PHYSIOLOGY

New Aspects of Heparin Effects

M. V. Kondashevskaya, V. S. Kudrin*, P. M. Klodt*, N. E. Chepurnova, and S. A. Chepurnov

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The effects of a 5-day heparin treatment (10 kD, 64 IU/kg, intraperitoneally) on food-procuring behavior and spatial memory in a 12-arm radial maze were studies on Wistar rats. The maximum reinforcement scores in heparinized rats were attained by day 7 and in control rats only by day 16. In total, 75% heparinized and 45% control rats successfully learned the task for 24 days. On day 25 the contents of major transmitters and their metabolites in various brain structures and in the small intestine of control and experimental rats were determined. The rats treated with heparin showed increased concentrations of norepinephrine in the hypothalamus, homovanillic acid in the striatum, and serotonin in the small intestine. Our findings indicate that heparin exhibits a wide range of activities in addition to its anticoagulant effect.

Key words: *heparin; learning; radial maze; transmitters*

During recent decade human activities changed markedly, which give rise to new professions with significant emotional stress. Adaptation to information overflow, communication problems, time pressure, *etc.* is associated with integrative physiological and biochemical changes. Negative emotions often impair regulation of different systems and organs. The nervous, cardiovascular, and blood coagulation systems response most quickly to emotional stress. This probably explains high prevalence of myocardial infarctions and thromboses of the cerebral, pulmonary, and peripheral vessels in the world and, especially, in Russia and former USSR. These pathologies are associated with hyperactivation of the blood coagulation system and require anticoagulant therapy with fibrinogenesis inhibitors. Heparins (interacting with blood coagulation factors, *i.e.* direct anticoagulants) are most commonly used in clinical practice over the world.

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University; *Laboratory of Neurochemical Pharmacology, Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. **Address for correspondence:** marilu-ka@mail.ru. Kondashevskaya M. V.

Heparins are glycosaminoglycans of different molecular weights consisting of sulfated D-glucosamine and D-glucuronic acid residues. In humans and animals, these macromolecules are produced and contained primarily in mast cells.

In mast cells, heparin (predominantly high-molecular-weigh heparin) is contained together with histamine and often (but not always) with serotonin. This combination is probably not occasional. There is evidence that mast cells can release heparin with low anticoagulant activity [6], which suggests that endogenous heparin can possess some unknown activities, including its possible central effect.

Our aim was to study the effect of chronic administration of high-molecular-weight heparin in low doses on food-procuring behavior and spatial memory in a 12-arm radial maze (RM), and on the content of the major transmitters in the brain and small intestine in Wistar rats.

MATERIALS AND METHODS

Male Wistar rats weighing 250-280 g (n=60) were kept in a vivarium (10 animals per cage). Experimen-

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tal rats were daily injected with high-molecular-weight heparin (Serva) in a dose of 0.36 mg/kg (64 IU/kg, intraperitoneally in 0.85% NaCl) for 5 days. Controls received physiological saline.

Before training the animals were adapted to RM as described elsewhere [3,4] to diminish their exploratory activity (5 successive days, 30 min per day). The experimental and control groups were adapted separately. After adaptation to RM, the rats of the experimental and control groups received 5 daily injections of heparin and physiological saline, respectively.

The experiments were started one day after the last heparin injection. The rats were deprived of food for 23 hours before training and than placed in RM, where each arm contained a reinforcement (sunflower seeds). The rat must visit all 12 arms and get rewards for 10 min. Repetitive visiting of the same arm and missed arms were counted. Recognition threshold (RT) and the time of successive correct choices (TSCC) [1] were evaluated. RT was defined as the ratio of 12/E, where E was the number of errors. The dynamics of these parameters was studied for 24 days.

On day 25, the contents of transmitters and their metabolites were measured by HPLC with electrochemical detection. Specimens of the small intestine (1.5 cm), hippocampus, pituitary, and striatum were isolated on ice, snap frozen and kept in liquid nitrogen before measurements. Norepinephrine, dopamine,

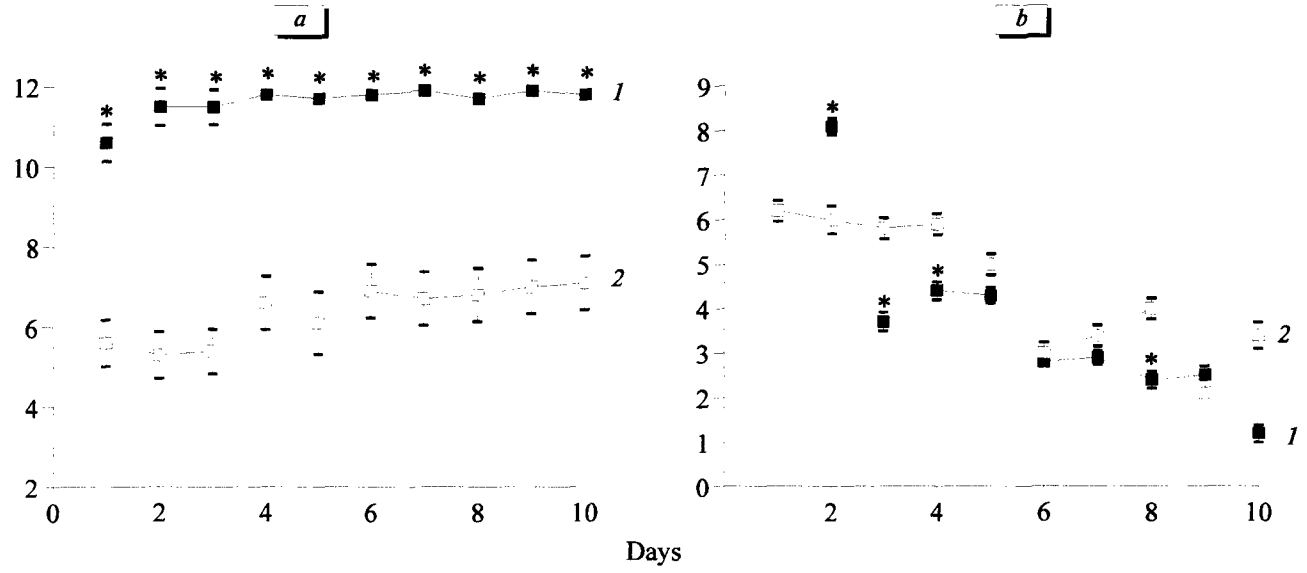

Fig. 1. Food reinforcement (*a*) and errors (*b*) in experimental (*1*) and control (*2*) rats. Here and on Fig. 2: *$p<0.01$ compared to the control. The data are presented for the first 10 days of training (period of most pronounced differences between experimental and contrd groups).

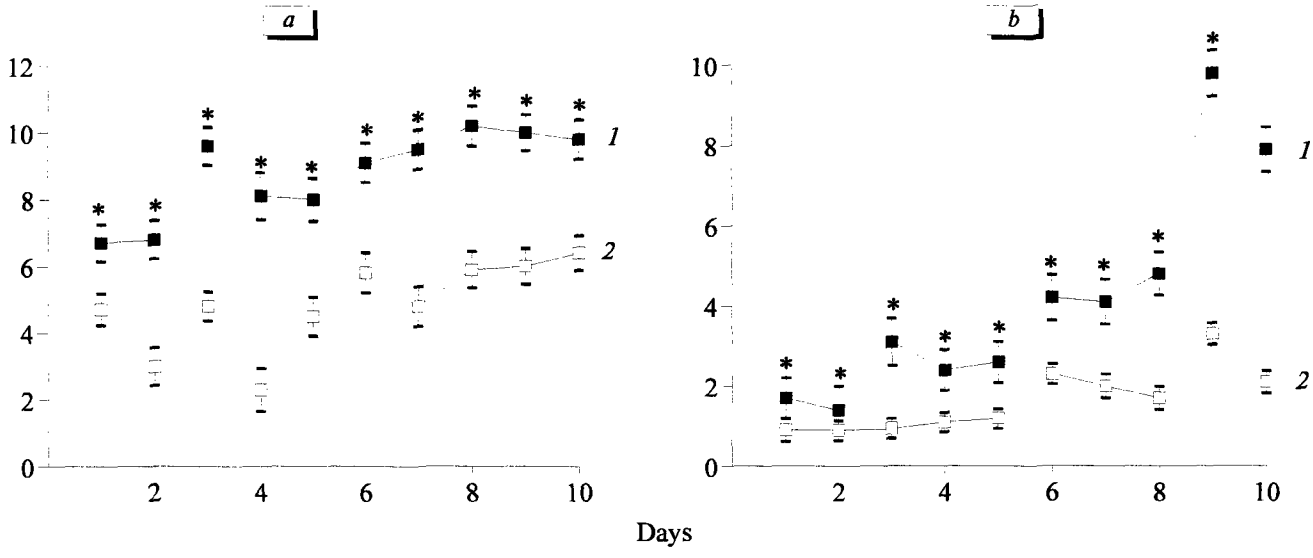

Fig. 2. The time of successive error-free choices (*a*) and recognition threshold (*b*) in experimental (*1*) and control (*2*) rats.

TABLE 1. Effects of Heparin on Transmitters in Brain Structures and Small Intestine of Wistar Rats (*M±m*)

Mediator	Hypothalamus		Hippocampus		Striatum		Small intestine	
	C	E	C	E	C	E	C	E
Norepinephrine	6.3±0.8	8.9±0.9**	3.6±0.5	3.8±0.3	1.4±0.4	1.9±0.8	0.95±0.20	0.93±0.30
5-Hydroxytriptamine (5-HT)	6.3±0.6	6.8±0.7	3.5±0.8	3.8±1.0	4.8±0.6	5.1±0.8	25.2±3.7	36.5±4.2**
5-Hydroxyindolacetic acid (5-HIAA)	6.2±1.1	6.9±1.2	4.8±0.9	5.2±1.1	5.9±0.9	6.9±1.3	0.44±0.10	0.52±0.10
5-HIAA/5-HT	0.98±0.02	1.00±0.03	1.37±0.10	1.37±0.10	1.2±0.2	1.35±0.20	0.017±0.001	0.014±0.002
Dopamine (DA)	1.5±0.3	1.5±0.2	0.50±0.09	0.50±0.03	45.2±3.1	44.5±2.3	0.24±0.05	0.24±0.04
Homovanillic acid (HVA)	0.69±0.16	0.65±0.14	0.24±0.12	0.21±0.06	2.59±0.34	5.63±0.94*	0.55±0.18	0.62±0.29
HVA/DA	0.46±0.09	0.43±0.02	0.48±0.02	0.42±0.03	0.06±0.09	0.12±0.02*	2.29±0.90	2.58±0.60

Note. *$p<0.001$, **$p<0.05$ compared to control. C: control, E: experiment.

serotonin, and their metabolites (3,4-dihydroxyphenylacetic, homovanillic, and 5-hydroxyindoleacetic acids) were determined by standard methods [2].

The data were analyzed statistically using the Fisher—Student test and Fisher's precise test.

RESULTS

The number of correct visits (rewards) in the experimental group 1.7-fold surpassed the control starting from the first day of individual training ($p<0.001$). The maximum level of reinforcement (after visiting of all 12 maze arms) was attained by day 7 and remained high with minor deviations throughout the experiment. Controls showed the maximum scores only by day 16, minor fluctuation of this parameters were also observed in following 8 days of individual training (Fig. 1, *a*). It should be noted that 55% rats in the control group «refused to learn» and exhibited passive defense reactions, while in the experimental group 90% rats actively explored the maze and got rewards.

The number of errors in the experimental group (by day 3) decreased more rapidly than in the control group (by day 14, Fig 1, *b*). After 3 training sessions, heparinized rats completed the task (visited all 12 arms and got maximum reward) before the end of session (10 min) and spent 1.3-1.5-fold less time in RM compared to controls ($p<0.001$).

In case of successful learning, RT and TSCC tended to 12. In our experiments, 45% experimental rats visited 10 to 12 arms without errors after 7-8 training sessions (Fig. 2, *a*). At this term, only 25% control rats visited 5 to 7 arms without errors. By the end of learning this parameter increased to 35%, but none control rat visited more than 7 arms without errors. Thus, TSCC in the control group did not exceed 7. On the other hand, 75% heparinized rats attained maximum RT on day 9, while in the control group only 45% rats approached this level by the end of the experiment (Fig. 2, *b*). Other rats in the control group were inactive: they did not explore the maze and got no food (RT=0).

Thus, 75% heparinized and 45% control rats were successfully trained during 24 days of the experiment ($p<0.001$).

The contents of transmitters in the pituitary, striatum, and small intestine of experimental and control rats were different (Table 1). In the experimental group, the contents of homovanillic acid in the striatum and norepinephrine in the hypothalamus 2.1- and 1.4-fold surpassed the control values ($p<0.001$ and $p<0.05$, respectively). No differences in the content of the test transmitters and their metabolites in the hippocampus were revealed between the experimental and control groups.

Interestingly, the content of serotonin in the small intestine of experimental rats 1.5-fold surpassed the

control ($p<0.05$), while its level in the brain was similar in both groups.

Thus, heparin in low (subtherapeutic) doses induced various changes in CNS persisting for at least 25 days. Heparin stimulated behavioral reactivity and concentration in rats, improves and accelerates learning. These effects were similar to those produced by psychostimulatory drugs. We assumed that heparin affects some central mechanisms responsible for the formation of optimal behavioral strategy during training. This mechanism can be mediated by opioid receptors, which is confirmed by an analgesic effect of heparin [5].

Our experiments and published data indicate that heparin exhibits a broad range of effects which cannot be confined to only its anticoagulant activity. This opens new vistas for its use in clinical practice.

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