ANTIHYPOXIC ACTION OF PYRACETAM

V. M. Gukasov, M. M. Rasulov,
S. N. Efuni, E. Ya. Kaplan,
V. I. Smiryagina, and M. V. Velikaya

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Pyracetam (α-pyrrolidone-acetamide) is effective in various forms of cerebral hypoxia, alcoholism, and poisoning [5, 7, 8]. Previous investigations have shown that pyracetam increases the resistance of the fetus to intrauterine hypoxia, lowers neonatal mortality and reduces the number of abortions, and improves the energy metabolism of the brain [6]; however, the mechanisms responsible for these effects of the drug are still unknown.

In the investigation described below some aspects of the mechanism of the antihypoxic action of pyracetam were studied.

EXPERIMENTAL METHOD

The region of the pedicle of the left kidney was infiltrated with 2% procaine solution in acute experiments on noninbred albino rats under pentobarbital anesthesia (40 mg/kg body weight). The blood vessels and ureter were exposed and clamped for 15, 30, 60, 120, and 240 min, thus producing a model of ischemia. Biopsy of kidney tissue was then undertaken at various stages of ischemia. The biopsy material, weighing 10-20 mg, was homogenized and used for investigation of lipid peroxidation (LPO). The kinetics of LPO was studied by the chemiluminescence (CHL) method [1]. The rate of rise of the initial, exponential part of the curve characterizing the stage of the slow flash of CHL, was investigated. In a special series of experiments in vitro on models of liposomes made from egg yolk the effect of the drug on the parameters of LPO was studied, compared with that of the known antioxidant α-tocopherol (TP). The effect of pyracetam on the rate of deoxygenation of the erythrocytes was studied by a polarographic method on the apparatus described previously [4]. Blood diluted with physiological saline in the ratio of 1:3 (pH 7.4) was added in a volume of 0.1 ml to the measuring cell. The blood solution was saturated beforehand with a mixture of 95% O_2 + 5% CO_2 for 2 min. The current in the cell was measured at 36°C. To determine the affinity of the erythrocytes for oxygen, the value of the semisaturation current was calculated graphically. In a special series of experiments the effect of prolonged administration of the drug on the respiration rate of liver mitochondria from Wistar rats, receiving pyracetam in a dose of 50 mg/kg daily for 30 days with their food, was investigated. Respiration of the liver mitochondria (MCH) was determined polarographically with the aid of an oxygen electrode of Clark type. Mitochondrial preparations were isolated by the method in [3] in G.P. Kirillova's modification: the first centrifugation was carried out at 1800g (10 min, 4°C), the supernatant was centrifuged under the same conditions at 12,000g, the MCH were sedimented again under the same conditions and then resuspended in isolation medium containing fraction 5 of bovine serum albumin, purified from fatty acids (Serva, West Germany). The suspension of MCH contained 80-100 mg protein in 1 ml. Rats not receiving the solution of pyracetam served as the control. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The study of the rate of deoxygenation of the blood under the influence of pyracetam showed that it lowers the values of the semisaturation current (Fig. 1), evidence of an increase in the affinity of hemoglobin for oxygen under the influence of the compound.

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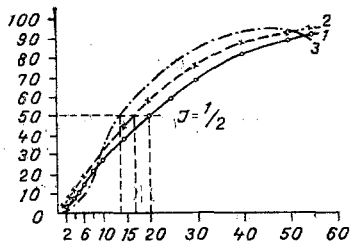

Fig. 1

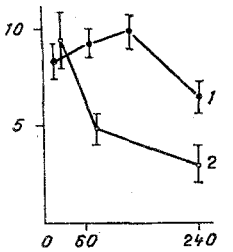

Fig. 2

Fig. 1. Effect of pyracetam on affinity of hemoglobin for oxygen. Abscissa, saturation current; ordinate, hemoglobin saturation with oxygen (in %); 1) control (whole blood); 2) blood + pyracetam in a dose of 0.05 mg/ml; 3) blood + pyracetam in a dose of 0.25 mg/ml.

Fig. 2. Effect of pyracetam on velocity of Fe^{++}-induced CHL in homogenates of ischemic rat kidney tissue. Abscissa, time (in min); ordinate, intensity of CHL (in relative units). 1) Dynamics of CHL in intact nephrons; 2) dynamics of CHL of nephrons during administration of pyracetam in a dose of 400 mg/kg.

TABLE 1. Effect of Pyracetam on Respiration of Rat Liver Mitochondria

Statistical parameter	Parameter of mitochondrial respiration				
	V_0	V_3	V_4	respiratory control (V_3: V_4)	V_{dnp}
Range of variation	12,6—19,5	38,1—58,5	15,6—27,4	2,37—4,1	45,6—74,3
		Expt.			
Percentage difference	9,6—19,1	30,1—46,5	12,6—24,3	1,58—3,24	33,2—60,8
	29,1±6,9	34,8±5,3	25,4±5,3	56,2±7,4	42,5±6,3

Legend. Rate of respiration (V) of mitochondria determined in nanomoles O_2/min/mg protein; V_0) original respiration rate of MCH in the presence of glutamate and malate; V_3) respiration rate in Chance's state 3; V_4) respiration rate of MCH in Chance's state 4; V_{dnp}) rate of uncoupled respiration of MCH in the presence of dinitrophenol.

Investigation of the respiratory function of the hepatocytes in rats treated for a long time with pyracetam revealed in the first, pilot series of experiments, that all the basic parameters of respiration of MCH were depressed (compared with the control) in rats receiving the compound (Table 1). Incidentally, these data were obtained when the pH of the isolation medium was 7.5, the pH of the incubation medium of MCH was 7.2, and the incubation temperature was 37°C. To discover any possible general rules governing the processes studied, it was decided that in later experiments the pH of the isolation medium (in some experiments pH 7.4), and of the incubation medium (pH 7.0, 7.2, and 7.4 in different series) should be changed, by altering their composition. The incubation temperature of MCH was lowered to 28°C. Under these circumstances in all cases just as in the previous series of experiments, all the parameters of mitochondrial respiration studied in rats receiving pyracetam were reduced.

In the study of the kinetics of LPO in kidney tissue homogenates, prophylactic peroral administration of pyracetam in a dose of 400 mg/kg 1 h before the beginning of renal ischemia lowered the rate of CHL to the stage of a slow flash of luminescence, evidence of reduction of the effect of lipid peroxidation under the influence of pyracetam, i.e., the drug inhibited free-radical peroxidation processes (Fig. 2).

In experiments in vitro on models of liposomes the antioxidative activity of pyracetam, calculated by the method in [2], was $5.3 \cdot 10^2$ M^{-1}, which does not differ significantly from the corresponding value for TP, namely $1.5 \cdot 10^3$ M^{-1}.

The following connection can be postulated between the phenomena described above: injection of the drug increases the affinity of hemoglobin for oxygen, and naturally this leads to minimization of oxidation processes in the mitochondria and a change in the respiratory electron transfer chain through the lipid bilayer of the membranes, in which free-radical LPO is correspondingly inhibited.

One of the components of the intimate mechanisms of the antihypoxic action of pyracetam is therefore its ability to inhibit LPO.

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ANTIHYPOXIC ACTIVITY OF HEME-PEPTIDES OF CYTOCHROME C

A. S. Losev, Yu. G. Bobkov, A. I. Lysko, A. M. Arutyunyan, A. F. Mironov, V. D. Rumyantseva, and R. P. Evstigneeva

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The search for effective antihypoxic agents among natural and synthetic compounds is necessitated by the importance of the hypoxic factor in the genesis and development of diseases of varied etiology. The hypoxic factor also plays an important role in the recovery period after exposure of the healthy individual to extremal factors of varied nature. Among pharmacological agents which increase working capacity may be mentioned antihypoxic agents of the electron carrier group [1], which possess strong electron-acceptor properties, such as, for example, cytochrome c.

There are data in the literature on the antihypoxic properties of cytochrome c [8, 9]. It has proved effective in the treatment of massive blood loss and during resuscitation measures [4, 7].

There is also evidence of the antihypoxic action of a heme-octapeptide obtained from cytochrome c by enzymic hydrolysis [14] This suggests that the clinical effect of cytochrome c is due to the action of heme-peptides, which are its metabolic products.

The aim of this investigation was to compare the antihypoxic activities of several heme-peptides of cytochrome c when given prophylactically and therapeutically in the recovery period after acute hypobaric hypoxia (AHBH) to mice with different types of hypoxic resistance.

EXPERIMENTAL METHOD

In a model of AHBH [2] on male mice (BALB/c × B10CW; CW × ASn × CC57W tetrahybrids) weighing 16-22 g, in the experiments of series I the antihypoxic activity of five heme-containing compounds was compared: hemin (Sigma, USA), heme c, heme-nonapeptide, heme-peptide 1-65,

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR. Research Institute for Biological Testing of Chemical Compounds, M. V. Lomonosov Moscow Institute of Fine Chemical Technology.

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