Impact of Heat Shock on Endothelium-Mediated Reactions of Isolated Rat Aorta

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In this study of the impact of heat shock on the functional state of vascular endothelium, injection of rats with the NO synthase blocker Nω-nitro-L-arginine immediately after heat shock results in a much lower fall in arterial pressure than in control animals, and the heat shock increases the inhibitory action of endothelium on both the force and rate of norepinephrine-induced contractions in isolated rat aorta, without any alterations in the sensitivity of adrenoreceptors; the endothelium-dependent aortal relaxation in response to acetylcholine also increases. It is shown that enhancement of endothelium-dependent vasodilator responses and inhibition of vasoconstrictor responses may contribute to the fall of arterial pressure and the development of collapse-like states in heat shock.

Key Words: *heat shock; endothelium-dependent relaxation; arterial pressure; nitrogen oxide; NO synthase blocker*

Disorders of cardiovascular functions are among the most severe and most dangerous consequences of heat shock and may lead to collapse and even to death. Whereas the response of the cardiovascular system to mild shock can be described as one of hyperdynamic type, its response to more intensive and longer-lasting heat exposures involves falls in cardiac output, peripheral vascular resistance, and arterial pressure (AP) [12]. The factor triggering collapse in the latter situation is considered to be the reduction in vascular resistance which precedes the development of hypotension and cardiac failure [10].

Although the mechanisms by which vascular tone is impaired in heat shock are poorly understood, several lines of evidence from recent studies suggest that these mechanisms involve participation of endothelial regulatory factors and, in particular, excessive production of nitric oxide (NO), a potent endogenous vasodilator. It has been shown that hyperthermia leads to a marked and sustained increase in the urinary excretion of nitrates [4] and to enhancement of the electron paramagnetic resonance signal from the NO heme in the blood [7].

Recently, our direct measurements demonstrated that heat shock indeed induces a sharp and generalized increase in NO production in the body [1]. However, the endothelium-mediated vascular reactions of isolated aorta remained unexplored. In the present study, therefore, we investigated endothelium-mediated vasodilator and vasoconstrictor responses of isolated rat aorta and AP in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 230-250 g were used. Heat shock was produced in one group by heating awake rats in a thermostat to a rectal temperature of 41°C. When this temperature was reached, the animals continued to be heated for another 15 min. The total duration of heating did not exceed 30 min. Rats of another group were injected intraperitoneally with the NO synthase inhibitor N-nitro-L-arginine (Sigma) in a dose of 200 mg/kg immediately after they were heated as described above.

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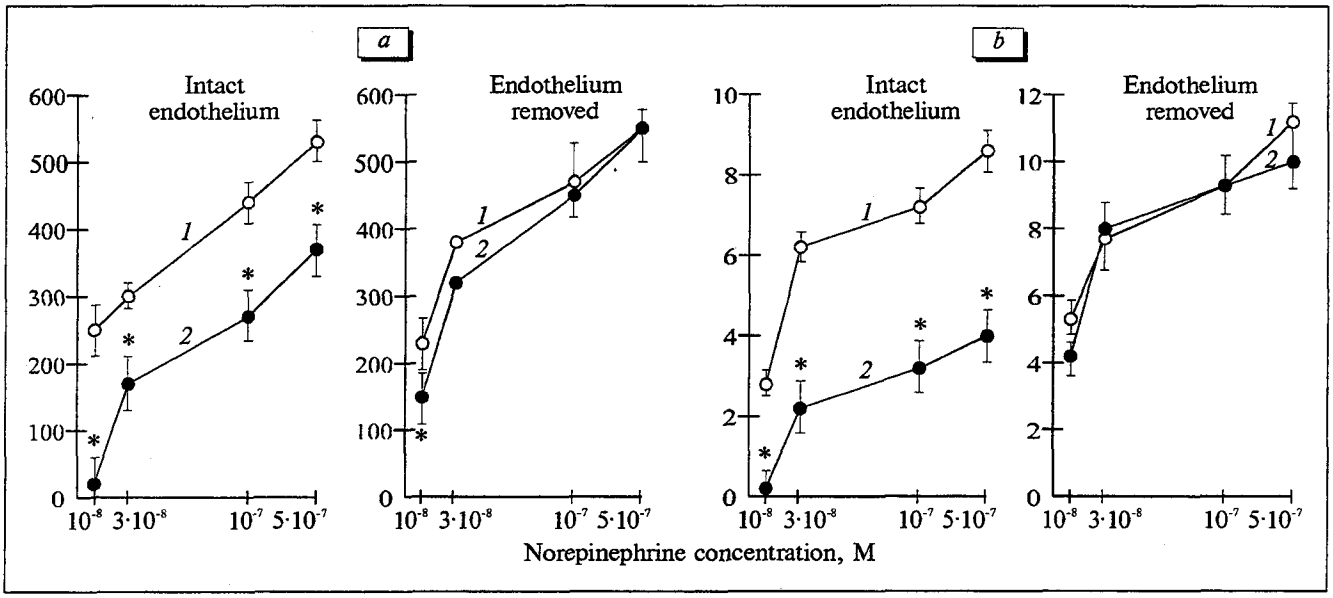

Fig. 1. Effect of heat shock on the force (*a*) and rate (*b*) of norepinephrine-induced contraction by isolated rat aorta. *1*) control rats; *2*) rats with heat shock. Ordinates: contraction force in mg (*a*) and contraction rate in mg/sec (*b*). *$p<0.05$ in comparison with the control group.

One hour after the exposure to heat was completed, AP was measured on the tail artery in awake rats by a bloodless method with a Physiograph DMP-4F apparatus (Narco Bio-Systems). This particular time was chosen for AP measurement because NO production had been shown to be at its peak 60 min after heat exposure [1]. Rats were then decapitated and their abdominal aorta was removed. A ring-shaped aortal preparation 3 mm wide was placed in a thermostated chamber filled with a continuously oxygenated Krebs solution of the following composition (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, 37°C) at an initial stretching force of 1.2 g. Contractions of the intact and de-endothelialized preparations were recorded concurrently on a two-channel Gemini recorder (Ugo Basile) using a DY-1 isometric force sensor. Endothelium was removed mechanically using a special catheter. Aortal contractions were induced with norepinephrine at 10^{-8}-5×10^{-7} M. Smooth muscle adrenoreactivity was estimated from the ED_{50} value calculated using the Ligand computer program. Endothelium-dependent aortal relaxation was induced with acetylcholine (10^{-8}-10^{-5} M) after the contractile response to norepinephrine had reached plateau (5×10^{-7} M).

The results were statistically analyzed by Student's *t* test.

RESULTS

Rectal temperature decreased in rats from 41°C to 39.5°C 30 min after their heating was discontinued and returned to the control level (37°C) 30 min later, i.e., by the time when they were decapitated.

During 1 h after heat exposure, the AP fell from 110±2.2 to 89.8±2.3 mm Hg ($p<0.05$). Rats injected with N-nitro-L-arginine immediately after the heat shock showed a lesser fall in AP: to 104±1 mm Hg ($p<0.05$). This indicates that heat shock activated NO synthase and that the NO produced in large amount made a substantial contribution to the fall in AP.

Figure 1 shows dose—response curves for contractile response to norepinephrine of aortal preparations with intact and removed epithelia. It can be seen that heat shock shifted the curve downward for intact preparations but not for de-endothelialized ones. The preparations of both types showed no significant change in the sensitivity of adrenoreceptors, the ED_{50} being 28±2 and 25±4 for intact preparations in the control and test groups, respectively, and 17.1±1.6 and 18±2 for de-endothelialized preparations in the two groups, respectively. This means that heat shock reduced the force (Fig. 1, *a*) and rate (Fig. 1, *b*) of contractile aortal responses. Inhibition of constrictor reactions in heat shock occurred at the endothelial rather than at the smooth muscle level, and this effect was not mediated by alteration in adrenoreceptor sensitivity.

The curves shown in Fig. 2 enabled us to evaluate the inhibitory influence of endothelium on the aortal contraction force (Fig. 2, *a*) and rate (Fig. 2, *b*) in the control rats and those after heat shock. Removal of endothelium resulted in increased contractile aortal responses to norepinephrine, which is reflected in the upward shift of the dose—response

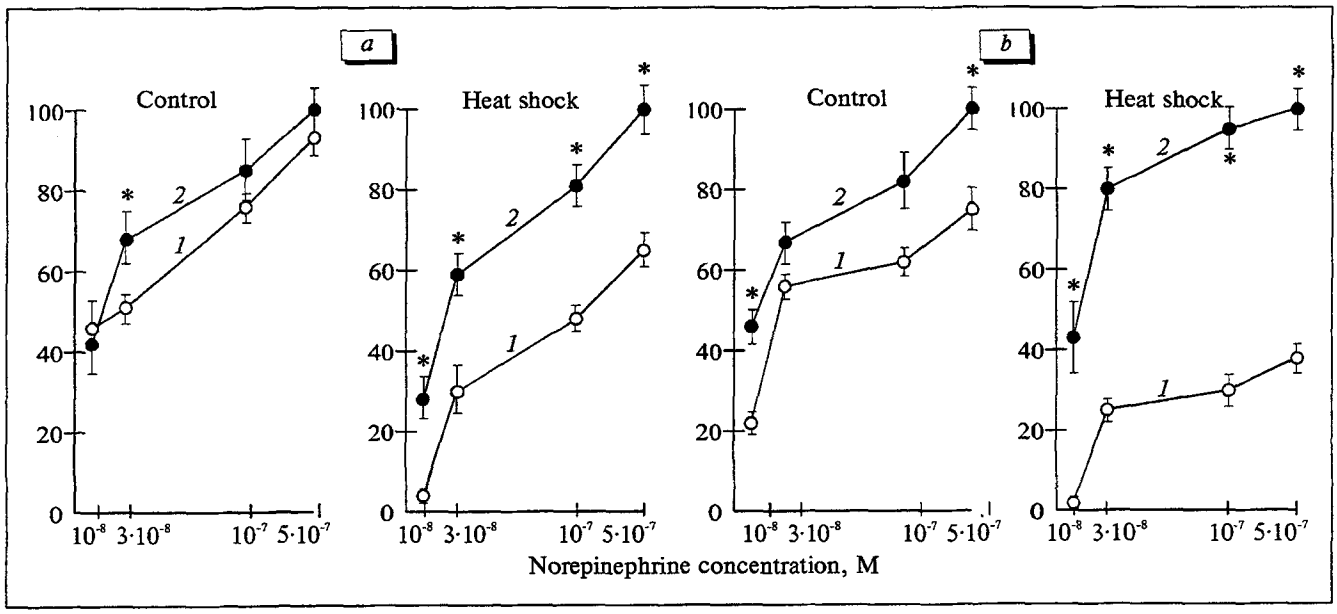

Fig. 2. Enhanced inhibitory effects of endothelium on the force (*a*) and rate (*b*) of contraction by isolated rat aorta after heat shock. Preparations with preserved (*1*) and removed (*b*) epithelium. Ordinates: contraction force (*a*) and rate (*b*) in % of their maximal values. *$p<0.05$ in comparison with the control group.

curves. Heat shock therefore enhanced the inhibitory effects of endothelium on the force and rate of norepinephrine-induced aortal contraction, the effect on contraction rate being stronger than that on contraction force. The difference between these two effects of heat shock can probably be explained by the observation that an increase in the contraction of smooth muscle is not accompanied by any change in the phosphorylation of the light myosin chain within a certain concentration range of intracellular Ca^{2+} [15]. For this reason, contraction force is a much less sensitive indicator than contraction rate of intracellular Ca^{2+} elevation as a result of endothelium removal [5].

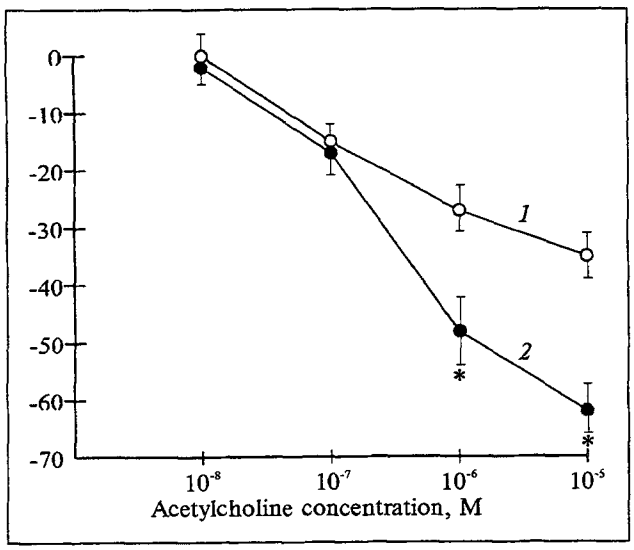

Fig. 3. Impact of heat shock on endothelium-dependent relaxation of isolated rat aorta. *1*) control; *2*) heat shock. Ordinate: endothelium-dependent relaxation, in % of the contraction induced by norepinephrine in a concentration of 5×10^{-7} M.

The endothelium-dependent relaxation of isolated aorta after heat shock significantly increased ($p<0.05$), the greatest relaxation caused by acetylcholine in a concentration of 10^{-5} M, being 62±3.6% after heat shock *vs.* 37±2.2% in the control group (Fig 3).

In considering the possible mechanisms of the phenomena described above it should be borne in mind that heat shock is a powerful stressor and is accompanied, as any stress, by strongly marked activation of free-radical oxidation [2]. Oxygen radicals have been shown to activate, via one of the transcription factors (NFκB protein), the synthesis of various proteins, including NO synthase [15]. NO synthase may also be activated by Ca^{2+} that enters the cells in excess through their membranes damaged as a result of intensified lipid peroxidation [3]. The activation of NO synthase should lead to augmented NO production in the body, which is what actually happens in heat shock [1].

Moreover, the increases in AP and cardiac output observed in the initial phase of heat shock may lead to greater NO release and to enhanced endothelium-dependent vascular relaxation as a result of increased shear stress on the vessel wall [3,14].

Regardless of what mechanism(s) underlies NO hyperproduction, the latter brings about generalized vasodilation. This vasodilation is caused, on the one hand, by the direct endothelium-dependent relaxa-

tion of vascular smooth muscle and, on the other, by the inhibition of vasoconstrictor sympathetic activity at the central level and the NO-mediated vascular hyperactivity in response to vasoconstrictor stimuli at the local level. This chain of events that causes the systemic AP to fall was previously demonstrated for septic shock which, as heat shock, is characterized by pronounced NO hyperproduction [14]. In the study described here we found that the fall in AP occurring in heat shock was accompanied by enhancement of endothelium-dependent relaxation and inhibition of vasoconstrictor responses. This finding, together with our previous observation that NO synthesis is enhanced in heat shock [1], suggests that the above-mentioned phenomena may contribute to the pathogenesis of vascular tone abnormalities in heat shock.

Clearly, NO hyperproduction is not the only cause of AP reduction in heat shock. However, the fact that the fall in AP we observed could be greatly reduced (although not prevented completely) by blocking NO synthase points to a leading role of NO in the hypotension caused by heat shock.

In the case of mild or short-term exposure to heat, when the developing cardiovascular response is of the hyperdynamic type, the rise in NO production is undoubtedly adaptive in nature. Blockade of NO production with N-nitro-L-arginine considerably increases the hyperthermia in response to high ambient temperatures as well as to pyrogens, because the action of NO is directed to improving heat exchange through peripheral vasodilation [6]. In addition, NO can limit the stress reaction by inhibiting activation of the sympathetic nervous system [3], and it also can limit free-radical oxidation by exerting a direct antioxidant action [9] and by inducing synthesis of antioxidant enzymes [11]. With more intensive and longer-lasting exposures to heat, this reaction is no longer adaptive but becomes excessive, leading to various injuries.

However, as the enhancement of NO generation is initially an adaptive response, caution should be exercised when the possibility of preventing a fall in AP by means of NO synthase blockers is considered. Indeed, attempts to raise the reduced AP in animals with septic shock, in which AP reduction is also due in large measure to NO overproduction, led to the development of ischemia, thrombosis, and ultimately to increased mortality among the animals [8]. Both the compensatory and damaging effects of NO should therefore be taken into account in searching for methods by which the body can be protected against heat shock.

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