Compensatory-Adaptive Mechanisms during Nitric Hypoxia in Rats

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Nitrates, nitrites, and nitric oxides are the most widespread compounds in the environment of modern society, since these substances enter the human organism with water, food, air, and drugs [1]. During the last ten years, nitric oxide (NO) has been established to take part in the regulation of the intracellular Ca^{2+} concentration by activating soluble heme-containing guanylate cyclase and ADP-ribosyl transferase [4]. The guanidine nitrogen of L-arginine is the source of NO. In the presence of O_2, superoxide anion radicals (O_2), hydroperoxide (H_2O_2), and OH^- radicals, NO can be oxidized into nitrites (NO^-_2) and nitrates (NO^-_3). Thus, NO, as well as NO^-_2 and NO^-_3 ions, are not only chemical compounds found in the human and animal environment, but also actual constituents of the internal medium of mammals. In inflammatory processes, disturbances of the gastrointestinal tract, circulation disorders, and ischemic and hypoxic states, the processes of formation of NO and products of its metabolic transformation (NO^-_2 and NO^-_3 ions) are intensified in the human and animal organism [1, 4, 13]. Thus, a study of the effect of nitric compounds on the mammalian organism is of prime importance in modern biology and medicine. NO is known to be one of the active compounds commonly grouped under the term "endothelium-derived relaxing factor" (EDRF). Being able to reduce the intracellular Ca^{2+} concentration in smooth muscle cells, NO causes vasodilation [5]. Endothelin (ET), a vasoactive peptide, exhibits the opposite effect (vasoconstriction). It has been suggested that ET largely contributes to the regulation of the regional blood flow under hypoxic conditions [7]. The greatest density of cells containing ET mRNA is observed in the hypothalamus, this attesting to the regulatory role of ET in the brain tissue [8]. ET actively contributes to the elevation of the intracellular Ca^{2+} content, due to the increased entry of extracellular Ca^{2+} and the release of calcium from the intracellular pool [10]. ET is known to stimulate the release of certain hormones, eicosanoids, and EDRF [12]. Hence, NO and ET are active vasoregulators and a disturbance of their balance in the blood may play an important role in the pathogenesis of vascular disorders, leading to circulatory disturbances and thence to the development of hypoxic states [11]. The aim of the present study was to investigate the compensatory-

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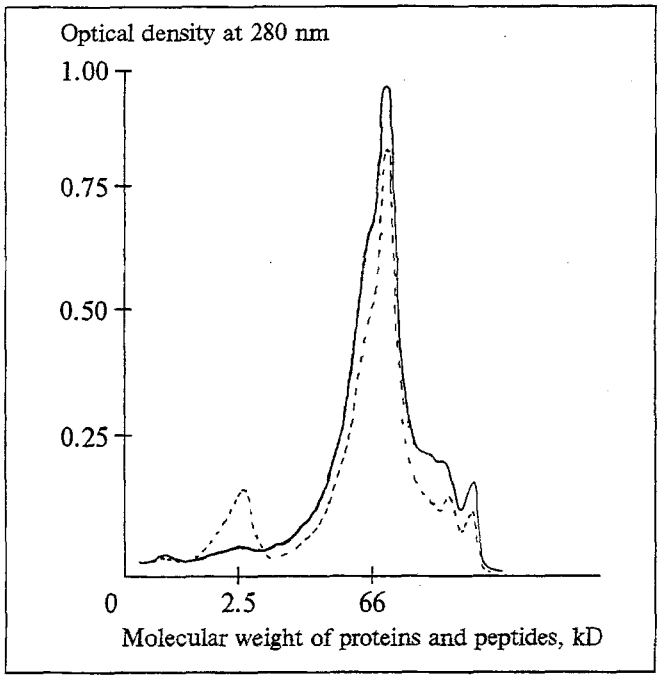

Fig. 1. Chromatogram of serum proteins and peptides in control rats (continuous line) and in rats injected with $NaNO_2$ (broken line) in a dose of 5 mg/100 g.

adaptive mechanisms coming into play during nitric hypoxia in rats.

MATERIALS AND METHODS

The study was carried out on male rats (71 non-pedigree rats and 12 Wistar rats). $NaNO_2$ solution was intraperitoneally administered in a dose of 0.5 mg/100 g body weight in a volume of 0.5 ml. Blood was taken one hour postadministration, when the methemoglobin level had attained its maximum for the given $NaNO_2$ dose. The protein content in the blood serum was determined after Lowry, albumin content by the colorimetric method using Bromcresol Green [2], hemoglobin (Hb) and methemoglobin (Met-Hb) by unified methods [6], and total α-amino nitrogen after Rubinstein and Pryce [14]. Serum proteins and peptides were separated by high-performance liquid chromatography (HPLC) on a Waters system (USA) with ultraviolet detection at 280 nm [13] (Fig. 1). The blood was taken from the jugular vein for endothelin and cGMP content determination, and from the caudal vein for Hb, Met-Hb, total protein, and albumin content determination and for chromatographic separation of the proteins. The blood samples were collected in tubes containing 7.5 mM EDTA (for cGMP determination) and, additionally, contrical in an amount of 100 kiU/ml blood (for ET determination). Plasma was obtained by blood centrifugation at 2000 g for 10 min at 4°C and stored at -20°C prior to the investigations. ET was extracted and purified from plasma acidified with 0.25 ml 2 M HCl by consecutive elution with 5 ml of 0.1% trifluoroacetic acid (TFA) and 2 ml of 80% methanol containing 0.1% TFA on Amprep 500 mg C2 columns (Amersham). The last fraction containing ET was dried under nitrogen and used for ET content determination with radioimmunoassay kits (Amersham). The cGMP content was determined in the plasma by ethanol extraction followed by drying under nitrogen and by spectrophotometric measurements at 492 nm with the aid of Ekros enzyme immunoassay kits. Electron paramagnetic resonance (EPR) spectra (Fig. 2) of the blood were recorded on a reflection EPR-spectrometer with double modulation of the magnetic field [3].

RESULTS

One hour postadministration, NO^-_2 in a dose of 5 mg/100 g causes maximal (for the given dose of nitrites) Met-Hb formation in the blood (68.73%, $p<0.01$) (Table 1), and therefore hypoxia attains its maximum at this time. An array of biochemical parameters which characterize the adaptive changes in rats during nitric hypoxia are presented in Tables 1 and 2. One hour after $NaNO_2$ injection, the total protein content and the Hb content were reliably reduced (by 33 and 22%, respectively, $p<0.001$) in the serum.

TABLE 1. Protein Content and Total α-Amino Nitrogen Content in the Blood Serum of Rats after Administration of $NaNO_2$ in a Dose of 5 mg/100 g Body Weight

Parameter	Control			$NaNO_2$			p
	n	$M\pm m$	σ	n	$M\pm m$	σ	
Total protein, g/liter	(12)	89.9±2.5	8.2	(11)	60.9±2.6	8.4	<0.001
Albumin, g/liter	(13)	49.2±2.0	7.0	(13)	24.8±0.7	2.5	<0.001
Hemoglobin, g/liter	(18)	14.6±0.3	1.3	(6)	11.5±0.4	0.9	<0.001
Methemoglobin, % of hemoglobin	(8)	1.5±0.1	0.3	(12)	68.7±1.3	4.1	<0.001
α-amino nitrogen, mg %	(15)	8.6±0.2	0.6	(13)	13.5±0.6	1.9	<0.001

Note. Here and in Table 2: n = number of animals examined.

Along with the decrease of the protein content, a 57% rise ($p<0.001$) of the α-amino nitrogen level was simultaneously observed in the serum. Activation of endopeptidases and aggravation of destructive alterations in the proteins under the influence of free radicals (NO and NO_2 formed during nitric hypoxia) may be among the causes of the changes observed [1,3]. Protein and peptide separation by HPLC (Fig. 1) confirmed the results of the spectrophotometric studies. The most pronounced decrease in the protein content was noted for the fractions with a molecular weight of 60-70 kD (albumin). On the other hand, the content of peptides with a molecular weight of 2.0-4.0 kD increased, the maximum increase being observed for the fraction with a molecular weight of 2.5 kD. This fraction may contain a rather large amount of physiologically active peptides, the content of which may alter as the compensatory-adaptive mechanisms are enacted during nitric hypoxia. One of the peptides whose molecular weight is 2.492 kD is ET. This peptide, as mentioned above, produces a vasoconstrictive effect and is released during hypoxia and enhanced NO synthesis in the mammalian organism [7, 9]. Therefore, the ET content in the blood can be expected to alter in nitric hypoxia.

Determination of the ET content in the plasma showed a more than twofold increase in the concentration of this peptide in rats one hour after $NaNO_2$ administration (5 mg/100 g body weight) as compared to the control animals (42.34±7.76 mmol/liter in the experiments and 20.1±1.84 mmol/liter in the control, $p<0.01$) (Table 2). At this time, the formation of Hb-NO complexes was noted; their EPR spectra are shown in Fig. 2. The percentage of these complexes was 10-15% of the total Hb content and amounted to 2×10^{-4} M. Hence, during increased delivery of nitric compounds to the organism, the intensive formation of Hb-NO complexes occurs, this in turn being attended by an increased synthesis of ET. Recently, oxyhemoglobin has also been shown to stimulate ET synthesis [11], which may be due to the ability of NO, O_2, O^-_2, and CO_2 to interact with heme-containing proteins, including soluble guanylate cyclase, and to raise the cGMP level.

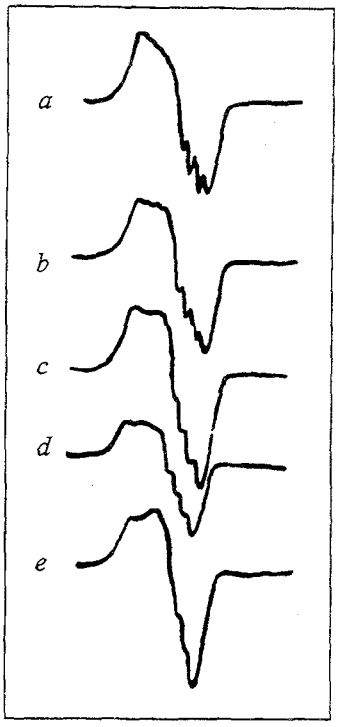

Fig. 2. EPR spectra of Hb–NO complexes in blood one hour after administration of $NaNO_2$ in a dose of 5 mg/100 g to rats (*a–e*).

Simultaneous determination of the cGMP concentration and ET content in the blood plasma showed a 3-fold increase in the concentration of this nucleotide after $NaNO_2$ administration (42.8±12.9 mmol/liter in the experiments and 14.0±2.77 mmol/liter in the control, $p<0.05$ (Table 2). Such a change of the cGMP content in the rat plasma may result from NO-induced activation of soluble guanylate cyclase in the platelets and other cells of the organism's tissues [4]. The results obtained provide evidence that nitric hypoxia and vasodilatory changes of vascular tone, noted by many researchers against the background of nitric preparations, are attended by activation of the compensatory systems, in which ET, a peptide with a vasoconstrictive effect, is one of the active elements. It is known that, by binding to peptidergic receptors, ET activates the Ca^{2+}-mobilizing system and along with it the synthesis of nitric oxide; the latter, interacting with soluble heme-containing guanylate cyclase, increases its activity and raises the level of cGMP [4,10,12].

Thus, the increase in the content of substances with a vasoconstrictive effect (ET) leads to enhanced synthesis of compounds which are able to

TABLE 2. ET and cGMP Content in the Rat Serum after Administration of $NaNO_2$ in a Dose of 5 mg/100 g Body Weight

Parameter, pmole/liter	Control			$NaNO_2$			p
	n	$M\pm m$	σ	n	$M\pm m$	σ	
ET–1,2	(5)	20.1±1.8	3.7	(6)	42.3±7.8	17.4	<0.01
cGMP	(5)	14.0±5.5	2.8	(6)	42.8±12.9	25.9	<0.05

induce vasodilation (NO and cGMP), while the boost of NO production during nitric hypoxia activates ET synthesis. Such give-and-take between the regulatory systems is evidently required by the organism to provide for effective regulation involving negative feedback. The latter is known to underlie the maintenance of homeostasis in the living organism and is, as a rule, realized on the basis of multiparametric regulation.

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Effects of Inhibition of Ca^{2+} Mobilization and Ca^{2+} Chemocontrolled Entry by 15-Hydroxyeicosatetraenoic Acid on the Modulation of Cholinoreceptor Plasticity in *Helix lucorum*

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Arachidonic acid and its noncyclic derivatives have long been recognized as secondary messengers [6,10]. Together with other secondary messengers, eicosanoids are involved in the intracellular regulation of the plasticity of neurons [8,9] and their cholinoreceptors (CR) [1-4]. It cannot be ruled out that endogenous 15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE) participates in the intracellular regulation of CR plasticity, since previously we have shown that exogenous 15-HETE

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