BIOPHYSICS AND BIOCHEMISTRY

Differences in NO Generation during Heat Shock in Genetically Different Populations of Rats

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

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The content of NO in the kidneys, heart, and spleen of intact August rats surpasses that of Wistar rats. After heat shock the content of NO rises: in the kidneys 15.5-fold, in the liver 3.2-fold, in the heart 10-fold, in the spleen 6.4-fold, in the intestine 2.8-fold, and in the brain 1.9-fold. Thus, August rats, which are less resistant to heat shock than Wistar rats, are characterized by a more pronounced activation of NO synthesis in response to heat shock.

Key Words: *nitric oxide; heat shock; Wistar rats; August rats*

Heat shock (HS) is accompanied by marked hypotension, convulsions, tissue damage, and dysfunction of various organs. Severe and long-term HS may even cause death [9]. Vascular collapse is assumed to be one of the main causes of death in HS [6]. There is good reason to believe that an important role in the development of vascular collapse is played by enhanced production of nitric oxide (NO), a potent vasodilator. In HS this phenomenon has been shown to be generalized in nature, since NO production builds up both in the blood [5] and in all the vital organs [2].

We showed earlier that resistance to HS varies in different animal strains. For instance, mortality caused by HS in August rats was found to surpass that of Wistar rats 6-fold [1]. These data suggest that various genetic populations may differ in respect to HS-induced activation of the NO-generation systems.

Laboratory of Physical Chemistry of Biopolymers, Institute of Chemical Physics, Russian Academy of Sciences; *Laboratory of Membrane Mechanisms of Adaptation; **Laboratory of Genetic Mechanisms of Adaptation, Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

The aim of the present study was to verify this assumption by comparing quantitatively the HS-induced accumulation of NO in tissues and organs of Wistar and August rats.

MATERIALS AND METHODS

The experiments were conducted on Wistar and August rats weighing 200-220 g. HS was reproduced by heating alert animals in an incubator until the rectal temperature attained 41°C, 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 electron paramagnetic resonance (EPR) signal with the g-factor being $g_{\perp}=2.035$ and $g_{||}=2.012$ and a 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 was evaluated by the intensity of the EPR

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signal, which was calculated by double integration using a solution of paramagnetic dinitrosyl iron complex with thiosulfate of a known concentration as a standard.

For the accumulation of MNIC in the organism, 30 min after HS the rats 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 in the femur) and 30 min later the animals were decapitated. The liver, kidneys, heart, spleen, intestine, and brain were isolated 1 hour after HS, since this time

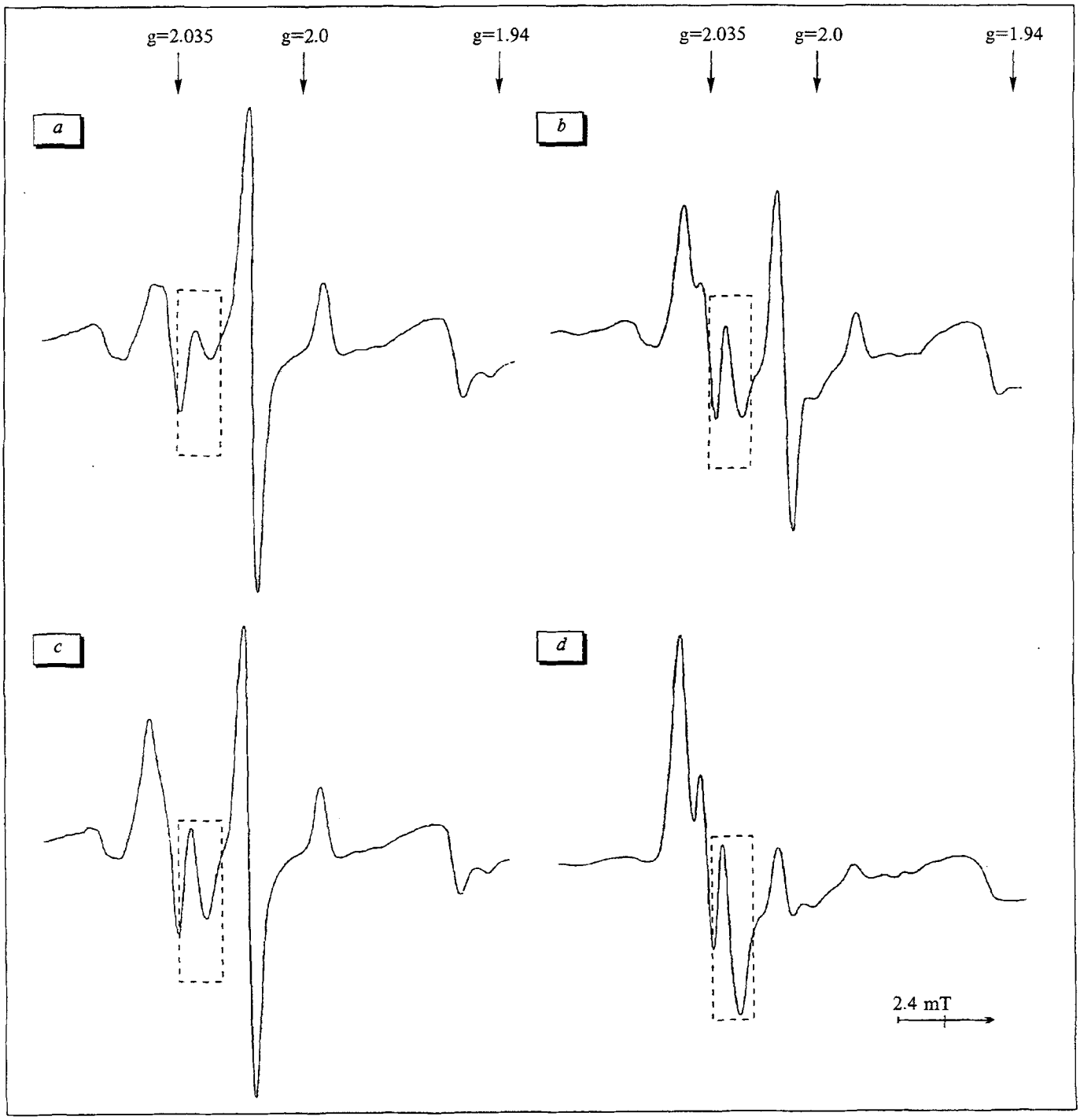

Fig. 1. Electron paramagnetic resonance (EPR) signals of MNIC–DETC in the liver of control and heat shock (HS)–subjected Wistar and August rats. Dotted rectangle shows the component of triplet hyperfine structure of the EPR signal, the amplitude of which served as the measure of the content of MNIC–DETC complexes in the tissue. Wistar rats before (*a*) and after (*b*) HS; August rats before (*c*) and after (*d*) HS.

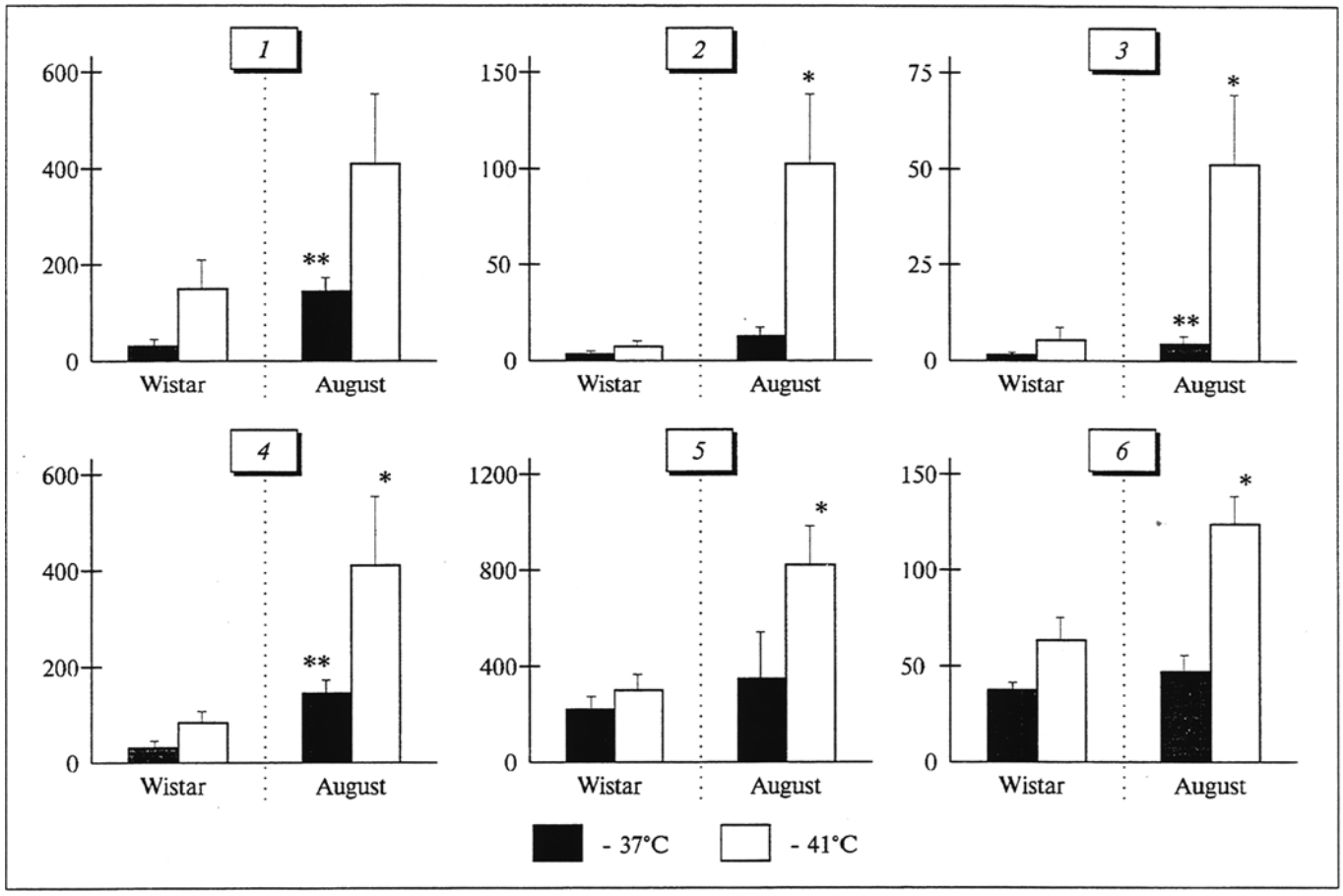

Fig. 2. Differences in NO induction in Wistar and August rats subjected to het shock (HS). *1*) liver; *2*) kidneys; *3*) heart; *4*) spleen; *5*) intestine; *6*) brain. Ordinate: content of MNIC–DETC complexes in tissue (ng NO/g tissue). Reliability of differences between Wistar and August rats: $^{*}p<0.05$ after HS, $^{**}p<0.01$ basal NO content.

has been shown to correspond to the maximal increase of the NO content in Wistar rats. The organs were minced, frozen in a press-mold, and stored in liquid nitrogen.

The EPR signal was recorded on a Radiopan modified EPR radiospectrometer at 77°K, modulation amplitude 0.5 mT, and SHF-power 10 mW.

RESULTS

Characteristic EPR spectra recorded in the liver of Wistar and August rats before and 1 hour after HS are presented in Fig 1. In both strains the amplitude of the EPR signal from the MNIC-DETC complexes, reflecting the content of NO, is increased in hyperthermia. However, in August rats this increase is much more pronounced.

The histograms in Fig. 2, constructed on the basis of statistical processing of the intensity of signals from the MNIC-DETC complexes, make it possible to compare the content of NO in Wistar and August rats not only in the liver but also in other organs before and after HS. In control August rats before HS the content of NO in the kidneys, heart, and spleen was reliably higher than in Wistar rats.

HS induced a more marked increase in the content of NO in different organs of August rats in comparison with Wistar rats: in the kidneys it was 15.5 times higher (115±51 *vs.* 7.4±1.2 ng/g, $p<0.05$), in the liver 3.2 times higher (434±177 *vs.* 133.6±71 ng/g, $p<0.05$), in the heart 10 times higher (54.5±17 *vs.* 5.4±2.3 ng/g, $p<0.05$), in the spleen 6.4 times higher (411±121 *vs.* 63.7±16.1 ng/g, $p<0.05$), in the intestine 2.8 times higher (878±213 *vs.* 307.9±63.7 ng/g, $p<0.05$), and in the brain 1.9 times higher (130±20 *vs.* 67±9.9 ng/g, $p<0.05$).

Thus, in the less HS-resistant August strain HS induces a more pronounced activation of NO synthesis.

NO is involved in numerous physiological processes, including relaxation of smooth muscles, neurotransmission, regulation of immune responses, and regulation of intestinal and microvascular permeability [10]. On the other hand, increased NO production has been implicated in many pathological states such as septic and hemorrhagic shock [10].

Overactivation of the NO-generating systems in August rats may result in a situation where NO no longer acts as a factor of physiological regulation and becomes a pathogenetic factor in the development of vascular collapse and endotoxemic and hemorrhagic complications in HS.

The fact that marked differences in NO generation were found in rats with different levels of resistance to HS suggests that resistance to HS is related to genetic mechanisms of NO generation.

The essence of this hypothetical mechanism is as follows: HS activates lipid peroxidation and stimulates production of free radicals [3]. NFkB protein, NO-synthase gene transcription factor, is an intracellular target for these radicals [7]. This activation of NO-synthase results in the accumulation of NO in various organs in HS. Earlier we demonstrated that the NO content rises rapidly after HS and also rapidly returns to the control level [2]. This attests to negative feedback regulation of the NO-generating system, which limits NO hyperproduction. This negative feedback is presumably mediated through the soxS gene encoding the antioxidant defense enzymes. NO has been found to activate the expression of this gene [8]. Thus, the accumulation of NO activates the antioxidant system via the above-described mechanism, and consequently reduces the content of free radicals activating NO synthesis. This mechanism safeguards the organism against NO hyperproduction. In Wistar rats the capacity of the antioxidant system does indeed increase in response to HS [4].

In August rats, however, the generation of NO is less effectively controlled, evidently due to some peculiarities of expression of the genes encoding the antioxidant defense enzymes.

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