EXPERIMENTAL PAPERS

Preventive Administration of the Heat Shock Protein Hsp70 Relieves Endotoxemia-Induced Febrile Reaction in Pigeons (*Columba livia*) and Rats

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Abstract—The stress-inducible 70 kDa heat shock protein (Hsp70) can exert a protective effect on endotoxemia and sepsis due to its ability to interact with immune cells and modulate the immune response. However, it remains unknown whether Hsp70 is able to relieve endotoxemia-induced fever. We carried out a comparative study of the effects of preventive administration of the human recombinant Hsp70 (HSPA1A) on lipopolysaccharide (LPS)-induced endotoxemia in pigeons and rats with preimplanted electrodes and thermistors for recording the thermoregulation parameters (brain temperature, peripheral vasomotor reaction, muscular contractile activity). Additionally, we analyzed the dynamics of the white blood cell (WBC) count in rats under the same conditions. It was found that preventive administration of Hsp70 relieves the LPS-induced febrile reaction in pigeons and rats and accelerates the restoration of the WBC count in rats. The data obtained suggest that these warm-blooded animals share a common physiological mechanism that underlies the protective effect of Hsp70.

INTRODUCTION

Endotoxemia is a condition caused by an increase in the blood level of lipopolysaccharide (LPS), which is the cell wall-derived endotoxin of Gram-negative bacteria. First of all, LPS affects the functions of immunocompetent and vascular endothelial cells, leading to the activation of the

immune system, increased synthesis of pro-inflammatory cytokines, development of a febrile reaction (fever), functional changes in the nervous, endocrine and other systems of the organism [1−3]. Fever is an ancient protective and adaptive mechanism, which is characteristic of ectothermic and endothermic vertebrates and aimed at providing optimal conditions for the functioning of the immune system and elimination of the pathogen [2, 4]. In ectothermic animals, the development of the immune response is accompanied by a behavioral fever, which makes these animals to choose a warmer place in order to increase their body temperature [2, 4]. In endothermic animals (mammals and birds), a controlled increase in body temperature, induced by LPS or other pathogens, occurs due to heat transfer limitation via peripheral vasoconstriction and an increase in shivering and non-shivering thermogenesis [5−8]. It should be noted that when studying changes in thermoregulation during endotoxemia and other conditions accompanied by a fever, the main focus is usually on the assessment of body temperature, while much less attention is paid to the brain temperature (T_{br}). A number of literature data indicate that an increase in T_{br} during hyperthermia and LPS-induced endotoxemia can increase the permeability of the blood-brain barrier, affect the activity of neurons and brain metabolism, and enhance the production of beta-amyloid [9−13]. It is assumed that an increase in T_{br} is especially critical for patients with craniocerebral traumas, ischemic and hemorrhagic strokes, as well as young children with a propensity to febrile seizures [14, 15]. Such a scenario can be dangerous for septic patients with a chronic febrile reaction, for example, during an increase in the blood LPS level and the presence of bacteremia or the development of a "cytokine storm" and viral sepsis, which often occur in severe cases of SARS-coV-2 infection [16, 17].

The available literature data indicate that LPS-induced endotoxemia in members of diverse animal groups (mammals, birds, fish, echinoderms) increases the expression of the stress-inducible heat shock protein with a molecular weight of 70 kDa (HSP70) in various tissues [18−22]. Hsp70 refers to the evolutionary ancient chaperone system, which has a protective effect at cellular and organismal levels against various stress factors and is characterized by an extremely conserved structure [23]. Hsp70 is able to enter the systemic circulation and modulate the activity of immunocompetent cells; however, the mechanisms of its interaction with various types of cells have not yet been fully understood. It was believed that extracellular Hsp70, dubbed a "chaperokine", can act as an alarm signal due to its interaction with various receptors on monocytes/macrophages, dendritic cells and neutrophils, such as Toll-like receptors (TLR2 and TLR4), CD91, CD40, Lox-1, etc., causing thereby the production of pro-inflammatory cytokines and NO, and also participating in antigen presentation [24−27]. Intravenous administration of exogenous Hsp70 helps reduce mortality in sepsis, corrects some hemodynamic and coagulation parameters, normalizes bilirubin and creatinine blood levels; in in vitro experiments, it reduces NO production by macrophages, decreases the generation of reactive oxygen species and partially normalizes neutrophil apoptosis in a model of endotoxin sepsis [28−31]. The above data attest to the ability of exogenous Hsp70 to influence immune response parameters and exert a protective effect in sepsis models, however, they do not allow concluding whether, in this case, the changes in body temperature, peripheral vasomotor reaction, and shivering thermogenesis, characteristic of the effect of LPS, are also corrected. Studies addressing the effect of exogenous Hsp70 on thermoregulation parameters during febrile reactions have not yet been carried out. Previously, it was shown that exogenous Hsp70, when delivered into the cerebrospinal fluid, is able to cause a decrease in T_{br}, shivering thermogenesis, and heart rate in pigeons under control conditions [32, 33]. These changes were associated with an increase in the duration of episodes and total time of slow-wave sleep, which has an anti-stress function. These data provided the rationale for studying the effects of exogenous Hsp70 on thermoregulation parameters of the febrile response and indicators of systemic inflammation (leukocytosis) in avian (pigeons *Columba livia*) and mammalian (Wistar rats) models of endotoxemia.

A comparative physiological investigation of

Hsp70 effects in endotoxemia will help elucidate the contribution of the stress-inducible protein Hsp70 to the regulatory mechanisms of the endotoxemia-induced febrile response and reveal common and specific patterns in the manifestation of putative protective effects of Hsp70 under the effect of bacterial endotoxins.

The goal of this study was to carry out a comparative analysis of the effects of exogenous Hsp70 on febrile reaction parameters, such as T_{br}, peripheral vasomotor response, muscle contractile activity (MCA), and white blood cell (WBC) count, in LPS-induced endotoxemia in pigeons *Columbia livia* and Wistar rats.

MATERIALS AND METHODS

Animals. Electrophysiological experiments were carried out on adult male and female pigeons *Columba livia* weighing 350−420 g and male Wistar rats weighing 280−320 g. WBC count was performed on male Wistar rats of the same weight and age. Animals intended for electrophysiological experiments, 7−10 days prior to surgery, were placed in a sound- and lightproof experimental chamber in order to let them adapt to the maintenance conditions (12 h light/12 h dark photoperiod, temperature 23 ± 1°C) During the experiment, the animals were in a transparent organic-glass cage. The distribution of pigeons into experimental groups was carried out by simple randomization, while rats were distributed based on stratified randomization using the open field test and the assessment of rats' response to handling. Animals that demonstrated high exploratory activity and low anxiety level were selected and distributed into experimental groups.

The inactive phase of the day was dark for pigeons and light for rats. Experiments on pigeons were started 4 h before the onset of the dark phase of the day, while on rats they commenced at the beginning of the light phase. The rats intended for WBC count were kept in a vivarium at a temperature of 23 ± 1°C. Food and water were available ad libitum. All manipulations with animals were carried out in compliance with the regulations of the Basel Declaration and protocol approved by the Ethics Committee of the Sechenov Institute of Evolutionary Physiology and Biochemistry (IEPhB) in accordance with national and international requirements.

Reagents. The *Escherichia coli* LPS endotoxin (0111:B4, Sigma Aldrich, USA) was used to induce endotoxemia in pigeons and rats. LPS was dissolved in apyrogenic 0.9% isotonic physiological saline and injected intravenously (i.v.) at a dose of 100 μg/kg in a volume of no more than 0.2 mL.

The purified recombinant human heat shock protein Hsp70 (HSPA1A) was obtained from the extract of *E. coli* transformed with a pMSHsp70 plasmid. Hsp70 was isolated by ion exchange chromatography using DEAE Sepharose (GE Healthcare) with subsequent ATP affinity chromatography using ATP agarose (Sigma, USA). Next, using a polymyxin-B gel (Sigma, USA), the bacterial LPS admixture was removed. The amount of LPS in the purified protein solution was estimated using a LALA E-Toxate test (Sigma Aldrich, USA). The final Hsp70 protein solution contained less than 0.5 EU/mg LPS [34, 35]. The resultant preparation was dissolved in saline and administered i.v. to animals 15 min before LPS (or control solution) administration at a dose of 85 μg/kg in a volume of no more than 0.1 mL.

To exclude the influence of contaminants (mainly LPS) in the Hsp70 preparation on experimental results, a series of experiments was carried out using thermodenatured Hsp70 (Hsp70den) at the same dose. For this, Hsp70 was heated in a water bath to 100°C for 5 min.

As a control solution, the animals were injected with saline used as a vehicle for LPS, Hsp70 and Hsp70den preparations. Saline was injected in a volume of 0.1 and 0.2 mL as a control to Hsp70 and LPS, respectively.

Experimental design. In order to analyze and compare the effect of Hsp70 on the LPS-induced dynamics of thermoregulation parameters, the following series of studies with i.v. administration of reagents at the above doses were carried out on pigeons ($n = 6$) and rat ($n = 6$):

(1) control group, saline (i.v.), twice at a 15-min interval;

(2) saline (i.v.), 15 min before LPS (i.v.);

(3) Hsp70 (i.v.), 15 min before saline (i.v.);

(4) Hsp70 (i.v.), 15 min before LPS (i.v.).

In order to exclude the effect of residual LPS

contamination of Hsp70 on physiological parameters in endotoxemia, a series of experiments was carried out on pigeons ($n = 5$) and rats ($n = 5$) using Hsp70den at the same dose.

To study the number of leukocytes (WBC count), blood was sampled from the rat caudal vein 1 h and 5 h after the administration of control solutions and Hsp70 and LPS preparations, as well as preventive Hsp70 administration prior to LPS. The reagents were administered at the same dose and in the same volume as in the case of studying electrophysiological parameters. This series of experiments included the following groups:

(1) control group, saline (i.v.), twice at a 15-min interval, before LPS (i.v.) ($n = 8$);

(2) saline (i.v.), 15 min before LPS (i.v.) ($n = 8$);

(3) Hsp70 (i.v.), 15 min before saline (i.v.) ($n = 8$);

(4) Hsp70 (i.v.), 15 min before LPS (i.v.) ($n = 8$);

(5) Hsp70den (i.v.), 15 min before saline (i.v.) ($n = 6$);

(6) Hsp70den (i.v.), 15 min before LPS (i.v.) ($n = 6$).

Surgical manipulations were carried out under general anesthesia using zoletil 100 (tiletamine hydrochloride + zolazepam; Virbac, Carros, France): 55 mg/kg to pigeons and 85 mg/kg to rats.

Changes in T_{br} and skin temperature (T_{sk}) were assessed using miniature thermistors (BetaTherm cat 2K7 MCD1, 0.46 mm in diameter, 1.5 kΩ resistance, USA). In pigeons, a mini thermistor for measuring T_{br} was implanted above the hypothalamic area according to the following coordinates: 9 mm rostral to the zero point, 2 mm lateral to the midline, and 6 mm below the skull surface [36]. In rats, a mini thermistor was implanted at the following coordinates: 0.5 mm caudal to the bregma, 2.5 mm lateral to the midline, and 5 mm below the skull surface [37]. A mini thermistor for registering T_{br} in both pigeons and rats was attached to the skull surface using Akrodent self-hardening dental plastic (Stoma, Ukraine). Mini thermistors to record T_{sk} (to assess the peripheral vasomotor reaction) were fixed on the unfeathered part of the pigeon leg and at the base of the rat tail. To record an electromyogram (EMG), hook-shaped gilded electrodes were implanted into the pectoral muscle of pigeons and cervical muscle of rats. The wires from the mini-thermistors and electrodes were pulled beneath the skin to the point where a connector was attached. In pigeons, the wires from the implanted electrodes and mini thermistors were pulled beneath the skin and connected to a microcircuit, a miniature "backpack" (15 g) that was put on the bird's back. In rats, connectors for cable connection were installed on the head and fixed with Acrodent plastic. The wires from EMG-recording electrodes and T_{sk} were also pulled beneath the skin and connected to a common connector. The signals from the "backpack" or head connector were switched to a cable traveling to a preamplifier, and then, through an analog processing and digitizing unit, fed into a computer for archiving and processing. The presence of an electrical rotary joint prevented the twisting of the wires and allowed the animals to move freely. The experiments began after complete postoperative recovery of animals (after 12−14 days).

Signal processing. The electrophysiological data array was recorded for 24 h in pigeons and 12 h in rats. The original computer system SASR-8800 (USA) was used to record and analyze electrophysiological parameters [38]. The primary signal amplification (by a factor of 100) was carried out in a preamplifier. Then, the signals were fed into the analog processing unit for further amplification. For this, an analog-to-digital converter with 8 analog inputs, an input voltage range of ±5 V, and a 0.01% noise level was used. The microcomputer sampled each signal 120 times per second using a 12-bit ADC simulator. The full-scale voltage range for the electrodes was ±1 mV. The EMG frequency range was 60−1000 Hz, with EMG signals being averaged in such a way as to give 10 measurements per second. All the obtained averages were stored as binary integers with a specific sign and a resolution of 0.25 mV [38]. The experimental setup and computer software enabled a temperature to be measured with an accuracy of 0.01°C. An increase in the temperature measurement accuracy was achieved by careful plotting a calibration curve. The thermistors were calibrated over a wide temperature

range, and each temperature point was obtained by averaging 10 measurements per second.

Electrophysiological data were processed using a set of programs developed previously by D. Ross (University of Tallahassee, USA) [38] and A.L. Zimin (IEPhB, St. Petersburg). In our experiments, average T_{br} and T_{sk} were calculated for 30 min. To assess the MCA level, EMG, the amplitude of which was proportional to the degree of muscle tension and the distance between the lead electrodes, was processed. Using the software, the mean integrated EMG of the pectoral and cervical muscles was calculated for 1 h.

White blood cell count. Total WBC count was performed using a Goryaev chamber. WBC nuclei were visualized by 3% acetic acid with Methylene blue. To count the number of WBCs, blood was diluted 10-fold.

Statistics. Data were statistically processed and illustrations were prepared using GraphPad Prizm 8. To check the normal distribution, the Shapiro–Wilk test was used. Changes in T_{br}, T_{sk}, and MCA was analyzed using a repeated measures ANOVA. One-way ANOVA for independent samples (with post-hoc analysis using the Tukey's test) was used to process the WBC count data. Data in the figures are presented as the mean ± standard error of the mean ($M \pm SEM$).

RESULTS

Effect of Hsp70 on thermoregulation parameters during endotoxemia in pigeons and rats

Our study showed that intravenous Hsp70 administration caused no changes in the T_{br}, peripheral vasomotor response, and MCA compared to the control in both pigeons and rats (Figs. 1a–1f).

LPS administration caused the development of fever in pigeons, as manifested in an increase in T_{br} compared to the control during the period from the 3rd to 8th hours by an average of $1 \pm 0.1°C$ ($p < 0.05$) (Fig. 1a). During the first 3.5 h, a decrease in T_{sk} by an average of $1.7 \pm 0.2°C$ ($p < 0.05$) was revealed, indicating the development of vasoconstriction leading to a limitation of heat transfer and contributing to an increase in T_{br} (Fig. 1b). 5.5 h after LPS administration, T_{sk} increased by an average of $1.9 \pm 0.2°C$ ($p < 0.05$),

indicating that vasoconstriction was replaced by vasodilation, which promoted an increase in heat dissipation. In addition, an increase in T_{br} during endotoxemia was accompanied by an increase in MCA by an average of 73% ($p < 0.05$) compared to the control level, indicative of an increase in heat production (Fig. 1c).

At the end of the active phase of the day, T_{br} in rats was, on average, 2°C lower than in pigeons (Fig. 1d). Upon LPS administration, an increase in T_{br} (by an average of $1.1 \pm 0.08°C$, $p < 0.05$) began in rats by 1 h earlier than in pigeons and persisted for the next 5 h (Fig. 1d). The increase in T_{br} was accompanied by a change in T_{sk} with a similar dynamics, however, the period of vasoconstriction development in rats was shorter than in pigeons (within 2 h), while the vasodilation period, on the contrary, was longer (from the 3rd to 7th hours) (Fig. 1e). Like in pigeons, in rats exposed to LPS, MCA intensified by 82% ($p < 0.05$), which contributed to an increase in heat production (Fig. 1f).

Preventive administration of Hsp70 to pigeons relieved LPS-induced fever; an increase in T_{br} by an average of $0.52 \pm 0.06°C$ ($p < 0.05$) was observed only for 1 h (Fig. 1a). Hsp70 application did not prevent the development of peripheral vasoconstriction in pigeons, but led to a reduced duration of the vasodilation period (Fig. 1b). In addition, Hsp70 completely eliminated the LPS-induced enhancement of MCA, which could contribute to a decrease in heat production and a severity of the febrile reaction (Fig. 1c).

In rats, preventive Hsp70 administration under endotoxemic conditions led to a decrease in the T_{br} increment from $1.1 \pm 0.08°C$ ($p < 0.05$) to $0.56 \pm 0.05°C$ ($p < 0.05$) (Fig. 1d); however, the period of increasing T_{br} in rats remained by 2.5 h longer than in pigeons (Fig. 1d). Hsp70 did not eliminate the development of peripheral vasoconstriction, characteristic of the effect of LPS, however, the duration of the vasodilation period decreased (Fig. 1e). In rats, by contrast to pigeons, Hsp70 did not prevent the development of LPS-induced enhancement of MCA, but promoted a reduction in the period of increasing this parameter compared to the effect of LPS alone (Fig. 1f).

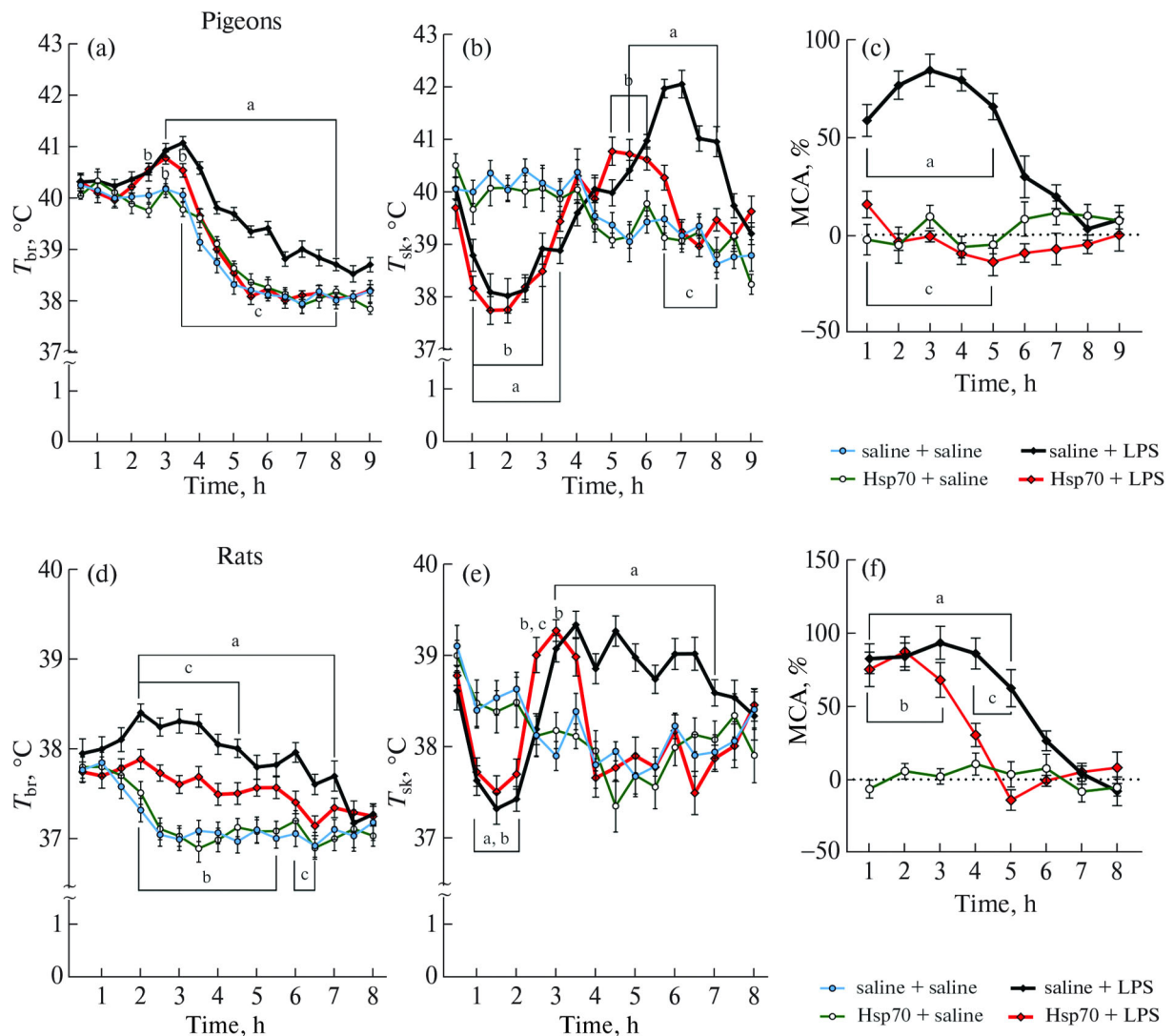Despite the fact that after isolation Hsp70 was

Fig. 1. Effect of Hsp70 on thermoregulation parameters during endotoxemia in pigeons (a−c) and rats (d−f). In (a), (b), (d) and (e): changes in brain temperature (T_{br}) and skin temperature (T_{sk}). *Ordinate*: T_{br} and T_{sk} values in control (saline+saline), upon the administration of Hsp70 (Hsp70+saline) and LPS (LPS+saline), and preventive Hsp70 administration before the induction of endotoxemia (Hsp70+LPS). *Abscissa*: time (h). (a) $F_{(3, 20)} = 19.20$, $p < 0.0001$; (b) $F_{(3, 20)} = 3.855$, $p = 0.0251$; (d) $F_{(3, 20)} = 7.398$, $p = 0.0016$; (e) $F_{(3, 20)} = 5.855$, $p = 0.0026$. In (c) and (f): changes in the level of muscle contractile activity (MCA) in pigeons and rats under the same exposures vs. control level. (c) $F_{(2, 15)} = 314.3$, $p < 0.0001$; (f) $F_{(2, 15)} = 334.3$, $p < 0.0001$. [a] $p < 0.05$—significant difference of LPS effects vs. control; [b] $p < 0.05$—significant difference of effects of preventive Hsp70 administration before LPS administration vs. control; [c] $p < 0.05$—significant difference of LPS effects vs. effects of preventive Hsp70 administration before LPS administration.

purified from endotoxin using polymyxin-B, it was necessary to check for the presence of a residual LPS contamination, which could affect the results of the experiments. LPS is known to be resistant to high temperatures, and in order to exclude the possibility of its influence on the results of our study, an additional series of experiments was undertaken using Hsp70den. This reagent was subjected to heat, which caused denaturation and loss of Hsp70 activity, leaving the properties of the possible LPS contamination unaffected. Our experiments showed that i.v. Hsp70den administration had no effect on changes in thermoregulation parameters characteristic of endotoxemia (Figs. 2a−2f). These data indicate that the fever relief in pigeons and rats,
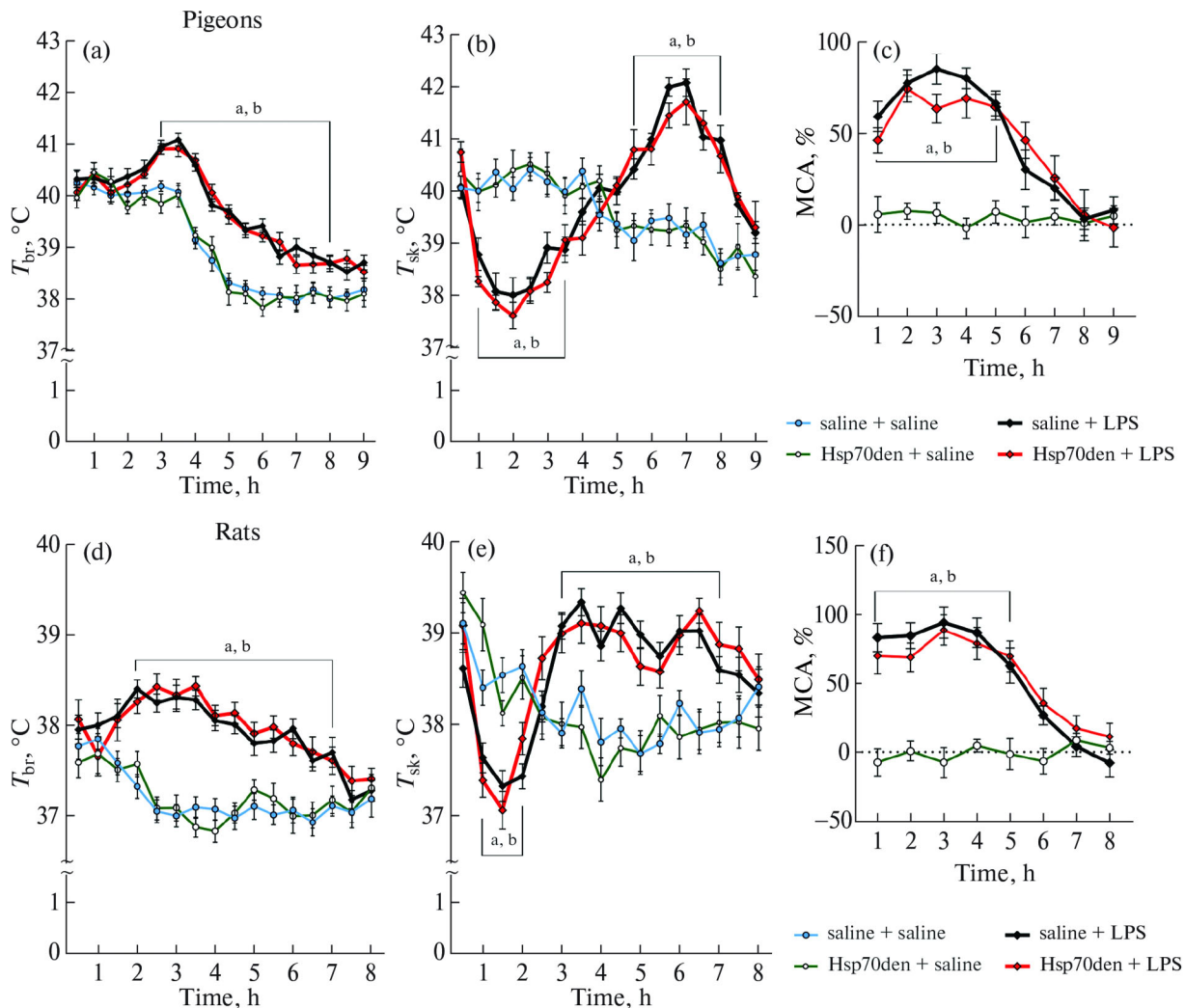
Fig. 2. Effect of Hsp70den on thermoregulation parameters during endotoxemia in pigeons (a–c) and rats (d–f). In (a), (b), (d) and (e): changes in brain temperature (T_{br}) and skin temperature (T_{sk}). *Ordinate*: T_{br} and T_{sk} values in control (saline+saline), upon the administration of thermodenaturated Hsp70 (Hsp70den+saline) and LPS (LPS+saline), and preventive administration of thermodenaturated Hsp70 before the induction of endotoxemia (Hsp70den+LPS). *Abscissa*: time (h). (a) $F_{(3, 16)} = 18.14$, $p < 0.0001$; (b) $F_{(3, 16)} = 8.42$, $p = 0.0014$; (d) $F_{(3, 16)} = 10.32$, $p = 0.0005$; (e) $F_{(3, 16)} = 8.44$, $p = 0.0014$. In (c) and (f): changes in the level of muscle contractile activity (MCA) in pigeons and rats under the same exposures vs. control level. (c) $F_{(2, 12)} = 210.1$, $p < 0.0001$; (f) $F_{(2, 12)} = 323.9$, $p < 0.0001$. [a] $p < 0.05$ − significant difference of LPS effects vs. control; [b] $p < 0.05$ − significant difference of effects of preventive Hsp70den administration before LPS administration vs. control.

which resulted from the preventive Hsp70 administration, was an intrinsic effect of this reagent.

Effect of Hsp70 on white blood cell count during endotoxemia in rats

Numerous studies indicate the ability of Hsp70 to modulate the functioning of immunocompetent cells. However, our experiments demonstrated that intravenous administration of exogenous Hsp70 does not cause significant changes in the number of WBCs either 1 h or 5 h post injection (Fig. 3a). LPS administration to rats caused an increase in the WBC count by 56% and 58% after 1 h and 5 h, respectively (Fig. 3a). With the preventive Hsp70 administration, a decrease in the WBC count was observed after 1 h compared to the effect of LPS alone. After 5 h, the values of this parameter reached the control level (Fig. 3a).

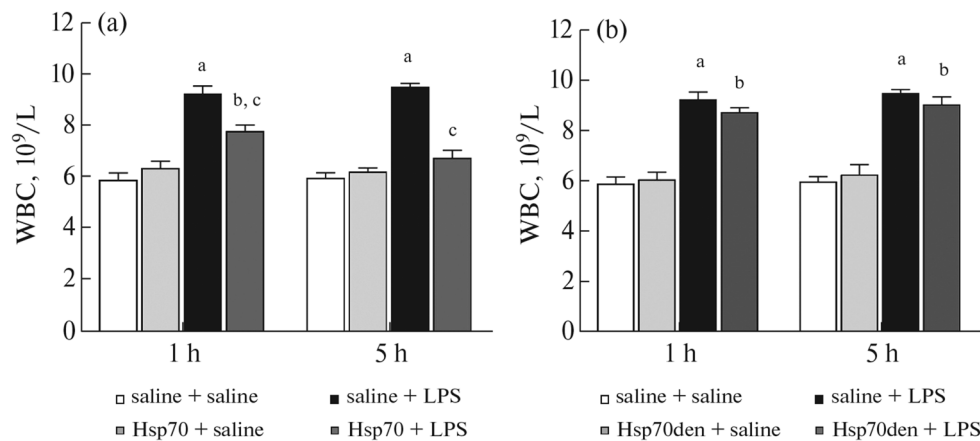Our experiments showed that the administra-

Fig. 3. Effect of Hsp70 and Hsp70den on the white blood cell (WBC) count in rats 1 h and 5 h after the induction of endotoxemia. (a) Changes in the WBC count in control (saline+saline), upon the administration of Hsp70 (Hsp70+saline), LPS (LPS+saline), and preventive Hsp70 administration before the induction of endotoxemia (Hsp70+LPS); (b) same in control (saline+saline), upon the administration of thermodenaturated Hsp70 (Hsp70den+saline), LPS (LPS+saline), and preventive administration of Hsp70den before the induction of endotoxemia (Hsp70den+LPS). (a) $F(3, 28) = 38.4$, $p < 0.0001$ (after 1 h), $F(3, 28) = 70.11$, $p < 0.0001$ (after 5 h); (b) $F(3, 26) = 48.2$, $p < 0.0001$ (after 1 h); $F(3, 26) = 50.1$, $p < 0.0001$ (after 5 h). [a] $p < 0.05$ − significant differences of LPS effects vs. control; [b] $p < 0.05$ − significant difference of effects of preventive Hsp70 or Hsp70den administration before LPS administration vs. control; [c] $p < 0.05$ − significant differences of LPS effects vs. effects of preventive Hsp70 or Hsp70den administration before LPS administration vs. control.

tion of Hsp70den did not cause a change in the WBC count, and its application before the induction of endotoxemia did not correct leukocytosis, which is characteristic of the effect of LPS (Fig. 3b). These data confirm that the ability of Hsp70 to attenuate LPS-induced leukocytosis, as revealed in this study, is an intrinsic property of exogenous Hsp70.

DISCUSSION

The endotoxin LPS is a widely used agent for modelling endotoxemia and studying changes in various thermoregulation parameters. Our study demonstrated that the administration of LPS in both pigeons and rats caused an increase in T_{br}, accompanied by the development of vasoconstriction and an increase in the MCA level. Vasoconstriction is a peripheral vascular reaction aimed at limiting heat dissipation and promoting an increase in T_{br}. The enhancement of MCA in endotoxemia represents a mechanism aimed at increasing heat production and promoting an increase in body temperature [6, 8]. The subsequent development of vasodilation, as observed in both pigeons and rats, may have been a mechanism limiting a further increase in T_{br}. Thus, in

our study, the development of endotoxemia in pigeons and rats was characterized by similar changes in thermoregulation parameters, which indicates the community of the thermoregulatory mechanisms underlying the response to endotoxin in pigeons and rats. The differences in this reaction to LPS between pigeons and rats consisted in the fact that the febrile response in rats developed earlier than in pigeons. In addition, the period of T_{br} elevation, as well as the periods of vasoconstriction and vasodilation, had different durations.

In our study, we performed for the first time a comparative physiological study of the effect of exogenous Hsp70 on the development of a febrile reaction in members of the two classes of vertebrates. Our experiments showed that in pigeons, Hsp70 administration reduced an increase in T_{br} and significantly accelerated its reversion to the control values. In rats, in contrast to pigeons, Hsp70 reduced the magnitude of the febrile response, but the increase in T_{br} persisted longer than in pigeons. In both pigeons and rats, peripheral vasoconstriction persisted, but the subsequent phase of vasodilation was less pronounced, which may have been associated with a lower intensity of the febrile reaction. A more pronounced anti-

pyretic effect of Hsp70 administration in pigeons is further evidenced by the absence of an increase in MCA, which probably contributed to a decrease in heat production. In contrast to pigeons, in rats, the increase in MCA persisted for 3 h after LPS administration. However, the restoration of this parameter to the control values occurred earlier than with the effect of LPS alone, and may have promoted a decrease in both heat production and T_{br}. It can be assumed that a more pronounced antipyretic effect of Hsp70 in rat LPS-induced endotoxemia requires a higher dose of this protein agent.

We established that LPS administration led to the development of leukocytosis in rats. An increase in the number of WBCs is one of the hallmarks of the inflammatory process [39]. As early as 1 h after the preventive Hsp70 administration, we observed a decrease in the WBC count, while after 5 h, this parameter normalized. Apparently, the decrease in WBC recruitment after the preventive Hsp70 administration may attest to anti-inflammatory properties of Hsp70. Thus, our data indicate the ability of Hsp70 to correct thermoregulation parameters of the LPS-induced febrile response, as well as the inflammatory response, in both pigeons and rats.

The well-known major participants in the triggering of fever in endotoxemia are pro-inflammatory cytokines IL-1β, IL-6, TNF-α, as well as a principal downstream mediator of fever prostaglandin E_2 (PGE$_2$) [6, 40]. However, the exact mechanisms behind the transmission of a LPS pro-inflammatory signal in birds remains obscure, since the bulk of studies devoted to this issue have been carried out on mammals. It is assumed that in birds, in contrast to mammals, IL-6 plays a more important role in triggering fever, while the involvement of PGE$_2$ appears to be less pronounced [41]. It has been shown that exogenous Hsp70 can interact with receptor complexes of immunocompetent cell membranes (CD11b/CD18, TLR2, TLR4) and modulate their activity. This leads to a decrease in the production of reactive oxygen species, TNF-α, IL-6, expression of TLR4 on the membrane surface, and IL-1β mRNA level [42−45]. There is evidence that the anti-inflammatory effect of Hsp70 may also be due to the involvement of the anti-inflammatory

cytokine IL-10 [46]. In in vivo experiments, it was found that preventive Hsp70 administration promotes an increase in the survival rate of animals upon the injection of high LPS doses, corrects a number of hemodynamic and biochemical parameters of blood (concentrations of bilirubin, creatinine, etc.) [28−31]. The totality of these data suggests that preventively administered Hsp70 can compete with LPS for binding to TLR2 and TLR4, while blocking the activation of the pro-inflammatory cascade with the involvement of the NF-kB factor. This assumption is supported by the data that the therapeutic application of Hsp70 (after the administration of a high dose of LPS) did not eliminate the development of tachycardia nor reduced the mortality rate [28]. It is well known that one of the key participants in the triggering of the febrile reaction is PGE$_2$ produced by peripheral macrophages [6], and it seems likely that the ability of Hsp70 to interact with these cells leads to a decrease in PGE$_2$ production and attenuation of the febrile response.

It should be noted that the above data on the protective effects of exogenous Hsp70 were obtained using cell cultures and laboratory mammals (mice and rats). The birds were found to have the same types of immunocompetent cells, as well as CD11b receptors and major mediators of inflammation (IL-6, IL-1β, TNF-α, PGE$_2$) [47, 48]. Apparently, the antipyretic effect of Hsp70 in pigeons and rats could have been realized via the similar mechanisms, being a result of the effect on the functioning of immunocompetent cells and production of pro-inflammatory cytokines. There is an opinion that birds are more tolerant to the effect of LPS than mammals. The TLR4 complex was found in both birds and mammals. However, in birds, neither LPS-binding protein, nor the free form of the CD14 receptor involved in signal transduction in mammals, nor the TLR4-mediated signaling cascade ensuring the production of interferons were found [49, 50]. Probably, the more pronounced antipyretic effect of Hsp70 in pigeons is associated with this feature.

Thus, our study showed that Hsp70 administration reduces the magnitude of the febrile response to LPS in pigeons and rats, and also contributes to the normalization of the number of WBCs in the rat blood. These results indicate the ability of

Hsp70 to attenuate the systemic inflammatory response. It can be assumed that mammals and birds, at least some of their representatives, share the common mechanisms of realization of the anti-inflammatory effect of Hsp70 during endotoxemia. The data obtained can provide a fundamental rationale for further research in this direction in order to assess the therapeutic potential of exogenous Hsp70 in endotoxemia and sepsis, which will be important for developing novel treatment strategies for these conditions.

AUTHORS' CONTRIBUTION

K.V.L.: conducting experiments, data processing and analysis, writing a manuscript; I.V.G.: obtaining Hsp70 protein, editing a manuscript; I.V.E.: conceptualization, experimental design, data analysis, editing a manuscript.

FUNDING

CONFLICT OF INTEREST

The authors assure that they have neither evident nor potential conflict of interest related to the publication of this article.

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