PHYSIOLOGY

Heat Shock Protein HSP70 Increases the Resistance of Cortical Cells to Glutamate Excitotoxicity

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Preincubation of cultured slices of the olfactory cortex of rat brain with heat shock protein in a concentration of 1 μg/ml protected the pre- and postsynaptic mechanisms of glutamatergic synaptic transmission from glutamate excitotoxicity (50 mM) inducing blockade of excitatory postsynaptic function and reducing presynaptic processes. It was hypothesized that heat shock protein protects AMPA and NMDA receptor-mediated processes.

Key Words: *heat shock protein; cultured slices; protection*

Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS) of mammals. This substance plays a key role in various adaptive functions, including learning, memory, emotional reactivity, sensory perception, and control of locomotion [6]. However, serious stress factors (*e.g.*, trauma and ischemia) induce massive release of intracellular glutamate and aspartate into the extracellular space [11]. It results in hyperactivation of glutamate receptors, impairment of glutamate reuptake, massive calcium entry into cells, initiation of cascade reactions associated with mitochondrial dysfunction, and activation of endogenous proteases. These processes accompany glutamate excitotoxicity and cause cell death [3,10,13]. It was established that glutamate excitotoxicity underlies the pathogenesis of various CNS pathologies, including hypoxic/ischemic injury, trauma, epilepsy, brain dysfunction during hypoglycemia and amyotrophic lateral sclerosis, and Alzheimer's disease [12]. Neuronal resistance to excitotoxicity is determined by expression of additional glutamate receptors and ability of cells to remove or bind excess Ca^{2+}, prevent oxidative stress, and stabilize mitochondrial function [10]. Glutamate receptors are the main target for toxic concentrations of glutamate. N-Methyl-D-aspartate (NMDA) receptors play a key role and, therefore, serve as the most promising target for new protective drugs.

It was found that NMDA receptor antagonists protect cells from excitotoxicity [7,9,15]. However, these preparations produce serious side effects, which limits their use in clinical practice [8]. Our recent studies revealed a protective effect of heat shock protein with a molecular weight of 70 kDa (HSP70). Application of this protein to cultured slices of the olfactory cortex from rat brain protected nerve cells from severe 10-min anoxia [1]. Here we studied the protective effect of HSP70 on the model of glutamate excitotoxicity.

MATERIALS AND METHODS

Experiments were performed on cultured slices of the olfactory cortex from male Wistar rats. Slices (500 μ) were preincubated in a medium containing 124 mM NaCl, 5.0 mM KCl, 2.6 mM $CaCl_2$, 1.24 mM KH_2PO_4, 1.2 mM $MgSO_4$, 3.0 mM $NaHCO_3$, 10.0 mM glucose, and 23.0 mM Tris-HCl. The solution was saturated with oxygen. Incubation was performed at 37°C and pH 7.2-7.3. After 2-h preincubation the slices were transferred into a flow chamber (perfusion rate 2 ml/min).

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Orthodromic stimulation of the proximal part of the lateral olfactory tract was performed with rectangular pulses (0.05-0.10 msec, 1-3 V) was delivered via platinum bipolar electrodes using an ESU-1 stimulator. Local potentials (LP) in slices were recorded using glass microelectrodes filled with 1 M NaCl (resistance 1-5 mΩ). Recording was performed in the site of maximum activity at a depth of 270-300 μ from the pial surface. The reference silver electrode was placed in a chamber. LP were amplified and digitized on a MD 32 analog-digital device (quantization frequency 20 kHz) and subjected to computer processing. We estimated the amplitude of LP excitatory components characterized by a certain mechanism of development and mediated by different receptors: total action potential of the lateral olfactory tract (presynaptic component of LP) activated by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and designated as the AMPA receptor-mediated excitatory postsynaptic potential; and non-N-methyl-*D*-aspartate (non-NMDA) and NMDA receptor-mediated components activated by NMDA and designated as the NMDA receptor-mediated excitatory postsynaptic potential. The amplitude of these components was measured from the isoline to peak. The amplitude of the NMDA receptor-mediated excitatory postsynaptic potential was measured at 7-8 msec. Controlled study was performed to standardize the experimental conditions.

We studied the protective effect of the protein containing constitutive and inducible isoforms of HSP70 (Hsc/Hsp70) from bovine muscles (Institute of Cytology, Russian Academy of Sciences). The protective effects of HSP70 were studied using 1 μg/ml protein. This protein concentration was selected taking into account published data on the effective protection of slices from anoxia [1]. The protein was *ex tempore* dissolved in the incubation medium. Brain slices were perfused with the solution after recording of control LP. Glutamate excitotoxicity was produced by 60-min perfusion of slices with an incubation medium containing 50 mM L-glutamate (Sigma). After recording of control LP, control slices (n=12) were perfused with L-glutamate-containing incubation medium (60 min) and washed (30 min). To study the protective effect of HSP70 (n=16), LP were recorded under control conditions and in the presence of HSP70 (20 min). The slices were perfused with a solution containing L-glutamate (without HSP70) for 60 min followed by 30-min washout. The lateral olfactory tract was stimulated under control conditions and during treatment with the test substances at 5-min intervals.

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

RESULTS

The model of glutamate excitotoxicity is extensively used in experimental studies. However, there is no standard concentration of glutamate: its concentrations vary from several hundreds to tens millimoles. Under normal conditions extracellular glutamate concentration in the brain is 30 μM [4,13]. Glutamate concentration in astrocytes and neurons is higher by 1000 times [5]. The estimated concentration of glutamate in ischemia can attain or surpass 600 μM [14]. However, this concentration of glutamate can be insufficient to produce total hyperactivation of NMDA receptors and

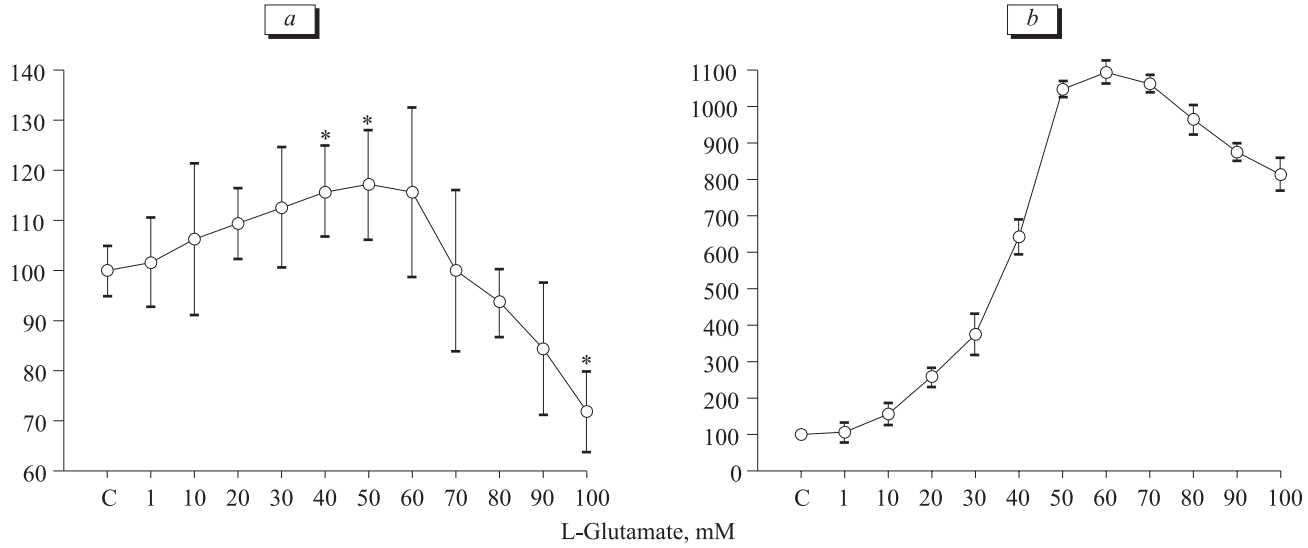

Fig. 1. Effect of L-glutamate in various concentrations on the amplitude of excitatory components in the excitatory postsynaptic potential (EPSP). Ordinate: amplitude of AMPA (*a*) and NMDA (*b*, % of the control). C, control (n=9). L-glutamate in each concentration was applied for 20 min. Each slice was tested with 1 concentration of L-glutamate (n=9 for each concentration). *$p \leq 0.05$ compared to the control.

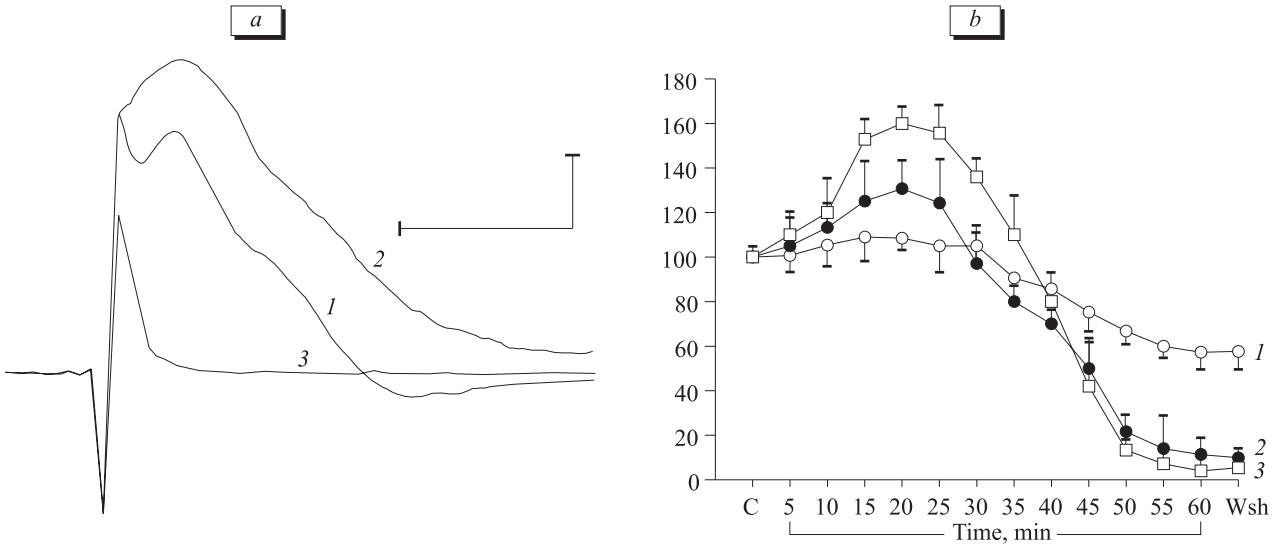

Fig. 2. Effect of L-glutamate in toxic concentration (50 mM) on glutamatergic synaptic transmission in cultured slices of rat brain olfactory cortex. *a*: changes in the local potential after application of L-glutamate: control (*1*); effect of glutamate over a 60-min period (*2*); after 30-min washout (*3*). *b*: effect of 50 μM L-glutamate on the amplitude of the action potential in the lateral olfactory tract (*1*); AMPA (*2*) and NMDA receptor-mediated excitatory postsynaptic potential (*3*). Here and in Fig. 3: ordinate, amplitude of the corresponding component in the local potential (% of the control). C, control. Wsh, washout.

to impair the mechanism of glutamate reuptake in slices. To select the effective toxic concentration of glutamate, we measured the amplitude of the AMPA and NMDA receptor-mediated excitatory postsynaptic potential at various concentrations of extracellular glutamate (n=9, Fig. 1). Activation of AMPA receptors was most significant at L-glutamate concentration of 50 mM. Total activation of NMDA receptors was maximum at L-glutamate concentration of 50-70 mM. Increasing the concentration of L-glutamate was accompanied by a less significant activation of receptors. In further experiments we used exogenous glutamate in a concentration of 50 mM to study the protective effect of HSP70 in cultured brain slices on the model of glutamate excitotoxicity.

L-Glutamate in a concentration of 50 mM produced phasic changes in glutamatergic synaptic transmission (initial activation followed by the decrease or irreversible blockade, Fig. 2, *a*, *b*). The amplitude of the AMPA and NMDA receptor-mediated excitatory postsynaptic potential increased 1 min after L-glutamate treatment (latent period). Most significant potentiation of LP was observed 20 min after treatment with the agonist (Fig. 2, *b*).

The amplitude of the AMPA and NMDA receptor-mediated excitatory postsynaptic potential progressively decreased after 25-min treatment with L-glutamate. The effect of this agonist persisted in this period (Fig. 2, *a*, *b*). Generation of excitatory postsynaptic components of LP was blocked after 50-min treatment (Fig. 2, *a*, *b*). Presynaptic processes were also inhibited by the end of this period. The amplitude of the action potential in the lateral olfactory tract decreased by 40% compared to the control. However, transmission of excitatory impulses along fibers of the lateral olfactory tract was not completely blocked (Fig. 2, *a*, *b*).

The effect of L-glutamate in a toxic concentration of 50 mM on fibers of the lateral olfactory tract (axons of mitral cells) was studied on thin slices (200 μ) [2]. Exogenous glutamate inhibited function of rapidly conducting fibers Aδ (by 30%), but had no effect on

TABLE 1. Effect of L-Glutamate (50 mM) and Preincubation with HSP70 (1 μg/ml) on Activity of Fibers of the Lateral Olfactory Tract

Conduction rate, m/sec	Group of fibers	Control	Glutamate, 50 mM, 50 min	HSP70, 1 μg/ml (50 min); glutamate, 50 mM (50th minute)
18-19	Aδ	+	—(30%)	+
6-10.3	B	+	+	+

Note. +, recorded activity; —, no activity in fibers of the lateral olfactory tract.

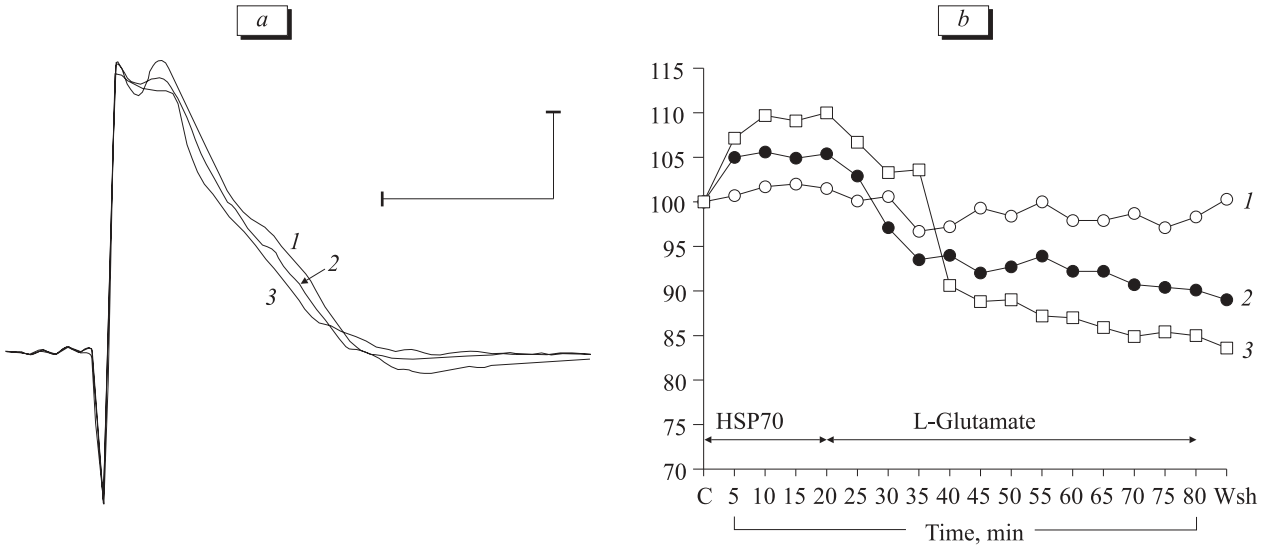

Fig. 3. Protective effect of heat shock protein (HSP70) on glutamatergic synaptic transmission in slices of rat brain olfactory cortex (relative to glutamate excitotoxicity). *a*: changes in the local potential: control (*1*); exposure of HSP70-pretreated slices (20 min) to 50 mM L-glutamate for 20 (*2*) and 30 min (*3*). *b*: Effect of pretreatment with 1 μg/ml HSP70 and application of 50 mM L-glutamate on the amplitude of the action potential in the lateral olfactory tract (*1*); AMPA (*2*) and NMDA receptor-mediated excitatory postsynaptic potential (*3*).

slowly conducting fibers B (Table 1). These data indicate that L-glutamate in toxic concentration impairs function of not only synaptic structures, but also conducting fibers.

Glutamate in a toxic concentration of 50 mM caused irreversible blockade of excitatory postsynaptic function. Washout for 30 or 60 min (n=3) did not restore this parameter (Fig. 2, *b*). Blockade of presynaptic function was not abolished after washout (Fig. 2, *b*).

Persistent and irreversible blockade of postsynaptic function and reduction of presynaptic function reflect glutamate excitotoxicity in cultured slices of the olfactory cortex. The mechanisms of glutamate reuptake are probably blocked in this period. Hyperactivation of NMDA receptors determines massive Ca^{2+} entry into cells, activation of intracellular proteases, and irreversible damage to nerve cells.

Pretreatment with 1 μg/ml HSP70 before administration of L-glutamate in a toxic concentration of 50 mM considerably modified changes in synaptic processes in cultured slices. Potentiation of the AMPA and NMDA receptor-mediated excitatory postsynaptic potential observed in the early period after L-glutamate treatment (without HSP70, Fig. 2, *b*) did not exceed 5 and 10%, respectively (Fig. 3, *a*, *b*). Therefore, these changes were not accompanied by hyperactivation of NMDA receptors, massive Ca^{2+} entry into cells, and their destruction. At later terms of L-glutamate exposure excitatory components of LP were reduced by 10 and 15% for the AMPA and NMDA receptor-mediated excitatory potential, respectively (Fig. 3, *b*), *i.e.* these processes were not irreversibly blocked under these conditions. Activity of presynaptic fibers remained practically unchanged after treatment of slices with HSP70.

Our findings show that application of exogenous HSP70 protects the mechanisms of glutamatergic synaptic transmission from glutamate excitotoxicity. HSP70 probably protects function of glutamate receptors (mainly NMDA receptors). This conclusion is supported by published data on protective effect of NMDA receptor antagonists in experiments with the prevalence of glutamate excitotoxicity [7,9,15].

The protective effect of HSP70 on presynaptic function can be explained by maintenance of structural conformations of ion channel proteins in conducting fibers of the lateral olfactory tract.

Our experiments showed that application of exogenous HSP70 increases the resistance of nerve cells to glutamate excitotoxicity, which serves as the major reaction of nerve structures to stress factors (*e.g.*, anoxia, ischemia, and trauma). Further studies with protein treatment under conditions of glutamate excitotoxicity are required to evaluate therapeutic activity of HSP70.

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