Volume 314, number 3, 264–266 FEBS 11840 December 1992

Air saturation of the medium reduces the rate of phosphorylating oxidation of succinate in isolated mitochondria

V.A. Khazanov[a], A.N. Poborsky[a] and M.N. Kondrashova[b]

[a]*Institute of Pharmacology, Russian Academy of Medical Science, Tomsk, Russia* and [b]*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia*

Received 15 October 1992

Rat brain mitochondria were isolated and their respiration was polarographically measured without contact with air oxygen. Gas-saturated experimental mixtures close to the in vivo partial oxygen pressure (normoxic) were compared with the air-saturated, i.e. hyperoxic, mixtures. The rate of phosphorylating oxidation of added succinate under normoxic conditions was found to be 70–100% higher compared with hyperoxic ones. The addition of succinate dehydrogenase activators results in a more than two-fold stronger stimulation of succinate oxidation under normoxia than under hyperoxia. Thiol group donors are shown to stimulate respiration under hyperoxia and not under normoxia. Hyperoxic conditions prevented oxidation of the low succinate concentrations corresponding to the physiological ones.

Oxygen stress; Succinate oxidation; Mitochondria

1. INTRODUCTION

Biological oxidation studies are usually carried out in incubation mixtures saturated with air oxygen. Under such conditions, pO_2, 150 mmHg, exceeds considerably those in living cells (50 mmHg and lower). This may induce some damage of the studied systems due to an oxygen stress [1,2]. In particular, oxidation of the widely used substrate succinate, may be decreased, as succinate dehydrogenase is highly sensitive to the oxygen tension. Earlier we have observed that the rate of succinate oxidation in rat liver mitochondria was raised when pO_2 was lowered to tissue level [3]. In those experiments however mitochondria were not protected from air oxygen during their isolation. In this work we protected mitochondria from air oxygen during all the procedures of isolation of the organelles.

2. MATERIALS AND METHODS

Fed male white rats of mixed breed (170–220 g) were used. Two pO_2 levels in media were compared: usual air saturated media (hyperoxic), and media saturated with a gas mixture of 4% O_2 (pO_2, 30 mmHg), 8% CO_2 and 88% N_2 (normoxic). In normoxic experiments, the homogenizer, centrifuge test tubes and other vessels were blown with the gas mixture and closed with hermetic covers. Rat brain mitochondria were prepared by differential centrifugation; washing was exempted. Rat brain mitochondria suspension contained 50–60 mg protein/ml and was investigated within 30–40 min after isolation [4,5].

The medium for homogenization and suspension contained 300 mM sucrose, 0.2 mM EDTA, 10 mM HEPES, pH 7.4. Incubation medium contained 170 mM sucrose, 40 mM KCl, 0.2 mM EDTA, 8 mM $KHCO_3$, 5 mM KH_2PO_4, and 10 mM HEPES, pH 7.4. Other additions were: 5 mM succinate, 3 mM glutamate, β-hydroxybutyrate, α-glycerophosphate, and malate, 1 mM aminooxyacetate.

Respiration was recorded polarographically in a 1 ml chamber using a Clark electrode at 26°C. The following parameters were measured: V_4, rate of State 4; V_3, rate of State 3; V_3–V_4, phosphorylating respiration; t_p, duration of the ADP phosphorylation process, and ADP/O ratio [6]. Protein concentration was measured with Folin reagent [7].

Correspondence address: M.N. Kondrashova, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia.

3. RESULTS

It was observed that pO_2 lowering in isolation and incubation mixtures results in an increase in all the measured rates of succinate oxidation. This effect proved to be the most pronounced for phosphorylating respiration reaching 100%. Correspondingly, t_p was shortened (Table I).

Partial oxalacetate inhibition of succinate oxidation is typical of the brain. It may be abolished by succinate dehydrogenase activators such as glutamate, isocitrate, α-glycerophosphate, and β-hydroxybutyrate [8,9]. The extent of succinate oxidation stimulation in the presence of succinate dehydrogenase activators under normoxia is increased even more than with succinate alone. Its absolute value reaches 270% for V_3–V_4 and the activator-induced increment of V_3 (ΔAct) is very much higher. The increase in the respiration rate in the presence of activators is due also to stimulated succinate oxidation, as glutamate + malonate, glutamate + malate, malate + pyruvate oxidation is not accelerated under normoxia. In all cases the ADP/O ratio remained constant.

In the experiments described, the succinate concen-

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Table I

The parameters of succinate oxidation under hyperoxic and normoxic conditions

Parameter	Succinate		Succinate + Glutamate		Succinate + α-glycerophosphate + β-hydroxybutyrate	
	Hyperoxia	Normoxia	Hyperoxia	Normoxia	Hyperoxia	Normoxia
V_4	17.1 ± 1.1	25.1 ± 1.3	19.1 ± 0.7	30.2 ± 1.1	23.9 ± 1.3	30.6 ± 2.3
V_3	35.9 ± 2.6	61.1 ± 1.3	35.4 ± 2.3	91.5 ± 1.8	40.2 ± 2.5	81.5 ± 1.7
V_3–V_4	18.8 ± 1.6	36.0 ± 1.1	16.3 ± 1.9	60.3 ± 1.3	16.2 ± 1.8	50.9 ± 1.3
t_p	171 ± 4	102 ± 3	174.4 ± 4	72.3 ± 3.3	153.2 ± 3.0	74.8 ± 3.1
ADP/O	1.9 ± 0.04	1.93 ± 0.02	1.96 ± 0.04	1.97 ± 0.02	1.95 ± 0.04	1.97 ± 0.03
ΔAct			− 0.5	30.4	+ 4	+ 20.4

In this and further tables data are means ± S.D., $n = 8$. Values under normoxia are significantly different from hyperoxia, P is <0.05 for V_4, V_3, V_3–V_4 and t_p.

tration was 5 mM. This is a commonly used one, in in vitro experiments being, however, non-physiologically high (succinate level in tissues, ~0.3–0.5 mM). Succinate excess may diminish the succinate dehydrogenase inhibition. Therefore we investigated succinate oxidation at low (physiological) concentrations of this substrate, maintained by glutamate transamination limited with partial aminooxyacetate inhibition. As shown in Table II, in this case the inhibitory effect of hyperoxia was greater than at higher succinate concentration. Under hyperoxia, V_3 fell in the course of ADP phosphorylation to a level lower than under normoxia. Therefore t_p became longer. The ADP/O ratio was not changed. The fall of V_3 under hyperoxia was apparently due to the strong oxalacetate inhibition as it is abolished by the succinate dehydrogenase activation.

The considerably higher effect of succinate dehydrogenase activators under normoxia shows that some other mechanisms than oxalacetate inhibition are also involved in narrowing the range of respiration in air-saturated media. Apparently some part of succinate dehydrogenase activity is completely lost probably because of oxidation of succinate dehydrogenase SH groups. As shown in Table III, the addition of thiol-group donors resulted in a 10–15% increase in the respiration rate and in shortening of t_p only in hyperoxia. The ADP/O ratio was not changed.

4. DISCUSSION

The described data show a strong inhibitory effect of atmospheric oxygen on 5 mM succinate oxidation and complete inhibition when a low succinate concentration was oxidized. Both effects may be considered as an oxygen stress in mitochondria. Coenzyme Q reduction is known as mitochondrial antioxidant. Its protective effect is realized in air-saturated media but is lost at higher oxygen concentrations [10]. Succinate oxidation supports most effectively the coenzyme Q reduction. These effects were observed in submitochondrial particles using 15 mM succinate. Our experiments in intact mitochondria show that oxygen under atmospheric pressure and lower succinate concentrations also damage the respiratory chain.

The considerable acceleration of succinate oxidation under normoxia may be autocatalytically supported by

Table II

The parameters of oxidation of transaminase generated succinate under hyperoxic and normoxic conditions

Parameter	Conditions	Glutamate + malate + aminooxyacetate	Glutamate + malate + aminooxyacetate + α-glycerophosphate + β-hydroxybutyrate
V_3	hyperoxia	9.95 ± 0.3→5.0 ± 0.1	17.2 ± 0.2
	normoxia	7.2 ± 0.1*	16.0 ± 0.3
t_p	hyperoxia	188 ± 27→576 ± 22	217 ± 16
	normoxia	546 ± 7	231 ± 2
ADP/O	hyperoxia	3.02 ± 0.03	2.83 ± 0.02
	normoxia	3.03 ± 0.03	2.80 ± 0.02

Values under normoxia are significantly different from hyperoxia ($P < 0.05$; $^*P \ll 0.01$).

Table III

The effect of cysteine and mercaptoethanol on rat brain mitochondria respiration under hyperoxic and normoxic conditions

Parameter	Conditions	Succinate	Succinate + cysteine + mercaptoethanol
V_4	hyperoxia	15.6 ± 0.2	20.8 ± 0.3
	normoxia	22.1 ± 0.3	22.8 ± 0.3
V_3	hyperoxia	32.6 ± 0.3	39.1 ± 0.2
	normoxia	60.7 ± 0.4	61.0 ± 0.3
V_3–V_4	hyperoxia	16.9 ± 0.2	18.2 ± 0.2
	normoxia	38.1 ± 0.3	38.2 ± 0.2
t_p	hyperoxia	170 ± 2	143 ± 3
	normoxia	102 ± 1	100 ± 1

Cysteine and mercaptoethanol, $5 \cdot 10^{-5}$ M.

coenzyme Q reduction which is higher than in air-saturated mixtures. Therefore, the succinate domination in the respiratory chain observed in air-saturated mixtures is apparently even greater in vivo under low pO_2.

On the other hand, the high rate of succinate oxidation under low pO_2 supports the view that transaminase-generated succinate serves as an energy source for intensive physiological activity [3,11,12] which is accompanied by a transient, oscillatory hypoxia under physiological conditions.

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