METHODS

Effects of Long-Term Exposure of Nerve Cells to Autoblood in *In Vitro* Model of Hemorrhagic Stroke

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Changes in bioelectrical activity of nerve cells after their long-term exposure to autoblood were studied *in vitro* on cultured brain slices. This model simulated the events characteristic of a hemorrhagic stroke. Brain slice was placed into a glass vial with autoblood for 60-420 min, after which the slice was transferred into a perfusion chamber and after washing from autoblood their focal potentials were recorded. The level and reversibility of disorders in nerve cell activity were detected by comparing the parameters of focal potentials with the control values. Delayed effects of autoblood were detected, manifesting in the progress in disorders of nerve cell activity with prolongation of exposure in the blood, and the period after which they could be restored was determined.

Key Words: *cultured slices; hemorrhagic stroke; modeling*

We previously proposed a model of hemorrhagic stroke on cultured slices of the olfactory cortex for studies of changes in the neuronal bioelectrical activity after application of autoblood or its fractions [4]. This model cannot be used for tracing modifications of cell electrogenesis and synaptic transmissions under the effect of blood during a long period, because this study requires great volumes (1-1.5 liter) of perfusion medium containing autoblood for application to brain slices. In addition, long-term exposure to autoblood in the model necessitates the use of heparin for preventing blood clotting.

The aim of this study was *in vitro* simulation of hemorrhagic stroke for studies of molecular and cellular processes in the nerve tissue in delayed periods after the start of exposure to autoblood until the moment of irreversible death of neurons. This approach helps to determine the duration of the so-called "therapeutic window" [2] for evaluation of the efficiency of drugs capable of restoring the activities of neurons exposed to autoblood.

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MATERIALS AND METHODS

Experiments were carried out on 24 slices of the cerebral olfactory cortex of hypertensive SHR rats. The slices [3] after preparation were placed into 1-ml glass vials with autoblood (without heparin) for 60-420 min, then transferred into perfusion chamber, washed from autoblood with incubation medium (124 mM NaCl, 5 mM KCl, 2.6 mM $CaCl_2$, 1.24 mM KH_2PO_2, 1.2 mM $MgSO_4$, 3 mM $NaHCO_3$, 10 mM glucose, 23 mM Tris-HCl), and focal potentials evoked in the slices by electrical stimulation of the proximal end of the lateral olfactory tract (LOT) were recorded. The amplitudes of stimulatory and inhibitory components of focal potentials with the corresponding mechanisms of genesis and mediated by various receptor mechanisms were analyzed: summary action potential of LOT (presynaptic component of focal potentials), AMPA and

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NMDA glutamatergic receptor components of the stimulatory postsynaptic potential (SPSP), and the late inhibitory postsynaptic potential (IPSP), generated during activation of $GABA_B$ receptors.

The degree of neuronal damage and the probability of cell recovery were evaluated by comparing the parameters of cell activity after exposure to autoblood with the control values (without exposure to blood) obtained in a special group of slices (n=5).

The results were statistically processed using nonparametrical Mann—Whitney U test. The differences were considered significant at $p<0.05$.

RESULTS

Phenomenologically our model should be regarded as an analog of a extensive hemorrhagic stroke, corresponding to the classification of cerebrovascular lesions [1] (development of pathological processes in neuronal functioning under conditions of exposure to autoblood released into the cerebral parenchyma).

Using this method, we studied the dynamics of modification of some mechanisms of electrogenesis in slices under the effect of autoblood in different periods, which enabled us detect the presence and determine the length of the therapeutic window. The choice of intervals of blood exposure is dictated by clinical data indicating that the period of actual restorative therapy of neurons ("therapeutic window") for ischemic stroke does not exceed 6 h, while after 24-72 h, if no measures are taken, the cells die [2,4].

In order to evaluate the therapeutic window, we studied the following periods of autoblood exposure: 60, 120, and 420 min. Exposure of slices in autoblood for 60 min (n=8) increased the amplitude of LOT action potentials by 19% (U=10, $p<0.05$). Postsynaptic processes (SPSP AMPA and NMDA) reacted similarly to autoblood (were inhibited). The amplitude of SPSP AMPA decreased by half in comparison with the control (U=7, $p<0.05$), while activity of NMDA-dependent processes was blocked (U=3, $p<0.05$). Activity of IPSP was inhibited by half during exposure to autoblood (U=5, $p<0.05$; Table 1).

Exposure to blood for 120 min (n=10) resulted in a more pronounced effect of autoblood on cell activity. During this period, autoblood exhibited various effects on the studied mechanisms of neuronal electrogenesis in the slices (Table 1). The amplitudes of presynaptic processes (evaluated by the LOT action potentials) increased by almost 50% (U=11, $p<0.05$). In contrast to presynaptic processes, postsynaptic ones (SPSP AMPA and NMDA) were inhibited. Pronounced depression of SPSP AMPA amplitude was observed (30% *vs.* control; U=15, $p<0.05$). Activity of NMDA-related processes was completely blocked (U=7, $p<0.05$). The inhibitory GABAergic processes (SPSP) decreased significantly, but were not blocked (U=6, $p<0.05$).

Hence, activity of presynaptic processes increased from minute 60 to minute 120 of incubation, in other words, LOT transmitting fibers (mitral cell axons) were activated. In parallel with this, autoblood reduced activities of postsynaptic mechanisms (stimulatory and inhibitory). However, they demonstrated different stability. Inhibition of AMPA glutamatergic processes depended on the duration of exposure to autoblood, while NMDA-dependent mechanisms proved to be the most sensitive and their activity was blocked under the effect of autoblood. The inhibitory GABAergic mechanisms were inhibited by autoblood exposure, but not blocked. These data indicate survival of the main mechanisms of bioelectrical activity in brain slices and hence, presumably, the period of up to 120 min from the moment of tissue plunging in autoblood until its removal can be regarded as the therapeutic window, during which neuronal activity can be restored to initial values by activation of endogenous mechanisms or therapy.

The next period of exposure to autoblood in our study was 420 min (n=9). During 420 min

TABLE 1. Parameters of Focal Potentials in Surviving Slices of the Rat Cerebral Olfactory Cortex in the Control and after Incubation in Autoblood without Heparin

Parameters	Control, μV	After incubation in autoblood, μV
60 min incubation, n=8		
LOT action potentials	270	320*
SPSP AMPA	260	110*
SPSP NMDA	60	0*
IPSP	20	10*
120 min incubation, n=10		
LOT action potentials	280	410*
SPSP AMPA	240	80*
SPSP NMDA	90	0*
IPSP	30	10*
420 min incubation, n=9		
LOT action potentials	300	150*
SPSP AMPA	250	20*
SPSP NMDA	100	0*
IPSP	30	0*

Note. *$p<0.05$ compared to the control.

autoblood exerted similar negative effects on the studied mechanisms of the neuronal electrogenesis mechanisms in the slices: pronounced depression of stimulatory and inhibitory electrogenesis mechanisms was observed (Table 1). The amplitude of LOT action potentials decreased 2-fold after exposure to autoblood (U=13, p<0.05) in comparison with the control. The SPSP AMPA amplitude decreased more than 10-fold (U=9, p<0.05). Activity of SPSP NMDA was blocked (U=7, p<0.05). In addition, activities of inhibitory mechanisms (IPSP, GABAergic processes) were blocked (U=5, p<0.05).

It should be noted that even a 2-fold increase of electrical stimulation of nerve cells after 420-min incubation of slices in autoblood did not restore the amplitudes of LOT and SPSP AMPA and NMDA action potentials. This means that the main mechanisms of electrogenesis in the olfactory cortex slices were damaged during this period. One more fact which confirmed the severity of disorders in the tissue is that the slices were greatly swollen after long-term incubation in autoblood, which was shown by weighing the slices before and after incubation in autoblood. This indicates the development of edema in nerve tissue of the slices by that time.

Hence, incubation in autoblood leads to progressive suppression and blockade of the main mechanisms of bioelectrical activity in cells by the 420th min of exposure. Presumably, this period (from the start of exposure to autoblood until its discontinuation) is the terminal point (threshold) for the therapeutic window, when urgent effective therapy is needed for restoration of neuronal activity.

Importantly that stable results were obtained by the proposed method in all slices, this indicating 100% reliability of this model of hemorrhagic stroke. In addition, this method demonstrates the dynamics of the development of the initial stage of inhibition and subsequent irreversible disorders in electrogenesis mechanisms, which helps to evaluate the therapeutic window in hemorrhagic stroke by activity of neurons.

Using this method, it is possible during this period to screen the drugs, which can effectively protect the neurons from the negative consequences of hemorrhagic stroke.

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