Effect of Single and Repeated Administration of High-Molecular-Weight Heparin in Low Doses on Brain Content of Neurotransmitters in Wistar Rats

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We demonstrated high sensitivity of the brain neurotransmitter system to high-molecular-weight heparin in Wistar rats. Single and repeated administration of heparin modulated the content of monoamines and neurotransmitter amino acids in the hippocampus, striatum, and cerebral cortex. Antistress activity of high-molecular-weight heparin and its positive effects on memory under experimental conditions suggest that this preparation in low doses can be used in the therapy of neurological and age-related diseases.

Key Words: *high-molecular-weight heparin; monoamines; neurotransmitter amino acids; brain structures; Wistar rats*

In humans and animals heparins are synthesized and stored in mast cells and basophils [8]. High-molecular-weight heparin is often used as an anticoagulant in the therapy of patients with various diseases associated with thrombosis or risk of thrombus formation (myocardial infarction, stroke, surgical interventions, *etc*.) [2,6].

Repeated treatment with high-molecular-weight heparin in low subtherapeutic doses improves learning in a food-procuring task and facilitates extinction of erroneous behavior. Cognitive, behavioral, and psychoemotional characteristics of experimental animals treated with heparin suggest that it improves associative process of conditioned activity and has a positive effect on the mechanisms of skill performance [3,5]. Improvement of psychoemotional characteristics induced by heparin is probably associated with changes in neurotransmitter content, because a correlation was revealed between the behavioral response to the drug and concentration of neurotransmitters in the brain [1].

We found no published data on the effect of high-molecular-weight heparin on neurotransmitter systems in the brain. Here we measured the content of monoamines and neurotransmitter amino acids in brain structures of Wistar rats after single and repeated administration of high-molecular-weight heparin.

MATERIALS AND METHODS

Experiments were performed on 60 male Wistar rats weighing 200-250 g and obtained from Stolbovaya nursery. Group 1 animals received intramuscular injection of high-molecular-weight heparin (Serva) in a single dose of 64 U/kg. Group 2 animals received daily injections of heparin in the same dose for 5 days. Intact animals served as controls. Psychophysiological studies showed that the course of treatment with physiological saline is followed by the formation of defensive behavior [5].

For biochemical analysis, the rats were decapitated 24 h after the last heparin injection. The

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hippocampus, hypothalamus, frontal cortex, and striatum were isolated on ice. The concentrations of monoamines norepinephrine, dopamine (metabolites of 3,4-dihydroxyphenylacetic [DOPAC] and homovanillic acids), and serotonin (5-HT, metabolite of 5-hydroxyindoleacetic acid) and neurotransmitter amino acids γ-aminobutyric acid (GABA), glycine, taurine, aspartate, and glutamate were measured by high-performance liquid chromatography [4,7].

The results were analyzed by Student's *t* test and Mann—Whitney *U* test (Biostatistica software).

RESULTS

The concentrations of serotonin and 5-hydroxyindoleacetic acid in the hippocampus of group 1 rats increased by 25 ($p<0.05$) and 32% ($p<0.05$), respectively. Serotonin content in the hippocampus of group 2 animals did not differ from the control (Table 1). Glutamate concentration in the striatum of group 1 rats decreased by 26% ($p<0.05$, Table 2). Taurine content in the cerebral cortex of these animals decreased by 28% ($p<0.05$, Table 2).

Glycine concentration increased by 30% only in the hippocampus of group 2 rats ($p<0.05$, Table 2). The concentrations of norepinephrine, dopamine, and serotonin in the cerebral cortex and hypothalamus of group 1 and 2 rats did not differ from the control (Table 1). The content of dopamine increased by 20% ($p<0.5$), while the concentrations of dopamine metabolites DOPAC and homovanillic acid in the striatum did not differ from the control (Table 1).

Our results suggest that high-molecular-weight heparin in a subtherapeutic dose of 64 U/kg modulates the content of neurotransmitters in rat brain. These changes persist for at least 1 day. Single and repeated administration of heparin increased dopamine content in the striatum. It should be emphasized that the effect of single treatment was not potentiated under conditions of repeated administration.

This composition of neurotransmitters was typical of rats receiving high-molecular-weight heparin immediately before the start of maze learning. Our previous studies showed that repeated administration of heparin in a dose of 64 U/kg considerably increased the number of animals demonstrating effective learning and stable performance of food-procuring behavior in radial (12-arm) and multi-alternative mazes. Heparin treatment led to disappearance of all manifestations of psychoemotional strain in rats [3,5]. Psychoemotional studies showed that heparin in low doses improves memory. Heparin produced an antistress effect and prevented behavioral changes in experimental animals under stress conditions (maze test). The results of behavioral experiments suggest that heparin in low doses pro-

TABLE 1. Concentration of Monoamines and Their Metabolites in Brain Structures of Wistar Rats 24 h after Administration of High-Molecular-Weight Heparin (nmol/g tissue, *M*±*m*)

Brain structure, group of animals	Norepinephrine	5-HT	Dopamine	DOPAC	HVA	5-HIAA
Hypothalamus						
control	9.102±1.125	3.431±0.192	1.843±0.111	0.068±0.027	0.630±0.036	3.380±0.223
group 1	11.615±2.093	3.600±0.196	2.186±0.268	0.085±0.032	0.590±0.034	3.875±0.284
group 2	9.754±1.116	3.474±0.282	2.022±0.161	0.050±0.019	0.670±0.038	3.452±0.246
Hippocampus						
control	3.863±0.785	1.356±0.078	0.309±0.042	0.031±0.001	0.206±0.008	1.458±0.117
group 1	3.127±0.727	1.696±0.074*	0.303±0.039	0.027±0.001	0.218±0.007	1.924±0.183*
group 2	3.425±0.716	1.414±0.060	0.223±0.036	0.028±0.001	0.222±0.007	1.513±0.117
Striatum						
control	1.207±0.260	3.211±0.132	56.490±3.329	4.191±0.186	2.847±0.134	7.306±0.392
group 1	1.102±0.218	3.474±0.149	67.610±2.912*	4.597±0.172	2.842±0.153	8.177±0.493
group 2	0.972±0.142	3.187±0.156	67.730±1.833*	4.200±0.209	2.689±0.194	7.492±0.481
Cortex						
control	3.42±0.181	5.704±0.303	0.682±0.066	0.043±0.002	0.358±0.082	1.629±0.184
group 1	3.370±0.162	6.088±0.450	0.954±0.217	0.049±0.002	0.780±0.243	1.695±0.178
group 2	3.49±0.164	6.261±0.306	0.859±0.135	0.053±0.003	0.736±0.192	1.557±0.128

Note. DOPAC, dopamine metabolite; 5-HIAA, 5-hydroxyindoleacetic acid (5-HT metabolite); HVA, homovanillic acid (dopamine metabolite). Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Concentration of Neurotransmitter Amino Acids in Brain Structures of Wistar Rats 24 h after Administration of High-Molecular-Weight Heparin (μmol/g tissue, *M*±*m*)

Brain structure, group of animals		Aspartate	Glutamate	Glycine	Taurine	GABA
Hypothalamus	control	1.187±0.211	3.877±0.432	3.267±0.464	2.807±0.367	4.547±0.503
	group 1	1.139±0.143	4.540±0.587	3.418±0.162	3.295±0.508	4.588±0.628
	group 2	1.225±0.208	3.815±0.526	3.248±0.398	2.854±0.440	4.280±0.520
Hippocampus	control	0.781±0.060	4.470±0.176	5.160±0.572	6.323±0.413	2.563±0.126
	group 1	0.981±0.084	5.017±0.367	4.054±0.280	7.154±0.321	2.559±0.159
	group 2	0.942±0.156	4.661±0.317	6.725±0.476*	6.743±0.424	2.608±0.167
Striatum	control	1.300±0.068	6.031±0.691	5.152±0.852	24.192±4.986	3.643±0.570
	group 1	1.078±0.144	4.452±0.423*	3.990±0.555	18.025±3.784	3.013±0.466
	group 2	1.435±0.176	6.112±0.726	6.607±1.050	26.242±4.711	2.774±0.588
Cortex	control	0.559±0.031	2.172±0.087	1.988±0.339	3.648±0.270	0.625±0.040
	group 1	0.592±0.091	1.976±0.140	2.029±0.325	2.630±0.153*	0.550±0.037
	group 2	0.600±0.119	1.726±0.236	2.096±0.427	3.601±0.223	0.515±0.055

motes functional rearrangements in the monoaminergic and aminoacidergic systems in rat brain.

We conclude that neurotransmitter systems of the brain are highly sensitive to low doses of high-molecular-weight heparin. Heparin exhibits antistress activity and improves memory, which widens the range of its application.

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