d.
	4	n.d.	n.d.	n.d.	n.d.	2106**
Basal amygdala	1	1516	—	6 651**	1518	1613
	2	n.d.	—	956**	n.d.	n.d.
	3	n.d.	—	1 184**	n.d.	n.d.
	4	n.d.	—	n.d.	6 580**	2070**
Corticomedial amygdala	1	849	—	38 457**	1 718*	865
	2	n.d.	—	3 667**	304**	n.d.
	3	n.d.	—	3 930**	n.d.	n.d.
Periaqueductal system						
A11	1	2335	—	24 883**	125**	3833*
	2	n.d.	—	4 253**	2 802**	403**
	3	n.d.	—	1 567**	n.d.	n.d.
Hypothalamus						
Anterior	1	1240	—	43 943**	1 439	1339
	2	n.d.	—	838**	n.d.	575**
	3	n.d.	—	11 627**	n.d.	n.d.
	4	n.d.	—	n.d.	8 514**	2853**
Mediobasal	1	908	—	39 655**	1 884*	2518**
	2	n.d.	—	35 281**	n.d.	n.d.
	3	n.d.	—	12 097**	n.d.	n.d.
	4	n.d.	—	n.d.	51 180**	3291**
Posterior	1	2895	—	24 991**	2 146	2273
	2	n.d.	—	1 167**	1 580**	n.d.
	4	n.d.	—	730**	8 830**	1628**
Serotoninergic system of nuclei raphe						
Nuclei raphe (B7 + B8)	1	2317	85**	11 619**	90**	3620*
	2	n.d.	235**	1 521**	2 792**	323**
	4	n.d.	690**	n.d.	n.d.	1186**
Immunocompetent organs and adrenals						
Bone marrow	1	10	—	10	10	78**
	4	n.d.	—	10	10	36 360**
Spleen	1	1100	—	10**	10**	150**
	4	n.d.	—	n.d.	n.d.	14 102**
Thymus	1	977	—	10**	10**	110**
	4	n.d.	—	n.d.	n.d.	11 280**
Adrenals	1	10	—	10	10	8 340**
	4	n.d.	—	n.d.	n.d.	39 350**

Legend. CA) Catecholes, 1) dopamine, 2) DHPAA, 3) HVA, 4) 3-MT; n.d.) not detected. Because of restriction on size of the table, the error is not shown, but only values of the mean (M) and significance of differences from the controls. $*p < 0.05$; $**p < 0.01$.

HCl was then added to the contents of the tubes, to yield a concentrated solution containing dopamine and its metabolites. Catecholes were determined by high-performance liquid chromatography (HPLC) with electrochemical detection ("LKB," Sweden). The conditions of chromatography were as follows: stainless steel column measuring 250 × 4 mm, packed with adsorbent for reverse phase chromatography (Lichrosorb RP-18 with particle diameter 5 μm, from "LKB"), mobile phase made up in bidistilled water, containing 0.1 M NaH_2PO_4, 1 mM EDTA, 10 mM NaCl, 5 mg/liter of sodium octyl sulfonate (SOS), and

10% (by volume) of methyl alcohol, pH 4.0 [9]. The rate of elution was 1 ml/min. The potential of the glass-carbon electrode was set at +0.65 V. The solution containing catecholes was introduced through an injector ("Rheodyne," Inc., USA) with a 20-μl loop into the column for HPLC.

To calculate the content of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DHPAA), 3-methoxytyramine (3-MT), and homovanillic acid (HVA) 50-80 mg (20 μl) of 3,4-dihydroxybenzylamine (DHBA) and isoproterenol (IP) were added during homogenization [8].

To determine catecholes in the immunocompetent organs and adrenals, the tissues were prepared by the method described above, the volume of homogenizing mixture being correspondingly increased, and the homogenization time lengthened to 4-5 min.

Of the reagents used, DA, DHPAA, 3-MT, HVA, DHBA, IP, and SOS were obtained from "Sigma" (USA). All other reagents were of Soviet manufacture and either of the highly pure grade or recrystallized.

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

EXPERIMENTAL RESULTS

Data on changes in the levels of dopamine and its metabolites (DHPAA, HVA, 3-MT) in the brain structures at different times after immunization of the rats with SRBC are given in Table 1.

So far as the dopamine level in the immunocompetent organs and adrenals is concerned, during the first few hours after immunization no changes were observed in the bone marrow or adrenals of the immunized rats; in the thymus and spleen the dopamine level fell sharply compared with intact rats (Table 1). However, 24 h after injection of the antigen the dopamine concentration rose sharply in the adrenals and bone marrow, but continued low in the spleen and thymus.

In the brain structures 2 min after immunization the dopamine level in the caudate nucleus rose; the concentration of its principal metabolite (DHPAA) remained at the control level, however. A significant decrease in the dopamine concentration was observed in the mesolimbic dopaminergic system (nucleus accumbens). An extremely sharp fall of the dopamine level (almost 30-fold) was observed at this time in the mesencephalic nuclei raphe.

By the 20th minute of the immune response dopamine metabolism (synthesis, release, DHPAA and HVA levels) in the brain became extraordinarily intensive. The dopamine content continued to increase in the caudate nucleus, the DA level and that of its principal metabolites DHPAA and HVA rose in cell group A9, in the mesolimbic (nuclei A10 and accumbens), and in the periaqueductal (nuclei of A11) dopaminergic systems, and also in zones to which the nigrostriatal and mesolimbic systems project their axons – the amygdaloid complex, hypothalamus, especially mediobasal and anterior, and the hippocampus. In this period the DHPAA level and HVA appeared in the caudate nucleus and the nucleus accumbens, evidence of intensification of dopamine metabolism in the nigrostriatal and mesolimbic systems.

Starting with the 20th minute the dopamine and DHPAA levels rose in the mesencephalic nuclei raphe also.

Later, toward the 60th minute, dopamine was detected in nuclear groups A9, A10, A11, and B7 + B8 and in the hippocampus in a concentration below the control level (intact animals), but the dopamine metabolite DHPAA was observed at a higher level than in the control, evidence that a high level of dopamine turnover still remained at this period. After 60 min a considerable concentration of dopamine, DHPAA, and HVA was observed in the caudate nucleus, a structure in which the most intensive metabolic activity was observed in the present experiments.

After 24 h, the dopamine content in the above-mentioned nuclear formations was an order of magnitude higher than its level after 60 min, and exceeded its concentration in intact animals, while at the same time, the DHPAA level was lowered, i.e., dopamine turnover was retarded. Thus, during the first few minutes after injection of the antigen neurochemical changes took place in the brain, primarily in the form of activation of the nigrostriatal dopaminergic system, for 2 min after the beginning of the immune response the first changes in dopamine metabolism appeared in the caudate nucleus of the neostriatum; after 20 min these changes became more marked in the nigrostriatal and other dopamine-containing regions of the brain. These neurochemical changes explain the fact obtained previously that operative destruction of the compact part of the substantia nigra, where the A9 nuclei are located, causes suppression of the immune response [4, 5]. The results are evidence of rapid involvement of the central activating mechanisms of immunomodulation in response to injection of an antigen and of the leading role of the nigrostriatal dopaminergic system in this process.

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EFFECT OF SEROTONIN ON ANTIGEN-NONSPECIFIC THYMUS SUPPRESSORS

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There is now considerable evidence to show that the serotoninergic system is involved in neuroimmunomodulation. It has been found that the biogenic amine serotonin has an inhibitory influence on the immune response due to the participation of suppressor cells of the thymus, spleen, and bone marrow [1]. The antigen-nonspecificity of suppressor T and B cells in these same organs has been demonstrated during activation of the serotoninergic system with the aid of two antigens: corpuscular (sheep's red blood cells — SRBC) and soluble (human serum albumin — HSA) [2].

An important role in autoregulation of immunogenesis is played by antigen-specific and antigen-nonspecific suppressor cells; the contribution of these suppressor clones, moreover, can vary considerably depending on the action of modulating agents such as hormones, peptides, and chalones [10]. Accordingly, to elucidate the mechanisms of serotoninergic regulation of the formation of antigen-nonspecific components of suppression, it is important to study the specific component of thymic suppressors in the inductive phase of the immune response.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 20-25 g, aged 2-3 months, in a syngeneic adoptive cell transfer system. Six groups of mice were used in the experiments, in which the donors of thymocytes were the following groups of animals: 1) mice receiving serotonin in a dose of 100 mg/kg, once, subcutaneously, in Freund's incomplete adjuvant, 30 min before immunization with SRBC (5×10^8 cells); 2) mice immunized intravenously with SRBC (5×10^8) and receiving serotonin in a dose of 100 mg/kg 30 min before immunization, and also 2-deoxyguanosine (dGUA) in a dose of 1 mg per mouse [7] from

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