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Control of OXPHOS efficiency by complex I in brain mitochondria

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Abstract

In the present work we have analysed the efficiency (P/O ratio) of energy production by oxidative phosphorylation (OXPHOS) in rat brain, liver and heart mitochondria. This study has revealed tissue-specific differences in the mean values of P/O ratios and ATP production rates. A marked dependence of the P/O ratio on the respiration rates has been observed with complex I (NADH:ubiquinone oxidoreductase), but not with complex II (succinate dehydrogenase) respiratory substrates. The physiological impact of the P/O variations with complex I substrates has been further confirmed by extending the analysis to brain mitochondria from three independent groups of animals utilized to study the effects of dietary treatments on the age-related changes of OXPHOS. The general site-specificity of the rate-dependent P/O variability indicates that the decoupling, i.e. decreased coupling between electron transfer and proton pumping, is likely to be mostly due to slip of mitochondrial complex I. These findings suggest an additional mechanism for the pivotal role played by the energy-conserving respiratory complex I in the physiological and adaptive plasticity of mitochondrial OXPHOS.

Keywords: Brain; Mitochondria; Oxidative phosphorylation (OXPHOS); Complex I; P/O ratio; Aging; Antioxidant dietary treatment

1. Introduction

Mitochondrial oxidative phosphorylation (OXPHOS) represents the major source of ATP in mammalian cells relying on aerobic energy metabolism. The mitochondrial respiratory complex I (NADH:ubiquinone oxidoreductase), complex III (ubiquinol:ferricytochrome *c* oxidoreductase) and complex IV (cytochrome *c* oxidase) build up a transmembrane electrochemical potential ($\Delta\mu H^+$ or Δp) by coupling their electron transfer activities to H^+-translocation from the matrix (negative, N) to the outer (positive, P) side of the inner mitochondrial membrane. The electrochemical gradient is then utilized backward for ATP synthesis by the OXPHOS complex V (ATP synthase).

The coupling mechanism between electron transfer and proton pumping activities of the mitochondrial respiratory complexes has been the object of intense research since many years and several models have been proposed to explain the peculiar properties of this process which remains, however, still unsolved (Hosler et al., 2006; Papa et al., 2006; Wikstrom, 2004). Related to this topic, a specific controversial aspect concerns the possible physiological variability of the energy conservation efficiency of the redox-driven proton pumps. The yield of mitochondrial ATP production by OXPHOS can be influenced by several factors among which uncoupling (leak) and decoupling (slip) play a major role in the modulation of the protonmotive force (Brown, 1992; Harper and Brand, 1993; Kadenbach, 2003; Luvisetto et al., 1991; Murphy, 1989; Murphy and Brand, 1987; Pietrobon et al., 1983). Uncoupling is mostly due to modifications of the permeability properties of the mitochondrial inner membrane, resulting in an increased leak of protons, caused by physical changes in the composition and structure of the lipid bilayer as well as by the presence of uncoupling agents and/or proteins (UCPs) (Krauss et al., 2005; Lowell and Spiegelman, 2000). Therefore, uncoupling manifests itself as a general (i.e. not site-specific)

Abbreviations: COX, cytochrome oxidase; OXPHOS, oxidative phosphorylation; NAC, *N*-acetylcysteine

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decay of the mitochondrial energy conservation efficiency. On the other hand, decoupling of specific redox-driven proton pumps derives from "slippage" events that could be caused by the activation of intramolecular electron transfer routes not associated with proton translocation, or by mechanistic/kinetic alterations of the coupling pathways/reactions. This situation would appear as a site-specific decrease of the H^+/e^- stoichiometry and of its associated ATP production efficiency. While several examples of physiological uncoupling of mitochondrial OXPHOS can be found (Kadenbach, 2003), it is not yet established whether or not OXPHOS efficiency can be naturally modulated by decoupling mechanism(s). In particular, slip of proton pumps has been proposed to occur at high values of the electron transfer rates and/or of the protonmotive force (Luvisetto et al., 1991; Murphy and Brand, 1987; Pietrobon et al., 1983), with these two parameters being functionally related to each other.

A classical way to study the efficiency of mitochondrial OXPHOS is based on the measurement of the P/O ratio, namely the ATP produced per oxygen atom reduced by the respiratory chain. Also in this case, the mechanistic values of the P/O ratios, are still in question and have been recently reviewed (Hinkle, 2005; see also Lee et al., 1996). At any event, the analysis of the P/O ratio represents a more direct and physiological approach to the study of OXPHOS efficiency, as compared with the measurements of the H^+/e^- stoichiometries of the proton pumps. The P/O ratio has also been shown to change by modulating the electron flux through the respiratory complexes (Fitton et al., 1994; Hinkle and Yu, 1979).

At a more general level of energy metabolism, the trade-off between yield and rate of ATP production has been recently discussed in terms of its involvement in evolutionary transition from unicellular to multicellular undifferentiated heterotrophic organisms and in their environmental competition for food utilization (Pfeiffer et al., 2001).

In the present work we have analysed the rate dependence of the P/O ratio in brain, heart and liver mitochondria from young (2 months) rats. Tissue-specific differences in the ATP production efficiency (P/O ratio) have been observed with complex I but not complex II substrates, with the variability of the P/O ratio correlating with the mitochondrial respiratory rate. The analysis has been then extended to brain mitochondria isolated from rats belonging to three additional independent groups utilized as animal models of different physiological studies. The rate-dependent variability of the P/O ratio by complex I substrates was remarkably present, within the same tissue, in brain mitochondria from old (28 months) rats and old rats subjected to a long-term (16 months) dietary treatment with the antioxidant *N*-acetylcysteine (NAC) (Cocco et al., 2005) or, respectively, to a long-term (16 months) hypocaloric diet.

2. Experimental procedures

2.1. Animals and diet

Animals (male Wistar rats), purchased from Harlan, Italy, were housed two to three animals per cage and maintained on a 12 h light/dark cycle in a temperature controlled ($22 \pm 1\,^{\circ}C$) room. Treatment with modified diets started at 12 months of age and continued until sacrifice. Young (2 months) and old (28 months) control rats were fed ad libitum with standard maintenance pellets, while the NAC-treated group was fed ad libitum with pellets containing 0.3% (w/w) of *N*-acetylcysteine (NAC) as reported by Miquel et al. (1995). The hypocaloric regimen followed the 'every-other-day (EOD) feeding' method, which is equivalent to a reduction of caloric intake to about 60% as compared with the ad libitum fed age-matched controls (Goodrick et al., 1983). All experiments were performed in accordance with local and national guidelines covering animal experimentation. Animals, not showing macroscopic evidence of pathologies, were sacrificed by decapitation and tissues were rapidly removed, rinsed free of blood and placed in ice-cold mitochondrial isolation buffer. Pooled tissues from two to three rats were used for each mitochondrial preparation.

2.2. Isolation of brain cortex non-synaptic mitochondria

Free non-synaptic cerebral cortex mitochondria were isolated essentially as in Nagy and Delgado-Escueta (1984). All steps were carried out at 0–4 °C. Brain cortex was washed with SHE medium (0.32 M sucrose, 5 mM K-Hepes, pH 7.4, 0.1 mM EDTA, 0.2 mM phenylmethylsulphonyl fluoride (PMSF from Sigma)) and homogenized in 10 volumes of the same medium. The homogenized suspension was centrifuged at $1200 \times g$ for 4 min and the resulting supernatant was then centrifuged for 10 min at $18{,}000 \times g$ to obtain the crude mitochondrial pellet and the cytosolic fraction as supernatant. The mitochondrial pellet was resuspended in 1 ml of SHE medium, diluted with 7.5 ml of 8.5% Percoll/sucrose solution (8.5% (v/v) Percoll in 0.25 M sucrose, 5 mM K-Hepes, pH 7.4, 0.1 mM EDTA) and finally layered into centrifuge tubes onto a preformed two-step discontinuous (8 ml of 10%, 8 ml of 20%) Percoll/sucrose gradient. After centrifugation at $22{,}000 \times g$ for 25 min, the "free" mitochondria were obtained as a pellet at the bottom of the tube. The pellet was washed three times with SHE medium and one time in BSA medium (0.32 M sucrose, 5 mM K-Hepes, pH 7.4, 0.1 mM EDTA, 0.05% fatty acid free bovine serum albumin, BSA) and finally resuspended in SHE buffer at a concentration of 8–10 mg protein/ml.

2.3. Isolation of heart mitochondria

Heart mitochondria were isolated by differential centrifugation, essentially as previously described (Di Paola et al., 2000). The final pellet was resuspended in 0.25 M