Electron Paramagnetic Resonance Study of Nitric Oxide Production during 5-HT2 Receptor Blockade in the Blood, Heart, and Liver of Rats with Myocardial Infarction

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The effect of serotonin 5-HT2 receptor blockade on nitric oxide production in rats with myocardial infarction was evaluated by the method of electron paramagnetic resonance. The spectra were recorded in samples of the heart tissue (left and right chambers), liver, and blood. 5-HT2 receptor blockade during myocardial infarction was followed by a decrease in the total content of nitrosyl complexes in the spin trap and R and T conformers of Hb—NO. The percent of T conformers increased in the remaining complexes of Hb—NO. These changes were observed in the heart and, particularly, in the blood. The amount of spin-trap complexes was lower in the liver. Hence, nitric oxide molecules were primarily associated with the spin trap in liver tissue. The decrease in the number of Hb-NO complexes in the blood probably reflects the decrease in the severity of hypoxia due to myocardial infarction. A correlation was found between these changes and physiological state of rats. 5-HT2 receptor antagonist improved general state of rats with infarction and increased survival rate.

Key Words: *heart; myocardial infarction; nitric oxide; electron paramagnetic resonance*

Nitric oxide (NO) plays an important role in the function of the cardiovascular system. For example, this compound regulates vascular tone, blood pressure [5], and myocardial function [3]. Activation of NO synthase and release of NO in endothelial cells are realized via 5-HT2 receptors and contribute to arterial dilation [6]. Serotonin has various effects on the cardiovascular system. On the one hand, this agent increases the strength of myocardial contraction [2], causes vasoconstriction, and elevates blood pressure via activation of 5-HT2 receptors. Therefore, 5-HT2 receptor blockade is used in the therapy of children with heart diseases and pulmonary hypertension to prevent blood pressure increase. On the other hand, serotonin can decrease the heart rate and blood pressure [3]. This effect of serotonin is realized via the increase in NO production. Tissue NO concentration is directly measured by means of electron paramagnetic resonance (EPR).

Serotonin and NO produce reciprocal effects in the regulation of cardiovascular function. Moreover, these compounds play a role in the development of myocardial infarction (MI). The effect of 5-HT2 receptor blockade on NO production in tissues of rats with MI was evaluated by means of an EPR study.

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MATERIALS AND METHODS

Experiments were performed on 10 outbred albino rats. We measured the amount of complexes of NO with the spin trap and heme iron. Heme iron enters the composition of hemoglobin and serves as a natural spin trap in the organism. Tissue samples were obtained from control rats (group 1), animals with MI (group 2), and rats with MI receiving 5-HT2 receptor antagonist cinanserin hydrochloride (Tocris, group 3). Experimental MI in rats was induced by ligation of the posterior right coronary artery ($^2/_3$ from the cardiac base). Sodium diethyldithiocarbamate (DETC) was used as a spin trap for NO [4]. Na-DETC in a dose of 500 mg/kg was dissolved in 2.5 ml water and injected intraperitoneally. The solution of iron citrate (II) (iron sulfate (II), $FeSO_4 \times 7\ H_2O$, 37.5 mg/kg, Sigma; and sodium citrate, 187.5 mg/kg) was injected subcutaneously. The interaction of Na-DETC with iron citrate ($FeSO_4$+sodium citrate) led to the formation of water-insoluble complexes of iron and DETC. This complex interacts with NO, which results in the formation of a stable complex of $(DETC)_2$—Fe—NO. This complex is detected by EPR.

The rats were decapitated 40 min postinjection. The tissues were rapidly isolated, dried, and frozen in liquid nitrogen using disposable syringes. The weight of samples was 100 mg. EPR study was conducted with samples of heart tissue (left and right chambers), liver, and venous blood. EPR spectra of samples were recorded on an ER-200E-SRS X-range spectrometer (Bruker) at 77 K.

RESULTS

All spectra included 3 types of paramagnetic complexes of Fe^{2+} and NO: complex with the spin trap, $(DETC)_2$—Fe—NO [4] (Fig. 1, *a*); and 2 complexes of heme iron and NO, R and T conformers (Fig. 1, *b*, *c*). These complexes are interpreted as the 6- and 5-coordinated complexes of heme iron in hemoglobin [7]. The ratio of signals from iron nitrosyl complexes significantly varied in tissues. The integral intensity of EPR signals was proportional to the number of paramagnetic centers that determine these signals. Variations in the relative number of NO-containing complexes were estimated from the integral intensity of 3 components in the total EPR spectrum of tissues.

EPR spectra of blood samples were similar in group 1 and 2 rats, but differed in intensity (Fig. 2). This type of the spectrum belongs to the R conformer of nitrosohemoglobin [7], which represents a linear combination of 2 spectra from 6-coordinated α-Hb—NO and β-Hb—NO [7]. In this complex of hemoglobin, iron atoms are localized in the heme plane. Accumulation of these complexes in the blood reflects hypoxia [1].

EPR spectra significantly changed in group 3 rats (Fig. 2). The signal of Hb—NO decreased by an order of magnitude. Against the background of the R conformer signal, hyperfine splitting of the ligand was pronounced at $g \approx 2.01$ (17 E) and less significant at $g \approx 2.06$. These changes in the spectrum are probably associated with the T conformer structure of Hb—NO. This complex is characterized by a significant decrease in the strength or loss of the bond between iron and nitrogen ions in distal histidine. Iron ion is shifted from the heme plane and has high ability to bind and transfer O_2. The observed changes are accompanied by a decrease in the severity of hypoxia. The signal of the T conformer was greater in blood samples from MI rats, receiving 5-HT2 receptor antagonist and were characterized by longer life span. Figure 2 *2* represents the spectrum of superposed signals from R and T conformers.

Tissue spectra of the left and right heart were identical in signal shape and ratio of various complexes with NO. They included all 3 initial components (Fig. 3). The R conformer produced the strongest signal. The amplitude of this signal exceeded that of the spin trap. The intensity of signals in the left heart was 10-20% higher than in the right heart. The intensity of signals in the heart tissue decreased after 5-HT2 receptor blockade (Fig. 3). The spectra of heart tissue from group 3 rats with longer life span included the T structure. This structure was nearly undetected in dead animals. The ratio of R conformers was higher in these specimens. Our results indicate that NO production differs in the right and left heart. Moreover, 5-HT2 receptor blockade is followed by a decrease in NO concentration in the heart of rats with MI.

Examination of the liver tissue showed that the EPR signal of NO complexes is highest for the NO-spin trap complex. Hence, the spin trap was associated with the majority of NO.

The concentration of NO in the liver tissue of rats with MI was higher than in control animals. Administration of 5-HT2 receptor antagonist reduced NO concentration. In the rat survived longer than others after 5-HT2 receptor blockade, the EPR spectrum included only the NO-spin trap complex. The signal of the T conformer was maximum in the blood from this rat.

Studying the EPR spectra of tissues from rats with MI showed that administration of 5-HT2 receptor antagonist decreased the amount of nitro-

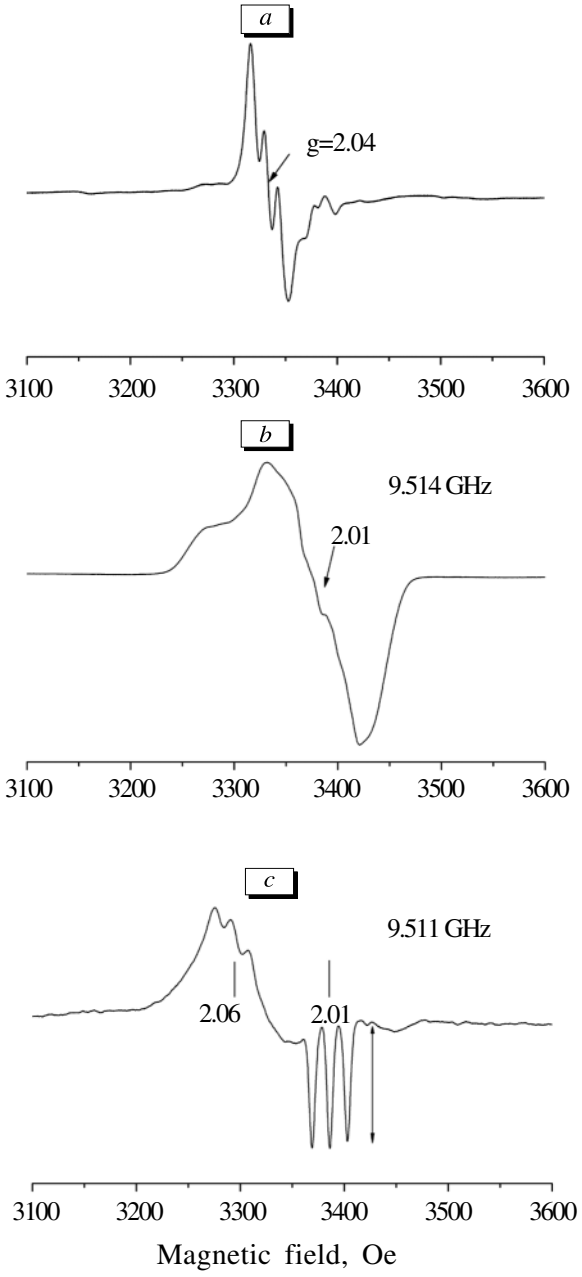

Fig. 1. EPR spectra of NO-containing complexes. Complex with the spin trap, $(DETC)_2$—Fe—NO (*a*); R conformer of Hb—NO (*b*); and T conformer of Hb—NO (*c*).

sohemoglobin complexes. These data show that serotonin does not activate NO production. The ratio of T conformers increased in the remaining Hb—NO complexes. This effect was most significant in the blood. The longer was the life span of rats with MI, the greater was the amount of T conformers of Hb—NO complexes.

Serotonin concentration is high in patients with MI. 5-HT2 receptor antagonists (*e.g.*, ketanserin) are used to prevent coronary artery spasm in these patients. The T structure was revealed in the spectra of heart tissue from MI animals, which exhibited the longest life span after 5-HT2 receptor blockade. However, this structure was nearly undetected in dead animals. The ratio of the R conformer was higher in dead animals. These features illustrate overproduction of NO during MI. High-intensity production of NO during MI is related to activation of inducible NO synthase. The observed changes result in production of peroxynitrites that produce a damaging effect on the heart. 5-HT2 receptor blockade is probably accompanied by the formation of NO in physiological concentrations. This agent causes coronary vasodilation, which increases the life span of rats with MI. Therefore, 5-HT2 receptor blockade is followed by a decrease in NO concentration in the heart of MI rats. This effect was less significant in other tissues and undetected in the liver.

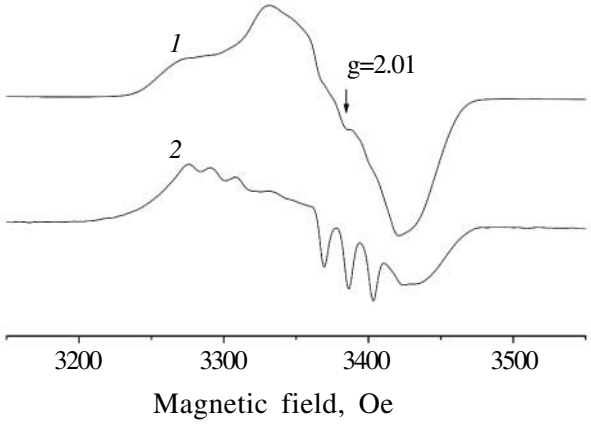

Fig. 2. EPR spectra of blood samples from the rat with MI (*1*) and animal with MI and 5-HT2 receptor blockade (*2*). R conformer of Hb—NO (*1*); and R and T conformers of Hb—NO (*2*). For pictorial presentation of EPR signal shape (*2*), the amplitude was increased by 10 times.

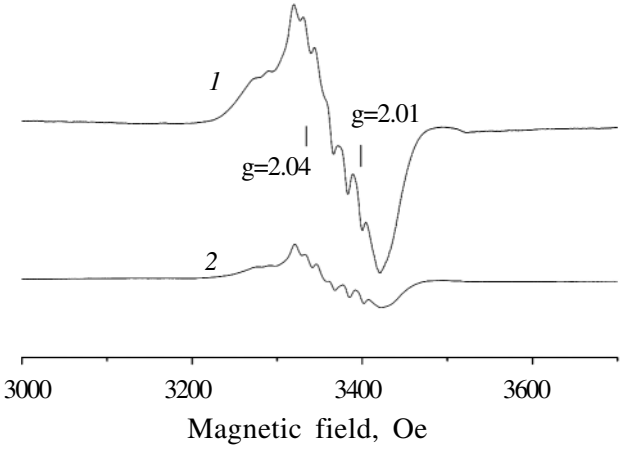

Fig. 3. EPR spectra of heart tissues from the rat with MI (*1*) and animal with MI and 5-HT2 receptor blockade (*2*).

The decresase in the number of Hb—NO complexes in the blood probably reflects alleviation of hypoxia. The spectra of R and T conformers are recorded in the blood during nitrate hypoxia of low severity. Severe hypoxia is accompanied the presence of R conformers [1]. The R conformer of Hb—NO is resistant to oxygenation. Therefore, NO is not replaced with oxygen. However, in T conformer of Hb—NO NO easily replaces O_2. Only the R conformer is present during severe hypoxia. This fact can be explained by periodic respiration, which results in the delivery of additional O_2 and substitution of NO for oxygen in the T conformer of Hb—NO. The amount of T conformers decreases, while R conformers are preserved in the blood. Apart from R conformers, the spectrum of T conformers is detected after 5-HT2 receptor blockade. The observed changes reflect a decrease in the severity of hypoxia. These changes correlate with the physiological state of rats. 5-HT2 receptor blockade improved the state of rats with MI and increased the survival rate of these animals. The signal of the T conformer was higher in survived rats than in dead animals. Dead specimens were characterized by a significant increase in the ratio of R conformers and absence of the signal from T conformers.

EPR study of tissues and blood from rats with MI showed that 5-HT2 receptor blockade is followed by a decrease in the amount of R conformers and conformational changes in the remaining nitrosyl complexes of heme iron in hemoglobin (transition from the R state to the T state).

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