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PHYSIOLOGY

Lipopolysaccharide-Free 70-kDa Heat Shock Protein Has Hypotherimic and Somnogenic Effects

Yu. F. Pastukhov*, I. V. Ekimova*, K. A. Hudik*, and I. V. Guzhova**

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The intracellular expression of 70-kDa heat-shock protein (HSP70) is one of the most important molecular mechanisms that protect cells and various organisms (from bacteria to humans) from heat and many other stresses (see [1, 2] for review). After the appearance of exogenous HSP70 preparations (both natural and recombinant), the development of new HSP70-based drugs is attracting much attention. However, the therapeutic potential of HSP70 has not been evaluated thus far, because some problems have not been solved. First, *in vitro* experiments have shown that intracellular HSP70 inhibits the synthesis of proinflammatory cytokines, whereas exogenous HSP70, conversely, stimulates and mediates the inflammatory signal transduction from the bacterial endotoxin lipopolysaccharide (LPS) causing fever [3 etc.]. Whether LPS-free HSP70 per se stimulates innate immunity is still debated (see [4] for review). Second, the protective properties of exogenous HSP70 were studied mostly on cell cultures [1, 2, 5, 6]. In 2002–2003, studying physiological functions of whole organisms was initiated [7, 8]. When rats, mice, and pigeons received intravenous, intaperitoneal, and intracerebral injections of LPS-containing HSP70, their somatovisceral parameters changed like in animals with fever. With increase in body and brain temperature, slow sleep (SS), which is considered an antistress factor [10], also increased [9]. However, cytokines also induce SS prolongation [11]; therefore, it is still unclear whether HSP70 or LPS causes the somnogenic effect.

We attampted to determine changes in thermoregulation, sleep, and wakefulness of pigeons that received central microinjections of exogenous LPS-free HSP70 preparations. This major stress protein proved to have pronounced hypothermic and somnogenic effects. When administered into the third ventricle of the pigeon brain, the preparation reduced muscle tone, brain temperature, and heart rate and increased the duration of SS episodes, total time of SS, and contribution of the latter into the suppression of somatovisceral parameters. These changes suggest that HSP70 has a neuroprotective effect, which promotes the antistress SS function and poststress restoration of the brain and body homeostasis. The anterior hypothalamus neurons responsible for thermoregulation and sleep are most likely to control the hypothermic and somnogenic HSP70 effects. Our results suggest that LPS-free HSP70 preparations have a curative effect.

Five pigeons (*Columba livia*) were used in our experiments. The birds were kept under the conditions not restricting their behavior at 24–25°C and a photoperiod of 12 : 12 h; food and water were provided ad libitum. They were anesthetized with Nembutal and operated seven to ten days before the experiments. The operation technique, computer recording for 12–24 h, the analysis (with a 1-s epoch) of brain temperature, leg skin (for evaluating peripheral vasomotor reaction), as well as the procedures of encephalography, electrooculography, electrocardiography, and electromyography, have been described previously [7, 12]. The temporal and visceral parameters of wakefulness, drowsiness, SS, and fast sleep were determined according to standard criteria and those developed in our laboratory with the use of computer software. The HSP70 preparation, which was a mixture of inducible and constitutive isoforms (3 : 2), was isolated from a bovine slow muscle and passed through a polymixin B column to remove LPS, the content of which was determined using the LAL-test [5, 6]. In *in vivo* experiments, HSP70 preparation was administered through a cannula into the third ventricle of the brain at a dose of 1.5 μg in a volume of 0.7 μl 5 min before the onset of the dark phase. Phosphate buffer or apyrogenic physiological solution was similarly administered to control animals. The results of our experiments were processed statistically; differences were estimated using Student's *t* test and considered significant at $p < 0.05$.

In control animals, brain temperature reduction from 39.5 ± 0.2 to 38.0 ± 0.1°C ($p < 0.001$) continued

* *Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, pr. Morisa Toreza 44, St. Petersburg, 194223 Russia*

** *Institute of Cytology, Russian Academy of Sciences, Tikhoretsii pr. 4, St. Petersburg, 194064 Russia*

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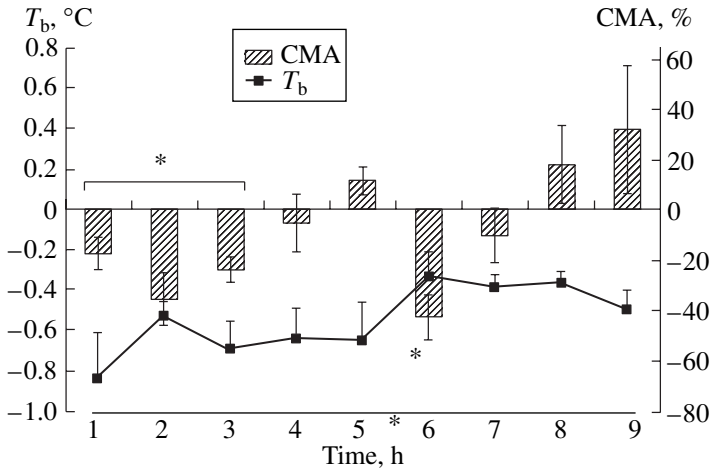

Fig. 1. Changes in brain temperature (T_b) and contractile muscle activity (CMA) of the pigeon pectoral muscle after HSP70 microinjections into the third ventricle of the pigeon brain as compared to control values obtained after the administration of apyrogenic physiological solution (the zero line). The coordinates are shown according to the atlas by H.J. Karten, W. Hodos (Baltimore, Maryland: J. Hopkins, 1967, pp. 1–194): A 7.5–7.25; L 0; H 10–11. Here and in Figs. 2 and 3: X axis, time after the microinjection; asterisks, differences from the control are significant at $p < 0.05$–0.001.

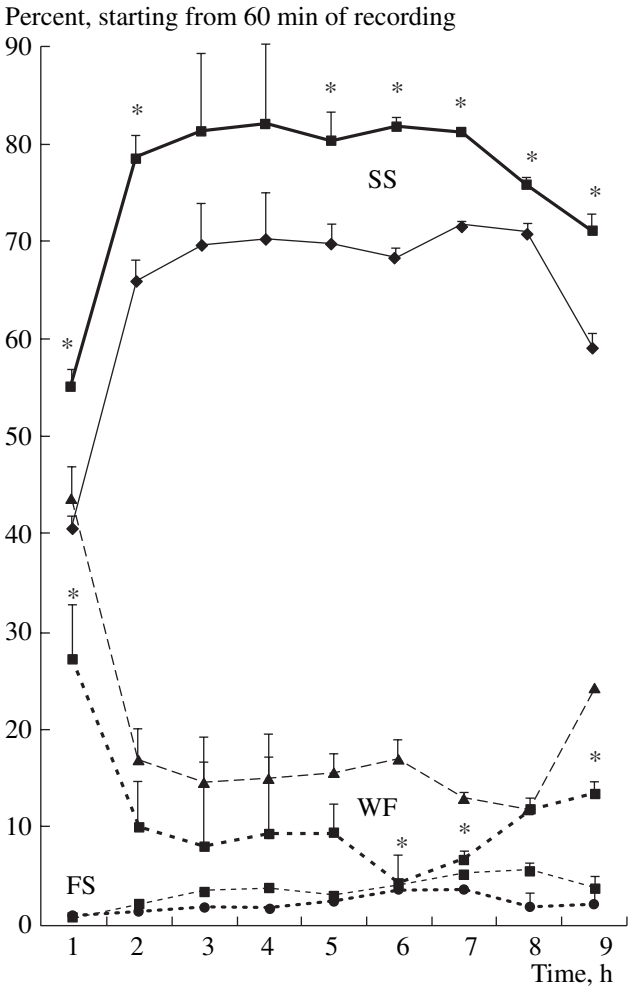

Fig. 2. Changes in the total time of wakefulness (WF), slow sleep (SS), and fast sleep (FS) after the microinjections of apyrongenic physiological solution (thin lines) and HSP70 (thick lines) into the third ventricle of the pigeon brain. The differences from the control values with respect to FS are significant for 2–4 and 8 h.

during the first 5 h of the dark phase, whereas during the "night plateau" (6–9 h), the temperature remained unchanged at the level of 37.6 ± 0.1°C. After HSP70 microinjection, the temperature reduction was 0.7 ± 0.2°C during the first 5 h and 0.4 ± 0.1°C during subsequent 6–9 h as compared to the control, which is presumably related to a decrease in the contractile muscle activity (Fig. 1). In addition, the heart rate was also reduced and a trend toward the peripheral vasoconstriction was observed.

During the first 5–7 min after HSP70 administration to pigeons, the animals assumedsleeping positions and displayed drowsiness episodes. The onset of the first SS episode with a duration of 7–20 s occurred significantly earlier (on average, within 8 min) than in the control (within 13.7 min). HSP70 caused a significant increase in SS time during the first, second, and fifth to ninth hours (by 35, 19, and, on average, 15%, respectively) (Fig. 2). As compared to the control, the average time of wakefulness was reduced by 42% (by 43 min during 9 h) at the expense of a 29% lower number of episodes and a 33% lower duration of the latter. The fast-sleep time was 33% reduced, mostly at the expense of the reduced number of episodes (by 24%). The total SS time was 17% increased (by 61 min during 9 h) only at the expense of a 35% higher episode duration.

Hence, after central microinjections of HSP70, the somnogenic effect of the latter is mediated by the mechanisms that support the SS duration and inhibit wakefulness and fast-sleep triggering. During SS, the brain temperature reduction (Fig. 3), as well as that of contractile muscle activity and heat rate, was more significant after HSP70 administration compared to the control.

LPS-containing HSP70 preparations were previously shown to increase the brain and body "core" tem-

perature, as well as the muscle activity after both peripheral and central injections to rats, mice, and pigeons [7–9]. These effects were inhibited with decreasing the HSP70 dose and its LPS content. Administration of the LPS-free HSP70 inhibited the muscle contractile activity significantly and induced hypothermia (Fig. 1). When compared, these data suggest that changes in thermoregulation parameters depend on the LPS content of HSP70 preparations. However, even LPS-free HSP70 can bind LPS when added to a cell culture or when it enters the blood [4], which may alter this protein activity. We studied the effects of HSP70 after the administration into the brain, which is protected from LPS by the blood–brain barrier. Our results suggest that the hyperthermic HSP70 effect is related to the LPS contaminant, whereas the hypothermic effect is related to the properties of HSP70 itself. LPS is known to increase the rate of the synthesis of proinflammatory cytokines and the expression of intracellular HSP70 in various tissues (see [13] for review). Since cytokines induce both pyrogenic and somnogenic effects [11], an increase in SS after the administration of LPS-containing HSP70 into the brain might be caused by both HSP70 itself and cytokines [9]. A significant increase in SS (by 61 min during 9 h of recording) after the administration of LPS-free HSP70 (Fig. 2) suggests that HSP70 has its own somnogenic effect.

The rapid development of the effects studied, which were the most pronounced during the first hour after the HSP70 administration into the third ventricle of the brain, suggests that the neurons of periventricular structures of the anterior hypothalamus are primarily responsible for the hypothermic and somnogenic HSP70 effects, because these cells control sleep, thermoregulation, and some related visceral functions. The HSP70 effects are likely related to its modulating influence on the synaptic processes, because synapses were shown to contain endogenous HSP70, which regulates the neurotransmitter release [1, 2], whereas exogenous HSP70 was capable of protecting the synaptic function of GABA, glutamate, and glycine during heating of surviving brain sections [14]. Exogenous HSP70 was absorbed by human neuroblastoma cells within 1–3 h and increased their stress resistance [6]. In our experiments, the long-term HSP70 effects (up to 6–9 h) were presumably caused by gradual HSP70 accumulation in nerve cells.

SS is known to be primarily responsible for brain "cooling," an increase in peripheral vasodilatation and a decrease in contractile muscular activity and heart rate [15]. HSP70 enhances this natural SS contribution into the development of pronounced muscular rest and suppression of visceral system activity, which testifies to the anti-stress SS function. The above changes are presumably caused by the neuroprotective HSP70 action resulting in the restoration of homeostasis of the brain and entire body after stress. Thus, with respect to the development of new drugs for therapy of poststress syndromes and diseases, the HSP70 preparation studied holds more promise than LPS-contaminated HSP70.

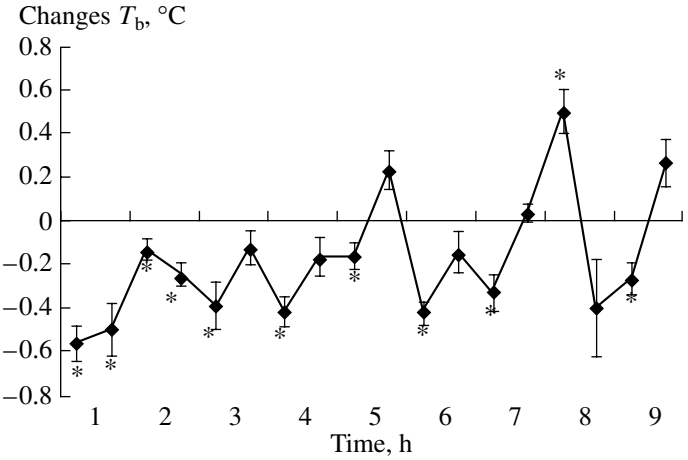

Fig. 3. Changes in brain temperature (T_b) during SS episodes (for every 30 min) after the HSP70 injection into the third ventricle of the brain as compared to the same parameter in the control (zero line).

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