Adaptation to Stress Prevents Acute Hypotension and Endothelial Hyperactivation in Heat Shock

E. B. Manukhina, D. A. Pokidyshev, and I. Yu. Malyshev

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Adaptation to stress reduces animal mortality in heat stress from 57 to 8% and prevents a decrease in blood pressure as well as excessive inhibition of constrictory and potentiation of dilatory reactions of isolated aorta associated with hyperproduction of NO. It is assumed that beneficial effect of adaptation is due to accumulation of a reserve pool of NO, which either has a direct effect, or mediates activation or synthesis of another protective factor.

Key Words: *nitric oxide; adaptation; heat shock; endothelium-dependent relaxation; blood pressure*

Acute hypotension accompanied by inhibition of vascular reaction to constrictory stimuli is the most dangerous cardiovascular disturbance in heat shock (HS). In severe HS, hypotension can become irreversible and cause death. A mechanism of this disturbance is related to hyperproduction of nitric oxide (NO), a potent endogenous vasodilator [3]. This phenomenon underlies hyperactivation of vascular endothelium, i.e., excessive potentiation of endothelium-dependent relaxation (EDR) and excessive inhibition by endothelium of vascular constrictive reactions [4]. Hyperproduction of NO is a common stage in the pathogenesis of various shocks, including septic [14], hemorrhagic [7], cardiogenic [1], and other shocks.

On the other hand, NO is an important regulator of many physiological functions of the organism and normally acts as a protective factor against ischemia, thrombosis, malignant tumors, infections, and other pathological states [11], and therefore, it is important to prevent excessive production of NO but not reduce its basal generation.

This can be achieved, for instance, with measured adaptation to environmental factors. We have found that short-term adaptation to nondamaging stress factors [10] effectively prevents the decrease in blood pressure (BP) and endothelium hyperactivation in experimental myocardial infarction. Therefore, in the present study we explored the possibility of using an analogous protection of the cardiovascular system in HS. To this end we compared changes in systemic BP, dilatory and constrictive endothelium-mediated reaction of isolated vessels, and mortality after HS of nonadapted and adapted to stress experimental animals.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 230-250 g. The animals were adapted to immobilization stress by fixation in the supine position. The duration of immobilization was 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min on day 4. Subsequent immobilization sessions lasted 60 min and were performed every other days. A total of 8 immobilization sessions were performed.

Forty-eight hours after the last immobilization session, the animals were subjected to HS: heating in a thermostat for 15 min till rectal temperature attained 41°C. The total period of heating did not exceeded 35 min. BP was measured on the caudal artery by the indirect bloodless method using an Physiograph DMP-4F device (Narco Biosystem) 1 h after HS.

Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

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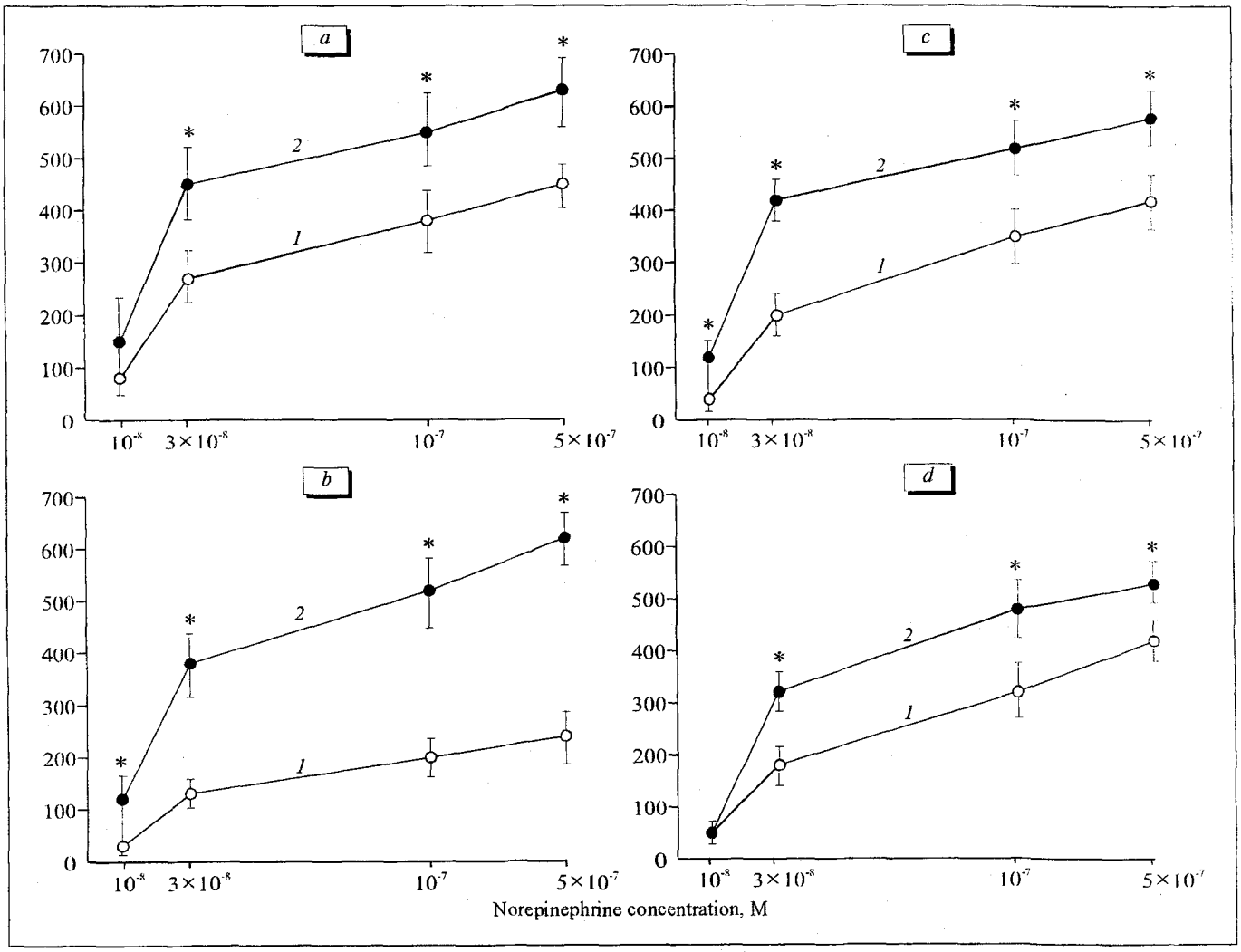

Fig. 1. Effect of adaptation to stress on contraction force of isolated aorta from control rats and animals subjected to heat shock. *a*) control; *b*) heat shock; *c*) adaptation; *d*) heat shock after adaptation; *1*) intact preparation; *2*) blockade of basal endothelial NO production. Ordinate: force of contraction, mg. *$p<0.05$.

The animals were sacrificed immediately after determination of BP, and the thoracic aorta ring preparation was placed into a thermocontrolled chamber (37°C) filled with oxygenated Krebs solution containing (in mM): 130 NaCl, 11 glucose, 14.9 $NaHCO_3$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 1.18 KH_2PO_4 (pH 7.4) at initial tension of 1.2 g. All recordings were performed with a DY-1 isometric transducer using a dual-channel Gemini recorder (Ugo Basile).

Contraction of the preparation was induced by cumulative addition of norepinephrine (NE, 10^{-8}-5×10^{-7} M). Each subsequent concentration of NE was added after the preceding reaction attained the maximum. EDR of the aorta preparation was induced by acetylcholine (10^{-8}-10^{-5} M) when the contractile reaction to 5×10^{-7} M NE attained a plateau. After a 30-min washout, the production of endothelial NO was blocked by a 20-min incubation in the presence of Nω-nitro-L-arginine (L-NNA, 10^{-5} M), and the above-described procedure was repeated. The inhibiting effect of endothelium on constrictory reaction were assessed by the shift of the dose—effect curves after incubation with L-NNA.

The data were processed statistically using the Student's *t* test. The significance of the differences between the areas between the dose—effect curves was assessed by the method of paired comparisons, while the significance of the differences in animal mortality was evaluated using the Fischer test.

RESULTS

One hour after HS we observed a drop in BP from 111±1 to 81±3 mm Hg ($p<0.05$), animal mortality after HS being 57% during the first 24 h. Adaptation to stress had no effect on BP, but completely pre-

vented its decrease after HS (105±3 mm Hg) and markedly reduced HS-related mortality (to 8%, $p<0.05$).

Figure 1 shows dose—effect curves of aorta constriction under conditions of preserved and inhibited endogenous NO production. Elimination of the inhibiting effect of the endothelium on smooth muscle contraction resulted in an upright shift of the dose—effect curve. The area between curves increased significantly after HS ($p<0.05$, Fig. 1, *b*) in comparison with the control (Fig. 1, *a*) due to suppression of the constrictive response of the preparation with preserved NO production. Adaptation to stress had no effect on the constrictive reaction in intact animals (Fig. 1, *c*), but prevented excessive suppression of these reactions in HS (Fig. 1, *d*). The area between curves in both intact and subjected to HS rats did not differ from the control.

As in our previous experiments [2], HS considerably enhanced EDR of the aorta to acetylcholine (Fig. 2): the maximum EDR induced by 10^{-5} M acetylcholine in control animals was 34.8± 4.4%, while after HS it increased to 66.9±4.5% ($p< 0.05$). Adaptation had no significant effect on EDR of the aorta from intact animals, but completely prevented the HS-induced rise of EDR: maximum EDR in animals subjected to HS after preadaptation was 36.2±2.3%, i.e., did not differ from the control (Fig. 2).

Thus, adaptation to stress considerably reduced mortality in animals subjected to HS, prevented the drop in BP and excessive suppression of constrictory and potentiation of dilatory reactions induced by hyperproduction of NO.

Hyperproduction of NO in HS is most probably related to activation of free-radical oxidation [6]. Oxygen radicals via transcription NFκB factor trigger the expression of inducible NO-synthetase (iNOS) in macrophages and vascular smooth muscle cells, which produces excessive amounts of NO for a long time [15]. This results in a sharp decrease of the vascular tone and suppression of vasoconstrictory reactions.

Some investigators suggest that shock-related hypotension is more likely due to activation of constitutive NO-synthetase (cNOS) rather than induction of iNOS. Unlike iNOS, cNOS is constantly present in vascular endothelium and is normally responsible for the balance between contraction and relaxation of smooth muscles [11]. This conception is based on the observation that NO-mediated hyporeactivity of vessels to adrenergic stimulation in shock precedes the induction of iNOS [14]. This assumption is also confirmed by the fact that in our experiments HS potentiates endothelium-dependent suppression of vascular constriction in response to NE and enhances EDR in the presence of acetylcholine.

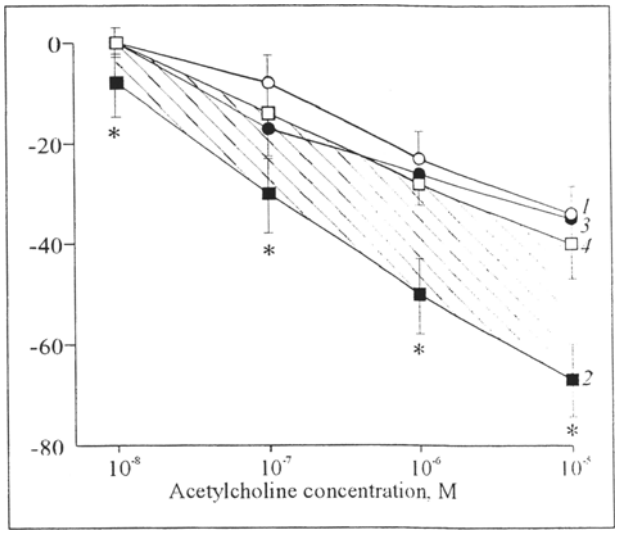

Fig. 2. Effect of adaptation to stress on endothelium-dependent relaxation of isolated aorta from control rats and animals subjected to heat shock. Ordinate: endothelium-dependent acetylcholine-induced relaxation, % of contraction induced by 5×10^{-7} M norepinephrine. *1*) control; *2*) heat shock; *3*) adaptation; *4*) heat shock after adaptation. Hatched zone: protective effect of adaptation.

This points to activation of basal and stimulated NO production, respectively, which is executed by endothelial cNOS [11].

Regardless the contribution of each NOS isoform, their activation can lead to acute and often irreversible circulation failure, which is probably the cause of high animal mortality in HS observed by us. Under these conditions, adaptation to stress effectively prevented both the BP drop and animal mortality. Hence, it can be assumed that this adaptation limits activation and/or expression of NOS.

It has been previously found that adaptation to stress increases the potential of NO-producing systems in the organism [4]. Since adaptation by itself had no effect on the studied functional parameters of the endothelium, it can be hypothesized that excessive NO produced during adaptation is accumulated as a biologically active store [12], and then it is released from the store and either exerts a direct protective effect or mediates activation or synthesis of another protective factor.

The direct protective effect of NO consists in a feed-back inhibition of NOS. This mechanism represents a natural NOS regulation in the organism, which can be successfully reproduced under experimental conditions using NO donors [13]. Moreover, accumulation of asymmetric dimethylarginine, an endogenous inhibitor of NOS, has been demonstrated in stress [7].

Apart from its direct protective effect, NO induces accumulation of other protective factors, in particular heat shock proteins [9]. These proteins are

accumulated in various organs, including blood vessels [8], during adaptation to stress and play an important role in the adaptive protection of the organism [5].

Our findings suggest that adaptation to stress can be regarded as a protective factor under pathological conditions characterized by hyperproduction of NO.

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