BRAIN AND LIVER ARGINASE AND POLYAMINES IN THE MECHANISM OF THE PROTECTIVE ACTION OF ARGININE AGAINST HYPEROXIA

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The study of the mechanism of action of substances increasing resistance of the organism to the toxic action of oxygen has become a matter of urgent importance because of the widening of the field of practical application of hyperbaric oxygen. The resistance of the organism to hyperoxic conditions can be increased by means of natural metabolites. A promising protector is the amino acid arginine [1, 2], whose protective effect includes a direct action on the activity of various enzymes [2] and also an effect on metabolism of products of the arginase reaction — urea and ornithine. The antihyperoxic effect of urea and the mechanism of its protective action have been demonstrated in the authors' laboratory. Under hyperoxic conditions urea stabilizes biological membranes and helps to maintain metabolism at a near-normal level [4, 5]. An important aspect of the protective action of arginine may be its effect on the level of the polyamines spermidine and spermine, which are formed with the participation of ornithine, the second product of the arginase reaction. The content of polyamines in the brain and liver falls significantly under the influence of hyperoxia [6].

The object of this investigation was to study the effect of protective concentrations of arginine on arginase activity and on the level of the polyamines spermidine and spermine in the rat brain and liver under hyperoxic conditions.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-150 g. The rats were placed in a pressure chamber into which pure oxygen was pumped at a constant rate of 2 atm/min up to a pressure of 6 atm. Under these conditions the rats developed convulsions on average after 20 ± 10 min. The following groups of animals were used: 1) intact (control); 2) animals receiving an intraperitoneal injection of 1 ml of arginine solution (100 mg arginine/100 g body weight) 50 min before decapitation; 3) animals developing convulsions due to oxygen poisoning; 4) animals receiving the above-mentioned dose of arginine 30 min before exposure to oxygen and placed in the pressure chamber at the same time as the control animals. The animals took part in the experiments at the time when unprotected rats developed convulsions.

The sample for determination of arginase activity contained 25 mg brain tissue or 0.5 mg liver tissue, 2.5 μM $MnCl_2$, 20 μM arginine in 1 ml glycine buffer, pH 9.5. Samples with all components but inactivated by the addition of 12% TCA served as the control. The control and experimental samples were incubated for 30 min at 37°C. Arginase activity was judged from the quantity of urea formed, which was determined by the urease method [7].

The content of polyamines was determined by the method of Russell et al. [13] with modifications. Brain tissue from three animals (4.5 g) and liver tissue (1.5 g) were homogenized in the cold in a Potter's homogenizer with Teflon pestle in 2 volumes of distilled water. Proteins were precipitated by boiling and allowed to stand for 1 h to allow complete extraction of polyamines. The samples were then centrifuged for 15 min at 12,000g. The supernatant was alkalified with NaOH solution of pH 9.5-10.5, after which the polyamines were extracted with n-butanol (3 volumes relative to the initial quantity of tissue) for 1 h with constant shaking. The butanol phase was withdrawn and evaporated on a water bath at 80°C. The dry residue was dissolved in 0.1 ml 0.1 N HCl and applied to strips of mark C chromatography paper, measuring 20 × 2 cm, at a distance of 20 cm from the cathode. Electrophoresis was carried out in citrate buffer, pH 3.4, for 3.5 h with a current of 1.5 mA/cm. The strips were dried and stained

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TABLE 1. Arginase Activity (in μmoles urea/g tissue/30 min) and Polyamine Content (in nmoles/g tissue) in Rat Brain and Liver after Hyperoxia and Treatment with Arginine (M ± m)

Test object	Indicator of metabolism	Control	Hyperoxia	Arginine	Arginine + hyperoxia
Brain	Arginase	12,28±0,32	10,41±0,34‡	13,85±0,32‡	13,30±0,31
	Spermidine	119±6	68±8*	142±7†	125±2
	Spermine	40±1	22±2*	50±2*	45±2
Liver	Arginase	282±16	201±10*	413±10*	253±8
	Spermidine	778±47	686±31	901±39	919±46‡
	Spermine	248±20	213±16	261±21	254±16

* $P<0,001$.
† $P<0,01$.
‡ $P<0,05$.

with a 2% solution of ninhydrin in acetone, acidified with acetic acid, for 5 min at 100°C. The stained fractions of spermidine and spermine were cut out, eluted with 5 ml 10% acetone for 30 min in darkness, and then subjected to colorimetry on a Specol (East Germany) spectrophotometer at 560 nm. Spermidine trihydrochloride and spermine tetrahydrochloride, from Merck (West Germany), were used as standards.

EXPERIMENTAL RESULTS

In animals exposed to hyperoxia arginase activity in the brain was reduced by 16% and in the liver by 29% (Table 1). The content of the polyamines spermidine and spermine in the brain also was reduced by 43 and 46%, respectively. The spermidine content in the liver at the time of onset of convulsions showed only a tendency to fall, but 4 h after the beginning of hyperoxia the change became statistically significant [6].

Administration of arginine to intact animals increased the arginase activity in the brain by 15% and in the liver by 55%. This increase in arginase activity was accompanied by an increase in the content of spermidine and spermine in the rats' brain by 19 and 25%, respectively, whereas the spermidine content in the liver showed only a tendency to rise and the spermine level was unchanged.

Injection of arginine increased the ornithine concentration in the rat liver [14]. Correlation between arginase activity and the increase in polyamine content was found in the lymphocytes and placenta [9, 11]. The increase in the polyamine content may have been due not only to activation of arginase, but also the effect of arginine on liberation of hormones (insulin, glucagon, growth hormone) [10] which, in turn, activate ornithine decarboxylase [12].

Consequently, in animals exposed to hyperoxia after injection of arginine, arginase was activated and the polyamine level raised. Protection by arginine during the session of hyperoxia was manifested by the fact that brain and liver arginase activity did not fall below the control level, the polyamine content in the brain likewise remained at the control level, and in the liver a small increase in the spermidine content actually was observed.

The results show that a characteristic feature of the brain is the close relationship between its arginase activity and polyamine level. The reason is that the source of arginine in the brain for arginase is mainly arginine liberated from proteins, and the arginase reaction is the sole supplier of ornithine. In the liver, where the ornithine cycle of arginine synthesis functions and ornithine is formed continuously, arginase activity is less closely linked with the polyamine dynamics.

Activation of arginase and the increase in the polyamine content play an important role in the correction of disturbances of metabolism developing during hyperoxia. In oxygen poisoning processes of peroxidation are intensified and profound changes are observed in the system of protein biosynthesis and destruction [3]. The product of the arginase reaction, namely urea, whose concentration rises after administration of arginine, exhibits antioxidant properties, stabilizes lysosomal membranes, and prevents intensification of hydrolysis of biopolymers and, in particular, of proteolysis [5].

The main function of polyamines is activation of protein-synthesizing systems [13]. An elevated polyamine level can prevent a decrease in the intensity of protein biosynthesis under hyperoxic conditions. Polyamines have been shown to exert an antioxidant effect [8]; this is

probably connected with their ability to form complexes with metals and to reduce their prooxidant properties. This property of the polyamines is of great importance for correction of the trigger mechanisms of development of oxygen poisoning.

The effectiveness of arginine as a protective agent against hyperoxia is thus determined by the broad spectrum of its normalizing action.

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STUDY OF DEOXYRIBONUCLEASE I ACTIVITY IN A DEOXYRIBONUCLEASE I—INHIBITOR SYSTEM

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Despite intensive research in various laboratories of the world the functional role of deoxyribonuclease (DNase) I in eukaryotes has not been unequivocally established [11]. One reason for this is evidently that DNase I in the course of evolution has acquired another (regulatory) function in addition to its catalytic (hydrolytic) function. Functional heterogeneity, due both to the chemical properties of the molecules and participation of the same enzyme in different processes (repair, recombination, replication, restriction) is a characteristic feature of the nucleases [4, 9].

Besides nucleases, their inhibitors also are found in prokaryotes and eukaryotes. In animals inhibitors of both DNase I and DNase II have been found [8, 13].

A study of the inhibitor of DNase I in warm-blooded animals showed that it is present not only in the serum, but also inside the cells and forms a specific inhibitor—enzyme complex [14]. It is stated in the literature that the inhibitor of DNase I belongs to the class of actin proteins [12]. It has accordingly been suggested that it may play a role in the proliferation of spleen cells [3].

The object of this investigation was to study the relations between serum DNase I and its intracellular inhibitor *in vivo*.

KEY WORDS: exogenous deoxyribonuclease I; splenic inhibitor.

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