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INHIBITORY EFFECT OF HIGH OXYGEN PRESSURE ON POTASSIUM-INDUCED ACTIVATION OF PYRUVATE DEHYDROGENASE AND GLUCOSE METABOLISM IN RAT BRAIN SLICES

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SUMMARY

The effects of high oxygen pressure on pyruvate dehydrogenase (pyruvate: lipoate oxidoreductase (decarboxylating and acceptor-acylating), EC 1.2.4.1) activity, tissue concentration of ATP, and CO_2 production from glucose were studied in rat brain cortical slices. The increase in pyruvate dehydrogenase activity and the lowering of cellular ATP, occurring during potassium-induced depolarization at 1 atm of oxygen, were reversed by increasing the oxygen pressure to 5 atm. When brain slices were incubated at 1 atm oxygen with [U-^{14}C]glucose, a high potassium medium approximately doubled the production of $^{14}CO_2$. Oxygen at 5 atm abolished this potassium-dependent increase in $^{14}CO_2$ production with no significant effect on glucose oxidation in normal Krebs-Ringer phosphate medium. Adding 4 atm helium to 1 atm oxygen did not interfere with the ability of potassium ions to activate pyruvate dehydrogenase, lower ATP, or increase glucose oxidation. The results show that toxic effects of hyperbaric oxygen, not manifest in "resting" tissue, may be revealed during stress such as potassium depolarization. The site of the toxic effects of oxygen is probably the cell membrane where excess oxygen appears to interfere with the action of the sodium pump, calcium transport or other processes stimulated by increased concentrations of extracellular potassium.

INTRODUCTION

The oxidative decarboxylation of pyruvate is catalyzed by the pyruvate dehydrogenase complex (pyruvate: lipoate oxidoreductase (decarboxylating and acceptor-acylating), EC 1.2.4.1) leading to formation of CO_2, followed by the sequential acetylation of co-enzyme A and the reduction of NAD [1]. The reaction may be regulated by the availability of substrate or coenzymes, by product inhibition [2–4], and by the reversible inactivation and activation of the apo-enzyme through phosphorylation and dephosphorylation respectively [5, 6]. We have found that pyruvate dehydrogenase is activated in cortical brain slices by potassium depolarization [7]. The finding demonstrates that the oxidation of pyruvate is rate-limiting in respiration of the brain, sub-

stantiating the hypothesis of Kini and Quastel [8]. Our interest in oxygen toxicity led to the investigation of the potassium-induced activation of pyruvate dehydrogenase and glucose metabolism under hyperbaric conditions.

METHODS

Brain slices were obtained from 100–150 g male Wistar rats fed ad libitum. The method for preparation of the slices and the procedures for incubation are described in a previous publication [7]. The slices were treated in pairs, each pair originating from the two hemispheres of the same animal, one of them serving as a control sample. Composition of incubation media and other experimental details are included with the tables. The experiments were carried out using a hyperbaric chamber (Model G15-APSP, Bethlehem Corp.) mounted on a shaker and fitted with a temperature control system. Before each experiment oxygen was used to flush out the air from the chamber. Pressure was raised at a rate of 1 atm/min, and abrupt decompression (approx. 1 atm/s) was used at the termination of the experiment. The samples were immediately frozen in liquid nitrogen and stored for subsequent analysis of pyruvate dehydrogenase activity or transferred into ice-cold 5 % perchloric acid in preparation for ATP analysis. To study the rate of CO_2 formation, brain slices were incubated in media containing [U-^{14}C]glucose in Warburg flasks with closed side-arms and sealed with rubber stoppers. A hypodermic needle was inserted through each stopper to allow pressurization of the interior of the flask. A paper wick soaked in 100 μl phenylethylamine served as the CO_2 trap in the center well. The reaction was stopped by tilting the chamber for transfer of 1 ml 5 % trichloroacetic acid from the side-arm to the reaction mixture. 20 min later, when the evolved CO_2 was completely absorbed, the chamber was decompressed at a rate of 1 atm/min. The paper wicks were transferred into liquid scintillation vials containing Hydromix to quantitate the amount of radioactive CO_2 produced. The brain slices were dried overnight at 90 °C for determination of dry weight. The results were converted to units/g wet weight using the value of 82 % water content for the tissue. (Dry weight/wet weight ratio was found to be 0.18 ± 0.015, $n = 25$. The wet weight was determined immediately after the slices were cut.) Pyruvate dehydrogenase activity was measured according to the method of Jungas [9]. ATP content was determined enzymatically [10], and the method of Lowry et al. [11] was used for protein analysis.

RESULTS

The effect of high oxygen pressure on pyruvate dehydrogenase activity in resting and potassium-depolarized tissue

In brain slices incubated in normal Krebs-Ringer phosphate medium at 1 atm oxygen, 50 % of pyruvate dehydrogenase was found to be in the active form [7]. In agreement with our previous experiments [7] potassium depolarization at 1 atm oxygen increased pyruvate dehydrogenase activity in the brain slices by 30 %. This potassium-induced activation of pyruvate dehydrogenase was reversed by high pressure oxygen.

The experimental design is illustrated in Fig. 1. Within 5 min, the activation of pyruvate dehydrogenase by 100 mM KCl was maximal, and this level of activity was

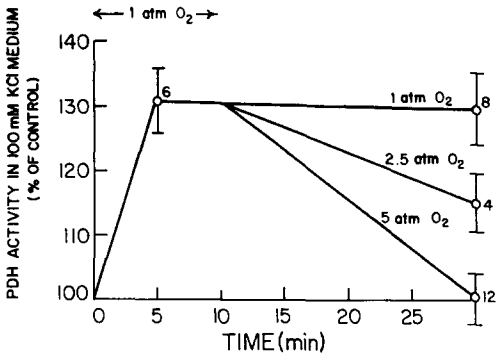

Fig. 1. Cortical brain slices were incubated in pairs as follows. Control samples: normal Krebs Ringer phosphate medium (5 mM KCl, 120 mM NaCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 16 mM sodium phosphate buffer (pH 7.4) and 6 mM glucose). Treated samples: high KCl medium (100 mM KCl, 25 mM NaCl; the remaining components as for control samples). Following 10 min at 1 atm, oxygen pressure was maintained at 1 atm or raised to 1.5, 2.5, 3.5 or 5 atm for 20 min. The figure shows the results obtained with 1 atm, 2.5 atm and 5 atm. The complete experiment is presented in Table I. Pyruvate dehydrogenase activity is expressed as mean ± S.E.M. (number of paired experiments).

maintained at 1 atm oxygen throughout the experiment. After 10 min, a group of samples were treated with high pressure oxygen for 20 min. The potassium-induced activation of pyruvate dehydrogenase in these samples was reversed by oxygen in direct proportion to the magnitude of pressure, and 5 atm oxygen completely reversed the effect of depolarization on the enzyme. Pyruvate dehydrogenase in the non-depolarized or resting tissue was only slightly affected by 5 atm oxygen. Increasing the hydrostatic pressure by helium at 1 atm P_{O_2} had no effect on the enzyme. These results are presented in Table I.

The mechanism of inhibition in the depolarized tissue was studied by replacing

TABLE I

REVERSAL OF POTASSIUM-DEPENDENT ACTIVATION OF PYRUVATE DEHYDROGENASE ACTIVITY BY HIGH OXYGEN PRESSURE

The experimental procedure and composition of solutions are described in legend to Fig. 1. Hyperbaric treatment began after 10 min incubation at 1 atm O_2 and continued for 20 min*. Pyruvate dehydrogenase activity is expressed in nM pyruvate decarboxylated/mg protein/min**. Values represent mean ± S.E.M. (number of paired experiments)***. n.s., not significant.

Treatment*			Pyruvate dehydrogenase activity**			
Time (min)	O_2 (atm)	He (atm)	5 mM KCl medium	100 mM KCl medium	*P*	
5	1	—	6.1 ± 0.42	7.8 ± 0.41	< 0.001	(6)***
30	1	—	6.2 ± 0.30	8.1 ± 0.63	< 0.001	(8)
30	1	4	5.8 ± 0.42	7.8 ± 0.61	< 0.001	(6)
30	1.5	—	5.9 ± 0.21	7.1 ± 0.14	< 0.001	(3)
30	2.5	—	5.5 ± 0.28	6.4 ± 0.14	< 0.025	(4)
30	3.5	—	5.8 ± 0.16	6.6 ± 0.22	< 0.020	(4)
30	5	—	5.1 ± 0.22	5.2 ± 0.42	n.s.	(12)

TABLE II

EFFECT OF SODIUM-FREE MEDIUM ON PYRUVATE DEHYDROGENASE ACTIVITY

The incubation procedure is described in legend to Fig. 1. NaCl is replaced by choline chloride in the high KCl medium. Pyruvate dehydrogenase activity is expressed as nM pyruvate decarboxylated/mg protein per min*. Values represent mean ± S.E.M. (number of paired experiments)**. Experiment A, 30 min incubation at 1 atm O_2; experiment B, 10 min incubation at 1 atm O_2 plus 20 min incubation at 5 atm O_2. n.s., not significant

Expt.	O_2 (atm)	Pyruvate dehydrogenase activity*			
		5 mM KCl medium	100 mM KCl, no Na medium	*P*	
A	1	6.1 ± 0.19	6.3 ± 0.16	n.s.	(8)**
B	5	5.5 ± 0.16	6.0 ± 0.20	n.s.	(4)

sodium with choline in the medium. As a result of the omission of sodium, activation of pyruvate dehydrogenase was only slight at 1 atm O_2, but the enzyme was protected against depression by 5 atm oxygen, in spite of maintained depolarization (see Table II).

The effect of high oxygen pressure on tissue ATP content

Since it has been demonstrated that an inverse relationship exists between tissue ATP content and degree of pyruvate dehydrogenase activation in brain tissue [7, 12], ATP levels and pyruvate dehydrogenase activity were determined at increased pressures of oxygen in parallel experiments. Results in Table III show that depolarization under oxygen tension of 1 atm led to a substantial reduction in the level of this nucleotide, in agreement with results of previous experiments [7, 13]. Raising the oxygen tension to 5 atm, 10 min after the start of the experiment, caused no change in tissue ATP content in the presence of the control medium. In the depolarized tissue, however, ATP was restored to the original level by the end of the experiment. The activation of pyruvate dehydrogenase was completely reversed at this time, as shown

TABLE III

EFFECT OF 5 atm OXYGEN ON TISSUE ATP LEVELS

Experimental procedure is identical to that used for determination of pyruvate dehydrogenase activity (Table I). Results are expressed as mean ± S.E.M. (number of paired experiments)*. n.s., not significant

Treatment	μM ATP/g wet tissue			
	5 mM KCl medium	100 mM KCl medium	*P*	
10 min at 1 atm O_2 plus 20 min at:	1.03 ± 0.04	0.60 ± 0.03	< 0.001	(7)*
1 atm O_2 + 4 atm He	0.95 ± 0.05	0.54 ± 0.08	< 0.005	(4)
5 atm O_2	1.01 ± 0.08	0.86 ± 0.06	n.s.	(5)
30 min at 1 atm O_2	0.96 ± 0.04	0.61 ± 0.04	< 0.001	(7)

in Table I. It is evident from these experiments that energy metabolism was modified by high-pressure oxygen in the depolarized tissue, but the inverse relationship between ATP and pyruvate dehydrogenase remained unchanged.

The effect of high oxygen pressure on oxidation of [U-^{14}C]glucose

Depolarization of brain slices by high KCl is known to increase the respiratory rate in the presence of glucose or substrates that are oxidized by a pathway involving pyruvate as an intermediate [14]. Since oxygen at high pressure reversed the activation of pyruvate dehydrogenase by potassium ions, it was expected that the stimulation of glucose oxidation by potassium would not be seen under these conditions. The rate of $^{14}CO_2$ formation from [U-^{14}C]glucose was, therefore, determined under 5 atm oxygen. In control experiments at 1 atm oxygen, $^{14}CO_2$ production from [U-^{14}C] glucose was markedly stimulated by 100 mM KCl. Both in normal Krebs-Ringer phosphate medium and in high potassium medium there was an initial lag period in the rate of $^{14}CO_2$ production for about 15 min. After this time $^{14}CO_2$ formation became linear with time. We chose 30 min for the incubation to avoid too long an exposure of the tissue to a high concentration of potassium ions. We found that under 5 atm oxygen the rise in CO_2 production in the potassium-depolarized tissue was decreased, with little or no effect by high pressure oxygen on the tissue incubated in normal Krebs-Ringer phosphate medium (see Table IV).

TABLE IV

EFFECT OF 5 ATM OXYGEN ON PRODUCTION OF $^{14}CO_2$ FROM [U-^{14}C]GLUCOSE

Brain slices were incubated for 30 min in normal Krebs-Ringer phosphate medium (5 mM KCl) or in high potassium medium (100 mM KCl), with proportional decrease in NaCl concentration to maintain isotonicity. The substrate was 6 mM [U-^{14}C]glucose. Results are expressed as mean ±S.E.M. (number of paired experiments)*.

Treatment		μM $^{14}CO_2$ produced/g wet weight per 30 min		
O_2 (atm)	air (atm)	5 mM KCl medium	100 mM KCl medium	
1	—	24.3±2.05	48.2±2.92	(7)*
—	5	25.0±2.79	49.5±1.28	(4)
5	—	20.9±1.12	28.9±1.69	(7)

DISCUSSION

Early experiments with brain minces and brain homogenates demonstrated that oxygen at elevated pressure inhibits respiration in the presence of glucose or pyruvate [15–17]. It was also observed, however, that brain slices are much more resistant to the toxic effects of oxygen [16, 17] than broken tissue preparations, since at oxygen pressure as high as 7 atm it took more than an hour for significant inhibition of oxygen uptake to occur. These findings are in agreement with our observation that 5 atm oxygen did not significantly alter the production of $^{14}CO_2$ from [U-^{14}C]-glucose when brain slices were incubated in normal Krebs-Ringer phosphate medium. In contrast to the earlier findings, however, we found that oxygen at high pressure has

marked and rapidly developing effects on metabolism when the tissue is depolarized by a high potassium medium. Our results show that at 5 atm the potassium-induced activation of pyruvate dehydrogenase in cortical slices is reversed by oxygen and the increase in $^{14}CO_2$ production from [U-^{14}C]glucose in depolarized tissue does not occur. The depression of pyruvate dehydrogenase activity is accompanied by an increase in tissue ATP.

Mayevsky et al. [18] recorded the fluorescent signal from brain cortex of unanesthetized rats during exposure of the animals to 6 atm oxygen. They observed an increase in the NAD:NADH ratio during the pre-convulsive state and concluded that high oxygen pressure produced a diminished flow of reducing equivalents through the mitochondrial electron transport chain. The limited availability of substrate at a high pressure was also indicated by their finding that administration of succinate to the animals delayed both the metabolic change and the onset of seizures [18]. Our observation that CO_2 production is inhibited in depolarized brain slices under 5 atm oxygen agrees with these findings. In addition, the fall in pyruvate dehydrogenase activity during hyperbaric oxygenation illustrates again the predominant role of this enzyme in the regulation of respiration.

The mechanisms by which potassium depolarization increases and high oxygen pressure reverses the activation of pyruvate dehydrogenase are not understood. The alterations in ATP content of the cell may be of major importance in both of these effects. Phosphorylation of the pyruvate dehydrogenase complex inactivates the enzyme [5, 6] and ADP is an inhibitor of pyruvate dehydrogenase kinase [19]. Thus, during potassium depolarization, both the decrease in ATP and increase in ADP can contribute to the observed increase in pyruvate dehydrogenase activity. Accordingly, the rise in ATP during exposure of the tissue to a high potassium medium at high oxygen pressures is expected to prevent the increase in pyruvate dehydrogenase activity. Furthermore, the requirement of sodium for the activation of pyruvate dehydrogenases by potassium indicates that the sodium pump may be involved in initiating these changes in ATP. Increased activity of the pump can lead to a decrease in cellular ATP and consequent activation of pyruvate dehydrogenase. High oxygen pressure may then act by decreasing the sodium pump activity during potassium depolarization.

Changes in intracellular free calcium concentrations may also be important in the phenomena described. Potassium depolarization is associated with an influx of calcium into the cell [20], and it has been observed that pyruvate dehydrogenase phosphatase, maximally activated by magnesium ion, is further stimulated by micromolar concentrations of calcium ions [21, 22]. Oxygen at high pressure may therefore also act to prevent the influx of calcium during potassium depolarization.

A fundamental problem in this field is the cause of the convulsions produced by oxygen at high pressure. J. D. Wood [23] has stated: "In view of the well-established ionic basis of nerve transmission, it is fairly safe to predict that changes in membrane permeability to ions such as sodium and potassium are the direct cause of seizures". Mayevsky et al. [18] gave strong support for this view as a result of their measurements in vivo of electroencephalogram and the pyridine nucleotide redox state in brains of unanesthetized rats. The changes in these parameters during exposure to hyperbaric oxygen prior to seizures were analogous to those produced by application of KCl to the cortex. In addition, considerable evidence from in vitro experiments suggests a

shift in electrolyte distribution at a high pressure of oxygen. Kaplan and Stein [24] as well as Joanny et al. [25] demonstrated an increase in intracellular sodium and a loss of potassium from cortical brain slices at 6 atm oxygen. It is noteworthy that these effects on electrolytes were significant in their experiments even though the brain slices were incubated in the presence of a normal Krebs-Ringer medium. Our experiments clearly show that such a medium, when compared with a depolarizing high KCl medium, offers considerable protection against the effects of high pressure oxygen.

Although the cause of the electrolyte changes is not known, results of our study strongly suggest that oxygen at elevated pressures acts at the cell membrane by inhibiting the sodium pump. The alterations in pyruvate dehydrogenase activity, ATP levels, and glucose metabolism in brain slices during hyperbaric oxygenation are similar to changes caused by ouabain [7, 13], an agent capable of causing convulsions upon brain intraventricular injection [26]. An action on sodium transport by high oxygen pressure was previously reported by Allen et al. [27], who observed, on a frog bladder preparation, that 10 atm oxygen reversed the stimulation in sodium transport elicited by aldosterone, without any effect on the basal level of activity. A similar "ouabain-like" action of excess oxygen in brain tissue may lead to an accumulation of cellular potassium in the extra-cellular space, increasing the excitability of the affected area.

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