Is Nitric Oxide Involved in the Adaptation to the Stress-Induced Damage?

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The iron dinitrosyl complex (a NO donor), adaptation to stress, and their combination suppress the stress-induced ulcer formation. Nω-nitro-L-arginine, a NO synthetase inhibitor, reduce the antistress effect of adaptation. Severe stress induces a sharp decrease in the NO production in the liver and brain. After adaptation to stress, the NO production in the liver and brain does not differ significantly from control levels. However, adaptation attenuates a decrease in the NO production in the liver caused by severe stress.

Key Words: *adaptation; nitric oxide; NO donor; NO synthetase inhibitor; stress; gastric ulcer; protection*

Social, physical, biological, and other stress factors play an important role in the pathogenesis of various diseases and their complications. There are two methods of increasing the body's resistance to stress. The first method uses of psychotropic and adaptive drugs [1]. The second method is based on the adaptation to various environmental factors [3]. The use of adaptation to repeated stress stimuli allows one to increase the body resistance to stress. The efficiency of this method was shown to be no less than that of pharmacotherapy [3]. On the other hand, pharmacotherapy is often accompanied by side effects. The mechanisms underlying the antistress effect of adaptation are unclear. This limits the possibilities of adaptation.

Nearly all major systems of the body (for example, the nervous and cardiovascular systems) are involved in the development of protective effects of adaptation to stress [3]. In the analysis of the antistress effects of adaptation, consideration must be given to one of the most common regulators of physiological functions of these systems, nitric oxide (NO) [9]. NO donors were shown to increase the body resistance to stress [7]. Long-term adaptation to stress (14 days) increases the NO production in various organs [4]. These data suggest that NO is involved in the antistress effect of adaptation to repeated stress stimuli.

Here we checked up this hypothesis and studied the influence of NO donor and NO synthetase (NOS) inhibitor on the antistress effects of adaptation to repeated stress stimuli and the NO production under stress and adaptation.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-220 g. Immobilization of the rats in individual plastic tubes (with neck fixation) was used as the stress procedure. The animals were immersed in water (21±1°C) up to the neck for 3 h. The resistance to stress was evaluated by calculating the area of the gastric mucosa ulcers. There were "early" ulcers with erosive surfaces and "developed" ulcers with necrotized bases [6]. The rats were killed by decapitation 1 h after the stress procedure. Adaptation to stress was performed by two 3-day immobilizations separated by a 2-day period. Each session was carried out according to the following protocol:

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the first day, 5-min immobilization; the second day, 10-min immobilization; and the third day, 15-min immobilization. The iron-dinitrosyl complex (IDNC; 200 μg/kg, i. v., 24 h before immobilization) was used as the NO donor [15]. Nω-Nitro-L-arginine (L-NNA, Sigma; 50 mg/kg, i. p., 1 h before immobilization) was used as the inhibitor of NOS. The NO production was estimated by electron paramagnetic resonance (EPR) [14]. The NO entrapping in the complexes containing exogenous Fe^{2+}-diethyldithiocarbamate (the NO spin-trapping reagent) was monitored. The NO trap was administered immediately after the stress or 30 h after the last adaptive procedure. All rats were killed 30 min later. The production of NO was evaluated by measuring its contents in the liver and brain. These organs differed from other organs in their relatively high initial levels of NO. Statistical analysis used the Wilcoxon—Mann—Whitney test (U criterion).

RESULTS

Adaptation to repeated stress suppressed the formation of ulcers with necrotized bases (Fig. 1). The NO donor did not affect the ulcer formation. Under the combined action of these factors, the NO donor potentiated the protective effect of adaptation. The NOS inhibitor practically did not affect the formation of ulcers with necrotized bases. The administration of this inhibitor during adaptation led to the formation of perforating ulcers in 40% of the animals. This effect was not observed in another groups (data not shown). The area of ulcers with necrotized bases in these rats was smaller than that in the control.

The NO donor and adaptation decreased by 80% the area of ulcers with erosive surfaces (Fig. 2). However, their combined use did not potentiate the protective effect. The NOS inhibitor did not affect the formation of ulcers with erosive surface. The area of ulcers with erosive surface decreased under combined effects of the NOS inhibitor and adaptation. However, the number of perforating ulcers increased in 40% of the animals (the data are not shown in Fig. 2). Thus, the NOS inhibitor decreases the area of erosive and necrotized ulcers, and inhibits the protective effect of adaptation.

Thus, the NO donor, adaptation to repeated stress, and their combination reduce the stress-induced ulceration. The NOS inhibitor alone does not affect the ulcer formation in stress and decreases the protective effect of adaptation. These results agree with the hypothesis that NO is involved in the mechanisms underlying adaptation.

Stress induced a sharp decrease in the NO production in the liver and brain from 46.9±8.9 ng NO/g tissue and 11.9±2.9 ng NO/g tissue, respectively, almost to zero (more precisely, to the lowest degree of EPR sensitivity). After adaptation, the production of NO in the liver and brain did not differ from the control level. However, adaptation to stress significantly attenuated a decrease in the NO production in the liver after a severe stress procedure (Fig. 3). These data confirm our hypo-

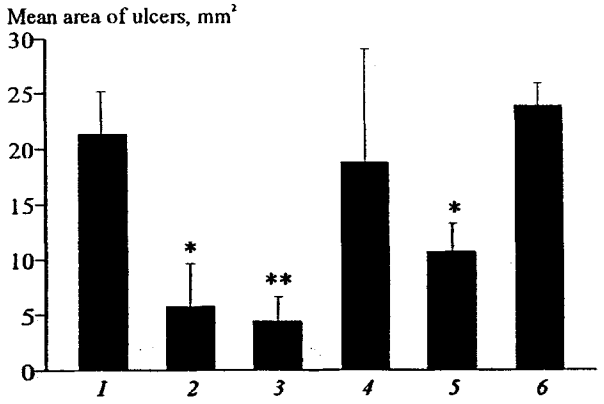

Fig. 1. Influence of NO donor (IDNC) and NOS inhibitor (L-NNA) on the antistressor effect of adaptation to repeated stress stimuli; the formation of gastric ulcers with necrotized base. Here and in Fig. 2: *1*) stress; *2*) adaptation+stress; *3*) adaptation+IDNC+stress; *4*) IDNC+stress; *5*) adaptation+L-NNA+stress; and *6*) L-NNA+stress. *$p<0.05$ and **$p<0.01$, respectively, compared with control (here and in Figs. 2-4).

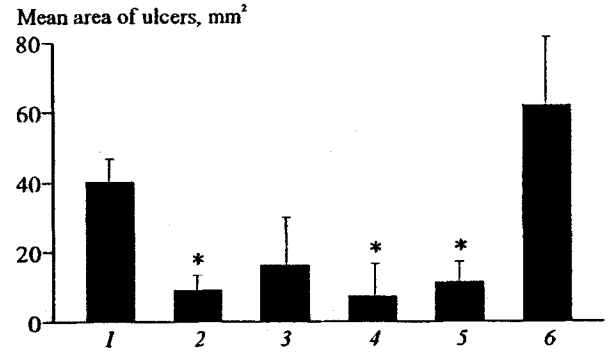

Fig. 2. Influence of the NO donor (IDNC) and NOS inhibitor (L-NNA) on the antistress effect of adaptation to repeated stress stimuli; the formation of gastric ulcers with erosive surface.

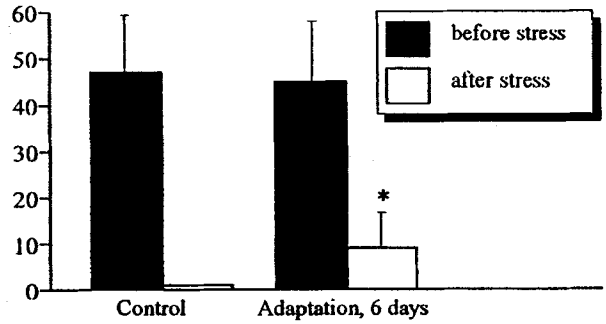

Fig. 3. Effect of severe stress on the NO production in the liver in control and stress adapted animals.

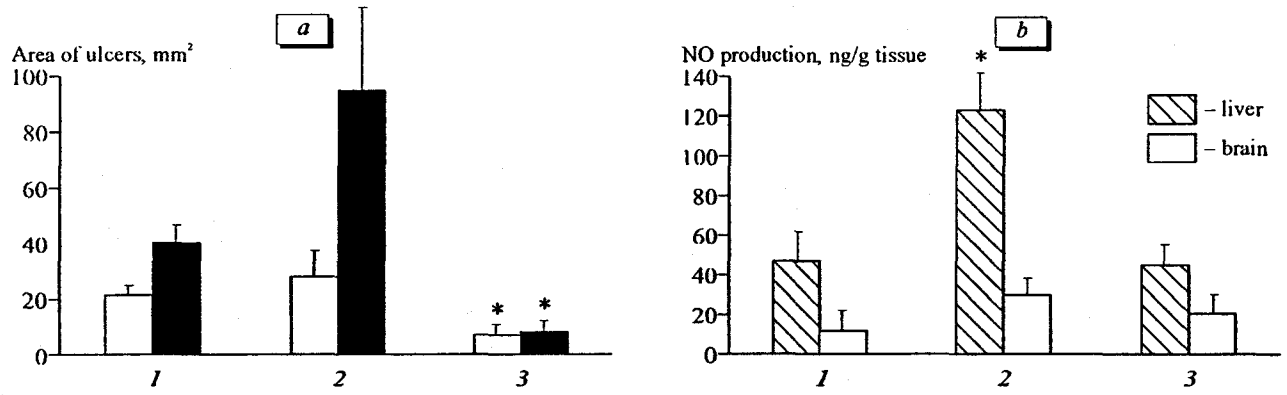

Fig. 4. Dynamics of the antistress effects and changes in NO contents in organs during adaptation to repeated stress. Light bars, the area of ulcers with necrotized base; dark bars, the area of ulcers with erosive surface. A: *1*) control+stress; *2*) 3-day adaptation+stress; and *3*) 6-day adaptation+stress. B: *1*) control; *2*) 3-day adaptation; and *3*) 6-day adaptation.

thesis that NO is involved in the protective effects of adaptation.

Then, we compared the dynamics of the protective effects with the dynamics of the NO production during adaptation to stress. On the third day of adaptation, the stress-induced erosive ulcers were 2.5 larger than in the control (Fig. 4). A strong antiulcer effect was observed on the sixth day of adaptation. It is interesting that the NO production in the brain and liver increased by 2.5 times after a 3-day adaptation and did not differ from control levels after a 6-day adaptation.

Thus, the dynamics of NO production during adaptation to stress is characterized by a transient increase on the third day of adaptation followed by its restoration to the initial level on the sixth day. The significance of this increase in the production of NO remains unclear. The first possibility is that overproduction of NO is a factor contributing to a decrease in the body resistance to stress-induced ulceration on the third day of adaptation. Another possibility is that an increase in the NO production is associated with activation of protective mechanisms formed on the sixth day of adaptation. The fact that the NOS inhibitor reduces the antistress effect of adaptation (Figs. 1 and 2) indicates the protective role of NO overproduction in adaptation.

Our studies demonstrate that an increase in NO production during adaptation precedes the development of its protective effects (Fig. 4). Therefore, the role of NO in the adaptive protection is obviously associated with its ability to activate endogenous protective mechanisms in the body rather than with direct protective activity of NO, for example, with the ability of NO to decrease the secretion of catecholamines [13], to provide normal trophic functions and motor activity of the stomach [9], and to inhibit stress-induced vasoconstriction of gastric arterioles. NO activates the synthesis of HSP70 proteins [8] which protect various organs from stress-induced damage [11] and are provably accumulated during adaptation to stress [5]. Moreover, NO is involved in the activation of antioxidant enzymes [10] and stimulates the synthesis of prostaglandins [12], which act as local factors of antistress protection.

The contribution of these NO-dependent mechanisms remains to be evaluated. However, our data suggest that NO plays an important role in the antistressor effects of adaptation to repeated stress. Modeling of NO generation systems will allow one to modify the resistance to stress.

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