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RESPIRATION OF RAT BRAIN MITOCHONDRIA DURING HYPEROXIA AND NORMOXIA

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Energy-producing reactions of mitochondria (Mch) depend on the partial pressure of O_2 and CO_2 in the medium surrounding the organelles [1, 3, 4, 9]. Traditionally the isolation of Mch and the study of their respiratory activity have been carried out in a hyperoxic medium saturated with air, in which the partial pressure of CO_2 is lower than in the intracellular medium [5]. However, the known effect of O_2 on activity of the respiratory chain, of dehydrogenases, and of other thiol enzymes of Mch [1, 2, 7, 10] suggests a considerable change in the functional state of the organelles during their isolation and study under hyperoxic conditions.

In order to determine the character of changes in respiration of Mch as the conditions of their functioning in vitro, with respect to O_2 concentration, are brought closer to those of existence in vivo, a comparative study was made of rat brain Mch on media saturated with air (hyperoxia) and saturated with a gas mixture containing O_2 in a concentration corresponding to the intracellular level (normoxia).

EXPERIMENTAL METHOD

Experiments were carried out on brain mitochondria from Wistar rats. The organelles were isolated by differential centrifugation, using methods preserving their native state [5, 7]. The isolation medium of Mch was: sucrose 0.3 M, HEPES 10^{-2} M, EDTA 10^{-4} M, pH 7.4, t = 0 ± 1°C. The composition of the incubation medium was: sucrose 0.17 M, KCl $4 \cdot 10^{-2}$ M, HEPES 10^{-2} M, KH_2PO_4 $5 \cdot 10^{-3}$ M, $KHCO_3$ $8 \cdot 10^{-3}$ M, EDTA 10^{-4} M, pH 7.4, t = 26°C. The respiratory activity of the organelles was recorded polarographically, as the rate of O_2 consumption of the Mch suspensions in different metabolic states after Chance [5]. The apparatus included a polarograph (PA-2, Czechoslovakia) and a thermostated cell of original design with membrane electrode of Clark type. The oxidation substrate was $5 \cdot 10^{-3}$ M succinate. The degree of

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TABLE 1. Parameters of Mitochondrial Respiration of Rat Brain under Normoxic and Hyperoxic Experimental Conditions ($\bar{x}$, n = 8)

Parameter studied	Oxidation substrate		
	SA	SA + α-GP + β-OHB	SA + GLU
V_{4n}	17,9/25,1*/ (40)	23,9/30,6* (28)	19,1/30,6* (63)
V_3	35,9/61,1* (70)	40,2/81,5* (103)	35,4/91,5* (158)
V_{4o}	17,1/25,1* (47)	23,9/30,6* (28)	19,1/31,2* (63)
V_3-V_{4n}	17,9/36,0* (101)	16,2/50,9* (214)	16,3/60,3* (270)
V_3-V_{4o}	18,8/36,0* (91)	16,2/50,9* (214)	16,3/60,3* (270)
ADP/O	1,95/1,93 —	1,95/1,97 —	1,96/1,97 —
T_p	171,4/101,6* (—41)	153,2/74,8* (—51)	174,4/72,3* (—59)

Legend. 1. V_{4n}, V_3, V_{4o}) Rates of respiration of Mch before, during, and after phosphorylation of added ADP, expressed in mμatoms O_2/min/mg protein; SA) succinic acid; T_p) phosphorylation time (in sec/mg protein). 2. Numerator gives parameter during hyperoxia, denominator during normoxia. Differences compared with control (hyperoxia), in per cent, given between parentheses. *$p < 0.05$ — difference from control significant.

inhibition of succinate-dependent respiration of the organelles was estimated by adding glutamate (GLU) $2 \cdot 10^{-3}$ M or a mixture of α-glycerophosphate (α-GP) and β-hydroxybutyrate (β-OHB), $3 \cdot 10^{-3}$ M of each, as succinate dehydrogenase (SDH) activators together with succinate to the incubation medium [6]. The concentration of mitochondrial protein in the cell was 3 mg/ml; ADP was added to a concentration of $5 \cdot 10^{-5}$ M. The media, substrates, and Mch suspension were aerated during the investigation with a gas mixture of the following composition: O_2 4%, CO_2 8%, N_2 88%. The aeration was monitored by an ABL-4 gas analyzer ("Radiometer," Denmark) up to complete saturation, which corresponded to a partial pressure of O_2 and CO_2 of 30 and 60 mm Hg respectively, and correlated with the partial pressure of these gases in brain tissue in situ [3, 4].

Mch isolated in a similar way on media equilibrated for gas composition with air (hyperoxia), i.e., providing the conditions usually adopted to study the functional state of organelles [5], were used as the control. The experimental results were subjected to statistical analysis by Student's t test [8].

EXPERIMENTAL RESULTS

The study of Mch under normoxic conditions showed considerable changes in their respiratory activity compared with the control (Table 1). Oxidation of succinate was characterized by an increase in the rate of respiration before, during, and after phosphorylation of the added ADP, an increase in the range of respiratory activity, while leaving unchanged the value of the coefficient of coupling of oxidative phosphorylation (ADP/O) and a decrease in the phosphorylation time. Addition of the SDH activators α-GP and β-OHB to the incubation medium together with succinate increased the degree of contrast of differences in the state of Mch during incubation under hyperoxic and normoxic conditions. In particular, they caused a 33% greater increase in the rate of phosphorylating respiration of the organelles in normoxia, with a corresponding 113% increase in the range of respiratory activity and a 10% decrease in the phosphorylation time compared with the hyperoxic state. Under these conditions GLU did not affect the rate of respiration during phosphorylation of the control Mch, while significantly activating the experimental organelles. Moreover, the response of Mch to it was more marked than to α-GP and β-OHB. The latter are known to be more powerful activators of SDH than GLU [7, 13], but these data were obtained under hyperoxic conditions. Under normoxic conditions, it is probable that not only the ability of GLU to abolish inhibition of SDH, as in the case of α-GP and β-OHB, on account of an increase in the degree of reduction of the respiratory chain in the NADH-dehydrogenase-coenzyme Q region, realized, but the contribution of this substrate to the transamination reaction also occupies a leading position [11, 12]. Inhibition of respiratory activity of Mch during hyperoxia, accompanied by narrowing of the range of respiratory activity, while the degree of coupling of oxidative phosphorylation remains unchanged, is evidently due to the accumulation of oxaloacetate (OAA), on the one hand, due to

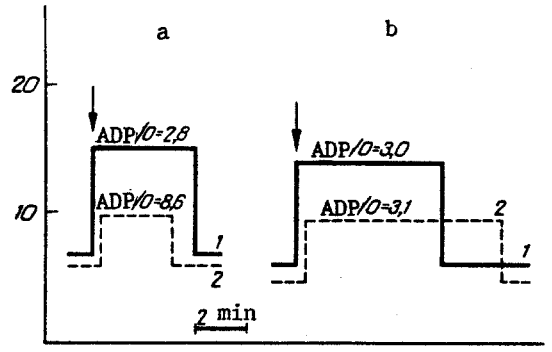

Fig. 1. Effect of hyperoxic (a) and normoxic (b) experimental conditions on rate of respiration of rat brain Mch during oxidation of mixture of substrates malate (3 mM) and glutamate (2.5 mM) separately (1) and in the presence of 1 mM AOA (2). Ordinate, rate of respiration of Mch (in mμatoms O_2/min/mg protein). Arrow indicates addition of ADP.

activation of the oxidase system by excess of O_2 but on the other hand, through the unsoundness of the pathways of inhibitory utilization and, in particular, the transamination reactions.

Further confirmation of this view is given by the experiments with the transaminase inhibitor aminooxyacetate (AOA) (Fig. 1). Under hyperoxic conditions, during utilization of a substrate mixture glutamate and malate by Mch, AOA significantly reduced the rate of phosphorylating respiration of the organelles, while increasing the calculated value of the ADP/O ratio above the theoretically possible level, and shortening the phosphorylation time. Under normoxic conditions the action of the inhibitor was to reduce the rates of respiration of Mch and to increase the phosphorylation time, while leaving the ADP/O ratio unchanged. The contribution of oxidation of endogenous succinic acid (SA) to the level of respiratory activity of Mch, oxidizing a mixture of NAD-dependent substrates [6], indicates that the differences revealed in the action of AOA were unconnected with the different degrees of coupling of oxidative phosphorylation processes in the organelles studied, but reflect different levels of accumulation of OAA and the inability of transamination pathways to remove the SDH inhibitor under hyperoxic conditions.

When the mechanism of the depriming effect of hyperoxic experimental conditions on parameters of mitochondrial respiration in the brain is considered, the possibility of direct oxidation by O_2 of the SH-groups of thiol enzymes and, in particular, of dehydrogenases, ATPases, and transaminases must be taken into account [2]. Cysteine and mercaptoethanol ($5 \cdot 10^{-5}$ M), donors of thiol groups, if added to the incubation medium of brain Mch, studied under hyperoxic conditions, increased by 10% ($p < 0.05$) the rate of respiration without any effect on coupling of oxidation and phosphorylation. In normoxia, these preparations had no effect on the respiratory activity of Mch. Hyperoxic experimental conditions thus significantly modulate the character of the metabolic reactions of the isolated Mch as a result of development of oxaloacetate inhibition of SDH and direct oxidation of thiol enzymes, which cannot be ignored during analysis of the fine gradations of the functional state of these organelles.

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ANTIEPILEPTIC EFFECTS OF NIFEDIPINE

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The antiepileptic effects of blockers of voltage-dependent Ca-channels have been discovered on various different models of epileptic activity (EPA) [1-4, 6-10] and also in man [11]. However, the particular features of the action of these preparations and of the abolition of EPA remain unclear and require further study. Their elucidation is of great importance for a solution to the problem of the use of these preparations in the treatment of epilepsy.

In the investigation described below the effect of nifedipine (1,4-dihydropyridine) on penicillin-induced focal EPA, an acute generalized seizure reaction induced by systemic administration of metrazol, and also the syndrome of increased sensitivity to an epileptogen during its chronic administration (metrazol kindling) and the seizure reaction of animals after the end of metrazol kindling.

EXPERIMENTAL METHOD

Experiments were carried out on 157 male Wistar rats. The animals were kept under ordinary animal house conditions and on a standard diet. A focus of EPA was created by application of filter paper soaked in a solution of the sodium salt of benzylpenicillin in a concentration of 20,000 IU/ml to the sensomotor cortex. By a method described previously [2] burr holes 2 × 4 mm in diameter were drilled 24 h before penicillin application in the animal's skull above symmetrical regions of the sensomotor cortex, the dura was removed, and monopolar cortical silver electrodes were applied

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