Effect of Blood Loss on Nitric Oxide Content in Liver and Mucosa and Muscle Layer of Small Intestine in Rats

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Moderate blood loss (20 ml/kg) increases NO content in the intestinal mucosa and muscle layer, and especially, in the liver, as revealed by *in vivo* nitric oxide (NO) binding with Fe^{2+}-diethyldithiocarbamate complex yielding paramagnetic mononitrosyl iron complexes. Enhanced NO synthesis after blood loss prevents vascular spasm in the mucosa, thus maintaining blood circulation and effective absorption in the small intestine.

Key Words: *absorption; small intestine; nitric oxide; blood loss*

Blood loss drastically decreases the volume of circulating blood, reduces arterial, venous, and portal pressure and cardiac output [2,3,7], rapidly and strongly increases norepinephrine concentration in the blood [2,3], without perturbation in mesenteric blood flow [3,4]. The absorption activity of the intestine is completely preserved [4]. The absence of perturbations in the mesenteric circulation is thought to be related to peculiarities of norepinephrine effect on the vascular tone in the intestinal mucosa; adrenergic nerve traffic is accompanied by autoregulation of the mesenteric blood flow and maintenance of the blood supply in the absorbing epithelium [7,13,19]. Blood flow in the small intestine decreases mainly due to circulatory restrictions in the muscular layer and cryptic zone of the mucosa [15,19].

There is evidence on the regulatory effect of nitric oxide (NO) on vascular tone. This agent is synthesized in the endothelium under the effect of biologically active substances and represent a nitrosylic compound, containing NO as an active component [18,20]. NO induces relaxation of vascular smooth muscles [12, 18,20], thus maintaining permeability of the mucosa during ischemia [17]. The enhanced production of NO during blood loss is effected by activation of constitutive NO-synthase (cNOS), localized predominantly in the vascular endothelium. NO can also be produced by neuronal NOS localized in nerve terminals of the small intestine [21].

This paper describes the effect of blood loss on NO content in the mucosa and muscle layer of the small intestine, and in the liver in rats. NO was assayed by its incorporation into mononitrosyl iron complexes with diethyldithiocarbamate (MNIC-DETC) [1,14].

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 400 g. Group 1 rats received food, while group 2 rats were fasted overnight. Blood (10 and 20 ml/kg) was taken at a rate of 0.8-1.0 ml/kg/min under hexenal narcosis (1% or 1 ml/100 g). DETC (500 mg/kg) was injected intraperitoneally simultaneously with subcutaneous injection of iron(II) citrate complex ($FeSO_4$+ sodium citrate, 20 mg+95 mg/kg). Narcosis was given 10 min postinjection, and after 10-15 min blood drawn. Tissue specimens were taken 30 min after the end of bloodletting. The rats were decapitated; liver and small intestine were extracted with subsequent isolation of the intestinal mucosa and muscle layer. Tissue specimens were placed into glass ampoules, stored in liquid nitrogen, and used for electron paramagnetic resonance (EPR) recordings. The control rats were subjected to the same procedure without bloodletting. The

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EPR spectra were recorded on a Bruker radiospectrometer at 77°K. To estimate MNIC-DETC level in specimens, we used a standard made of frozen solution of MNIC-DETC in dimethylformamide [10]. MNIC-DETC concentrations in specimens and the corresponding level of NO incorporated into these complexes (nmol/g wet tissue) were determined by comparing of EPR signals of MNIC-DETC in test and standard specimens. Significance of differences was analyzed as described previously [6] by comparing dispersion of experimental data for 10 animals.

RESULTS

Typical EPR spectra were recorded in specimens taken from liver, intestinal mucosa and muscular layer of the small intestine in test and control rats (Figure 1). The EPR signal of MNIC-DETC with $g_{\perp}$=2.035 and $g_{||}$= 2.020 is characterized by a triplet hyperfine structure (HS) at $g_{\perp}$ caused by interaction of unpaired electron with nitrogen nucleus of NO ligand. Some records revealed EPR signal generated by DETC complex with endogenous copper (Cu^{2+}), which at given magnetic intensity is characterized by a 4-component HS (A,B,C,D) resulted from interaction of the unpaired electron with Cu^{2+} nucleus [14]. In addition, all records show EPR signals of free radicals at g=2 and reduced iron-sulfuric proteins at g=1.94 [14].

The content of NO drastically decreased in specimens from group 2 rats. This drop was most pronounced in the intestinal mucosa from control and experimental rats (approximately by 8 nmol/g tissue, Table 1). In all examined tissues (small intestine from group 1 rats) blood loss of 20 ml/kg significantly increased NO level. This increase was less pronounced in group 2 with 10 ml/kg blood loss (such experiments were not carried out on fed rats). In group 1, NO content in the small intestine after blood loss of 20 ml/kg was maintained at a high level.

High NO content in examined tissues after relatively short-term (10-15 min) moderate (20 ml/kg) blood loss is presumably caused by activation of cNOS localized predominantly in vascular endothelium. Liver cells normally contain no cNOS. The synthesis of inducible NOS (iNOS) in the liver is usually activated only by various inflammatory agents 3-4 h after the contact [16]. The same is true for the small intestine [16]. Apart from endothelial cNOS, NO in the intestine can be produced by neuronal NOS localized in synapses at the intestine surface [21].

Moderate blood loss (20 ml/kg) does not impair absorption in the small intestine, and even enhances it [4]. It does not disturb circulation in the mucosa. As a result, circulation in the epithelium [7,13,19] and its absorption activity [4] are preserved after blood loss. Published data suggest that the absence of circulatory disturbances in the mucosa can be explained by a small number of adrenergic receptors responsible for the constrictor effect of norepinephrine [9,20]. Our data indicate that enhanced production of NO in this tissue observed in the control and after blood loss, plays a key role in the maintenance of circulation in the mucosa. Production of NO was decreased in experimental and control fasted rats presumably due to deficiency of L-arginine, the source of NO. However, it significantly increases during blood loss.

In the small intestine, the content of NO was lower in the muscle layer characterized by a large number of adrenergic receptors, than in the mucosa. In fasted rats it significantly increases after blood loss. However, this increase is insufficient for vasodilation and maintenance of blood circulation in the intestine. Vasospasm in the muscle layer of small intestine plays a pronounced physiological role under these conditions. Presumably, this spasm contributes to redistribution of cardiac output and to centralization of circulation [4,7].

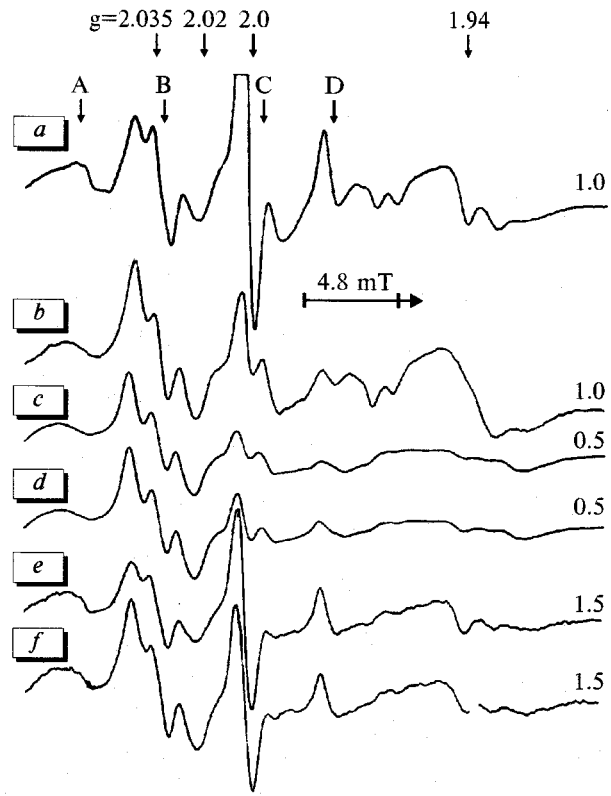

Fig. 1. Electron paramagnetic resonance (EPR) spectra in the specimens prepared from liver (*a, b*), mucosa (*c, d*) and muscle layer (*e, f*) of small intestine from fed rats in the control (*a, c, e*) and after blood-loss (*b, d, f*). The rats were injected with diethyldithiocarbamate (DETC) and iron citrate complex 30 min prior to decapitation. Records were obtained at 77°K. A-D are the components of the hyperfine structure of EPR signal from Cu^{2+}-DETC complex.

TABLE 1. Nitric Oxide Content (nmol/g Wet Tissue) in Liver and Small Intestine in Fed and Fasted Rats after Conditions Blood Loss ($M\pm m$, n=10)

Group		Liver	Small intestine	
			muscle layer	mucosa
Group 1	Control	1.3±0.5	2.1±1.7	9.0±7.0
	Blood loss, 20 ml/kg	9.0±4.0**	4.7±2.0*	11.0±4.7*
Group 2	Control	0.3±0.2+	0.2±0.1+	0.9±0.7+
	Blood loss, 10 ml/kg	0.5±0.3	0.4±0.2	1.7±0.7
	20 ml/kg	4.0±3.0**	1.1±1.1**	3.4±2.3**

Note. *p<0.05 and **p<0.01 compared with the control; +p<0.01 compared with group 1 control.

Similarly to the muscle layer of the small intestine, hepatic vessels, and in particular the portal vein, contain a large number of adrenergic receptors [5, 8,11]. The release of norepinephrine into the blood caused by a moderate blood loss provokes a powerful spasm of portal vessels and less pronounced spasm of hepatic arteries [4,8,11]. Portal circulation is drastically reduced, while arterial blood supply to the liver is little changed [4]. Simultaneously, there is a pronounced increase in NO concentration in hepatic vessels (Table 1). Presumably, the enhanced NO production in the liver is a response to constrictor effects of hormones, in particular norepinephrine. NO moderates spasm of hepatic vessels, thus maintaining absorption function and preserving circulation in the hepatointestinal region [4]. In this process an important role is played by arterial blood supply to the liver and intestinal epithelium [13,19].

Our data show, that endothelium of the intestinal mucosa, its muscle layer, and the liver increase NO production, which leads to vascular relaxation. The competitive effects of vasodilator NO and a number of vasoconstrictors (in particular norepinephrine) result in the maintenance of stable blood flow in the intestinal epithelium [9,14,19] and stable blood outflow from the intestine to the liver [4,17]. Activation of these adaptive mechanisms during the early posthemorrhagic period provides conditions for functional activity of the liver and intestine, redistribution of cardiac output, and centralization of circulation.

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