Endothelial Function and Nitric Oxide Production in Rats Adapted to Intermittent Hypoxia

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Adaptation to intermittent hypoxic hypoxia did not affect the endothelium-dependent relaxation of the aorta in rats, but significantly increased the relaxation of their tail artery. Following the adaptation, the NO level fell in the liver, intestine, and kidneys and remained unchanged in the spleen. Adaptation to hypoxia presumably limits NO synthesis and/or release in the vascular endothelium or enhances the capacity of this oxide to bind in a physiologically active depot.

Key Words: *adaptation to hypoxia; nitric oxide; endothelial relaxation factor; endothelium-dependent relaxation; tail artery*

Nitric oxide (NO), which is the active principle of endothelial relaxation factor, is known to play a pivotal role in the prevention of excessive vasoconstriction. Diminished NO production by vascular endothelium is an important factor in the pathogenesis of several diseases (e.g., hypertension and angina pectoris) in which vascular tone is increased [10]. On the other hand, excessive production of endothelial relaxation factor may lead to drastic falls in the vascular tone and systemic arterial pressure (AP), as is observed to occur in myocardial infarction and severe stress [1,4] and in different forms of shock [10]. Hence the importance of finding agents that can correct and prevent such conditions without disturbing the endothelium-mediated responses of vessels and systemic AP. One agent of this type is intermittent hypoxia, adaptation to which, as shown in animal studies, inhibits the development of experimental hypertension [5] and prevents AP from falling in acute myocardial infarction as a result of excessive NO production by the endothelium [1], but does not alter AP in normal animals [9]. However, the mechanisms of such dissimilar effects produced by adaptation to hypoxia remain unexplored. In the present study on rats we compared the effect of adaptation to intermittent hypoxia on endothelium-dependent vessel relaxation with its effect on the production of NO, the mediator of this relaxation.

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MATERIALS AND METHODS

Male Wistar rats weighing 300-330 g were used. They were adapted to hypoxic hypoxia in a hypobaric chamber. The adaptation course consisted of 35 daily sessions of 4 h each at an "altitude" of 1000 m on day 1, 2000 m on day 2, 3000 m on day 3, and 4000 m on days 4-35. Rats unadapted to hypoxia served as controls. The rats were killed by decapitation 48 h after the last session. In one series of tests, a 3-mm-wide circular preparation of the thoracic aorta from each rat was placed in the working chamber of an "organ-bath" system (Ugo Basile) filled with a continuously oxygenated Krebs solution at 37°C. The initial tension was 1200 mg. Endothelium-dependent relaxation of the aorta was

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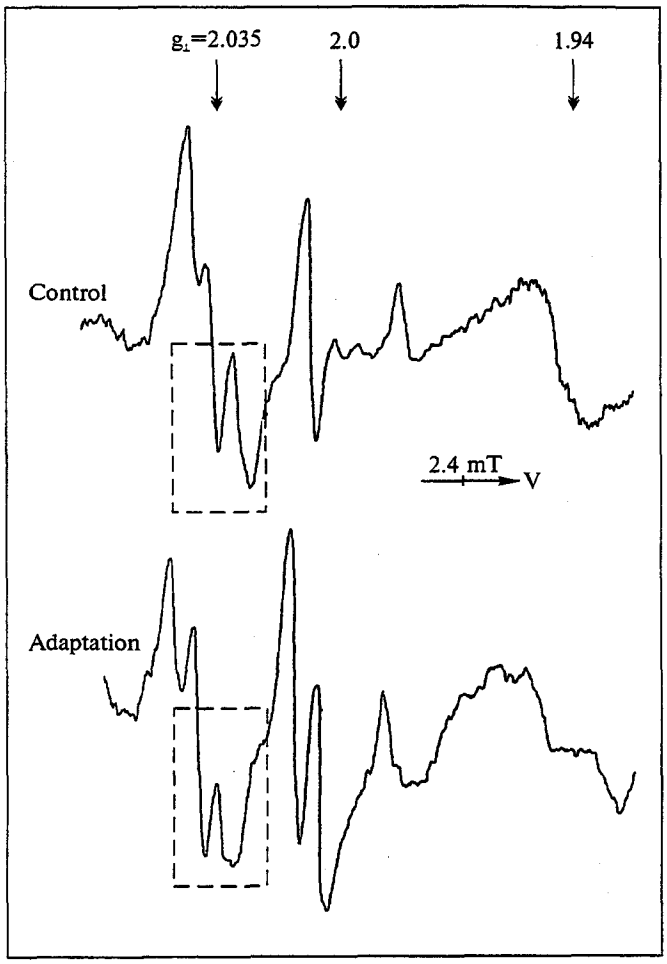

Fig. 1. EPR signals of MNIC–DETC complexes from the livers of control and hypoxia–adapted rats. The component of the signal's ultrafine triplet structure whose intensity was used as a measure of NO levels is boxed.

induced by acetylcholine (10^{-5} M) after its contraction produced by norepinephrine (5×10^{-7} M) had reached a plateau.

In another test series, an 8-mm-long segment of the tail artery from each rat was perfused with oxygenated Krebs-Henseleit's solution at 37°C and at a constant flow rate of 2 ml/min imparted by a roller pump. The response of the perfused vessel was evaluated by changes in the perfusion pressure recorded using a pressure sensor (Statham). An endothelium-dependent dilatation response was induced by acetylcholine (10^{-7} M) applied during the contractile response induced by norepinephrine (5×10^{-7} M).

The quantity of NO produced in rat tissues was estimated by measuring NO incorporation into Fe^{2+}-diethyldithiocarbamate (Fe^{2+}-DETC) complexes $(C_5H_{10}NS_2)_2Fe$) with the formation of paramagnetic mononitrosyl iron (MNIC)-DETC complexes. The latter complexes are characterized by an electron paramagnetic resonance (EPR) signal with g-factor values of 2.035 ($g_\perp$) and 2.02 ($g_{||}$) and by an ultrafine triplet structure at $g_\perp$ (Fig. 1). The amount of the MNIC-DETC complexes per sample and thus the quantity of NO incorporated into them was estimated from the intensity of this signal calculated by the method of double integration using a solution of paramagnetic dinitrosyl iron-thiosulfate complexes of known concentration as the reference.

To cause MNIC production in the body, rats were injected with a Na-DETC ($C_5H_{10}NS_2Na$) solution (500 mg/2.5 ml H_2O/kg body weight) intraperitoneally and with a $FeSO_4+C_6H_5O_2Na_3$ solution (20 mg+95 mg/2.5 ml H_2O/kg) subcutaneously. In addition, they received an intraperitoneal injection of carbachol (a long-acting synthetic acetylcholine analog) in a dose of 375 μg/2.5 ml H_2O/kg 10 min after Na-DETC to boost NO generation. Rats were killed by decapitation 30 min after the injection of Na-DETC, and their isolated organs (liver, kidneys, spleen, and small intestine) were minced, frozen in a press mold, and stored in liquid nitrogen until use.

EPR signals from the samples were recorded with an EPR radiospectrometer at 77°K, a field modulation amplitude of 0.5 mT, and a UHF power of 10 mW.

RESULTS

NO levels in the organs studied are shown in Table 1. In the hypoxia-adapted rats, the basal level of MNIC-DETC complexes, which reflects spontane-

TABLE 1. Amounts of NO Incorporated into MNIC–DETC Complexes in Organs of Control and Hypoxia–Adapted Rats ($M\pm m$)

Organ	NO generation, ng/g wet tissue			
	spontaneous (n=5)		carbachol-stimulated (n=8)	
	control rats	adapted rats	control rats	adapted rats
Liver	50±26	36±20	106±30	43±6**
Kidneys	0	0	6±3	0
Spleen	3±1	3±1	13±3*	13±3*
Intestine	390±20	223±36**	526±216	160±23**

Note. n = number of rats in the test series; $p<0.05$: *in comparison with spontaneous NO generation, **in comparison with control (unadapted) rats.

ous NO generation, was 28% lower in the liver and 43% lower in the intestine than in the unadapted controls. Kidney levels of these complexes in the adapted rats were below the detection limit of the method used, while their spleen levels were the same as in the controls. The EPR spectra of MNIC-DETC complexes shown in Fig. 1 permit visual assessment of NO generation for the liver: it can be seen that the EPR signal in the adapted group was lower than in the control group.

NO generation was enhanced by carbachol. After the adaptation to hypoxia, however, it was lower than in the control and even more so than spontaneous generation (by 43% and 70% in the liver and intestine, respectively). In the kidneys, carbachol-stimulated NO release was detected only in the control group, being below the detection limit in the adapted group.

Thus, the content of MNIC-DETC complexes and, hence, NO production were reduced in rat organs after the adaptation to intermittent hypoxia. The results obtained with isolated vessels were different in that the adaptation to hypoxia did not lead to a reduction in the endothelium-dependent relaxation of either conductance (Fig. 2, *a*) or resistance (Fig. 2, *b*) vessels. Rather, the adaptation tended to increase (albeit slightly) the endothelium-dependent relaxation of the aorta (Fig. 2, *a*) while significantly enhancing that of the tail artery (Fig. 2, *b*). Such adaptation has been shown not to affect systemic AP [9], whose level, like endothelium-mediated relaxation, depends on NO production [12].

These differences may be explained as follows. Hypoxia results in diminished Ca^{2+} accumulation in vessel cells [8], which limits the activation of constitutive NO synthase [12] and is apparently caused by the activation of antioxidant enzymes due to the adaptation to intermittent hypoxia [7]. This defense mechanism may prevent both a rise of the Ca^{2+} concentration in the cells and tissues of hypoxia-adapted animals with induced hypertension [5] and a fall in AP because of lowered NO levels in hypoxia-adapted animals with induced acute myocardial infarction [1]. A consequence of the low Ca^{2+} level in the tissues of hypoxia-adapted animals may be the lowered NO levels recorded in such animals. In isolated vessels, however, the entry of Ca^{2+} into enodothelial and smooth-muscle cells may increase because of the uncontrollable damage occurring to the vessels during their isolation and their functioning *in vitro*. As a result, the activation of constitutive NO synthase may be enhanced with consequent increases in NO synthesis and endothelium-dependent relaxation of the vessels.

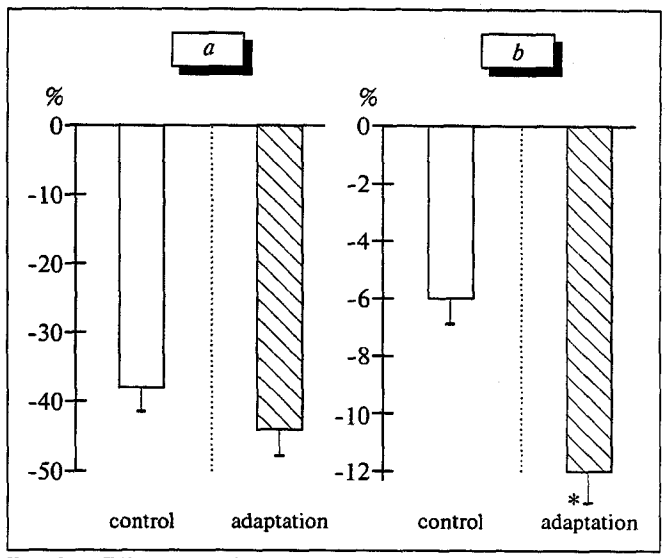

Fig. 2. Effects of adaptation to hypoxia on endothelium-dependent relaxation of the aorta (*a*) and tail artery (*b*). The values are percentages of the contractile response to norepinephrine. *$p<0.05$ in comparison with the control.

An alternative mechanism by which the formation of MNIC-DETC complexes decreases could be as follows. During adaptation to hypoxia, the thiol-disulfide equilibrium in proteins may shift in favor of thiols because oxidative reactions are weakened. As a result, NO incorporation into iron complexes with the protein thiol groups to form an NO depot may be more efficient than in hypoxia-unadapted animals [11]. If so, then the amount of NO released from the cells and capable of being trapped by Fe^{2+}-DETC complexes should decrease with a resultant fall in the level of MNIC-DETC complexes. It should be noted that hypoxia induces a sharp increase in the utilization of the NO precursor L-arginine [6]. The NO that then forms can bind to form stable complexes (NO depot) which will accumulate in the tissues. The subsequent utilization of NO from its depot may be regulated by low-molecular thiols that form low-molecular dinitrosyl iron complexes capable of being released from the cells [11]. Since they are a source of NO, these complexes can inhibit the development of hypertension [2]. In isolated vessels, the mechanism of NO depot formation operates less efficiently because of oxidative reactions, so that NO begins exiting in large amounts from the endothelial cells to cause effective endothelial-dependent relaxation of the vessels. This is precisely what was observed in our experiments.

Adaptation to hypoxia is likely to increase the power of NO-synthesizing systems and (apparently) even more so that of NO-depositing systems, which may explain why adaptation to hypoxia has been found capable of preventing circulatory disturbances

associated with excessive NO production (e.g., falls of vascular tone and AP in myocardial infarction) as well as those associated with inadequate production of this oxide (as, for example, in hypertension).

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