Increased Generation of Nitric Oxide in Tissues of Rats Following Their Adaptation to Short-Term Stress (An EPR Study)

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Adaptation of rats to short-term immobilization stress increases the ability of their isolated organs to generate nitric oxide (NO): its spontaneous release by the liver, gut, heart, and kidney tissues rises 2- to 4-fold and its carbachol-stimulated release by these tissues rises 4- to 5-fold. It is suggested that such adaptation leads to rapid NO generation in the adapted animal in response to exogenous or endogenous stimuli and thus increases the efficacy of defense reactions.

Key Words: *nitric oxide; adaptation to stress*

It has been established that increased (within physiological limits) generation of nitric oxide (NO) may play a substantial role in many defense reactions of the body. Thus, macrophage-generated NO is cytotoxic to blastomatous and bacterial cells [7], and the increased generation of NO from compounds introduced into the body results in the dilatation of coronary vessels and can thus eliminate or limit the damage caused to the heart by ischemia or reperfusion [11]. NO has also been shown to modulate sympathetic effects [6] and to exhibit antimitogenic activity [5]. On the other hand, adaptation to repeated stress factors, which is our concern here, not only protects from stress-induced damage as such [2] but also produces a wide range of protective cross-effects. It can, for example, protect the heart from ischemia- and reperfusion-induced damage [2], modulate the regulatory adrenergic effects on resistive vessels in favor of their dilatation [3], and exert a strong protective effect in sublethal hypoxia [4].

These similarities between the protective effects of stress adaptation and the physiological effects of NO suggest that adaptation to multiple noninjurious stress may increase the power of the NO-generating system in organs and tissues, thereby augmenting NO generation, both spontaneous and stimulated.

The purpose of this study was to explore how preliminary adaptation to short-term immobilization stress might influence spontaneous and carbachol-stimulated NO generation by tissues of internal organs in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 200-230 g were used for the experiment. A course of adaptation consisted of eight exposures on alternate days to stress produced by immobilizing the animals on the back with fixed limbs. The first exposure lasted 15 min, the second 30 min, the third 45 min, and the other five 60 min each. The tests were started 48 h af-

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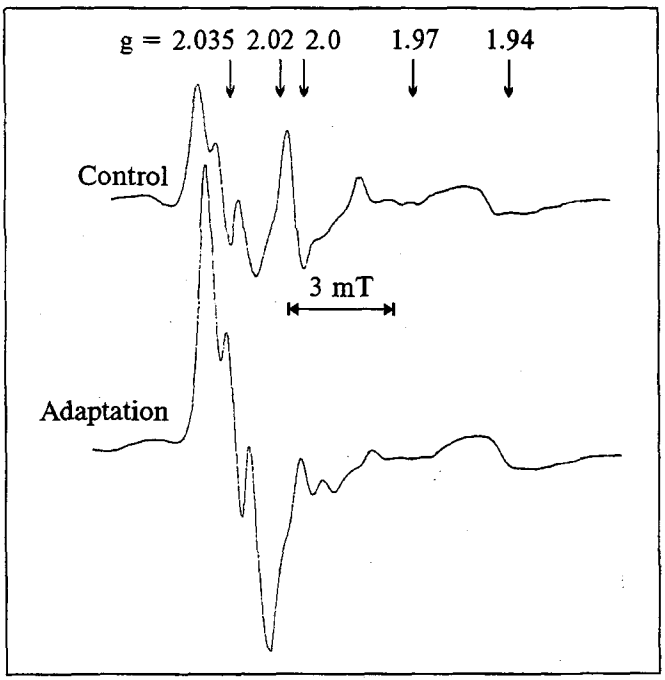

Fig. 1. Typical EPR signals of MNIC–DETC recorded for liver tissue from control and stress–adapted rats.

ter the last (8th) adaptation session. Intact rats served as controls.

The amount of NO produced in rat tissues was estimated by the incorporation of this oxide into complexes with Fe^{2+}-diethyldithiocarbamate [Fe^{2+}-DETC $(C_5H_{10}NS_2)_2Fe$] to form paramagnetic mononitrosyl iron complexes (MNIC) with DETC (MNIC-DETC). The latter complexes are characterized by an EPR signal with g-factor values of g_I=2.035 and g_{II}=2.012 and by a hyperfine triplet structure at g_I (Fig. 1). From the intensity of this signal, calculated by the method of double integration using as the standard a solution of paramagnetic dinitrosyl iron-thiosulfate complexes of a known concentration, the content of MNIC-DETC in each test sample and thus the quantity of NO incorporated into these complexes was estimated. The procedure used for the quantitation of NO in animal tissues has been described in detail previously [13].

MNIC formation was induced in rats by injecting them with a Na-DETC ($C_5H_{10}NS_2Na$) solution intraperitoneally (500 mg/2.5 ml H_2O/kg body weight) and a $FeSO_4$ + $C_6H_5O_7Na_3$ solution subcutaneously (20 mg + 95 mg/2.5 ml H_2O/kg body weight). To stimulate NO formation, the rats were injected, 10 min later, with carbachol intraperitoneally at 375 μg/2.5 ml H_2O/kg, which has been shown to increase both the production and release of NO [1]. Thirty minutes after the Na-DETC injection, the rats were decapitated and their organs (heart, liver, kidneys, spleen, and small intestine) were minced and the tissues obtained were frozen in molds and stored in liquid nitrogen prior to analysis.

EPR signals from the samples were recorded on a Rubin EPR radiospectrometer at 77°K, a field modulation amplitude of 0.5 mT, and an SHF power of 10 mW. The content of MNIC-DETC per tissue sample was estimated from the signal intensity.

RESULTS

Data on NO generation in the organs studied are presented in Table 1. NO-carrying MNIC-DETC were formed in both the control and adapted animals, but the adaptation led to enhanced spontaneous generation of these complexes in all organs so that the organs of adapted animals contained 2 to 4 times more MNIC-DETC than did those of the controls. The logic of the method used signified that NO generation was increased following the adaptation. The typical EPR spectra shown in Fig. 1 permit visual evaluation of this phenomenon with reference to the liver. It can be seen that the EPR signal recorded for the liver of an adapted rat was much stronger than that obtained for a control rat's liver.

The injection of carbachol, a long-acting synthetic analog of acetylcholine, led to a significant increase of NO generation in all organs with the exception of the spleen, where it decreased. This exception can probably be explained by the enhanced activity of hemoxygenase I in the spleen (where this enzyme functions to destroy erythrocytes) and will be discussed in a separate article.

Two important circumstances should be taken into consideration when evaluating the present results. First, as found in separate tests, NO generation by tissues of rats exposed to only one immobilization stress for 60 min and killed immediately thereafter did not differ from that recorded for the control group. Second, the rats used for the tests described above were killed 48 h after the last (8th) exposure to stress, and this implies that it was the adaptation to repeated stress which was responsible for the observed augmentation of NO generation. It is important to note that the increases in spontaneous and stimulated NO generation recorded in this study occurred after a fairly prolonged period of adaptation (16 days) rather than rapidly under the action of some stimulant.

At the basis of such long-term adaptation to various environmental agents, including multiple stress factors, is the activated synthesis of nucleic

TABLE 1. Amounts of NO Incorporated into MNIC–DETC Complexes in Organs of Control and Stress–Adapted Rats. The Values are Means±SEM

Organ	NO generation, ng/g wet tissue:			
	spontaneous (n=5)		carbachol-stimulated (n=8)	
	control rats	stress-adapted rats	control rats	stress-adapted rats
Heart	1±1	10±10	6±3	23±10
Liver	43±10	100±23	66±10	197±30**
Kidney	1±1	26±10**	6±3	30±10**
Spleen	16±10	77±26	6±3	27±6**
Gut	110±20	377±186	100±20	457±60**

Note. n – number of animals. Significant differences ($p<0.05$) from spontaneous NO generation and from the control are indicated by one and two asterisks, respectively.

acids and proteins, primarily of enzymes in the system responsible for adaptation [8].

In our particular case, the most likely factors underlying the observed increases in spontaneous and stimulated NO generation were activated synthesis and increased population of inducible NO-synthase (an enzyme catalyzing NO production through conversion of arginine into citrulline) [10]. Synthesis of NO-synthase is determined by several genes [9], whose expression could increase in the process of adaptation.

A fact which is crucial for the proper interpretation of our results is that spontaneously increased NO generation such as that detected in this study of isolated and disintegrated organs cannot take place in the living organism as a continuous phenomenon, as this would inevitably lead to severe systemic damage because of the well-known toxicity of NO [7,9]. Yet stress-adapted animals exhibit a high resistance to injurious agents. In this connection it should be borne in mind that NO can be stored in cells as a bioactive pool of S-nitrosylated proteins or dinitrosyl iron complexes [12].

It is therefore possible that during the progressive development of long-term adaptation, the potential strength of the NO-generating system increases along with the potential of the systems binding this oxide with the formation of depots. Consequently, when certain exogenous or endogenous stimulants act on the body, NO is rapidly generated (though over a time short enough not to cause damage), and this permits the development of efficient defense reactions and in particular of the above-mentioned protective effects of stress adaptation.

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