EFFECT OF HYPEROXIA ON CONTENT OF THE POLYAMINES SPERMINE AND SPERMIDINE IN RAT BRAIN AND LIVER

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The effect of an increase in the oxygen pressure (6 atm) on the content of the polyamines spermine and spermidine in the rat brain and liver was investigated. During the period of oxygen convulsions and 4 h after decompression the spermidine concentration in the brain and liver was reduced. The spermine concentration in the brain also was reduced during the period of convulsions, but 4 h after decompression it was significantly increased, although still below the control level. The spermine level in the liver was unchanged during oxygen convulsions, but rose sharply during the next 4 h. The role of polyamines in the regulation of protein biosynthesis during hyperoxia is discussed.

KEY WORDS: hyperoxia; spermine; spermidine.

Exposure of animals to a raised oxygen pressure causes profound disturbances of metabolism and the functions of the body. Prolonged exposure may lead to death. Meanwhile the practical use of hyperoxia is increasing and a close study of the mechanism of its toxic action is accordingly necessary.

In oxygen poisoning disturbances in the system of protein synthesis are observed. In the course of exposure for 1 h daily for 10 days to a pressure of 2 atm oxygen, on the third day the protein turnover (as [^{35}S]methionine) was found to be increased in the brain, liver, and kidneys [1]. A disturbance of the properties of DNA [5] and of the content and structure of RNA [4] has also been demonstrated in the brain during oxygen convulsions. One of the causes of development of changes in the nucleic acids during hyperoxia is a disturbance of the permeability of the lysosomal membranes and activation of acid deoxyribo- and ribonucleases of the brain [5]. A substantial decrease in the RNA content in motoneurons and the surrounding glia of the cerebral cortex and spinal cord during oxygen convulsions has been revealed by spectrophotometry [6].

Polyamines play an important, although insufficiently studied, role in the protein-synthesizing system. Incorporation of labeled leucine or phenylalanine into proteins of isolated cell nuclei of the cerebellum or cerebral hemispheres in vitro was intensified after the addition of spermine or spermidine. With an increase in the concentration of the latter, the stimulating effect was replaced by inhibition [8]. Depending on their concentrations, the polyamines stimulate or inhibit DNA synthesis catalyzed by DNA-polymerase [14]. The authors cited suggest that the stimulating effect of polyamines is due to their interaction with the DNA template. In experiments in vitro spermidine and spermine participate in the reaction catalyzed by DNA-dependent RNA-polymerase [7].

The object of this investigation was to study the effect of a raised oxygen pressure on the spermine and spermidine concentration in the brain and liver of rats.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-150 g. The rats were kept in a pressure chamber into which pure oxygen was pumped at a constant speed of 2 atm/min up to 6 atm. Under these conditions the rats developed convulsions on average after 25 ± 10 min. The following groups were investigated: 1) intact animals (control); 2) animals in a convulsive state; 3) animals which had developed convulsions and were tested 4 h after decompression (after-effect).

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The concentration of the polyamines was determined by the method of Russell et al. [10] with certain modifications. Brain tissue (4.5 g) from three animals or liver tissue (1.5 g) was homogenized in the cold in a Potter's homogenizer with Teflon pestle in 2 volumes of distilled water. Protein was precipitated by boiling and by centrifugation for 10 min at 10,000 g. The supernatant was alkalified with NaOH solution to pH 9.5-10, after which the polyamines were extracted with n-butanol for 1 h with constant stirring. The butanol phase was removed by suction and evaporated to dryness on a water bath at 80°C. The dry residue was dissolved in 0.1 ml 0.1N HCl and applied to strips of mark "C" chromatography paper measuring 30 × 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 for 5 min at 100°C with a 2% solution of ninhydrin in acetone, acidified with acetic acid. The stained fractions of spermine and spermidine were cut out, eluted with 5 ml 10% acetone for 30 min in darkness, and then examined colorimetrically at 540 nm. Spermidine trihydrochloride and spermine tetrahydrochloride (from Merck, West Germany) were used as the standard. The standard solutions were put through the same stages of processing as the tissue samples.

EXPERIMENTAL RESULTS

The spermidine concentration in the brain of the control animals in the present experiments was 119 nmoles/g and the spermine concentration 48 nmoles/g (Table 1). The concentrations of the polyamines in the brain as determined by different workers do not agree. According to Shaskan et al., the spermidine concentration in the rat brain is 186 nmoles/g [12], whereas the spermine concentration, according to Shaw [13] is 88 nmoles/g. The concentrations of spermine and spermidine in the present experiments were fairly close to these values.

According to other workers [9, 11], the concentrations of spermidine and spermine in the rat brain are 343-480 and 270-280 nmoles/g respectively. The spermidine and spermine concentrations in the liver of the control rats were 535 and 215 nmoles/g respectively (Table 1). This is significantly below the figures given by Raina (890 nmoles/g for spermidine and 700 nmoles/g for spermine) [9]. The disagreement could perhaps be due to the use of different methods of investigation. Electrophoresis on paper gave results closer to those now obtained. The higher results were observed during fluorimetric estimation of dansyl derivatives of the polyamines fractionated by thin-layer chromatography.

The spermidine concentration in the brain of the rats during convulsions was found to be significantly and sharply reduced (by 43%, Table 1). It had not returned to normal 4 h after decompression. The spermine concentration in the brain of the animals was reduced by 46% in the convulsive phase, but during the next 4 h the concentration increased again, although not up to the control level.

The spermidine concentration in the liver during the period of convulsions showed a tendency to decrease (Table 1). During the next 4 h its concentration continued to fall and reached 26% of the control value ($P < 0.02$). The changes in the spermine concentration were in the other direction. During the period of convulsions the spermine level remained unchanged, but after 4 h it had risen by 76%. Presumably this increase was due to activation of spermine synthetase, using spermidine as the precursor. However, the decrease in the spermidine level as observed could not have caused such a sharp increase in the spermine concentration. Possibly another mechanism of spermine formation unconnected with the addition of an aminopropyl group to the available spermidine exists. A similar conclusion was drawn by Shaskan [12], who studied the distribution of radioactive label in spermidine and spermine following administration of labeled putrescine.

The decrease in the spermidine concentration in the brain and liver and the spermine concentration in the brain of animals during hyperoxia cannot be explained purely by inhibition of the enzymes of their biosynthesis. The half-renewal time of spermidine in the liver is 4 days and in the brain 5 days [10]. This means that even if its biosynthesis is completely stopped for 4 h, its concentration could not fall so sharply. It can therefore be tentatively suggested that besides the inhibition of biosynthesis of spermidine in the liver and brain and of spermine in the brain during hyperoxic convulsions, sharp activation of the enzymes of their catabolism, especially diamine oxidases, takes place. During hyperoxia, degradation of the polyamines may perhaps take place through the intervention of monoamine oxidase (MAO). During hyperoxia the production of lipoperoxides in the tissues is increased [5]. According to Gorkin, lipoperoxides are a factor which can bring about the transformation of MAO, i.e., can modify its substrate specificity [2]. Such a transformation of MAO can take place in the brain during hyperoxia [3], and under these circumstances MAO becomes capable of deaminating putrescine, AMP, and glucosamine.

Another possible cause of the decrease in the polyamine concentration may be a change in permeability of the blood–tissue barriers. Under normal conditions the blood–brain barrier prevents the outflow of poly-

TABLE 1. Concentration (in nmoles/g tissue) of Spermidine and Spermine in Brain and Liver of Rats during Hyperoxia (M ± m)

Experimental conditions	Brain		Liver	
	spermidine	spermine	spermidine	spermine
Control	119±8 (9)	48±2 (6)	535±43 (14)	215±13 (15)
Convulsions.	68±8* (7)	26±2* (9)	473±21 (16)	213±16 (17)
After-effect (4 h)	51±7* (7)	34±3* (7)	394±43* (8)	379±29* (12)

*$P < 0.05$ compared with the control.

Legend. Number of experiments given in parentheses.

amines from the brain. In hyperoxia increased permeability of the blood–brain barrier is observed and spermidine and spermine can escape into the blood stream, where their concentration is much lower.

Many workers who have studied changes in the concentration of polyamines during development of the brain and after certain procedures (hepatectomy) have observed correlation between the polyamine concentration and the nucleic acid concentration. Changes in the spermidine level correlate with changes in the RNA concentration, whereas changes in the spermine concentration correlate with DNA [11]. Comparison of the polyamine concentrations in the brain and the nucleic acid concentrations during hyperoxia in the present experiments showed that changes in the spermidine level in the brain correlate closely with changes in the RNA concentration during hyperoxia.

During exposure to an extremal factor such as hyperoxia the protein concentration in the brain cells falls and destruction of the cells is intensified [5, 6]. At that moment there is a sharp decrease in the spermidine concentration in the brain and liver. Processes of destruction of the proteins of the brain and other tissues are intensified still further 4 h after exposure to hyperoxia, and this is accompanied by a further decrease in the spermidine concentration.

It can be postulated that the spermidine concentration is one of the mechanisms of regulation of protein biosynthesis at the RNA level. The increase in the spermine concentration in the brain and liver 4 h after exposure to hyperoxia is evidently connected with its role in repair processes at the level of DNA and RNA.

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