BIOPHYSICS AND BIOCHEMISTRY

Effect of Mexidol and Nitroglycerine on Iron-Sulfur Centers, Cytochrome P-450, and Nitric Oxide Formation in Liver Tissue of Experimental Animals

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In experiments on mice we studied the effect of individual or combined treatment with mexidol and nitroglycerine on iron-sulfur centers of the mitochondrial respiratory chain, cytochrome P-450 of the endoplasmic reticulum, and nitric oxide formation in the liver tissue. Mexidol had a potent effect on these parameters and protected iron-sulfur centers from oxidation, including that induced by nitroglycerine.

Key Words: *mexidol; nitroglycerine; nitric oxide; cytochrome P-450; iron-sulfur centers*

Atherosclerosis and coronary heart disease are accompanied by activation of lipid peroxidation (LPO) in membranes and decrease in blood antioxidant activity. Antioxidants are used for the correction of these changes [2,4]. These compounds include 3-hydroxypyridine derivatives mexidol and mexicor with a wide range of pharmacological activity [1,3].

Antioxidants are usually prescribed in combination with standard cardiac drugs, including nitroglycerine (NG) preparations increasing LPO and modulating the function of tissue metalloenzymes [5]. Nitric oxide (NO) is released during NG metabolism; it binds to iron-containing proteins with the formation of heme-NO nitrosyl complexes and iron-sulfur centers-NO (ISC-NO). These changes are followed by inhibition of mitochondrial ISC and cytochrome P-450 in the endoplasmic reticulum. It is interesting to evaluate the effect of combined treatment with NG and antioxidant mexidol on biochemical processes in mammalian tissues. Here we studied the effects of these preparations on mitochondrial ISC, cytochrome P-450 in the hydroxylation system of the endoplasmic reticulum, and NO formation in the liver tissue of mice.

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MATERIALS AND METHODS

Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) was used in concentrations of 2.5×10^{-3} and 2×10^{-2} M. The concentrations of NG were 6×10^{-6} and 6×10^{-4} M.

Liver tissue was isolated from male SHK mice weighing 20-22 g. The animals were maintained in a vivarium under standard conditions and had free access to water and food.

The liver was removed immediately after decapitation of animals, washed in physiological saline, cut into small pieces, and incubated with mexidol and/or NG. Incubation of samples with physiological saline served as the control. Variations in the amplitude of electron paramagnetic resonance (EPR) spectra for ISC, cytochrome P-450, and heme-NO

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complexes were studied after incubation of liver samples at room temperature for 0.5, 3, and 26 h. Tissue samples were taken and frozen at -196°C (height 30 mm, diameter 3 mm). EPR spectra in blocks were recorded on an ESP-300 X-range spectrophotometer (Bruker-Analitishe-Messtechnik) under the following conditions: MW power 20 mW, amplitude of magnetic field modulation 5 G, temperature -196°C.

RESULTS

Signals of ISC (g-factor 1.94, N-1b centers of the NADH-dehydrogenase complex) and cytochrome P-450 (g=2.42, g=2.25) are usually detected in the liver of intact animals. Apart from these signals, a strong signal of heme-NO nitrosyl complexes with a typical triplet splitting (g=2.01) [7] was recorded in liver samples after incubation with NG (Fig. 1). The presence of this signal in the EPR spectrum was related to nitrosyl complexes of 2 heme-containing proteins, Hb-NO and cytochrome P-450-NO. The appearance of heme-NO complexes indicates that NG biotransformation results in the formation of considerable amounts of NO. Incubation of liver samples with mexidol (2×10^{-2} M) and/or NG (6×10^{-4} M) at room temperature for 26 h was accompanied by a decrease in the EPR signal of ISC (Fig. 2). The maximum decrease (by 3.4 times) was associated with oxidation of ISC and observed in the presence of NG. Combined treatment with mexidol and NG also contributed to oxidation of ISC. However, the degree of ISC oxidation was lower under these conditions (2.9-fold decrease in ISC activity). ISC activity decreased only by 1.2 times after incubation with mexidol for 26 h. ISC activity in the control sample decreased by 3 times. Individual treatment with mexidol prevented oxidation of ISC. After 26-h incubation of liver tissue with mexidol, the signal of ISC was 1.5-fold higher compared to the control. The data indicate that mexidol protects ISC of the mitochondrial respiratory chain from oxidation, including that induced by NG. Mexidol maintains function of the mitochondrial electron transport chain and, therefore, provides energy supply to cells.

The EPR signal of cytochrome P-450 decreased after incubation of 2 liver samples with NG. These changes were probably related to the formation of cytochrome P-450-NO nitrosyl complexes. This conclusion is derived from experimental observations that treatment with NG in high doses results in the appearance of considerable amounts of NO. Mexidol had a positive effect on cytochrome P-450. The signal of cytochrome P-450

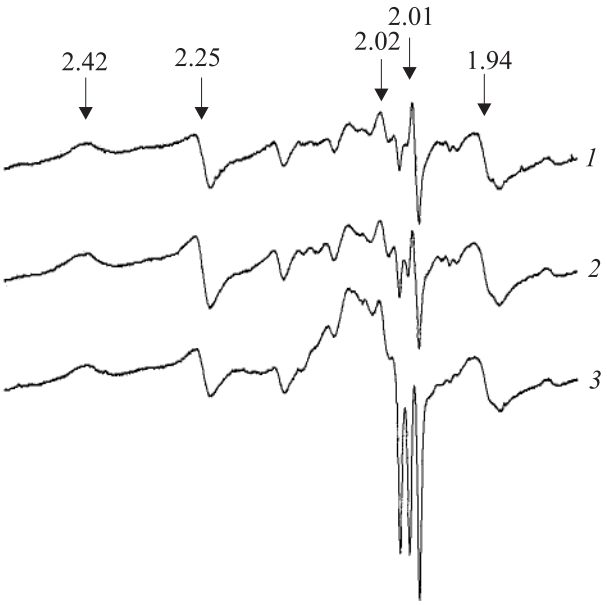

Fig. 1. EPR spectra of mouse liver samples after 30-min incubation with physiological saline (*1*), mexidol (2.5×10^{-3} M, *2*), and nitroglycerine (6×10^{-6} M, *3*).

increased by 1.4 times after 3-h incubation with mexidol. This signal decreased by the 26th hour, but remained above the basal level. This signal decreased in the control sample.

The EPR signal of cytochrome P-450 is related to the presence of active enzyme. Probably, the signal of active form is not associated with the direct effect of mexidol on the active center of enzyme. This signal is indirectly related to the protection of membranes from LPO.

The signal of heme-NO complexes increased by 1.5 times after 26-h incubation with NG. A 2.8-fold increase in this signal was detected by the 26th hour of incubation with mexidol and NG (Fig. 2, *c*). Therefore, the amount of newly formed NO was higher in experiments with mexidol. EPR spectra of samples were analyzed after incubation with mexidol. Control samples were incubated under similar conditions. We showed that incubation of liver samples with mexidol is accompanied by the formation of NO. The signal of heme-NO complexes was detected after subtraction of EPR spectra for control samples from EPR spectra for samples incubated with mexidol. The mechanisms for mexidol-induced formation of NO remain unclear. Mexidol probably activates the arginine-dependent pathway of NO formation with the involvement of inducible NO synthase (iNOS). It should be emphasized that nearly all cells in the liver tissue can express iNOS (hepatocytes, Kupffer cells, endothelial cells, and Ito cells). Mexidol also modulates iNOS activity in vascular endothe-

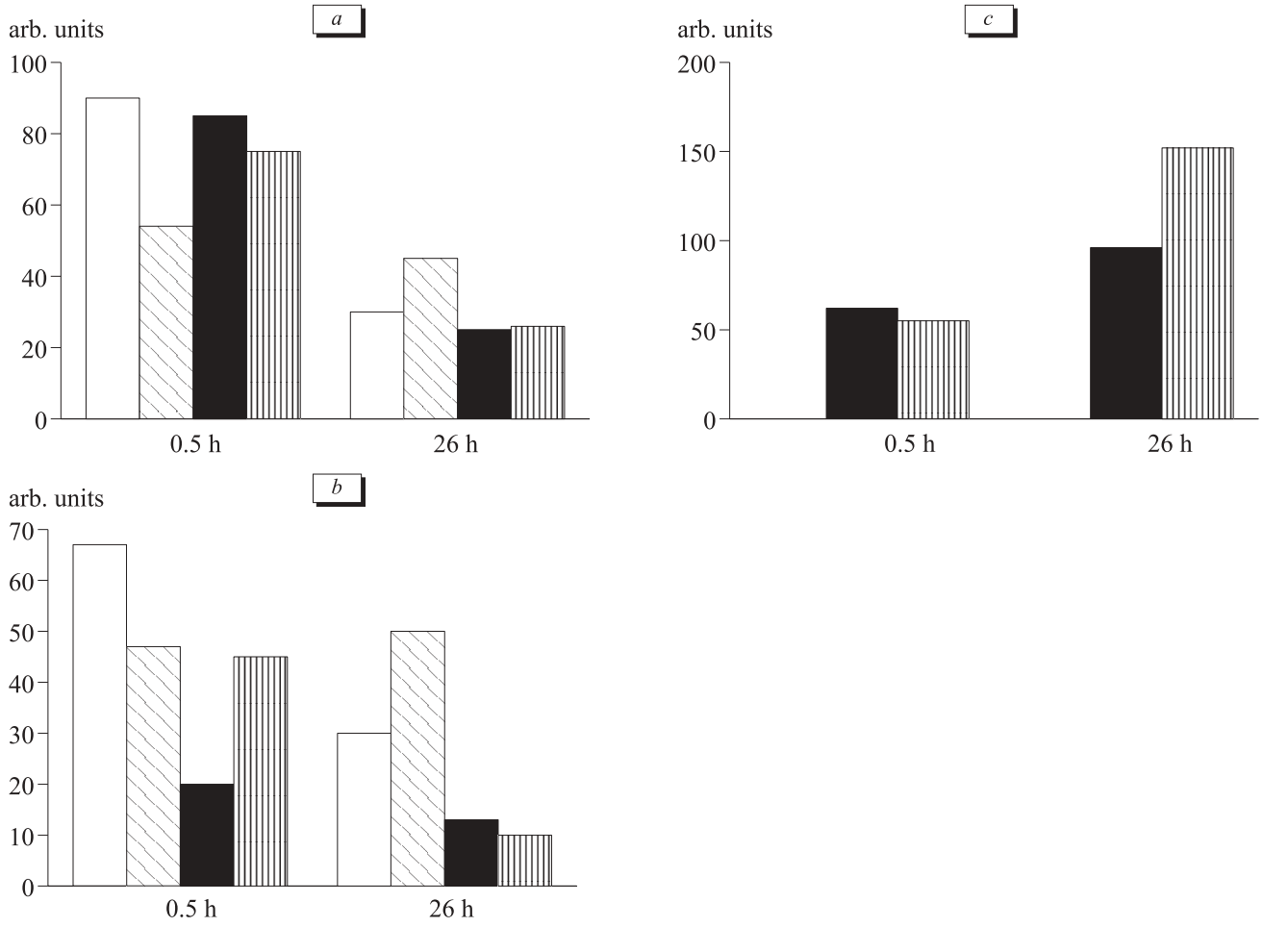

Fig. 2. EPR signals during incubation of liver tissue with mexidol (2×10^{-2} M) and/or NG (6×10^{-4} M). Light bars, control; shaded bars, mexidol; dark bars, NG; vertical shading, mexidol+NG.

lial cells. These cells certain not only endothelial NO synthase, but also iNOS [6].

Our results show that mexidol maintains ISC function in the mitochondrial respiratory chain and activity of cytochrome P-450, improves energy supply to cells, and protects liver tissue from oxidation.

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