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OXYGEN TOXICITY IN THE MAMMALIAN BRAIN

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The lactate dehydrogenase (LDH) activity of mouse brain homogenates was examined after exposure to hyperbaric oxygen (5763.8 mm Hg PO ₂) and compared to room air controls (158.8 mm Hg PO ₂). The effect of reduced glutathione on LDH activity after hyperbaric oxygen exposure was also examined. The activity of LDH after treatment with hyperbaric oxygen was significantly diminished when compared with controls. In the presence of reduced glutathione, homogenates exposed to hyperbaric oxygen demonstrated higher activity than did		

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homogenates incubated without glutathione. It is concluded that oxygen induced inhibition occurs through the oxidation of essential free sulfhydryl groups and that this oxidation can either be prevented by reduced glutathione or the disulfide bridges may be reduced to free sulfhydryl groups by the glutathione after oxidation.

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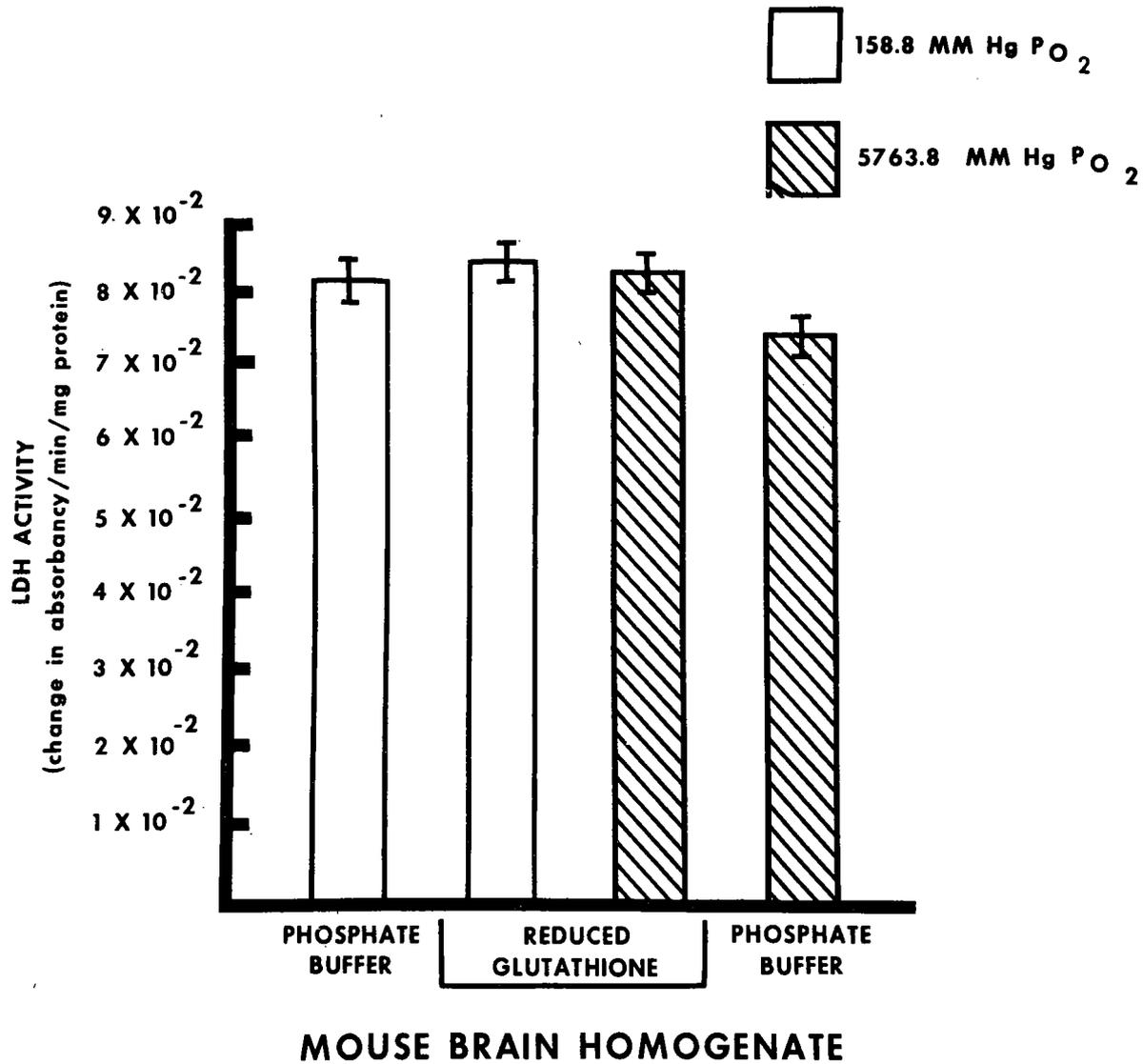
TABLE I

Mouse brain lactate dehydrogenase activity as influenced by hyperbaric oxygen. LDH activity determinations were made after 5 hours at 20°C and were expressed in terms of change in absorbancy/min/mg protein.

LDH Activity Mean \pm SE (N)	Incubation Media	PO ₂ (mm Hg)	% Change**
0.08230 \pm 0.00857(68)	Phosphate Buffer	158.8	-
0.08504 \pm 0.00719(68)	Phosphate Buffer + GSH	158.8	+3.22
0.08320 \pm 0.00736(68)	Phosphate Buffer + GSH	5763.8	+1.08
0.07460 \pm 0.00783(68)*	Phosphate Buffer	5763.8	-9.35

*Significant difference in LDH activity at the 5% level.

**Based on % change from room air control without reduced glutathione.



MOUSE BRAIN HOMOGENATE

FIGURE 1

Lactate dehydrogenase activity (change in absorbancy/min/mg protein) in mouse brain homogenate as influenced by five hour exposure to hyperbaric oxygen ($5763.8 \text{ mm Hg PO}_2$) or room air (158.8 mm Hg PO_2) at 20°C . Plotted are mean \pm S.E. Refer to Table I for statistical details.

INTRODUCTION

The toxic effects of oxygen on the central nervous system were first described by Bert¹ in the nineteenth century. It has since been found that oxygen mediated convulsions occur after a latent period that is inversely proportional to the partial pressure of oxygen.²

Although the exact mechanism of oxygen toxicity has not been firmly established, experimental results have led to a number of hypotheses. Lipid peroxidation, oxidation of pyridine nucleotides and free radical formation have all been suggested by Haugaard³ in an excellent review article. An increasing amount of evidence supports the hypothesis that oxygen causes a breakdown in oxidative metabolism resulting in a decrease in the amount of energy-rich phosphate bonds available to nervous tissue.⁴ The inhibition of oxidative metabolism has been explained by a direct toxic effect of oxygen on certain enzymes involved in the tricarboxylic acid cycle in mammalian brain and liver.⁵

There is a noticeable paucity of information in the literature concerning the toxic effects of oxygen on the enzymes involved in the glycolytic scheme. Horn, Haugaard, and Haugaard⁶ found that glyceraldehyde phosphate dehydrogenase was the only enzyme in the reactions from fructose-diphosphate to lactate that was rapidly inactivated by oxygen at 1 atm. Shaw and Leon⁷ found no significant change in LDH activity or triose phosphate dehydrogenase activity in the rabbit retina during exposure to oxygen at 1 atm. Lactate dehydrogenase of yeast, however, was found by Armstrong, Coates, and Morton⁸ to be so easily inactivated in air that it was difficult to purify. Thus, there is some controversy concerning the susceptibility of LDH to oxygen toxicity.

A number of different compounds have been used as protective agents against oxygen toxicity.^{4,9,10} Most of these studies have been concerned with acute oxygen toxicity which leads to convulsions preceding death in rats and mice. These in vivo studies have thus far not elucidated the mechanism of oxygen toxicity or the mechanism of protection against oxygen toxicity afforded by various compounds.

The present in vivo experiments were performed on mouse brain homogenates. The studies were conducted to determine: (1) if LDH is inhibited by elevated PO_2 , and (2) if protection against oxygen toxicity is afforded by reduced glutathione added to the brain homogenates.

MATERIALS AND METHODS

Male and female Swiss Webster mice (6 months old, approximate weight 35-40g) were used in all experiments. Mice were killed by cervical dislocation and the brain was removed. The brain was then sonified in 10 ml of phosphate buffer (0.034M, pH 7.40). The homogenate was centrifuged (Beckman, model LZ-50 ultracentrifuge) at 4°C, 20,000 RPM for 30 minutes and the supernatant was removed and placed on ice. Two 2.5 ml portions of the supernatant were taken and placed in two test tubes. To one tube was added 25 μ l of phosphate buffer and to the other 25 μ l of reduced glutathione (Calbiochem, Los Angeles, CA). The final concentration of reduced glutathione was 1.4 μ moles/g, a concentration closely approximating the concentration of reduced glutathione in the mouse brain in vivo.¹¹

Brain homogenates from the same animal were divided into two groups, the controls and the experimentals. Each incubation vial consisted of 100 μ l of the appropriate enzyme mixture. The control samples were exposed to room air (158.8 mm Hg PO₂) for five hours and the experimentals were exposed to 100% O₂ at 100 psi (5763.8 mm Hg PO₂) for five hours in a table top Bethlehem Environmental Chamber (model H-70-A, Bethlehem, PA). The control and experimental groups were further divided, i.e., one-half of the group consisted of enzyme plus phosphate buffer and the other half consisted of enzyme plus reduced glutathione.

Lactate dehydrogenase activity of the brain homogenates was determined by the method of Worthington (Worthington Biochemical Corp., Freehold, NJ). Following incubation the LDH activities of the homogenates were determined by measuring the spectral conversion of NADH₂ to NAD on a Cary 14 recording spectrophotometer (Cary Instruments, Monrovia, CA) at a wavelength of 340 m μ and a temperature of 20°C. The assay medium consisted of 0.1 ml NADH₂ (0.0027 M, pH 8.0), 2.7 ml phosphate buffer (0.034 M, pH 7.4), and 0.1 ml sodium pyruvate (0.01 M, pH 7.0). At time zero, the medium was added to the incubation vial and vigorously mixed with the enzyme. The mixture was then immediately placed in a quartz cuvette and its changing optical density was compared to a blank (assay medium plus 100 μ l of phosphate buffer).

The change in absorbancy was linear from 0.5 to 1.5 minutes with linearity over this range being obtained by adjusting the enzyme concentration. Enzymatic activity was expressed as change in absorbancy/min/mg protein. Protein determinations were done by a modification of the method of Lowry.¹²

RESULTS

An incubation period of five hours was found to be optimum in that LDH retained its activity in room air and when exposed to hyperbaric oxygen, allowed sufficient time for enzymatic inhibition. Preliminary studies showed that mouse brain LDH was not significantly inhibited after exposure to 100% O₂ at 7.6 atm for three-hour periods.

The experimental group exposed to hyperbaric oxygen without glutathione demonstrated a significantly reduced LDH activity as compared to the controls and the experimental group with glutathione (Table 1, Fig. 1). The control group with glutathione demonstrated a slightly higher, but insignificant, activity than the control group without glutathione. In order to determine if there was a possible oxidation with resultant inactivation occurring in the control without glutathione, brain homogenates both with and without glutathione were incubated in 100% N₂ for five hours. There was no significant difference in enzyme activity in the two groups after the incubation period, indicating the possibility of a partial oxidation occurring in the room air controls without glutathione.

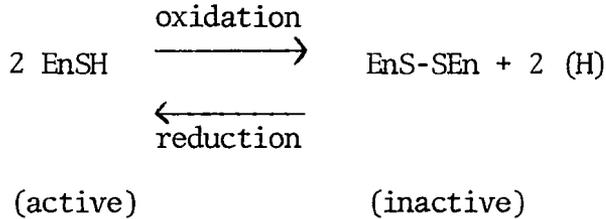
DISCUSSION

Lactate dehydrogenase activity is inhibited by exposure to hyperbaric oxygen in mouse brain homogenate. This fact demonstrates that oxygen can directly inhibit a glycolytic enzyme. It has been shown that oxygen can inhibit succinic dehydrogenase, an important enzyme of the tricarboxylic acid cycle (TCA), in neural tissue. The observation that oxygen inhibits LDH means that oxygen can interfere with the oxidative metabolism of the brain either by a direct action on a TCA cycle enzyme or indirectly by inhibiting an enzyme of the glycolytic scheme, thus diminishing the production of precursors needed for oxidative metabolism.

Fundy, et al., have established that four sulfhydryl groups per molecule are essential for the catalytic operation of lactate dehydrogenases from a number of animals.¹³ In vitro studies dealing with oxygen toxicity have provided strong support for the hypothesis that oxidation of sulfhydryl groups of important tissue constituents plays an important role in the production of the symptoms of oxygen toxicity in vivo.³ The tetrameric nature of the LDH molecule, along with the observation that four sulfhydryl groups per molecule are essential for catalytic operation, suggest the presence of one active site sulfhydryl group per subunit.

Upon treatment with mild oxidizing agents, including molecular oxygen, the inactivation of the hydrolytic enzymes is considered to

involve the formation of the disulfide form of the enzyme according to the equation:



where: En = enzyme

The oxidation by oxygen of SH groups to disulfide (S-S) linkages within a given chain of the enzyme molecule requires that the sulfhydryl groups be present in reasonably close juxtaposition in the peptide chain. In the LDH molecule, where the essential SH groups do not lie in the same chain, there are two possible mechanisms by which disulfide bridge formation may occur. First, it is possible that the SH groups from two different chains may be close enough for interchain S-S groups to be formed by oxidation with molecular oxygen. A second possibility is the occurrence of a dimerization between enzyme molecules as a result of intermolecular disulfide bridge formation.

There are a number of problems involved in the in vivo study of protective agents against oxygen toxicity. A major problem in comparing the protection afforded by different compounds administered via the intraperitoneal route is the relative time and rate of absorption from the peritoneal cavity. In addition, it is necessary to assume an often times unknown equilibration period for the protective agent following injection and prior to exposing the animal to hyperbaric oxygen. There are also a number of other factors that can influence the sensitivity of an animal to oxygen toxicity, such as fear and nutritional state. For the above reasons, an in vitro approach, in which the system being studied can be isolated, is best for studying the effects of protective agents on cellular oxygen toxicity.

Lactate dehydrogenase maintained activity equivalent with controls (159 PO₂) when incubated in vitro with reduced glutathione and exposed to hyperbaric oxygen (5764 PO₂). This observation provides evidence that inhibition occurs through the oxidation of essential free sulfhydryl groups, and this oxidation can be prevented by reduced glutathione. Glutathione may exert its protective effect by maintaining the free sulfhydryl groups in a reduced and viable state when exposed to the oxidizing atmosphere, or the disulfide bridges may be reduced to free sulfhydryl groups by the glutathione after oxygen induced oxidation.

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