Mechanisms of Reciprocal Control of Reactions and Processes Involved in Energy Production by Mitochondria

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Correlations between respiration rates in the mitochondria in different states are studied using various oxidation substrates. Specific features and integration between different functional cycles are substrate-dependent. It is suggested that variations of the mitochondrial function correspond to specific phases of pathological process.

Key Words: *mitochondria; respiration rates; function*

The understanding of the mechanisms whereby the reactions and processes involved in energy production are controlled and coordinated is crucial for purposeful regulation of mitochondrial metabolism. This calls for adequate quantitative evaluation of the organization of oxidative and phosphorylation processes. Solution of this problem is a principal step to unveil mitochondrial processes such as uncoupling of oxidation and phosphorylation [2] and maintenance of ion equilibrium [6].

The aim of the present study was a quantitative evaluation of the relationships between phosphorylation rate and oxygen uptake in the mitochondria in the presence of various oxidative substrates and dinitrophenol (DNP).

MATERIALS AND METHODS

Nonpedigree male rats weighing 180-200 g were used. The animals were decapitated, and the mitochondria were isolated from liver homogenate by differential centrifugation in an isolation medium containing 0.32 M sucrose and 1 mM EDTA (pH 7.4). Respiration was assayed polarographically using a coated platinum electrode of the Clarke type with a Teflon membrane in an incubation medium containing 200 mM sucrose, 20 mM KH_2PO_4, and 5 mM $MgCl_2$ (27°C). Respiration rate was measured in the presence of the following substrates: succinate (6 mM), β-hydroxybutyrate (7 mM), or a mixture of glutamate (3 mM) and malate (3 mM). State 3 was induced by the addition of 200 μM ADP. 2,4-DNP (40 μM) was added for uncoupling of oxidative phosphorylation. The protein content was measured by the biuret method [5].

Respiration rate in all metabolic states was expressed in natoms oxygen/mg protein×sec. The rate of phosphorylation of the added ADP was expressed in nmol/mg/sec. The index of respiratory control (RC) was determined as the ratio between rates of oxygen uptake in state 3 and state 4 [4]. The index of stimulation of respiration (SR) was calculated as the ratio between the respiration rates in states 3 and 4' (the state after the addition of substrate and prior to the addition of ADP). Paired correlation coefficients between RC, SR, V_p, and respiration rates in various states were calculated from experimental data.

RESULTS

Correlation analysis proved that the rate of phosphorylation and the rates of oxygen uptake in all respiration stages strongly correlated when succinate

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was the main substrate of oxidation (Fig. 1, *a*), predominantly due to the ability of mitochondria to oxidize native succinate. For example, the higher the rate of succinate oxidation at rest ($V_{4'}$), the higher the rate of succinate oxidation in state 3, and the higher the rate of ADP phosphorylation and the rate of uncoupled respiration. From the functional point of view, the utilization of succinate as the primary substrate of oxidation is attended by strict determination of the linear links between individual reactions underlying oxidative phosphorylation. In contrast, when β-hydroxybutyrate was used as an oxidative substrate (Fig. 1, *b*), the relationships between the rates of reactions were weakened (except for $V_3 \leftrightarrow V_p$).

Specific organization of the mitochondrial function was observed when glutamate and malate were used simultaneously as oxidation substrates (Fig. 1, *c*). Under these conditions some features of coordination processes typical of succinate oxidation ($V_{4'} \leftrightarrow V_p \leftrightarrow V_4 \leftrightarrow V_{unc}$) and, simultaneously, NAD-dependent substrates ($V_{4'} \leftrightarrow V_3 \leftrightarrow V_4$) were observed in the correlation pleiad.

The phenomenon of respiratory control is an important component of membrane bioenergetics [3]. Correlation analysis quantitatively proved the independent informative validity of RC and SR indexes for the evaluation of the functional state of the mitochondria.

Extensive experimental data obtained on the same mitochondria prompted us to study the relationships between respiration rates for various substrates. The most pronounced correlation was observed between respiration rates with succinate and β-hydroxybutyrate (data not shown).

Our findings for the first time describe quantitatively the mitochondrial function in the presence of substrates routinely used in the studies of mitochondria to assess the respiration and phosphorylation processes [1]. From these data it can be suggested that different substrates are responsible not only for different rates, but also for different relationships between various functional cycles in the mitochondria. Based on the nature of the correlation pleiads, three variants of mitochondrial organization can be recognized. Two of them can be regarded as relatively opposite, specifically, to the strong coordination of all reactions in succinate oxidation and, by contrast, as loose coordination of respiration rates in the oxidation of β-hydroxybutyrate. The third variant of respiration in the presence of glutamate and malate is an intermediate form of organization, and is likely to correspond to the transition state between succinate-dependent respiration to predominant oxidation of NAD-dependent substrates.

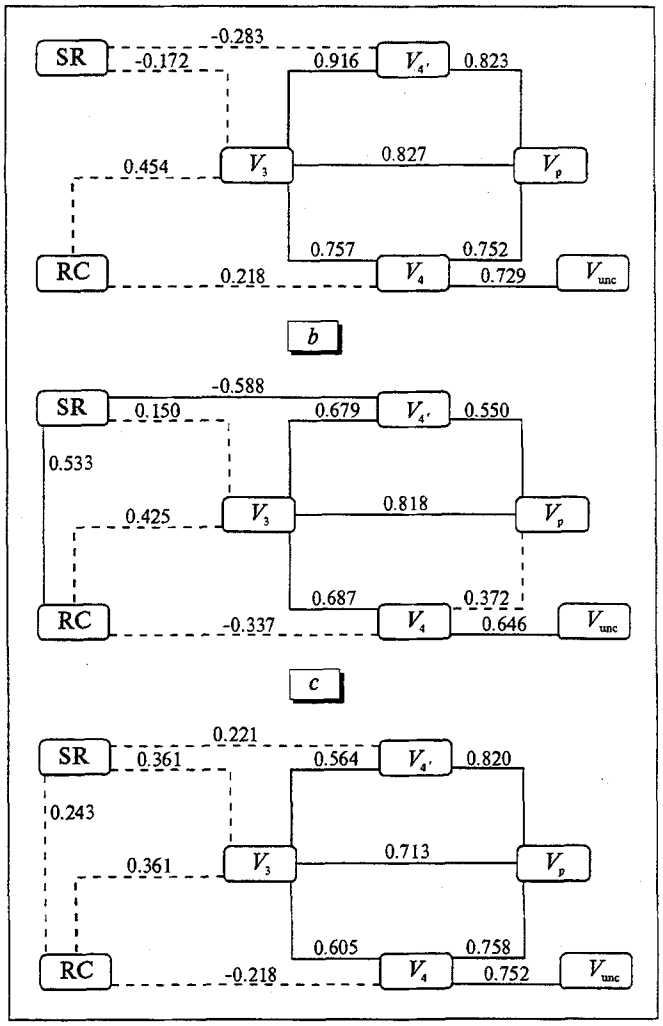

Fig. 1. Relationships between oxidation and phosphorylation rates, the indexes of stimulation of respiration (SR), and respiratory control (RC). Paired correlation coefficients were significant at $p<0.04$ (solid lines), other correlations were insignificant (dotted line). Substrates: *a*) succinate (30 determinations), *b*) β-hydroxybutyrate (29), and *c*) glutamate+malate (30).

From the physiological viewpoint, these variants of mitochondrial function may represent dynamic changes accompanying cell activation and return to the resting state. Previously, it was shown that the respiration boost in stimulated tissues is due to enhanced oxidation of succinate, the oxidation of NAD-dependent substrates being unchanged [3].

The succinate-dependent respiration undoubtedlyplays a key role in the mobilization of defense systems and functional activation of various tissues by adverse factors. Under these conditions, metabolic therapy supporting succinate-dependent respiration may considerably increase the organism's reactivity. As a pathological process decays, the mixed variant of mitochondrial function becomes more adequate, which it turn requires specific pathways of metabolic control. Finally, after normalization of biological

integration at all levels, the mitochondria switch to primary oxidation of NAD-dependent substrates, which is the most adequate form of respiration under these conditions.

REFERENCES

1. M. N. Kondrashova, *Biokhimiya*, **56**, No. 3, 388-405 (1991).
2. E. N. Mokhova, A. A. Starkov, and V. A. Bobyleva, *Ibid.*, **58**, No. 10, 1513-1522 (1993).
3. V. P. Skulachev, *Energetics of Biological Membranes* [in Russian], Moscow (1988).
4. B. Chance and G. R. Williams, *J. Biol. Chem.*, **217**, 383-438 (1955).
5. A. G. Gornall, C. C. Bardawill, M. M. David, *Ibid.*, **177**, 751 (1949).
6. T. E. Gunter and D. R. Pfeiffer, *Am. J. Physiol.*, **258**, C755-C786 (1990).