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ABSTRACT

The effect of hyperbaric oxygen tensions on the oxygen consumption of mouse liver homogenates was investigated. It was found that hyperbaric oxygen rapidly inhibits the oxidative metabolism of the mammalian liver. Mouse liver homogenate exposed to an oxygen tension of 3837.8 mm Hg for 30 minutes demonstrated a 50.6% reduction in oxygen consumption compared to controls exposed to nitrogen at ambient pressure.

The effects of reduced glutathione as a protective agent against hyperbaric oxygen toxicity were also examined. Liver homogenates pretreated with reduced glutathione and exposed to hyperbaric oxygen tensions demonstrated greater activity than untreated controls. It is concluded that: (1) Reduced glutathione protects important enzymes associated with oxidative metabolism by keeping them in a reduced and viable state, and (2) Reduced glutathione can stimulate oxygen consumption by increasing succinate formation through a reduced glutathione - succinate shunt.

INTRODUCTION

Studies of oxygen toxicity *in vitro* have provided strong support for the hypothesis that oxidation of sulfhydryl groups on important tissue enzymes plays an important role in the production of the symptoms of oxygen toxicity *in vivo* (Haugaard, 1968). The oxidation of sulfhydryl groups could either occur within a molecule or between different molecules resulting in disulfide bridge formation and consequent enzyme inactivation. One group of sulfhydryl-containing enzymes, the dehydrogenases, has been shown to be particularly sensitive to the toxic effects of oxygen. An important glycolytic enzyme, lactate dehydrogenase, from the amphibian retina has been shown to be inhibited by exposure to normobaric oxygen tensions for 24 hours (Baeyens and Hoffert, 1972). Inhibition of enzymes involved in oxidative metabolism would be reflected by a decreased oxygen consumption upon exposure to high oxygen tensions. In most *in vitro* studies dealing with oxygen toxicity, the inhibiting effects of oxygen on cellular metabolism have been demonstrated only after exposure of the tissues to oxygen for a relatively long period of time or by exposing them to extremely high oxygen tensions.

In the cell, reduced glutathione (GSH) is a major sulfhydryl group containing component. Reduced glutathione activates a number of enzymes, and it was proposed by Barron (1955) that the main function of this substance is to maintain sulfhydryl groups of enzymes in their reduced and active state. The early rationale for using GSH as a protective agent against oxygen toxicity was mainly because of this "sulfhydryl-sparing effect." More recently it has been observed that GSH may stimulate oxidative metabolism by another mechanism (Sanders et al., 1969). Glutathione can be converted to succinate by a number of different tissues, and this increased production of succinate can serve as a secondary support system for the maintenance of high energy levels.

The purpose of our study was two-fold. First, does oxygen at elevated partial pressures exert an inhibitory effect on the mammalian liver and secondly, if such an inhibitory effect does exist, can it be alleviated by the presence of GSH?

MATERIALS AND METHODS

Male and female adult Swiss Webster mice (approximate weight 35-40 g) were used in all experiments. Mice were killed by cervical dislocation and a piece of liver weighing approximately 850 mg was removed and the wet weight was recorded. The hepatic tissue was then homogenized in phosphate buffer for 15 seconds. A 2-ml sample of the liver homogenate was placed in each of two 50-ml Erlenmeyer flasks containing 22 mg of dextrose and 20 ml of mammalian Krebs-Ringer solution (pH=7.60, osmolarity 289.25 m Osm/Kg) modified to contain 2.94 g $NaHCO_3$. In addition, 78 mg of reduced glutathione was added to one of the flasks making the final concentration of GSH in the flask 12 m M/1. Two 10-ml portions were then withdrawn from both Erlenmeyer flasks and each portion was placed in a separate petri dish. The control petri dishes, one containing GSH and the other containing no GSH, were exposed to a nitrogen environment (P_{N_2}=757 mm Hg) for 0.5 hours. The two experimental petri dishes, one containing GSH and the other without GSH, were placed in a table-top Bethlehem Model H-70-A Environmental Chamber. The chamber was filled with medical oxygen at a pressure of 3837.8 mm Hg. The total incubation time was 30 minutes, including a 15 minute purge with pure oxygen at a flow rate of 8 1/min. to completely remove any residual gases.

Following the incubation period, the oxygen consumption of the control and experimental tissue homogenates was measured by means of a YSI Model 53 Biological Oxygen Monitor. The system consists of a Clark-type oxygen electrode and four tissue bath chambers for the oxygen consumption measurements. The temperature of the oxygen electrode and the tissue chambers was regulated at 20±.1°C by means of a constant temperature water bath. The electrode system was calibrated against an air-saturated solution of modified mammalian Krebs-Ringer of 3 ml vol. contained in one chamber of the tissue bath assembly. Each of the other three chambers contained 3 ml of air-saturated liver homogenate. The solutions were kept saturated with air by means of a magnetic stirer in each chamber. To determine oxygen consumption, meter readings were taken at the start and finish of a five-minute recording period. By substracting the concentration of oxygen remaining after five minutes from the initial concentration of oxygen at zero time and knowing the solubility coefficient of oxygen in mammalian Krebs-Ringer (=0.0310) (Umbreit et al., 1964) at 20°C, it was possible to determine the oxygen consumption of the tissue homogenate. Oxygen utilization was expressed as μl of oxygen consumed per hour per mg wet weight. All values were corrected to standard temperature and pressure dry (STPD). A student t-test was used to determine significant differences within and between control and experimental groups.

RESULTS

Preliminary experiments were performed to determine if exposing the control liver homogenates to 100% nitrogen at ambient pressures had any detrimental effect on oxygen consumption. There were no significant differences ($P<0.1$) in oxygen consumption between homogenates incubated in room air and those incubated in nitrogen at ambient pressure for 30 minutes.

Table I gives the mean values for liver oxygen consumption from 16 animals. A statistical analysis revealed that there were no significant differences ($P<0.1$) in liver oxygen consumption between animals. The toxic effects of oxygen on oxidative metabolism were demonstrated by a 50.6% reduction in oxygen consumption of the experimental homogenates exposed to hyperbaric oxygen for 30 minutes. This oxygen-induced reduction in oxygen consumption was significant at a $P<0.001$.

Control homogenates pretreated with GSH showed significantly higher ($P<0.01$) oxygen consumptions than controls containing no

Table I: Oxygen consumption of mouse liver as influenced by hyperbaric oxygen. Oxygen utilization determinations were made after 30 minutes at 20°C and were expressed in terms of l of oxygen consumed per hour per mg wet weight.

TISSUE PREPARATION	INCUBATION GAS	O_2 CONSUMPTION Mean ± S.E.(N)	% CHANGE***
CONTROL LIVER HOMOGENATE	P_{N_2}=756.7 mm Hg	63.25 ± 2.86(48)*	----
CONTROL LIVER HOMOGENATE 12mM GSH	P_{N_2}=756.7 mm Hg	109.17 ± 2.61(48)	+ 42.1
EXPERIMENTAL LIVER HOMOGENATE	P_{O_2}=3837.8 mm Hg	31.27 ± 1.94(48)**	- 50.6
EXPERIMENTAL LIVER HOMOGENATE 12mM GSH	P_{O_2}=3837.8 mm Hg	98.50 ± 3.36(48)	+ 35.8

*Significantly different from GSH treatments at 1% level.
**Significantly different from untreated control at 1% level.
***Based on % change from untreated control.

GSH. Experimental homogenates pretreated with GSH and exposed to hyperbaric oxygen also had higher rates of oxygen consumption ($P<0.01$) than untreated controls. Thus, GSH not only protected liver tissue against hyperbaric oxygen toxicity but also had the effect of stimulating oxygen consumption over that of the untreated control.

DISCUSSION

There has been a reluctance to conclude that the inhibitory effects of oxygen on metabolism are the direct cause of the symptoms of oxygen toxicity in the intact animal. The main reason for this reluctance is that the inactivation of enzymes *in vitro* appeared to be too slow in onset to account for the symptoms of oxygen toxicity in the intact animal. The present study demonstrates that the earlier views may have been too conservative. Oxygen is capable of reducing the oxygen consumption of liver homogenate by 50.6% in as little time as one-half hour at a relatively low pressure.

Numerous studies have demonstrated that various sulfhydryl group containing compounds afford protection against oxygen toxicity or at least delay the appearance of symptoms under some experimental circumstances. Baeyens (1975) has shown that one such compound, GSH, can maintain LDH activity after exposure of mouse brain homogenates to a P_{O_2} of 5764 mm Hg for five hours. The observation that GSH has a protective effect against oxygen toxicity provides support for the concept that oxidation of sulfhydryl groups plays a role in the mechanism of oxygen toxicity. Reduced glutathione protects important tissue constituents by a "sulfhydryl-sparing effect"; that is, by maintaining essential sulfhydryl groups in a reduced and viable state.

In a study dealing with GSH as a possible protective agent against oxygen toxicity, the GSH-glutamate-GABA-succinate pathway was implicated as a support system for the maintenance of brain and liver ATP levels in six different species of experimental animals exposed to hyperbaric oxygen (Sanders et al., 1969). This pathway, also called the GSH-succinate shunt, is shown in Figure 1. The physiological significance of the GSH-succinate shunt has not been clearly elucidated, but a possible function has been proposed. The shunt may function in bypassing the inhibition of α-ketoglutarate dehydrogenase of the Krebs cycle by removing α-ketoglutarate from the cycle by transamination with GABA to yield glutamate and the re-entry of the carbon chain at the succinate level. The possible physiological significance of the GSH-succinate shunt may be as an alternate source for the production of succinate.

Experiments in which rats have been exposed to high pressure oxygen have shown that prior injections of succinate resulted in normal and above normal concentrations of ATP being present in the cerebral hemisphere, liver and kidney (Sanders et al., 1965). In contrast, in the corresponding tissues of animals not pretreated with succinate, there was a significant reduction in the concentration of ATP. It was concluded that succinate protects against oxygen toxicity by restoring ATP concentration and metabolic function in these tissues.

When succinate is the substrate, only two molecules of ATP are formed per atom of oxygen used as compared to three atoms of ATP per atom of oxygen when α-ketoglutarate is converted to succinate in the Krebs cycle. Thus, succinate not only can result in an increased production of ATP, but also utilizes more oxygen than other oxidative phosphorylation substrates. This explains the increased oxygen consumptions of liver homogenates treated with GSH in our experiments. The GSH increases succinate production by means of the GSH-succinate shunt resulting in increased oxygen consumption by the tissue to maintain the energetic efficiency of ATP formation.

In conclusion, the 50.6% reduction in hepatic oxygen consumption in the experimental liver homogenates exposed to hyperbaric oxygen indicates that oxygen can directly inactivate an enzyme or enzymes of oxidative metabolism. The fact that liver homogenates pretreated with GSH demonstrated higher rates of oxygen consumption than the homogenates without GSH can be explained by two different mechanisms. First, GSH protects the enzymes of oxidative metabolism by maintaining them in a reduced and viable state. Second, and more importantly, the increased oxygen consumption in both control and experimental homogenates treated with GSH is due to increased succinate production by the GSH-succinate shunt.

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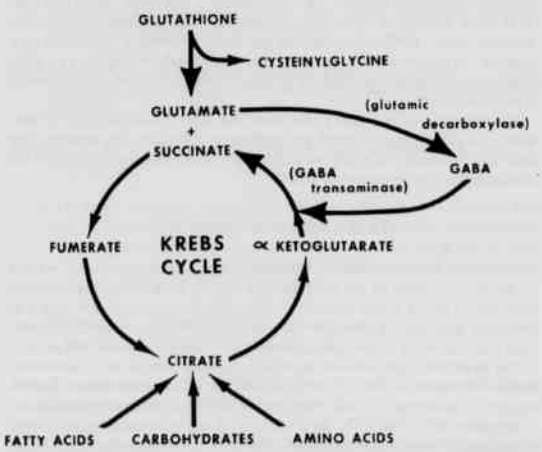

Figure 1. GSH-Succinate Shunt (shown by large arrows) and its relationship to the Krebs cycle.