Mitochondrial Respiratory Chain Adjustment to Cellular Energy Demand*

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Véronique Nogueira‡, Michel Rigoulet§, Marie-Astrid Piquet††, Anne Devin‡, Eric Fontaine‡, and Xavier M. Leerverve¶

From the ‡Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Joseph Fourier, 38041 Grenoble Cedex, France, the §Institut de Biochimie et de Génétique Cellulaires du CNRS, Université Bordeaux II, Bordeaux 33077, France, and the ¶Service d’Hépato-Gastro-Entérologie et de Nutrition, CHU Côte-de-Nacre, Caen 14033, France

Because adaptation to physiological changes in cellular energy demand is a crucial imperative for life, mitochondrial oxidative phosphorylation is tightly controlled by ATP consumption. Nevertheless, the mechanisms permitting such large variations in ATP synthesis capacity, as well as the consequence on the overall efficiency of oxidative phosphorylation, are not known. By investigating several physiological models in vivo in rats (hyper- and hypothyroidism, polyunsaturated fatty acid deficiency, and chronic ethanol intoxication) we found that the increase in hepatocyte respiration (from 9.8 to 22.7 nmol of O2/min/mg dry cells) was tightly correlated with total mitochondrial cytochrome content, expressed both per mg dry cells or per mg mitochondrial protein. Moreover, this increase in total cytochrome content was accompanied by an increase in the respective proportion of cytochrome oxidase; while total cytochrome content increased 2-fold (from 0.341 ± 0.021 to 0.821 ± 0.024 nmol/mg protein), cytochrome oxidase increased 10-fold (from 0.020 ± 0.002 to 0.224 ± 0.006 nmol/mg protein). This modification was associated with a decrease in the overall efficiency of the respiratory chain. Since cytochrome oxidase is well recognized for slippage between redox reactions and proton pumping, we suggest that this dramatic increase in cytochrome oxidase is responsible for the decrease in the overall efficiency of respiratory chain and, in turn, of ATP synthesis yield, linked to the adaptive increase in oxidative phosphorylation capacity.

In aerobic living systems, oxidative phosphorylation activity can vary widely to adequately match ATP synthesis to energy demand according to physiological or pathological conditions. In contrast to short-term adaptation, which relies only on a flux modulation through every functional unit of mitochondrial oxidative phosphorylation, chronic adaptation to various rates of ATP utilization can also be achieved by modifying the number of these functional units (mitogenesis). Indeed, in the light of the large physiological variations in ATP turnover observed in living systems, it is highly probable that the amount of enzymes involved in the oxidative phosphorylation pathway plays a significant role. Recently the trade-off between rate and yield of ATP synthesis in heterotrophic organisms has been highlighted as a possible major mechanism of cooperation and competition involved in the evolutionary aspects of energy metabolism (1).

By investigating the effect on the yield of ATP synthesis (ATP/O)1 after acute modulations of the flux through the oxidative phosphorylation pathway in yeast mitochondria, it has been shown that the decrease in flux was accompanied by an increase in efficiency when the flux was limited by substrate supply (2–4). Conversely, no change in ATP/O was observed when the oxidative phosphorylation flux was modulated by the inhibition of ATP synthesis (oligomycin) (2–4). Hence, depending on the mechanism permitting to modulate the flux through the respiratory chain, different consequences on the efficiency of this pathway were observed (4). In contrast to these clear effects on oxidative phosphorylation efficiency following acute modulations of the flux in vitro, the consequence of chronic adaptation to various rates of ATP turnover on the yield of ATP synthesis in vivo is not known since the mechanism permitting such adaptation is poorly understood as yet. This issue, however, is of major importance since a large disparity in mitochondrial oxidative phosphorylation activity is encountered between species, tissues, and in a given tissue according to physiological or pathological states. Indeed, if a change in the rate of oxidative phosphorylation pathway also affects its yield, a compromise between flux and efficiency must be achieved, and one main question pertains to the mechanism and the cost permitting such large changes in oxidative phosphorylation capacity.

In this work, we have studied the parameters of oxidative phosphorylation in isolated liver cells and liver mitochondria from rats subject to various physiological or pathological conditions known to affect oxidative phosphorylation: thyroid status (5–12), polyunsaturated fatty acid deficiency (13–17), and alcohol intoxication (18, 19). Hypothyroidy was obtained by adjunction of propylthiouracil, an inhibitor of the first step of thyroid hormone synthesis, in drinking water. We observed that the respiratory rates of isolated liver cells from these models varied from 9.8 to 22.7 nmol/min/mg dry cells, while maximal mitochondrial ATP synthesis capacity varied from 165 to 382 nmol/min/mg protein. These events were related to ATPase activity as well as cytochrome, ANC, and Pmit carrier

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† To whom correspondence should be addressed: Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Joseph Fourier, BP 53X, 38041 Grenoble Cedex, France. Tel.: 33-476-51-43-86; Fax: 33-476-51-42-18; E-mail: Xavier.Leverve@ujf-grenoble.fr.

1 The abbreviations used are: ATP/O, adenosine triphosphate synthesis (nmol/min/mg protein)/oxygen consumption rate (nanomol/min/mg); PUFA, polyunsaturated fatty acid; ∆ψ, electrical potential difference across the mitochondrial inner membrane; TPMP+, triphenylmethylphosphonium ion; ∆Gr, Gibbs free-energy difference in oxidation reaction (redox potential); DNP, 2,4-dinitrophenol; J(24), oxygen consumption rate; ANC, adenine nucleotide carrier.

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contents. The activation of oxidative phosphorylation rate was accompanied by a decrease in ATP/O ratio and was associated to a change in the composition of the respiratory chain since the proportion of cytochrome oxidase varied from 6% to 27% of total cytochrome content. Because cytochrome oxidase is well recognized to be the location of a slippage between redox reaction and proton pumping (2, 20) we propose the change in the proportion of cytochrome oxidase to be the major determinant of the modulation of oxidative phosphorylation in these physiological adaptive processes.

MATERIALS AND METHODS

Male weaning Wistar rats were divided into five groups: control, polyunsaturated fatty acid (PUFA)-deficient, hyperthyroid, hypothyroid, and alcohol. Rats were fed for 6 weeks a synthetic diet containing 72% of energy as carbohydrate, 22% as protein, and 6% as lipid (soya oil). In the PUFA-deficient group, this diet was supplemented with stearic and palmitic acid (3% of energy for each) instead of soya oil. Hyperthyroidy was induced by daily intraperitoneal injections of 3,3',5-triiodo-L-thyronine (15 μg/100 g of body weight in 0.05 ml NaOH in saline) for 10 days before killing. In the hypothyroid group, 0.05% (w/v) 6- n-propyl-2-thiouracil was given in drinking water for 6 weeks. In the ethanol group, ethanol was given in drinking water with progressively increasing concentrations from 5 to 30% during the 6 weeks (5% increase every week).

Hepatocytes were isolated by the method of Berry and Friend (34) as modified by Groen et al. (35), from rats fasted for 24 h. Liver cells (15 mg dry cells/ml) were incubated with 20 mM glucose as substrate. After a 25-min incubation, myxothiazol-sensitive oxygen consumption was measured, permitting to consider only mitochondrial respiration since myxothiazol is an inhibitor of complex III. Cell and mitochondrial matrix volumes were determined by subtracting either 14C(carboxymethyl)linulin or 3H]TPMP + and 36Cl −, respectively (36). The intramitochondrial NADH/NAD + ratio was determined by the metabolite indicator method (37) assuming the reaction catalyzed by β-hydroxybutyrate dehydrogenase (EC 1.1.1.30) is in near equilibrium. Kapp = [aconetic acid] × [NADH]/[β-hydroxybutyric acid] × [NAD +], i.e. 4.93 × 10 −5 (38).

Δέμ, represents the span of the redox potential between the electron donor couple (NADH/NAD +) and the final electron acceptor couple (O2/H2O) across the respiratory chain. It was calculated from Δέμ, = −ΔFm, − 61.5 log([NADH]/[NAD +]) with ΔFm, = 1.2 volt.

The overall efficiency of the respiratory chain was estimated by investigating the relationship between the rate through the global reaction (oxygen consumption) and the overall thermodynamic driving force applied to this reaction and expressed as 2ΔFm, nΔΨ, where ΔFm, is the difference in redox potential across the respiratory chain (see above), and n is the H +/O stoichiometry of the overall respiratory chain. It is accepted that n is equal to 10 for substrates giving their electrons to complex I (36, 39). The relationship between the rate of oxygen consumption and the overall thermodynamic driving force was established experimentally by modulating the ΔΨ in intact cells with small additions of DNP (20–75 μM).

Mitochondria were isolated from liver of freshly killed rats by the standard method (40). They were incubated at a concentration of 4 mg/ml in an oxygraph vessel equipped with a Clark electrode at 37 °C in a medium containing 125 mM KCl, 1 mM EGTA, 5 mM P2- Tris, and 20 mM Tris-HCl (pH 7.2) in the presence of 5 mM succinate-Tris, 0.5 mM malate-Tris, and 1.25 μm rotenone. After the addition of 1 mM ADP, mitochondrial oxygen consumption and ATP synthesis rates were measured. These conditions permitted to determine ATP/O at maximal state 3 respiratory rate. In a parallel experiment, oligomycin (1.25 μg/ml protein) was added to the mitochondrial suspension to determine the non-phosphorylating respiratory rate (state 4).

Mitochondrial preparations, and each determination was made in triplicate (mean ± S.E.).

TABLE I

Influence of physiological state on the cellular respiratory rate

| Respiratory state | ΔΨi(mV) | O2 uptake | H+ uptake |
|------------------|---------|-----------|-----------|
| Control          |         | 12.9 ± 0.6 | −151 ± 2  |
| Hypothyroidy     |         | 152 ± 1.2  | −142 ± 1  |
| Hyperthyroidy    |         | 22.7 ± 1.1 | ND        |
| Ethanol          |         | 11.9 ± 0.9 | ND        |
| PUFA deficiency  |         | 16.5 ± 0.5 | −152 ± 3  |

The spectra of fully oxidized (2 H2O2) versus fully reduced (few crystals

FIG. 1. The physiological state affects the oxidative phosphorylation capacity of liver mitochondria. Mitochondria (4 mg/ml), isolated from liver of five groups of rats: control group (A), PUFA-deficient group (D), hyperthyroid group (C), hypothyroid group (C), and alcohol group (V) were incubated at 37 °C in a medium containing 125 mM KCl, 1 mM EGTA, 5 mM P2- Tris, and 20 mM Tris-HCl (pH 7.2) in the presence of 5 mM succinate-Tris, 0.5 mM malate-Tris, and 1.25 μm rotenone. After the addition of 1 mM ADP, mitochondrial oxygen consumption and ATP synthesis rates were measured. A, relationship between state 3 oxygen consumption rate and state 3 ATP synthesis rate. B, relationship between state 3 respiration and oxidative phosphorylation efficiency (ATP/O). For each group, experiments were repeated for five separate mitochondrial preparations, and each determination was made in triplicate (mean ± S.E.).
of sodium dithionite) cytochrome. Respiratory chain cytochrome concentrations were also determined similarly in isolated hepatocytes. Wavelength pairs and absorbivity coefficients used were: cytochrome c + c1 (550–560 nm) e = 18 nm⁻¹ cm⁻¹, cytochrome b (563–575 nm) e = 18 nm⁻¹ cm⁻¹, and cytochrome a + a3 (605–630 nm) e = 24 nm⁻¹ cm⁻¹ (41, 42).

Determination of phosphate and adenine nucleotide carriers content was assessed in rat liver mitochondria in a medium containing 125 mM KCl, 1 mM EGTA, 5 mM Tris-Pi, a n d 1 mM ADP with 5 mM succinate as respiratory substrate and 20 mM Tris-HCl (pH 7.2, 37 °C). Before measurement, mitochondria were incubated 2 min in 0.35% (w/v) Triton X-100 with or without 1.25 μg/mg protein oligomycin. Oligomycin-sensitive ATP hydrolysis was assessed by determination of Pₐ production (43).

RESULTS

The Physiological State Strongly Affects the Oxidative Phosphorylation Capacity of Both Liver Cells and Mitochondria—As compared with control cells, thyroid status and PUFA deficiency significantly affected the respiratory rate of isolated liver cells; hyperthyroidy and PUFA deficiency increased Jₐ by 75 and 28%, respectively, while hypothyroidy decreased it by 25% (Table I). Alcohol intoxication had no significant effect. Hyperthyroidy was responsible for a significant decrease in the mitochondrial membrane potential. These results are in good agreement with data from isolated mitochondria where we found a large adaptive response among the five different conditions in both respiration (117–441 nanomol of O₂/mg of proteins) and ATP synthesis (165–382 nmol of ATP/mg of proteins) (Fig. 1A). It is striking to note that the increase in maximal oxidative phosphorylation activity was accompanied by a simultaneous decrease in the coupling. Indeed, as can be see on Fig. 1B, ATP/O ratio ranged from 1.6 to 0.9 nmol/nanomol with succinate as substrate.

Effect on the Respiratory Chain Efficiency—The different physiological conditions used in this study are known to affect the composition of the mitochondrial inner membrane, which may in turn affect the membrane permeability (13, 16, 17, 44–49). However, if a change in the membrane permeability to protons were the only mechanism responsible for the observed change in the oxidative phosphorylation yield, the intrinsic efficiency of the respiratory chain would not be affected. As previously shown, this hypothesis can be tested by determining experimentally the relationship between the flux through the respiratory chain and the related overall thermodynamic driving force applied to it (4, 50). The overall thermodynamic driving force can be determined in our conditions simultaneously with the flux through the reaction (oxygen consumption rate, see "Material and Methods" and also Ref. 36). Therefore, a relationship between the respiratory rate and the overall thermodynamic driving force can be established in each of our different conditions by modulating the protonotive force with small amounts of protonophore DNP. Since DNP is a pure protonophore, changes in respiratory rate induced by its addition are not related to any intrinsic effect at the level of the respiratory chain. Therefore, the relationship is characteristic of a given respiratory chain efficiency. As shown in Fig. 2, it appears that different relationships were observed according to the different physiological conditions, indicating a change in respiratory chain efficiency per se. It may be worth mentioning that the changes in the relationship between force and rate are consistent with the conclusion that the change does not simply result from an increase in the number of mitochondria per cell.

The different components of the oxidative phosphorylation pathway were determined in our conditions in isolated mitochondria (Table II). In hypothyroidy total cytochrome, ANC, and Pᵢ carrier contents were decreased approximately by 30% while ATPase activity was not significantly affected. Conversely, in hyperthyroidy, total cytochrome, ANC, Pᵢ carrier contents, and ATPase activity were significantly increased. In PUFA deficiency, total cytochrome content and ATPase activity were increased. ANC, Pᵢ carrier contents, and ATPase activity

| cyanide  | b   | c₁   | total | ATPase activity | Adenine nucleotide carrier content | Phosphate carrier content |
|---------|-----|------|-------|----------------|-----------------------------------|--------------------------|
| nmol/mg protein |     |      |       | mmol Pᵢ/min | pmo1/mg protein | nmol/mg protein |
| Control   | 0.097 ± 0.011 | 0.188 ± 0.015 | 0.210 ± 0.017 | 0.495 ± 0.033 | 413 ± 66 | 177 ± 15 | 10.50 ± 0.35 |
| Hypothyroidy | 0.020 ± 0.002 | 0.106 ± 0.008 | 0.215 ± 0.028 | 0.341 ± 0.021 | 403 ± 15 | 133 ± 29 | 7.45 ± 0.15 |
| Ethanol   | 0.044 ± 0.008 | 0.175 ± 0.015 | 0.233 ± 0.011 | 0.461 ± 0.011 | ND | ND | ND |
| PUFA deficiency | 0.413 ± 0.013 | 0.235 ± 0.011 | 0.209 ± 0.019 | 0.587 ± 0.032 | 719 ± 76 | 170 ± 19 | 10.67 ± 0.41 |

Fig. 2. Relationships between oxygen consumption rates and overall thermodynamic forces at the level of the respiratory chain in hepatocytes. Rat liver cells (15 mg/ml) were incubated in a Krebs-bicarbonate medium containing 20 mM glucose as described under "Material and Methods." After 25 min of incubation, myxothiazol-sensitive oxygen uptake (Jₐ) was determined. ΔE'ₜ is the difference in redox potential across the electron transport chain, and 10 is the proton/2 electrons stoichiometry of the respiratory chain. Data are mean ± S.E. from four separate cellular preparations. Control group (▲), PUFA-deficient group (■), hyperthyroidy (●), and hypothyroidy (○).

TABLE II

Influence of the physiological state on the content of oxidative phosphorylation pathway components

Liver mitochondria were isolated from control, hypothyroid, hyperthyroid, ethanol, and PUFA-deficient rats. Respiratory chain cytochrome contents were measured as described under "Material and Methods." Adenine nucleotide and phosphate carriers contents were determined by using quasi-irreversible inhibitor (carboxyatractylate and mersalyl, respectively), and in a parallel experiment, ATPase activity was determined as described under "Material and Methods." Results are mean ± S.E. from five separate cellular preparations. * p < 0.05 versus control. ND, not determined.
were not determined in ethanol group, and total cytochrome content was not affected. The extreme observed changes are roughly comparable within the different enzymes involved in this pathway of oxidative phosphorylation: e.g. between 2- and 3-fold increase in hyperthyroidy as compared with hypothyroidy. However, there are some remarkable exceptions showing qualitative modifications of the oxidative phosphorylation pathway. Actually, the main change occurred at the level of cytochrome oxidase, whose content ranged from 0.02 to 0.224 (nmol/mg protein), i.e. 11-fold, between hypothyroidy and hyperthyroidy. However, it should be pointed out that when compared with controls, the relative differences for the various proteins in hyperthyroidy are fairly limited to about 2-fold. As shown in Fig. 3, the changes in cytochrome aa₃ were closely related to changes in respiration both in state 4 and in state 3.

**Fig. 3. Relationships between cytochrome aa₃ content and respiratory rates.** Oxygen consumption was measured in the conditions described in Fig. 1. State 3 (A) and state 4 (B) respiration rates were measured after the addition of ADP (1 mM) and oligomycin (1.25 μg/mg protein), respectively. In parallel experiments, respiratory chain cytochrome aa₃ concentrations were measured as described under “Material and Methods.” For each group, experiments were repeated for five separate mitochondrial preparations and each determination was made in triplicate (mean ± S.E.). Control group (△), PUFA-deficient group (□), hyperthyroidy (●), hypothyroidy (○), and ethanol (◇).

**Fig. 4. Relationships between the yield of oxidative phosphorylation (ATP/O) and the proportion of cytochrome aa₃, b, and cc₁ at the level of the respiratory chain.** Oxygen consumption was measured in the conditions described in Fig. 3. In parallel experiments, respiratory-chain cytochrome contents were measured as described under “Material and Methods” and were expressed as percentage of total cytochrome content. For each group, experiments were repeated for five separate mitochondrial preparations, and each determination was made in triplicate (mean ± S.E.). Control group (△), PUFA-deficient group (□), hyperthyroidy (●), hypothyroidy (○), and ethanol (◇). A, relationship between ATP/O and cytochrome b. B, relationship between ATP/O and cytochrome c + cc₁. C, relationship between ATP/O and cytochrome a + a₃.
tion of cytochrome $c$, increased with the yield of oxidative phosphorylation (ATP/O) (Fig. 4B), while cytochrome $aa_3$ proportion decreased with ATP/O (Fig. 4C).

Since we found both quantitative (cellular $J_{O_2}$) and qualitative (mitochondrial cytochrome content expressed/mg of mitochondrial protein) modifications, the question arises of possible relationships between these parameters. As shown in Fig. 5, we found tight relationships between oxygen consumption rate and total cytochrome contents both in intact liver cells and in isolated mitochondria. On one hand, cellular oxygen consumption rate was related to the cellular content of total cytochromes (Fig. 5A), while on the other hand (Fig. 5B), the cellular oxygen consumption rate was also related to the qualitative changes of mitochondria (total cytochrome content/mg of mitochondrial protein). Furthermore, the increase in cellular oxygen consumption rate was significantly linked to a decrease in the yield of oxidative phosphorylation (ATP/O ratio, Fig. 5C).

DISCUSSION

In the different physiological conditions studied here, we found that liver cells can sustain very different rates of respiration and consequently of ATP turnover. Such a finding raises the question of the mechanism by which this energetic adaptation can occur. It should be noticed that these large variations were observed within a mitochondrion population of one given organ (liver) of one species (rat) and in conditions fully compatible with life. Such a physiological adaptation could be related to variations in the mitochondrial content of these liver
cells. In the light of the data presented here, it is rather diffi-
cult to address precisely this issue since the yield of mitochon-
dria and liver cell isolation cannot be directly compared due to
a difference in isolation procedures. It must be noted, however,
that the quantity of total cytochromes was dramatically af-
affected in the different physiological conditions both when ex-
pressed per mg of dry cells or mg of protein of isolated mito-
ochondria. Moreover, the strong relationships between the re-
spiration observed in intact cells and the mitochondrial cy-
tochrome content (see Fig. 5, A and B) favor the view that the
main modification permitting this adaptation is the change in
the oxidative phosphorylation complexes expressed by mg of
mitochondrial protein. In addition, since the intercept of these
relationships are close to zero, this suggests that whatever the
condition, the cellular respiratory activity per “unit of respira-
tory chain” is constant. It must be noted that the cellular
oxygen consumption considered here is purely related to mito-
ochondrial respiration since it is myxothiazol-sensitive.

Besides this adaptive change in the amount of oxidative
phosphorylation complexes, we also found a change in the
efficiency of the respiratory chain when assessed in intact cells
(see Fig. 2). It appears that when the cellular respiratory rate
increases, the efficiency of the respiratory chain decreases. The
data obtained in isolated mitochondria show that the increase
in cytochrome content is associated to a redistribution in the
respiratory chain units in chronic hyperthyroidy. This latter
effect being most probably responsible for a change in the effi-
ciency. Hence, as evidenced in Fig. 5C, the adaptive increase in cellular respi-
ration in our experimental conditions is accompanied by a
decrease in the efficiency of oxidative phosphorylation, and
vice versa. Thus, conversely to acute changes in ATP synthe-
sis, which are due to changes in oxidative phosphorylation
rate constant. It must be noted that the cellular
energy consumption considered here is purely related to mito-
ochondrial respiration since it is myxothiazol-sensitive.

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