Enhanced Production of Nitric Oxide in Rat Organs in Heat Shock

E. B. Manukhina, I. Yu. Malyshev,* V. D. Mikoyan, L. N. Kubrina,** and A. F. Vanin****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 520-523, May, 1996
Original article submitted March 6, 1995

Heat shock is shown to lend a marked boost to the production of nitric oxide (NO), which attains the maximal level 1 hour after exposure and returns to the initial level after 24 hours. The generation of NO in all studied organs is completely blocked by Nω-nitro-L-arginine, an inhibitor of NO synthase, both in the control and after hyperthermia. Thus, heat shock markedly stimulates NO synthesis. This generalized effect may underlie the drop in the peripheral vascular tone that is characteristic of heat shock.

Key Words: *nitric oxide; heat shock; electron paramagnetic resonance; inhibitor of NO synthase*

Heat shock is a severe systemic disorder, one of the most serious manifestations of which is pronounced vascular insufficiency leading to a dramatic drop of arterial pressure and to collapse [13]. These effects are thought to be caused by generalized vasodilation, the mechanism of which remains little studied [9,10]. Neither a reduced activity of the sympathetic nervous system, nor a drop of the blood level of catecholamines or injury to the contractile apparatus of the vascular smooth muscles are shown to be responsible for this vasodilation [5]. Therefore, it seems likely that this drop of the vascular tone is mediated through local humoral regulatory mechanisms, one of the most important mediators of which is nitric oxide (NO), a potent endogenous vasodilator. NO plays an important role in preventing excessive vasoconstriction, and its insufficient production largely contributes to the pathogenesis of hypertension, atherosclerosis, etc. [14]. On the other hand, hyperproduction of NO may lead to an excessive drop of the vascular tone and systemic arterial pressure, similar to those observed in septic and hemorrhagic shock [14] and in acute myocardial infarction [1].

Hyperthermia has been recently demonstrated to lead to a considerable and stable increase in the urinary excretion of nitrates [4]. Moreover, in rats subjected to heating the electron paramagnetic resonance (EPR) signal from NO heme in the portal blood has been shown to be increased [7]. In addition, the hyperthermal reaction has been found to be weakened by NO donors and enhanced by NO inhibitors [6]. In view of the above, it may be assumed that high temperatures stimulate NO production in the organism. However, this assumption has not been directly verified.

In light of the above, the objective of our study consisted in a direct quantitative determination of the dynamics of NO and its origin in different organs in rats exposed to hyperthermia.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 210-230 g. Heat shock was reproduced by heating the alert animals in a thermocontrolled chamber until the rectal temperature attained 41°C,

Laboratory of Membrane Mechanisms of Adaptation; *Laboratory of Genetic Mechanisms of Adaptation, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences; **Laboratory of Biopolymer Physical Chemistry, Institute of Chemical Physics, Russian Academy of Sciences, Moscow

0007-4888/96/0005-0471$15.00

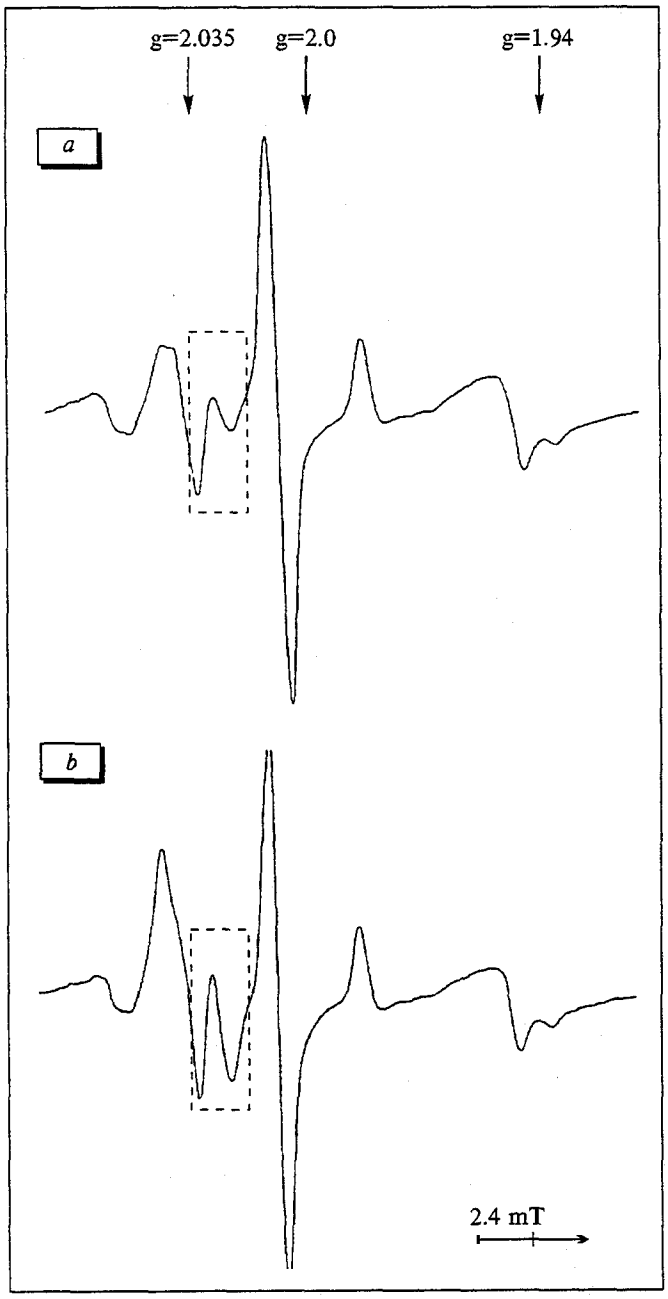

Fig. 1. Electron paramagnetic resonance (EPR) signals of MNIC–DETC in the liver of control rats (*a*) and rats exposed to heat shock (*b*). Dotted line shows the component of triplet hyperfine structure of the EPR signal, the amplitude of which served as the measure of the content of MNIC–DETC complexes in the tissue.

after which heating was continued for an additional 15 min. The total heating period did not exceed 30 min.

The amount of NO produced in rat tissues was evaluated from its incorporation in Fe^{2+}-diethyldithiocarbamate complexes [Fe^{2+}-DETC, $(C_5H_{10}NS_2)_2Fe$] yielding paramagnetic mononitrosyl iron complexes (MNIC) with DETC. These complexes are characterized by an EPR signal with the g-factor being $g_{\perp}=2.035$ and $g_{II}=2.012$ and triplet hyperfine structure at $g_{\perp}$ (Fig. 1). The amount of MNIC-DETC in the sample and, consequently, the amount of NO incorporated in this complex were evaluated by the intensity of the EPR signal, which was calculated by double integration using a solution of paramagnetic dinitrosyl iron complex with thiosulfate of a known concentration as a standard. The method used for evaluating the NO content in animal tissues has been described at length elsewhere [15].

For the accumulation of MNIC in the organism the animals were injected with solutions of Na-DETC ($C_5H_{10}NS_2Na$, 500 mg/2.5 ml H_2O/kg, intraperitoneally) and $FeSO_4$+sodium citrate (20 mg+95 mg/2.5 ml H_2O/kg, subcutaneously) 30 min before decapitation. The isolated organs (liver, kidneys, spleen, intestine, brain, and heart) were minced, frozen in a mold, and stored in liquid nitrogen.

The EPR signal was recorded on a Radiopam modified EPR radiospectrometer at 77°K, modulation amplitude 5 mT, and SHF-power 10 mW.

Another group of animals was injected with the inhibitor of NO synthase Nω-nitro-L-arginine (L-NNA, 200 mg/kg, intraperitoneally) immediately after hyperthermia and decapitated after 1 and 24 hours.

The data were processed statistically using the Student *t* test.

RESULTS

Heat shock markedly boosts the NO production in the organism. The dynamics of the NO content in different rat organs is presented in Fig. 2. The formation of NO carrier complexes was established in both the controls and animals exposed to heat shock. Hyperthermia sharply increased the concentration of MNIC-DETC complexes, which reflects the enhanced production of NO in all studied organs. The content of these complexes was maximal 1 hour after termination of the heating procedure, but started to drop after 4 hours and did not differ from the initial level after 24 hours. The EPR spectra of MNIC-DETC complexes presented in Fig. 1 make it possible to assess this phenomenon visually with respect to liver tissue. The EPR signal in the liver of animals exposed to heat shock is seen to be enhanced in comparison with the control.

In animals in which hyperthermia was followed by an injection of L-NNA, an inhibitor of NO synthesis, the EPR signal from MNIC-DETC was not recorded in any of the studied organs either in the control or 1 and 24 hours after hyperthermia.

Thus, heat shock was accompanied by a sharp transient enhancement of NO production. Since an

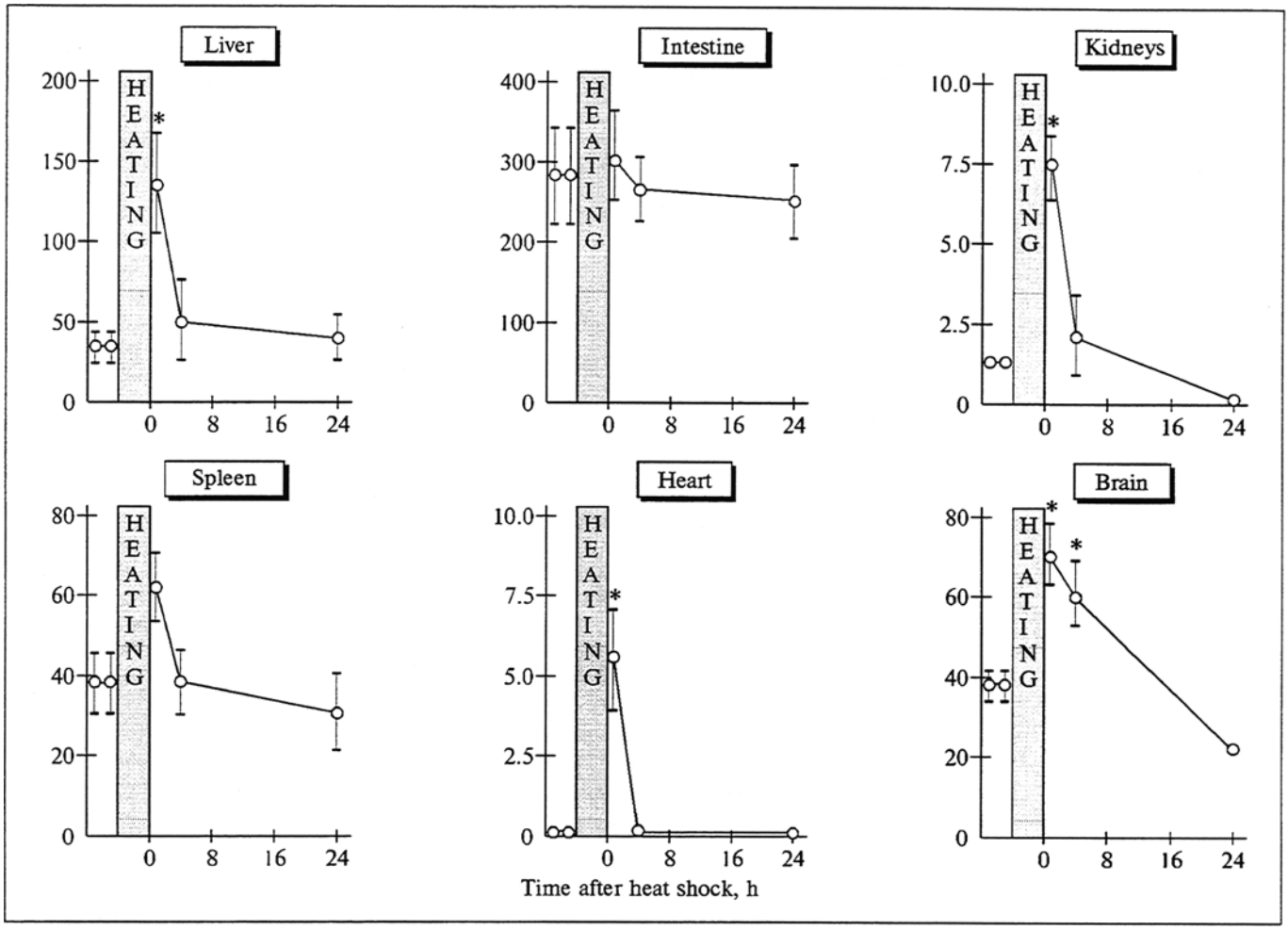

Fig. 2. Dynamics of NO production in rat organs after heat shock. Ordinate: content of MNIC–DETC complexes in tissue (ng NO/g tissue). *$p<0.05$ in comparison with the initial value.

increase in the concentration of NO-containing MNIC-DETC complexes was detected in all studied organs, this may be thought to be a generalized phenomenon. The fact that an injection of L-NNA completely inhibits NO production suggests that this NO is synthesized from L-arginine in the reaction catalyzed by NO synthase [12]. The mechanisms of hyperproduction of NO under these conditions remain unclear. It may be hypothesized that one such mechanism is a sharp activation of free radical oxidation, which accompanies heat shock [3]. If this is so, the chain of events preceding the activation of NO synthase is similar to that triggered by endotoxins [2]. The generation of oxygen radicals activates a transcription factor, NFkB protein, which in interacting with the genome induces the synthesis of various proteins, including NO synthase [11]. Moreover, the damage to the cell membranes due to enhanced lipid peroxidation stimulates Ca^{2+} influx into the cells, which in turn also activates constitutive NO synthase [8].

Our findings and previous reports suggest that stepped-up production of NO in response to overheating is an adaptive phenomenon. However, in the case of extremely long and intense heating this reaction can become excessive and may lead to long-term and stable peripheral vasodilation accompanied by a loss of vascular reactivity to constrictive stimuli, which is indeed observed in heat shock [13].

Thus, long-term exposure to high temperature stimulates the generation of NO from L-arginine in the reaction catalyzed by NO synthase. This effect is a generalized phenomenon and may lie at the basis of the pronounced and long-lasting drop of the peripheral vascular tone, which is characteristic of heat shock.

REFERENCES

1. A. F. Vanin, E. B. Manukhina, A. V. Lapshin, and F. Z. Meerson, *Byull. Eksp. Biol. Med.*, **116**, No. 8, 142-144 (1993).
2. V. D. Mikoyan, N. V. Voevodskaya, L. N. Kubrina, *et al.*, *Biokhimiya*, **59**, No. 5, 732-738 (1994).

3. A. P. Shepelev, *Vopr. Med. Khim.*, **22**, No. 1, 47-51 (1976).
4. E. A. Carter, T. Derojaswalker, S. Tamur, *et al., Biochem. J.*, **304**, Pt. 1, 201-204 (1994).
5. C. V. Gisolfi, R. D. Matthes, K. C. Kregel, and R. Oppliger, *J. Appl. Physiol.*, **70**, 1821-1826 (1991).
6. A. V. Gourine, in: *Thermal Balance in Health and Disease: Recent Basic Research and Clinical Progress*, Eds. E. Zeisberger *et al.*, Birkhauser Verlag AG (1994), pp. 491-495.
7. D. M. Hall, G. R. Buettner, B. D. Matthes, and C. V. Gisolfi, *J. Appl. Physiol.*, 77, No. 2, 548-533 (1994).
8. K. Hiki, Y. Yui, R. Hattori, *et al., Jpn. J. Pharmacol.*, **56**, 217-220 (1991).
9. A. J. Kielblock, N. B. Strydom, F. J. Burger, *et al., Aviat. Space Environ. Med.*, **53**, 171-178 (1982).
10. K. C. Kregel, P. T. Wall, and C. V. Gisolfi, *J. Appl. Physiol.*, **64**, 2582-2588 (1988).
11. A. Mülsch, B. Schray-Utz, P. I. Mordvintsev, *et al., FEBS Lett.*, **321**, No. 2-3, 215-218 (1993).
12. C. Nathan, *FASEB J.*, **6**, 3051-3064 (1992).
13. S. Shibolet, M. C. Lancaster, and Y. Danon., *Aviat. Space Environ. Med.*, **47**, 280-301 (1976).
14. R. A. Star, *Am. J. Med. Sci.*, **306**, 348-358 (1993).
15. A. F. Vanin, P. I. Mordvintsev, and A. L. Kleshchev, *Stud. Biophys.*, **107**, 135-142 (1984).

Concerning the Mechanism of Succinate Oxidation in Albino Rat Liver

S. E. Manoilov and N. V. Sedykh

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 524-525, May, 1996
Original article submitted October 27, 1994

Experiments with deuterated succinate (2,3-dideuteriumbutanediacid) prove that succinate is oxidized via the peroxide pathway. After the addition of deuterated succinate to liver centrifugate, heavy water is formed as a result of its dehydration.

Key Words: *deuterated succinate; heavy water; liver pellet; fumarate*

In investigating the role of the hydrogen peroxide—catalase system in tissue respiration, we hypothesized that succinate oxidation is associated with the formation of peroxides [1,2]. Dehydration of succinate in the process of tissue respiration is catalyzed by flavoprotein oxidases, which display peroxidase activity under aerobic conditions [9]. Consequently, succinate can be directly oxidized by molecular oxygen with the participation of flavoprotein enzymes [4,7,9]. To clarify this issue, experiments with deuterated succinate (DS) were performed, which allowed us to trace the fate of the succinate hydrogen in the α-position relative to the carboxyl group.

MATERIALS AND METHODS

Deuterated succinate was synthesized from sodium maleate with mercury as a catalyst. Sodium maleate (1 g) was dissolved in 4.2 ml 99% heavy water and vigorously shaken with sodium amalgam until sodium succinate crystals formed. Discoloration of potassium permanganate indicated the end of the reaction. The crystals were filtered out, dissolved in water (5 ml), and neutralized with HCl. Deuterated succinic acid was extracted with ether. The ether was distilled, and the residue dried and tested for purity. Its melting point proved to be 183°C. DS (2,3-dideuteriumbutanediacid) thus obtained was used in the experiments.

Livers (3 g) of intact outbred albino rats were homogenized in the cold in phosphate buffer (6 ml, pH 7.3) and centrifuged at 15,000 g, after which DS was added. Incubation was carried out for 4 h. Succinate and DS in phosphate buffer were used as controls.

After the incubation, proteins were precipitated, and the content of heavy water was determined in transparent centrifugate obtained by distillation under low pressure in special quartz equipment. The sensitivity of the method was ±0.04 mol/dl.

Veterinary Institute, St. Petersburg

0007-4888/96/0005-0474$15.00