Doklady Biological Sciences, Vol. 395, 2004, pp. 118–120. Translated from Doklady Akademii Nauk, Vol. 395, No. 4, 2004, pp. 551–553.

PHYSIOLOGY

Effects of Exogenous Heat Shock Protein (Hsp70) on Glutaminergic Synaptic Transmission in Rat Olfactory Cortex *in Vitro*

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Presented by Academician A.D. Nozdrachev June 26, 2003

Received August 23, 2003

Cells of all living organisms respond to stress factors by increased synthesis of the so-called heat shock or stress proteins. The genes of these proteins are activated not only in stress, but also during the main processes of cell vital activity, proliferation, differentiation, and apoptosis [1, 7]. All of them are identified as molecular chaperones. One of them, heat shock protein with a molecular weight of 70 kDa (Hsp70), appears in cells after exposure to high temperature. This protein is a product of a multigene family. The function of these proteins in the nervous tissue is unclear thus far. They have been found in the synapses of the mammalian central nervous system [8], which indicates the importance of Hsp70 for synaptic plasticity. The data that the antagonists of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and N-methyl-*D*-aspartate (NMDA) subtypes of the glutaminergic excitatory system of the brain of homoiotherms, e.g., MK-801, ketamine, phencyclidine, and UM90K, induce the expression of this protein in nervous cells [4, 9, 10] indirectly confirm that Hsp70 is involved in neurotropic processes.

We studied some neurotropic effects of the treatment with exogenous Hsp70 on neuronal activity in surviving slices of rat olfactory cortex.

The experiments were performed using surviving tangential slices of the olfactory cortex of male Wistar rats. The slices, 500 μm in thickness, were preincubated in the medium of the following composition (in mM): NaCl, 124.0; KCl, 5.0; $CaCl_2$, 2.6; KH_2PO_4, 1.24; $MgSO_4$, 1.2; $NaHCO_3$, 3.0; glucose, 10.0; Tris-HCl, 23.0 (pH 7.2–7.3). The solution was saturated with oxygen; a temperature of 37°C was maintained. After 2 h of preincubation, the slices were transferred into the recording continuous-flow chamber with a perfusion rate of 2 ml/min, with the incubation medium and the atmosphere above the slices being saturated with oxygen.

The lateral olfactory tract (LOT) was orthodromically irritated with 0.05- to 0.1-ms rectangular stimuli with an intensity of 1–3 V applied through platinum bipolar electrodes from an ESU-1 stimulator. We measured focal potentials (FPs) in the slices with the use of glass microelectrodes filled with 1 M NaCl with a resistance of 1–5 mΩ. The measurement point was located in the maximum-activity focus at a depth of 270–300 μm. The indifferent silver electrode was located in the chamber.

The FPs were amplified, digitized using an analog–digital converter with a quantization frequency of 20 kHz, and treated using a computer. We analyzed the amplitudes of the excitatory components of FPs with the corresponding mechanisms of genesis and mediated by different receptor mechanisms: the total LOT action potential (LOT AP; the presynaptic FP component), and the non-NMDA (activated by AMPA) and NMDA (activated by NMDA) components of the excitatory postsynaptic potential (EPSP) [2, 5, 6], hereinafter referred to as the AMPA and NMDA EPSP components, respectively. The amplitudes of these components were measured from the isoline to the peak. The neurotropic effects of the protein consisting of the constitutive and inducible Hsp70 forms (Hsc/Hsp70) isolated from bovine muscles [3] at concentrations of 0.7 and 70 μg/ml were estimated in the experiments with cortical slices. Fourteen slices of the olfactory cortex were used. A 0.7 μg/ml solution of bovine serum albumin (Sigma) was used as a reference preparation (control).

The nonparametric Wilcoxon–Mann–Whitney U test was used for statistical comparisons.

The perfusion of cortical slices with a 0.7 μg/ml Hsp70 solution for 20 min led to increase in the amplitudes of individual FP components (LOT AP, AMPA EPSP, and NMDA EPSP) caused by LOT irritation. If the Hsp70 solution was replaced with the control solution, the amplitudes of both postsynaptic responses

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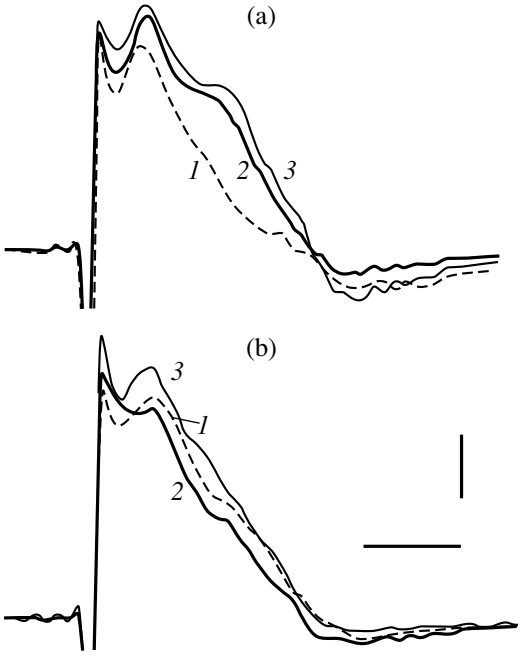

Fig. 1. Changes in FPs caused by Hsp70 at concentrations of (a) 0.7 and (b) 70 μg/ml: (*1*) control FPs; (*2*) FPs after 20 min of perfusion with Hsp70; (*3*) FPs after 20 min of washing Hsp70 off. Scale bars: 100 μV, 10 ms.

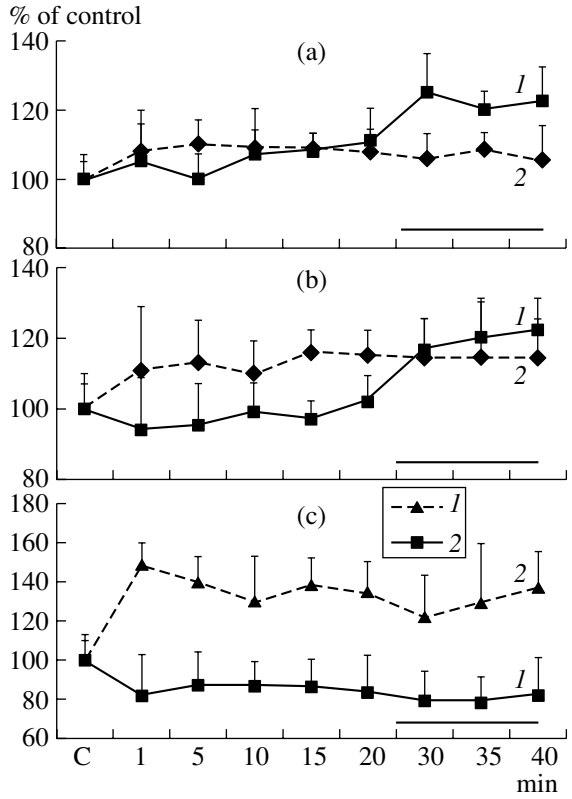

Fig. 2. The effects of (*1*) 0.7 and (*2*) 70 μg/ml Hsp70 on the dynamics of the amplitudes of individual FP components in neurons of the pyriform cortex: (a) LOT AP, the presynaptic component; (b) AMPA EPSP; and (c) NMDA EPSP. Abscissa shows the time of the perfusion of cortical slices with Hsp70 (a nonuniform scale). C, control; the horizontal bar shows washing off Hsp70. Ordinate shows the amplitude of the corresponding FP component (n = 7 for each concentration; $p \le 0.05$).

(AMPA and NMDA EPSPs) were slightly decreased (Fig. 1a).

At a higher concentration (70 μg/ml), Hsp70 induced decrease in the amplitudes of the postsynaptic responses (AMPA and NMDA EPSPs) and a slight increase in the presynaptic response (LOT AP). Washing the cortical slices with an Hsp70-free medium restored the amplitudes of these FP components (Fig. 1b).

Hsp70 at both concentrations increased the amplitude of the presynaptic response (LOT AP) by no more than 10% (Fig. 2a). However, when 70 μg/ml Hsp70 was washed off, the average amplitude of this component increased by 20%, whereas the LOT AP amplitude did not change upon washing off 0.7 μg/ml Hsp70 (Fig. 2a).

To determine the dependence of the glutaminergic neurotransmission in the pyriform cortex on Hsp70 concentration, we analyzed the postsynaptic responses (AMPA and NMDA EPSPs). For example, 0.7 μg/ml Hsp70 induced an increase in AMPA EPSP amplitude by 14%. This potentiation was retained throughout the period of treatment and after the protein was washed off (Fig. 2b).

Conversely, 70 μg/ml Hsp70 caused a decrease in AMPA EPSP amplitude, which increased, on average, by 20% after the protein was washed off (Fig. 2b).

Hsp70 exerted the strongest effect on the activity of NMDA-related processes, with the vector of these changes varying depending on the protein concentration used (Fig. 2c). For example, 0.7 μg/ml Hsp70 induced a prolonged, strong potentiation of NMDA EPSP, which increased, on average, by 45% compared to the control value; the potentiation was retained throughout the period of treatment and somewhat decreased (still remaining higher than the control value) after Hsp70 was washed off (Fig. 2c). The latent period of the induction of changes in the amplitudes of the pre- and postsynaptic responses did not exceed 1 min. The pattern of the changes in the amplitude and temporal parameters of the NMDA EPSP caused by Hsp70 (0.7 μg/ml) was similar to that of the changes accompanying the induction and development of long-term posttetanic potentiation caused by electric tetanization of the cells of the pyriform cortex [2]. This conclusion follows from the comparison of the latent period of the potentiation and its long-term retention in the cases of electric tetanization and Hsp70 treatment without tetanization.

We also found opposite changes in the activity of NMDA-related processes induced by the two Hsp70 concentrations used. At a concentration of 70 μg/ml, the

protein decreased the mean NMDA EPSP amplitude by 15% after a latent period of 1 min, this depression persisting for a long time. After the protein was washed off the slices, the suppression of responses remained about the same (Fig. 2c). The observed phenomena were phenomenologically similar to the development of the prolonged depression induced in the cells of the pyriform cortex by either electric tetanization or repetitive, rhythmic activation at a frequency of 10–30 Hz [2].

We think that the potentiating effect of exogenous Hsp70 at the lower concentration may be determined by the restoration and simultaneous removal of damaged protein components forming AMPA and, especially, NMDA receptor complexes, which increases the efficiency of these receptor subtypes involved in the glutaminergic excitatory neurotransmission. Apparently, the induction and maintenance of the long-term depression of neuronal activity in the rat pyriform cortex treated with the higher Hsp70 concentration is impossible to explain by the known characteristics of the protective effect of this protein alone; thus, this issue require further studies.

As noted above, bovine serum albumin was used as a reference preparation. The perfusion of the slices ($n = 5$) with the incubation medium containing 0.7 μg/ml albumin for 20 min was accompanied by progressive decrease in the AMPA EPSP and NMDA EPSP amplitudes (by 30 and 45%, respectively). The LOT AP amplitude was decreased by 10% on average. After Hsp70 was washed off for 15 min, the amplitudes of the measured FP components displayed only a tendency towards restoration. Therefore, the vectors of the effects of Hsp70 and albumin on glutaminergic synaptic transmission in the slices of olfactory cortex were different: Hsp70 activated these processes, whereas albumin suppressed them.

Thus, we have demonstrated that exogenous Hsp70 acts in the manner characteristic of an endogenous adaptive regulator, actively modulating the glutaminergic excitatory synaptic transmission (AMPA- and NMDA-related processes) in the rat olfactory cortex. The NMDA receptor mechanisms proved to be the most susceptible to exogenous Hsp70 application. Hsp70 activated these processes, which indicates that this protein facilitates the function of NMDA-related processes, thus mimicking long-term posttetanic potentiation induced by electric tetanization of these neurons [2].

In addition, Hsp70 actively affected the AMPA subtype of glutamate receptors, apparently, through the activation of sodium channels, which promoted depolarization of neurons and an increase in their excitability. The effect of exogenous Hsp70 was the strongest in postsynaptic structures and the weakest in presynaptic ones. These data contradict, to a certain extent, to the results of experiments with slices of the mouse medulla oblongata. The incubation of these slices in the presence of Hsp72 modulated the presynaptic mechanisms of the neurotransmitter release and had practically no effect on postsynaptic processes [7]. We believe that this discrepancy is related to the specificity of the Hsp70 and Hsp72 effects in different cerebral structures, although this issue requires special investigation.

Note that Hsp70 activated postsynaptic processes only at the lower of the two concentrations studied, whereas the higher concentration depressed them. Obviously, Hsp70, which possesses the chaperone activity, substantially modified the expression of the neurotropic and plastic characteristic of neural structures. These data add considerably to the information on the functional patterns of chaperones in the mammalian nervous system.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project nos. 03-04-49597 and 01-04-49097).

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