Protective Effect of Adaptation to Stress Against the Hemorrhagic Shock-Induced Damage: the Role of the Antioxidant System

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Modeling of hemorrhagic shock in rats adapted to immobilization stress required the removal of greater volumes of blood than that in control rats. The antioxidant system activation in adapted rats was accompanied by an increase in resistance to blood loss. The antishock effect of preliminary adaptation to stress was shown for the first time.

Key Words: *hemorrhagic shock; adaptation; stress; antioxidant protection*

The activation of free-radical reactions in various organs and tissues is a factor determining the severity of the damage caused by hemorrhagic shock. In spite of the fact that blood loss induces generalized ischemia in all body tissues, the severity of the damage to various organs is different. Free-radical oxidation in these tissues is activated to various degrees. Therefore, the activation of the antioxidant system is important in shock. Various organs require various degrees of protection against reactive oxygen species. Superoxide dismutase (SOD) and catalase are the most abundant antioxidant enzymes. A decrease in their activity may determine the severity of tissue damage. However, there are only several works devoted to the comparative study of the activities of these enzymes in various organs under hemorrhagic shock [5,15]. These data account for the increasing interest of researchers and doctors in antioxidant preparations and the search for new methods of the prevention and correction of hemorrhagic shock. For this purpose, SOD and catalase of various origin and SOD-like compounds have been extensively used. However, the administration of exogenous protein-like and nonprotein chemical compounds is not the only method for increasing antioxidant protection and general resistance of the body. Activation of antioxidant enzymes was observed after adaptation to various environmental factors [8,9,14]. Adaptation to short-term stress is the most effective in this respect. This process was shown to be accompanied by the activation of some components of the antioxidant system in the body.

Here we measured the activities of SOD and catalase, the concentration of SH-groups, and the content of lipid peroxidation (LPO) products in various organs of rats subjected to hemorrhagic shock. Adaptation to short-term stress was used as the method for prevention of shock-induced damage.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g. They were divided into four groups of ten animals each: control (group 1), hemorrhagic shock (group 2), adaptation to stress (group 3), and adaptation followed by hemorrhagic shock (group 4). After catheterization of the femoral artery under sodium ethaminal anesthesia, hemorrhagic shock was modeled by an hour fractional bleeding accompanied by a regular decrease in arterial pressure (AP) to 40-45 mm Hg (Fig. 1). The rats were adapted to a short-term stress by 1-h limb im-

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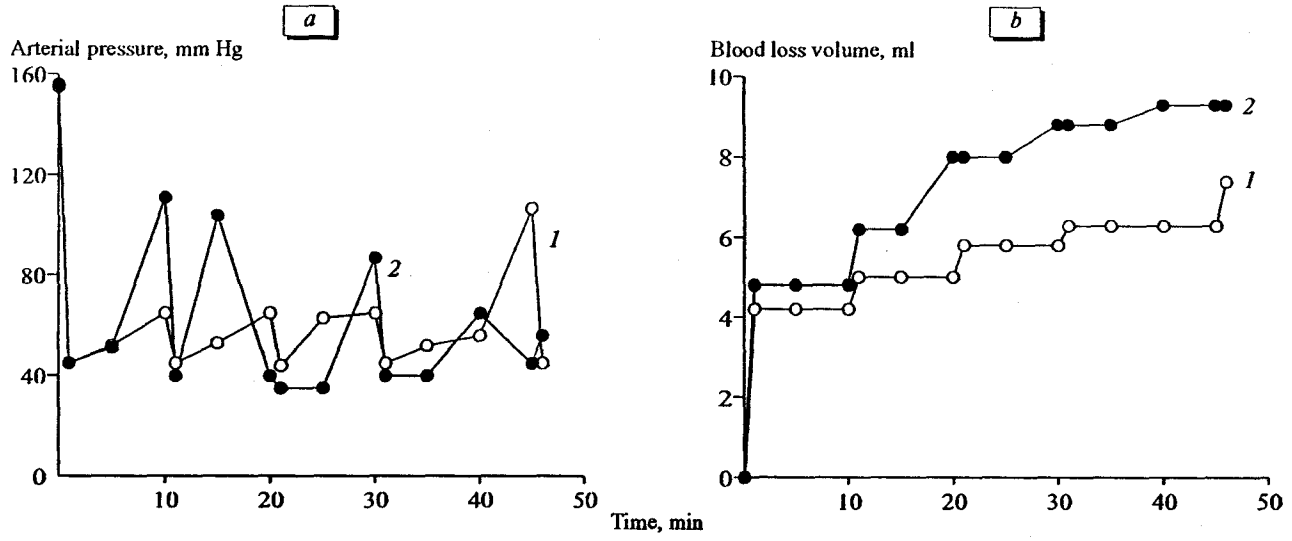

Fig. 1. Changes in *a*) arterial pressure and *b*) blood loss during fractional bleeding in control rat (*1*) and in the rat subjected to preliminary adaptation to immobilization stress (*2*).

mobilization procedures performed at daily intervals for 12 days. They were killed by decapitation 3 h after the beginning of bleeding. The heart, brain, and liver were removed and frozen in liquid nitrogen. Homogenates were prepared with an Ultra-Turrax knife or a Teflon-glass homogenizer. SOD activity was assayed by measuring the reduction of tetranitro blue tetrazolium with superoxide anion radicals at 560 nm [4]. The difference between the rates of the superoxide radical formation in the xanthine/xanthine oxidase system before and after the addition of tissue homogenate was estimated. A unit of SOD activity was defined as the amount of the enzyme providing a twofold inhibition of the reduction of tetranitro blue tetrazolium. Catalase activity was assayed spectrophotometrically by analyzing the composition of exogenous H_2O_2 at 240 nm. The method modified for tissue homogenates was used [6]. The activity was calculated with a molar extinction coefficient of 39.4 $mol^{-1} \times cm^{-1} \times liter$) and expressed as mmol H_2O_2/min/mg protein. The concentration of the common SH-groups was measured at 412 nm [13]. The content of TBARS was estimated spectrophotometrically by the absorption spectrum at 500-570 nm (the maximum at 532 nm) [10] and expressed as optical density units/mg protein.

The data were analyzed using the Student *t* test.

RESULTS

Figure 1 shows that modeling of hemorrhagic shock in rats adapted to immobilization stress required the removal greater volumes of blood (for maintaining AP at 40-45 mm Hg) than that in control rats (groups 2 and 4, respectively). During the first phase of hemorrhagic shock, AP was restored (after blood loss) more rapidly in rats adapted to emotional stress than in group 2 animals.

Hemorrhagic shock caused a 21.4% increase in the concentration of LPO products in the brain. However, the concentration of LPO products decreased by 35.7% in group 4 animals (Table 1). SOD activity increased in groups 2 and 3. The activity of catalase and the concentration of SH-groups remained unchanged in all groups.

In group 2 rats, a 31.2% increase in the concentration of LPO products and an increase in SOD activity in the liver were observed.

The heart catalase activity increased in the animals of groups 3 and 4.

Modeling of hemorrhagic shock is based on a decrease in AP to 40 mm Hg and its further maintaining at this level by gradual bleeding [1].

Adaptation of rats to stress increased their resistance to blood loss. This is reflected by increased volume of blood necessary for providing such a low AP as in nonadapted rats (Fig. 1). A smaller blood loss did not provide the AP level essential responsible for appropriate modeling of hemorrhagic shock. The compensatory mechanisms for the restoration of lowered AP were more effective in animals adapted to stress (especially at the early phase of hemorrhage, when AP was restored to 100 mm Hg over a few minutes after blood loss). The protection provided by preliminary adaptation to stress against the hemorrhagic shock-unduced damage is probably associated with the increase in the circulating blood volume [7] and adrenergic reactivity of blood vessels [3].

TABLE 1. Changes in the LPO and Antioxidant System Indices in Rats Subjected to Hemorrhagic Shock and Adaptation to Stress and in Adapted Rats Subjected to Shock (*M±m*)

Group	LPO, U optical density/mg protein	SOD, U/mg protein	Catalase, H_2O/min/mg protein	SH-groups
Brain				
Control, group 1	0.028±0.009	10.3±2.2	0.8±0.2	0.51±0.08
Shock, group 2	0.034±0.005*	13.3±3.8*	0.9±0.3	0.5±0.1
Adaptation, group 3	0.028±0.01	12.4±1.1*	0.8±0.3	0.5±0.08
Adaptation+shock, group 4	0.018±0.008*	12.4±1.6*	0.8±0.1	0.5±0.05
Liver				
Control, group 1	0.026±0.007	15.8±1.9	139.7±12.9	0.49±0.1
Shock, group 2	0.035±0.007*	20.2±3.1*	135.1±19.8	0.49±0.1
Adaptation, group 3	0.021±0.008	16.6±4.1	140.7±38.3	0.45±0.07
Adaptation+shock, group 4	0.028±0.01	17.8±3.8	151.1±22.7	0.43±0.06
Heart				
Control, group 1	0.04±0.02	12.4±2.6	5.6±1.7	0.32±0.07
Shock, group 2	0.04±0.01	11.6±1.4	6.2±1.3	0.33±0.06
Adaptation, group 3	0.05±0.01	12.0±1.9	7.5±1.5*	0.32±0.05
Adaptation+shock, group 4	0.04±0.01	12.7±2.8	7.2±0.8*	0.33±0.05

Note: *$p<0.05$ compared with control.

The activation of LPO under hemorrhagic shock was observed in the brain and liver (Table 1). SOD was activated in these organs during hemorrhagic shock. This may reflect the compensatory reaction of the body which decreases the intensity of free-radical processes. These data suggest that the generation of $O_2^{\cdot-}$ in hemorrhagic shock contributes to LPO activation. It is unlikely that H_2O_2 is involved in the key stages of LPO activation during shock because the activity of catalase in these organs remains at the control level.

Adaptation to stress increased SOD activity in the brain, while the level of LPO products remained constant [9]. The degree of SOD activation induced by shock and adaptation to stress (shock in preliminary adapted rats) is not higher than that when each factor acts individually. This is due to different causes of $O_2^{\cdot-}$ generation in the brain and temporal disconnection of these processes during shock and adaptation to stress. However, the levels of LPO products in the brain in adapted rats subjected to hemorrhagic shock were lower than those in unadapted rats and control rats. This was due to the protection provided by adaptation to stress against brain damage induced by shock.

The heart was the least susceptible to blood loss. LPO processes were not activated in this organ. The increase in catalase activity in the myocardium after adaptation to stress may result from a specific reaction of the heart to the increased level of catecholamines whose oxidation causes a free-radical tissue damage [12].

In parallel with the absence of changes in the catalase activity in the brain and liver, our results indicate that the generation of $O_2^{\cdot-}$ (but not of H_2O_2) is most important in shock. Therefore, the antishock effects of SOD-containing antioxidant preparations should more pronounced than those of catalase-containing preparations [11].

Thus, adaptation to short-term stress protects the brain and liver against the damage caused by hemorrhagic shock. This is due to activation of the antioxidant system and inhibition of LPO processes.

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