Effects of Heparin on Synaptic Activity in the Hemorrhagic Stroke Model *in Vitro*

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We studied the ability of heparin to modify synaptic activity on the original stroke model *in vitro*. Cultured slices of the brain slices from hypertensive rats were treated with the autoblood clot. Administration of heparin (2 mg/ml) before autoblood treatment had a protective effect on ionotropic glutamatergic and GABAergic receptors, whose activity was inhibited by the blood.

Key Words: *surviving slices; hemorrhagic stroke; focal potentials; heparin*

Heparin belongs to a family of sulfated polysaccharides or glycosaminoglycans. Heparin and heparan sulfates are present in basal membranes and extracellular matrix, as well as on the cell surface (in the composition of synaptic membranes in the peripheral nervous system and brain). Glycosaminoglycans and specific binding proteins have a lot of functions (*e.g.*, play an important role in cell-cell interaction). Specific receptors for heparan sulfates on the cell surface and presence of numerous heparin-binding adhesive structures indicate that these molecules have a modulatory effect on the cell [11].

Heparan sulfate proteoglycans are involved in activity of the brain extracellular matrix, which forms reticular structure around the nerve cell bodies and proximal dendrites in CNS [4].

Heparan sulfates and growth factor-associated heparins (*e.g.*, pleiotrophin) play a role in kinase signal pathways through the cell surface receptors and *in vitro* modulate the axonal growth, movement of axons, and synaptogenesis [12,15].

Heparin-related molecules play an important role in activity of excitatory synapses in the brain. One of the heparan sulfate proteoglycans, argine, is an essential component in synapse formation [8,16]. They are involved in the induction and intensity of postsynaptic processes [13]. Heparan sulfate proteoglycan syndecan-2 plays a role in postsynaptic specialization due to the interaction with the protein PDZ domain [7]. Heparan sulfates play an important role in synaptic plasticity and long-term potentiation (LTP) [6]. The effect of these compounds is realized through the excitatory NMDA and AMPA glutamate receptors. Heparin-bound growth factor provides a relationship between the growth and synaptic plasticity. Application of heparin-binding growth-associated molecule (HB-GAM) inhibits NMDA-dependent LTP in the adult hippocampus. Injection of heparin or removal of heparan sulfates inhibits LTP, which illustrates the dependence of LTP from endogenous heparan sulfate [10]. Heparan sulfate proteoglycans probably bind to AMPA receptors. Previous studies showed that heparin increases the probability of AMPA receptor opening [5].

In vitro changes in LTP correlate with animal's behavior. HB-GAM-deficient mice are characterized by a lower threshold of LTP induction, which increases and reaches the level typical of wild-type mice after HB-GAM application. The mice with HB-GAM overexpression demonstrate a rapid learning in the water maze [6]. Administration of heparin in low doses (lower than clinical doses) to rats induces the cascade of change in CNS, which is manifested in the concentration of attention, formation of the optimal strategy and acceleration of learning behavior [1], and antistress effect [2]. Moreover, the content of excitatory and inhibitory transmitter in some structures of the

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brain increases in heparin-treated animals [1,2]. These data indicate that heparan sulfate proteoglycans are involved in synaptic function and plasticity and probably play a role in learning and memory.

Molecular mechanisms of activity of heparin glycosaminoglycans should be studied in details, since heparin-containing products are extensively used for the prevention and therapy of strokes.

Here we studied the influence of heparin on neuronal synaptic processes in rat brain slices under normal conditions and during their contact with blood (*in vitro* model of hemorrhagic stroke).

MATERIALS AND METHODS

Our study was performed according to the European Communities Council Direction (86/609 EEC) and ethical principles for animal experiments.

The study was conducted on cultured slices of the brain olfactory cortex from male SHR hypertensive rats. The effect of heparin on glutamatergic and $GABA_B$-ergic synaptic transmission was studied electrophysiologically by measuring the focal potentials (FP) under long-term exposure to the autoblood (donor blood for reinfusion, autohemotransfusion, or autohemotherapy). The experiment was performed on the original model of hemorrhagic stroke *in vitro* [3].

We measured the amplitude of excitatory and inhibitory components of FP that are mediated by the corresponding mechanisms and realized through various receptors. The following parameters were evaluated: overall action potential of the lateral olfactory tract (AP LOT, presynaptic component of FP), AMPA- and NMDA-receptor components of the excitatory postsynaptic potential (EPSP), and late inhibitory postsynaptic potential ($IPSP_M$) generated upon activation of $GABA_B$ receptors.

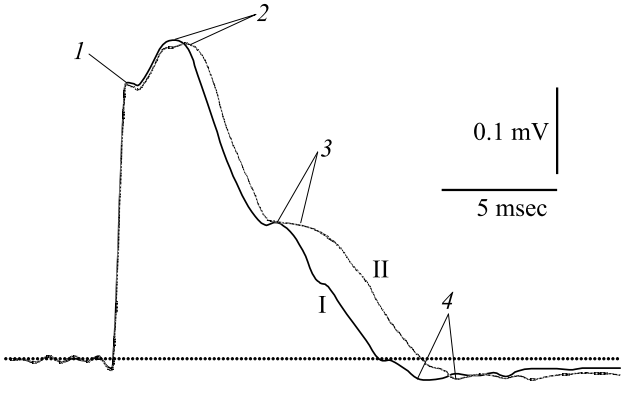

Fig. 1. Modification of FP components in the olfactory cortex of brain slices from SHR hypertensive rats after treatment with heparin sodium in a concentration of 2 mg/ml. Components: AP LOT (*1*), AMPA EPSP (*2*), NMDA EPSP (*3*), and $IPSP_M$ (*4*). Dotted line: isoline. I, control; II, heparin.

Slices of the olfactory cortex were preincubated with heparin sodium (2 mg/ml, Moscow Endocrine Plant) for 15 min. They were placed into a perfusion chamber for the registration of FP, which were generated in response to LOT electrostimulation (main afferent input in olfactory cortex neurons). The frequency of LOT stimulation was 0.003 Hz. In another series, the heparin-preincubated slices were put into autoblood samples that were obtained during the preparation of brain slices. The slice was maintained in 3 ml autoblood for up to 360 min (upper limit of the therapeutic window). Our previous studies showed that longer exposure leads to irreversible changes in synaptic activity [3]. The slice was transferred into a perfusion chamber, the blood was washed out, and FP components were recorded. A special series was performed to study the effect of autoblood (blood clot) on FP during this period. Autoblood inhibits all postsynaptic mechanisms. Activity of conductive fibers in LOT was suppressed by 60% (of the control level). Activity of the cells treated with autoblood for 360 min was compared with the control (special group of slices, n=6) to evaluate the degree of autoblood-induced damages and effect of heparin on these changes.

Aspirin (acetylsalicylic acid) served as the reference product. This agent is used as an antithrombotic drug for stroke therapy in clinical practice.

The results were analyzed by nonparametric Mann–Whitney U test. The differences were significant at $p \leq 0.05$.

RESULTS

Preincubation of slices with heparin sodium in a concentration of 2 mg/ml for 15 min had no effect on the amplitude of excitatory and inhibitory processes in electrogenesis (Fig. 1).

However, heparin sodium induced prolongation of excitatory and inhibitory postsynaptic processes. The duration of AMPA EPSP and NMDA EPSP increased by 16±4 ($p \leq 0.05$) and 27±6%, respectively. Activation of inhibitory $GABA_B$-ergic processes in $IPSP_M$ was prolonged by 23±5% (statistically significant). Prolongation of FP postsynaptic components (AMPA and NMDA EPSP) is realized via the lengthening of descending waves (*i.e.*, repolarization). The descending wave of AMPA EPSP is formed due to potassium channel activation. Therefore, prolongation of these processes can be interpreted as an increase in the period of channel opening. Prolongation of the descending wave of NMDA EPSP is probably related to increased calcium influx into the cell. This process is not followed by calcium overload, but prevents the cell form a negative effect of blood elements.

In the next series we studied the effect of heparin sodium under conditions of exposure of brain slices from the rat olfactory cortex to autoblood (modeling of hemorrhagic stroke; Fig. 2). Incubation of slices with autoblood was followed by inhibition of postsynaptic mechanisms for electrogenesis. Activity was preserved only in a small group of LOT conductive fibers (38%). This negative effect of autoblood was observed in our previous experiments [3].

Preincubation of slices in a heparin sodium solution had the protective effect on electrogenesis in neurons of slices that were later treated with autoblood. For example, activity of presynaptic structures was 97% of the baseline level (U=39, $p \leq 0.05$). Activity of excitatory and inhibitory postsynaptic mechanisms remained high. The amplitude of AMPA EPSP and NMDA EPSP was 90% (U=31, $p \leq 0.05$) and 81% (U=28, $p \leq 0.05$) of the baseline level, respectively. A protective effect on the inhibitory mechanisms was 75% of the level observed before autoblood treatment (U=27, $p \leq 0.05$; Fig. 2).

These data indicate that pre-exposure with heparin sodium has a protective effect on glutamatergic and $GABA_B$-ergic mechanisms of synaptic transmission in brain slices.

Aspirin (acetylsalicylic acid) was used as a reference preparation. This agent is used as an antiaggregant for the therapy of ischemic and hemorrhagic stroke in clinical practice. The effect of aspirin in a concentration of 0.02 mg/ml on brain slices was evaluated by various parameters of bioelectric activity (individual components of FP, 15-min exposure). Aspirin had little effect on the generation of FP components (Fig. 3). The intensity of presynaptic processes (evaluated from the amplitude of AP LOT) was 29%. The amplitude of postsynaptic components (AMPA and NMDA EPSP) was 28 and 5% of the control, respectively. The inhibitory mechanisms (evaluated from the amplitude of $IPSP_M$) were completely blocked. These differences from the baseline level were statistically significant. Hence, aspirin does not protect nerve cells from the negative effect of autoblood on this model of hemorrhagic stroke *in vitro*. These data are confirmed by the results of clinical observations. It was shown that single treatment with aspirin does not modify the mechanisms of synaptic transmission in the brain.

Our findings confirm the results of *in vivo* experiments demonstrating that heparin produces only a partial neurotrophic effect [1,2]. This agent does not influence the amplitude characteristics, but increased the duration of electrogenesis.

Our data on the effect of heparin on synaptic transmission are confirmed by the results of previous studies. *In vitro* experiments showed that heparin produces a modulatory effect on AMPA receptors.

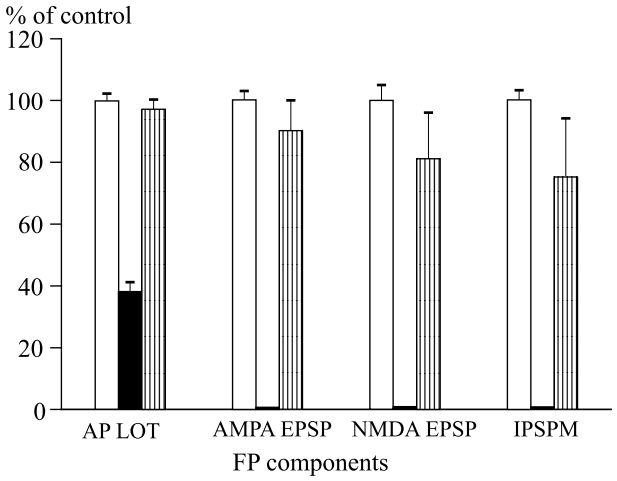

Fig. 2. Amplitude of FP components: after incubation of slices in the control medium (light bars, n=8); after incubation in 3 ml autoblood (dark bars, n=14); after preincubation with 2 mg/ml heparin sodium and subsequent incubation in autoblood for 360 min (shaded bars, n=12).

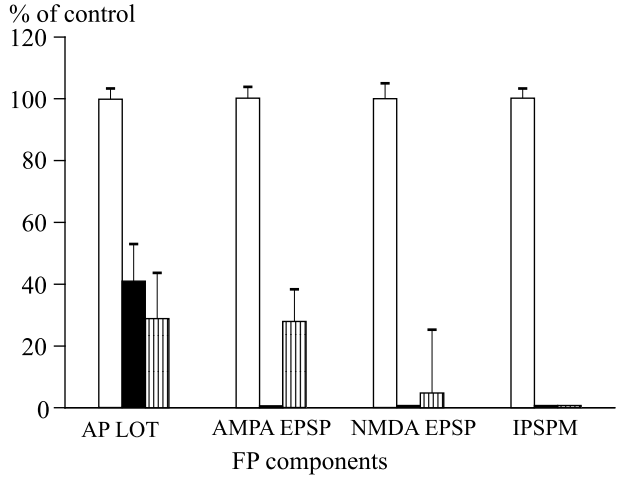

Fig. 3. Amplitude of FP components: after incubation of slices in the control medium (light bars, n=8); after incubation in 3 ml autoblood (dark bars, n=14); after preincubation with 0.02 mg/ml aspirin and subsequent incubation in autoblood for 360 min (shaded bars, n=12).

Heparin increased the cooperativeness between ion channels and prolonged the period of channel opening. However, heparin did not modify current amplitude in an individual channel. Moreover, heparin had no effect on functional activity of AMPA receptors in the presence of AMPA receptor antagonist CNQX. These effects of heparin were specific, since treatment with other polysaccharides (*e.g.*, dextran and glucosamine 2,3-disulfate) did not change activity of AMPA receptors. Hence, endogenous polysaccharides in synapses modulate functional activity of AMPA receptors in dependence on their concentration and degree of sulfation. Heparin-containing proteoglycans localized in the synapses probably interact with

AMPA receptors and modify their functional characteristics [5,9,14].

We revealed that heparin sodium modify functional activity of NMDA-dependent ionic glutamatergic receptors and inhibitory $GABA_B$ receptors. The effect of heparin sodium on receptors is manifested in the prolongation of their activity, which probably contributes to the protective effect of this agent under conditions of cytotoxic influence of the blood and products of its lysis on the model of hemorrhagic stroke *in vitro*.

We conclude that the pre-exposure of nerve cells in brain slices to heparin protects them from adverse effect of autoblood on the model of hemorrhagic stroke *in vitro*. Therefore, heparin can act as an agent protecting from negative consequences of hemorrhagic stroke under experimental conditions.

Heparin and its analogues with strong neurotropic activity can be used as biologically active additives or medicinal products for the prevention or therapy of consequences of hemorrhagic stroke.

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