Physiological diversity of mitochondrial oxidative phosphorylation


1INSERM, U688 Physiopathologie Mitochondriale, Université Victor Segalen-Bordeaux 2, Bordeaux, France; and 2IFR 31, Institut Louis Bugnard, BP 84225, UMR 5018 CNRS UPS, Toulouse, France

Submitted 20 April 2006; accepted in final form 7 June 2006


Hence, our study demonstrates that, in tissues, oxidative phosphorylation capacity is highly variable and diverse, as determined by different combinations of 1) the mitochondrial content, 2) the amount of respiratory chain complexes, and 3) their intrinsic activity. In all tissues, there was a large excess of enzyme capacity and intermediate substrate concentration, compared with what is required for state 3 respiration. To conclude, we submitted our data to a principal component analysis that revealed three groups of tissues: muscle and heart, brain, and liver and kidney.

Address for reprint requests and other correspondence: R. Rossignol, INSERM U688, Physiopathologie mitochondriale, Université Victor Segalen-Bordeaux 2, 146 rue Léo-Saignat, F-33076 Bordeaux Cedex, France (e-mail: rossig@u-bordeaux2.fr).
respiratory chain complexes, and 3) their intrinsic activity. We discuss these differences in regard to the variability of OXPHOS control and the dramatic tissue specificity of mitochondrial diseases (43, 50).

EXPERIMENTAL PROCEDURES

Chemicals. All chemicals used for mitochondrial preparations and enzymological and polarographic studies were purchased from Sigma. Chemicals for Western blot experiments were purchased from Bio-Rad.

Animals. Male Wistar rats weighing 180–200 g, having free access to water and standard laboratory diet, were used for this study. The animals were killed by cervical shock and decapitation. All of the procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals (Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects). Our experimental protocol was performed in accordance with the guidelines of the French National Institute for Science and Medical Research (INSERM).

Preparation of rat muscle mitochondria. Rat muscle mitochondria were isolated by differential centrifugation. Muscle from two hind legs were collected in isolation medium I (in mM: 210 mannitol, 70 sucrose, 50 Tris·HCl, pH 7.4, and 10 K+ EDTA) and digested by trypsin (0.5 mg/g of muscle) for 30 min. The reaction was stopped by addition of trypsin inhibitor (soybean 3:1 inhibitor to trypsin). The supernatant was centrifuged at 1,000 g for 5 min. The supernatant was strained through gauze and centrifuged at 7,000 g for 10 min. The resulting pellet was resuspended in ice-cold isolation medium II (in mM: 225 mannitol, 75 sucrose, 10 Tris·HCl, pH 7.4, and 0.1 K+ EDTA), and a new series of centrifugations (1,000 and 7,000 g) was performed. The last mitochondrial pellet was resuspended into a minimum volume of isolation medium II to obtain a mitochondrial concentration of between 50 and 80 mg/ml. Protein concentration was measured by the Biuret method using BSA as standard.

Preparation of rat liver and kidney mitochondria. Liver was collected in isolation medium A (in mM: 250 sucrose, 10 Tris·HCl, pH 7.6, and 1 K+ EGTA) and homogenized. The homogenate was centrifuged at 1,000 g for 5 min. The supernatant was strained through gauze and centrifuged at 7,000 g for 10 min. The resulting pellet was resuspended in ice-cold isolation medium B (in mM: 250 sucrose, 10 Tris·HCl, pH 7.6, and 0.1 K+ EGTA), and a new series of centrifugations (1,000 and 7,000 g) was performed. The last mitochondrial pellet was resuspended in a minimum volume of isolation medium B to obtain a mitochondrial concentration of between 50 and 70 mg/ml.

Preparation of rat brain mitochondria. Brain mitochondria were isolated from whole brain. Rats were killed by decapitation without stunning, and the brains were removed and homogenized in isolation buffer (in mM: 250 sucrose, 10 Tris·HCl, pH 7.4, and 0.5 K+ EDTA). The homogenate was centrifuged at 1,000 g for 5 min. The supernatant was strained through gauze and centrifuged at 7,000 g for 10 min. The resulting pellet was resuspended in ice-cold isolation buffer, and a new series of centrifugations (1,000 and 7,000 g) was performed. The crude mitochondrial pellet was resuspended in a final volume of 10 ml in 3% Ficoll medium (3% Ficoll, 250 mM sucrose, 10 mM Tris·HCl, pH 7.4, and 0.5 mM K+ EDTA). This suspension was carefully layered onto 20 ml of 6% Ficoll medium (6% Ficoll, 250 mM sucrose, 10 mM Tris·HCl, pH 7.4, and 0.5 mM K+ EDTA) and centrifuged for 30 min at 11,500 g. The mitochondrial pellet was resuspended in isolation medium and centrifuged for 10 min at 12,500 g. The mitochondria were made up to a concentration of ~50 mg protein/ml in the isolation buffer.

Transmission electron microscopy. To allow comparisons of the mitochondrial organization in muscle, heart, liver, kidney, and brain, we prepared sections from tissues obtained from the same animal. These were also submitted to the same procedure of fixation and coloration. First, the tissues were dissected immediately after death and fixed by immersion in solution A (2.5% glutaraldehyde, 4% paraformaldehyde, 4% saccharose, and 2% polyvinilpyrolidone in 0.1 M cacodylate buffer). These were sectioned in 40-μm-thick blocks, immersed for 1 h in fixative solution A, and rinsed with water. The blocks were recut into smaller samples of ~12 μm-thick and immersed in a second fixative (solution B: 2% osmic acid in 0.1 M cacodylate buffer) for 1 h. After dehydration, the blocks were embedded in epoxy (epon) resin and cut into longitudinal or transversal sections 0.1 μm thick. The different sections were stained with a solution of uranyl acetate and lead citrate. Sections were observed on a Philips CM10 microscope. Morphometric analysis was performed by randomly analyzing selected tissue sections of the different tissues (n ≥ 5) obtained from three different animals.

Western blotting. Samples were diluted into SDS-PAGE tricine sample buffer (Bio-Rad) containing 2% β-mercaptoethanol by incubation for 30 min at 37°C, and separated on a 10–22% SDS polyacrylamide gradient mini-gel (Bio-Rad) at 150 V. Proteins were transferred electrophoretically to 0.45-μm polyvinylidene difluoride membranes for 2 h at 100 mA in CAPS buffer (3.3 g CAPS, 1.5 liters of 10% methanol, pH 11) on ice. Membranes were blocked overnight in 5% milk-PBS + 0.02% azide and incubated for 3 h with primary antibodies purchased from Mitosciences. The antiprin antibody was purchased from Calbiochem. After three washes with PBS-0.05% Tween 20, the membranes were incubated for 2 h with horseradish peroxidase-conjugated goat anti-mouse antibody (Bio-Rad) diluted in 5% milk-PBS. This secondary antibody was detected using enhanced chemiluminescent Plus reagent (Amersham). The signal was quantified by densitometric analysis with the use of Image J (National Institutes of Health) software.

Respiration measurements. Mitochondrial oxygen consumption was monitored at 30°C in a 1-ml thermostatically controlled chamber equipped with a Clark oxygen electrode (Oxy 1, Hansatech) in respiration buffer [75 mM mannitol, 25 mM sucrose, 100 mM KCl, 10 mM Tris phosphate, 10 mM Tris·HCl, pH 7.4, 50 μM EDTA, plus respiratory substrates (10 mM pyruvate in presence of 10 mM malate)]. The mitochondrial concentration used for respiration measurements was 1 mg/ml, and state 3 was obtained by the addition of 2 mM ADP. Respiration rates were expressed in nanograms atom O per minute per milligram of proteins. The OCR is defined as the ratio of state 3 (in the presence of ADP) to state 4 (in the absence of ADP) respiratory rate. The uncoupling ratio was defined as the ratio of the uncoupled respiratory rate (measured in presence of 1–10 μM carbonyl cyanide 3-chlorophenyl hydrazone) to state 3.

Enzymatic determination. Assays of all respiratory chain enzyme activities were carried out spectrophotometrically at 30°C using a double-wavelength Xenius spectrophotometer from SAFAS (Monaco) and standardized reproducible methods as described previously (1). All activities were expressed in nanomoles per minute per milligram. The kinetic parameters (Vmax and Km) of complexes III and IV were obtained by fitting the experimental curve V = f[S], with the Michaelis-Henri equation, V = (Vmax × [S])/(Km + [S]), where [S] is substrate concentration and V is velocity, using Kaleida Graph 3.0.2 (Abelbeck software). Activity measurements were performed for complex III using 10 μg/ml of mitochondrial protein (muscle and liver) with a ubiquinone concentration ranging from 0 to 200 μM. For complex IV, we used 10 μg/ml of mitochondrial protein (muscle and liver) and a reduced cytochrome c concentration that also ranged from 0 to 200 μM.

Complex II (succinate dehydrogenase). The assay was performed by following the decrease in absorbance at 600 nm resulting from the reduction of 2,6-dichlorophenolindol-phenol in 1 ml of medium containing 60 mM KH2PO4 (pH 7.4), 3 mM KCN, 20 μg/ml rotenone, 20 mM succinate, and 10 μg mitochondrial protein. The reaction was initiated by the addition of 1.3 mM phenazine methasulfate and 0.18 mM 2,6-dichlorophenolindolone sodium salt hydrate. The extinction coefficient used for DCPIP was 21 M−1·cm−1.
Complex III (ubiquinol cytochrome c reductase). The oxidation of 6.5 mM decylubiquinol by complex III was determined by using cytochrome c (III) as an electron acceptor. The assay was carried out in basic medium supplemented with 2.5 mg/mL BSA, 15 μM cytochrome c (III), and 5 μg/mL rotenone. The reaction was started with 10 μg of mitochondrial protein, and the enzyme activity was measured at 550 nm. The extinction coefficient used for cytochrome c was 18.5 M⁻¹·cm⁻¹.  

Complex IV (cytochrome c-oxidase). Two methods were used for determining cytochrome c-oxidase activity. Initially, cytochrome c-oxidase activity was determined spectrophotometrically with cytochrome c (II) as substrate. The oxidation of cytochrome c was monitored at 550 nm at 30°C. The extinction coefficient used for cytochrome c was 18.5 M⁻¹·cm⁻¹. In the second method, we monitored the cytochrome c-oxidase activity by inhibiting the rest of the respiratory chain with rotenone and antimycin, using 3 mM ascorbate and 0.5 mM N,N,N',N'-tetramethyl-p-phenylenediamine, as an electron donor system.

Citrate synthase. The reduction of 5,5-dithiobis(2-nitrobenzoic acid) by citrate synthase at 412 nm (extinction coefficient of 13.6 M⁻¹·cm⁻¹) was followed in a coupled reaction with coenzyme A and oxaloacetate. A reaction mixture of 0.2 M Tris·HCl, pH 8.0, 0.1 mM acetyl-coenzyme A, 0.1 mM 5,5-dithiobis(2-nitrobenzoic acid), and 5–20 μg of muscle or brain mitochondrial protein was incubated at 30°C for 5 min. The reaction was initiated by the addition of 0.5 mM oxaloacetate, and the absorbance change was monitored for 5 min.

Determination of cytochrome b₈, b₉, b₁, c, c₁, and aa₃ absolute content by spectrophotometry. Mitochondria isolated from muscle and liver were used at 1 mg/mL in 1 mL of the respiratory buffer described above. Individual fully reduced (with excess sodium dithionite) or fully oxidized (with excess ferricyanide) absorbance spectra were recorded between 500 and 650 nm on a SAFAS Genius double-wavelength spectrophotometer (SAFAS, Monaco). The concentration of cytochrome aa₃ ([aa₃]) contained in the heme of cytochrome c-oxidase (complex IV) was determined from the difference spectrum [reduced − oxidized (red-ox)] at the maximum absorption value of 605 nm (A₆₀₅(red-ox₇,415)), normalized by the absorbance of the isobestic point at 630 nm (A₆₃₀(red-ox₇,630)). Values were calculated by the Beer-Lambert law (see Eq. 1), with an extinction coefficient ε₆₀₅-redox aa₃ of 24,000 M⁻¹·cm⁻¹ (40) and a cuvette length of 1 cm

\[\text{[aa₃]} = (A_{605\text{red-ox}} - A_{605\text{red}}})/\varepsilon_{605\text{red-ox aa₃}} \]  

The absolute content of c-type cytochromes (c + c₁) was determined from the same difference spectrum (reduced − oxidized) at the maximum absorption value of 550 nm (A₅₅₀(red-ox₇,550)), normalized by the absorbance of the isobestic point at 535 nm (A₅₃₅(red-ox₇,535)). The concentration of cytochrome c + c₁ was determined according to the Beer-Lambert law (see Eq. 2), with an extinction coefficient ε₆₃₀-redox c + c₁ of 18,500 M⁻¹·cm⁻¹ (26) and a cuvette length of 1 cm

\[c + c₁ = (A_{630\text{red-ox}} - A_{630\text{red}})/\varepsilon_{630\text{red-ox c + c₁}} \]  

The total content of cytochrome c was then calculated from this total content in cytochrome c + c₁ by subtracting the contribution of c₁, which was determined by considering its molar equivalence of 1:1, with cytochrome b₉ (22). This content in cytochrome b₉ was determined experimentally, using a detergent-based partial solubilization of mitochondrial proteins described in Ref. 40, which leads to the physical separation of complex II and complex III. In this manner, it is possible to determine the part of the absorbance of the different b-type cytochromes from the total (b₉ + b₈ + b₁), calculated from the maximum absorption peak at 562 nm (A₅₆₂(red-ox₇,562)), normalized by the absorbance of the isobestic point at 575 nm (A₅₇₅(red-ox₇,575)). The total extinction coefficient of b₁ + b₈ + b₉ (ε₅₆₂-redox b₁ + b₈ + b₉) equals was 56,100 M⁻¹·cm⁻¹ and that of b₉ (ε₅₆₂-redox b₉) was 26,000 M⁻¹·cm⁻¹ (40). The cuvette length was 1 cm. In this manner, the concentration of cytochrome c₁ was calculated from that of b₉ according to Eq. 3:

\[ [c₁] = 0.5 [A_{562,575}(1 - R)]/(ε_{b₁ + b₈ + b₉} × 1) \]  

\[ [c] = [c + c₁] − [c₁] \]
Mitochondrial structure in the different tissues. We analyzed several electron micrographs of various tissue sections, including slow-type and rapid-type skeletal muscle, heart, liver, kidney, and brain, all observed at the same magnification (Fig. 2). A large diversity in mitochondrial shape and organization according to tissue was observed, with an important variability of organellar section profile, intracellular localization, and heterogeneity. First, we compared two types of skeletal muscle, the soleus (Fig. 2A) vs. the peroneus digiti quarti (Fig. 2B). Accordingly, the former is a “slow type” and contained more mitochondria than the latter, which is a “rapid type.” In these two types of muscle, the mitochondria appeared to be contained within membranous compartments all along the myofibers and looked highly compacted. In heart (Fig. 2C), mitochondria were more numerous and presented with a larger section. In liver (Fig. 2D), they were difficult to distinguish, as they presented with a less clear envelope and fewer cristae. They also looked more perinuclear and were closely intricated with the Golgi apparatus. In kidney (Fig. 2E), there was a large number of mitochondria essentially located in the interdigitations of the tubules, where the ion-pumping activity is very active. In brain (Fig. 2F), mitochondrial sections were more dispersed throughout the cytosol, with an important variability in section profiles. The subsequent observation of mitochondrial internal organization revealed differences between tissues concerning the number of cristae and matrix density. For instance, there was more cristae per surface unit of mitochondrial section in heart (124 ± 27) than in muscle (107 ± 11), brain (97 ± 13), kidney (46 ± 6), and liver (26 ± 4). Measurement of the matrix density, expressed as the ratio of matrial vs. cytosolic densitometry, showed a higher value in heart (1.91 ± 0.42) than in brain (1.35 ± 0.32), soleus muscle (1.30 ± 0.17), kidney (1.23 ± 0.29), and liver (1.04 ± 0.30).

Variable enrichment in respiratory chain proteins on mitochondria isolated from different tissues. To extend the above detailed analysis at the organellar level, we have isolated the mitochondria from rat muscle, heart, liver, kidney, and brain. First, we looked at the relative expression level of each respiratory chain complexes I-V by Western blot using a set of seven monoclonal antibodies. This was performed on 5 μg of mitochondrial proteins under nonsaturating conditions, as shown in Fig. 3. To evaluate the respiratory chain content per mitochondrial compartment, we normalized their expression level to the porin band density (Fig. 3). It can be seen that mitochondria isolated from heart and muscle contained more respiratory chain complexes (normalized to porin) than shown in Fig. 3. To perform an absolute determination of the respiratory chain content in mitochondria isolated from the different tissues, we calculated their actual number of moles per milligram of mitochondrial proteins, from the absorbance of cytochrome b13, b1, or b1 and aa3, respectively. An example of difference absorbance spectra is given in Fig. 4. It can be seen that the different cytochromes of type b (b13, b1, and b1) formed a large peak at 562 nm. For this reason, we performed a physical separation of complex II and complex III that allowed us to...
determine the individual parts of cytochrome $b_H + b_L$ and $b_{II}$ in this peak (see histogram in Fig. 4). Calculation of the absolute content of these different cytochromes (Table 1) indicated a higher content (factor of 2–3) in heart and muscle than in liver, kidney, and brain. In contrast, the molar ratio between these different cytochromes was conserved between tissues. We chose to set the value of complex III content at 3 for an easier comparison to the results of the literature. In this manner, we obtained a conserved ratio $1:3:7$ between complexes II, III, and IV in the five tissues (Table 1).

Intermediate substrates of the respiratory chain: absolute content and stoichiometry with respective enzyme complexes in different tissues.

The total content in cytochrome $c$ was determined by spectrophotometry on mitochondria isolated from muscle, heart, liver, kidney, and brain (see spectra on Fig. 4). The results, expressed in picomoles of cytochrome per milligram of mitochondrial proteins, are given in Table 1. They show large differences between tissues, with higher values in heart and muscle than in liver, kidney, and brain. The total content of CoQ9 was determined by HPLC-EC on the same mitochondrial preparations (Table 1). It showed a large variability between tissues, with high values in muscle and liver compared with values in kidney, heart, and brain. From these values and the absolute content in respiratory chain complexes determined above, we calculated the stoichiometry between complex III and CoQ9 as well as complex IV and cytochrome.
of cytochrome c, respectively (Table 1). The mean values for CoQ9/complex III range from 7.9 ± 0.9 in heart to 44.9 ± 6.2 in liver and that of cytochrome c/complex IV from 1.4 ± 0.3 in liver to 4.4 ± 1.1 in brain.

**Respiratory chain complex-specific activity.** $V_{\text{max}}$ and $K_m$ of respiratory chain complexes II, III, and IV were measured on mitochondria isolated from the different tissues. Hence, they must be referred to as the apparent kinetic parameters ($V_{\text{max-app}}$ and $K_{m-app}$) because they were not measured on the pure enzyme. The maximal activity of citrate synthase was also determined on these mitochondrial preparations. All of the values obtained are listed in Table 2. Results show important differences between enzyme complexes, with complex IV presenting the highest rate of substrate oxidation, compared with complexes III, II, and I. Comparisons of these activities in the different tissues show that the heart presents with the highest velocities, compared with muscle, brain, liver, and kidney, respectively. The same hierarchy between tissues was also observed for the other complexes (Table 2). These differences in $V_{\text{max-app}}$ between tissues could be attributed to variations in the amount of respiratory chain complexes and/ or in their catalytic constant. Thus, to evaluate the catalytic constant ($k_{\text{cat}}$) of respiratory chain complexes II, III, and IV in tissues, we divided their maximal activity (Table 2) by their absolute content (see Table 1), determined on the same isolated mitochondria. In this manner, we obtained important differences in the $k_{\text{cat}}$ values of complex IV, which presented with a higher value in brain (17,232 ± 2,184 min$^{-1}$) than in liver (13,043 ± 1,497 min$^{-1}$), heart (10,900 ± 1,562 min$^{-1}$), muscle (8,339 ± 974 min$^{-1}$), and kidney (6,436 ± 748 min$^{-1}$). For complex III, we obtained the highest $k_{\text{cat}}$ value in brain (13,377 ± 1,544 min$^{-1}$) compared with muscle (6,371 ± 732 min$^{-1}$), heart (5,201 ± 497 min$^{-1}$), liver (6,337 ± 541 min$^{-1}$), and kidney (5,431 ± 630 min$^{-1}$).

**Respiratory chain functioning parameters at state 3.** The measurement of state 3 respiratory rate with pyruvate-malate as substrate revealed comparable values on the mitochondria isolated from the different tissues (Table 3). However, some significant differences ($P < 0.05$) were observed for the RCR values. On the mitochondria respiring at steady-state 3, we also monitored the redox status of the intermediate metabolites cytochrome c and CoQ9. It was expressed as the percentage of substrate content that was in the reduced form at state 3, compared with the total amount reducible by dithionite. For CoQ9, we observed that 61.4 ± 14.7% was reduced at state 3 in the liver, compared with kidney (55.6 ± 12.9%), brain (2.2 ± 0.8%), heart (1.9 ± 0.4%), and muscle (2.0 ± 0.7%). For cytochrome c, we obtained a percentage of reduction at state 3 of 69 ± 7.6% in heart, 63 ± 5.1% in muscle, 39.0 ± 6.8% in brain, 37.1 ± 4.1% in kidney, and 43.4 ± 4.5% in liver. These results are summarized in Table 3.

**PCA of mitochondrial parameters from different tissues.** To compare the respiratory chain features in different tissues, while taking into account all the parameters measured in our study, we performed a PCA that allowed data reduction and interpretation, while preserving as much of the original information as possible. For this, we used some compositional parameters measured on mitochondria isolated from the five different tissues (content in complexes II, III, and IV; $k_{\text{cat}}$ of complexes II, III, and IV; $K_m$ of complexes II, III, and IV) and some phenomenological parameters representative of the control of mitochondrial respiration (control coefficient and biochemical threshold values of complexes II, III and IV) determined previously (36, 37). Completion of the PCA on these different results gave the biplot presented in Fig. 5. It can be seen that the different tissues segregate within the three groups according to their abscissa (first principal component) and ordinate (second principal component) values: muscle and heart (bottom left), liver and kidney (top right), and brain (bottom right). This suggests that, in each group, the tissues share similar compositional features of the respiratory chain, as well as comparable metabolic behavior.

**DISCUSSION.**

Cellular adaptation to environmental and physiological constraints necessitates a fine tuning of the control of mitochondrial respiration, in response to changes in energy demand and

### Table 1. Absolute amounts of the different respiratory chain cytochromes in mitochondria isolated from different tissues

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total content in cytochrome $b_h$, pmol/mg protein</td>
<td>32±5</td>
<td>60±9</td>
<td>16±4</td>
<td>21±6</td>
<td>17±9</td>
</tr>
<tr>
<td>Total content in cytochrome $b_o$, pmol/mg protein</td>
<td>121±9</td>
<td>124±16</td>
<td>43±4</td>
<td>51±5</td>
<td>53±16</td>
</tr>
<tr>
<td>Total content in cytochrome $a+a_s$ pmol/mg protein</td>
<td>277±15</td>
<td>320±25</td>
<td>93±8</td>
<td>119±14</td>
<td>125±21</td>
</tr>
<tr>
<td>Total content in CoQ9, pmol/mg protein</td>
<td>2,348±104</td>
<td>975±95</td>
<td>1,929±97</td>
<td>1,291±78</td>
<td>967±123</td>
</tr>
<tr>
<td>Total content in cytochrome c, pmol/mg</td>
<td>489±25</td>
<td>509±21</td>
<td>129±12</td>
<td>307±35</td>
<td>548±47</td>
</tr>
<tr>
<td>Molar ratio CII/CoQ9/CI/CIIV</td>
<td>0.89:58.2:3.1:2.6:9.1</td>
<td>1.45:23.6:3.11.9:7.5</td>
<td>1.1:13.4:7.3:9.0:6.5</td>
<td>1.26:73.2:3.18:2.7:1</td>
<td>1.0:57.9:3.34:8:7.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. CoQ, coenzyme Q; CII, complex II; CIII, complex III; CIV, complex IV.

### Table 2. Respiratory chain complexes activity in mitochondria isolated from different tissues

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal activity of CS, nmol·min$^{-1}$·mg protein$^{-1}$</td>
<td>1,089±115</td>
<td>1,175±206</td>
<td>334±98</td>
<td>195±67</td>
<td>478±128</td>
</tr>
<tr>
<td>Maximal activity of complex III, nmol·min$^{-1}$·mg protein$^{-1}$</td>
<td>771±136</td>
<td>645±112</td>
<td>294±89</td>
<td>277±97</td>
<td>709±223</td>
</tr>
<tr>
<td>Maximal activity of Complex IV, nmol·min$^{-1}$·mg protein$^{-1}$</td>
<td>2,310±307</td>
<td>3,488±651</td>
<td>1,213±117</td>
<td>766±143</td>
<td>2,154±446</td>
</tr>
<tr>
<td>$K_m$ for complex III, μM</td>
<td>9.9±0.7</td>
<td>11.2±1.3</td>
<td>15.8±0.9</td>
<td>42.7±2.6</td>
<td>23.4±2.1</td>
</tr>
<tr>
<td>$K_m$ for complex IV, μM</td>
<td>28.2±2.7</td>
<td>27.2±1.8</td>
<td>27.9±3.9</td>
<td>19.2±2.3</td>
<td>11.3±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. CS, citrate synthase.
substrate delivery. Accordingly, the different tissues present with large differences in the composition of the OXPHOS machinery and the organization of mitochondria, which could reflect their variable physiological activity. In addition, mitochondria also participate in fundamental cell signaling and apoptotic processes, the regulation of which may also lead to changes in organellar content, composition, and functionality.

Recently, different studies have evidenced important differences in the mitochondrial proteome between tissues, but their functional implication was not assessed. Here, we have analyzed the diversity of mitochondrial oxidative phosphorylation between tissues and its consequences for the control of energy production. For this, we determined the compositional and functional features of the respiratory chain on mitochondria isolated from rat muscle, heart, liver, kidney, and brain.

First, we looked at the mitochondrial content in the different tissues. This simple question raised numerous issues concerning the accuracy of the different methods of quantification generally employed. For instance, the classic measurement of mitochondrial section area on electron micrographs is limited by the very complex three-dimensional organization of mitochondria as a global network (16, 31, 51), the morphometric parameters of which also vary widely on energy status (17–19, 23, 35). Accordingly, in our study, there was an important variability of mitochondrial morphology between tissues: in heart and skeletal muscle, they presented essentially with a regular quasi-crystalline organization (46), whereas in liver and kidney they looked more irregular and less compacted by tissue architecture. In brain, the mitochondria looked more like a collection of small and numerous ovoid sections. Moreover, we observed a strong heterogeneity of mitochondrial intracellular distribution within tissues, so that the number of organellar profiles varies dramatically with the localization of the section. For instance, in brain, there are few mitochondria around the nucleus, in the basal corpus, whereas they are omnipresent along the axone, reaching a centimeter range in length. Likewise, in kidney, there are more mitochondria in the tubule (microviliotuses) than in the basal corpus. In the other tissues, mitochondria present with a less heterogeneous organization. The internal organization of mitochondria, i.e., the form of the cristae, is also irregular and variable on physiological conditions (28). Accordingly, in our study, in tissue sections viewed by transmission electron microscopy, we observed important differences in the internal organization of the mitochondria. For instance, there was a variable number of cristae per organellar section, along with differences in their arrangement. The heart presented with the highest number of cristae per surface of mitochondrial section vs. the other tissues (factor of 1.3–4.8). Moreover, we observed variations in the density of the mitochondrial matrix, compared with the cytosol, with a again a high value in heart vs. that shown in the other tissues (factor of 1.4–1.9). A high number of cristae, as observed in heart, could indicate a higher mitochondrial content in respiratory chain complexes, in agreement with studies showing a preferential localization of these proteins in the cristae (11, 14). Accordingly, heart mitochondria also present with the darker matrix, which could indicate a higher state of respiration in vivo (17–19). Another method of mitochondrial quantification frequently used is the determination of mitochondrial-to-tissue protein ratio. However, in our

![Fig. 5. Principal component analysis of the main mitochondrial features in different tissues: biplot representation of the PCA results for muscle, heart, liver, and kidney. The variables correspond to the mean value of the following parameters obtained in the different tissues: CIII content, CIV content, CIII apparent (app) Km, CIV apparent Km, coenzyme Q9 (CoQ9) content, cytochrome c (cyt c) content, state 3 reduction level of CoQ9, state 3 reduction level of cytochrome c, biochemical threshold value of CIII (taken from Ref. 37), and biochemical threshold value of CIV (taken from Ref. 37). CS, citrate synthase.](http://ajpcell.physiology.org/)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Muscle</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>State 3 respiratory rate, ng atom·min⁻¹·mg⁻¹</td>
<td>184±10</td>
<td>229±21</td>
<td>149±17</td>
<td>156±21.3</td>
<td>176±11</td>
</tr>
<tr>
<td>Respiratory chain ratio</td>
<td>4.6±1.2</td>
<td>5.0±1.5</td>
<td>4.1±1.9</td>
<td>3.7±0.9</td>
<td>2.6±1.4</td>
</tr>
<tr>
<td>Reduced [CoQ9] at state 3, % of total</td>
<td>2.0±0.7</td>
<td>1.9±0.4</td>
<td>61.4±14.7</td>
<td>55.6±12.9</td>
<td>2.2±0.8</td>
</tr>
<tr>
<td>Reduced [cytochrome c] at state 3, % of total</td>
<td>63±5.1</td>
<td>69±7.6</td>
<td>43±4.5</td>
<td>37±4.1</td>
<td>39±6.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. Brackets indicate concentration.
conditions, it could not be used to compare accurately the mitochondrial content of the different tissues, given the important variability in the yield of the different methods of organelar isolation from the various tissues.

To avoid these errors, we determined the content of mitochondria on total lysates from the different tissues, by measuring 1) the citrate synthase activity and 2) the expression level of respiratory chain complexes I, II, III, and IV, as well as mtTFA. The results show that heart contains more mitochondria than muscle, liver, brain, and kidney. This is in good correlation with the expression level of mtTFA, suggesting the importance of that protein in determining the tissue OXPHOS capacity. We also observed a good correlation between citrate synthase activity and complex III or complex I content in tissues, confirming its utilization as a marker of the respiratory chain content in tissues. This also suggests that the citrate synthase expression level could be correlated with that of the respiratory chain complexes.

In the second part of our study, we questioned the possible qualitative differences in mitochondria isolated from the different tissues, regardless of their variable tissue content, as discussed above. The analysis of the relative expression level of seven proteins of the respiratory chain by Western blot indicates a higher content of complex I, II, III, IV, and V in heart- and muscle-isolated mitochondria than in liver, kidney and brain. For instance, the complex III core 2 subunit was more expressed in heart-isolated mitochondria (set as 100%) than in muscle (86 ± 17%), kidney (39 ± 9%), liver (34 ± 06%), and brain (25 ± 07%). This is also true for the other proteins analyzed, suggesting a diversity in intramitochondrial respiratory chain content between tissues. We also determined the absolute content in cytochromes b1, c1, c, c1, and a, as well as the content in CoQ9, on the isolated mitochondria. The results show that the highest absolute contents in respiratory chain complexes are in muscle and heart mitochondria, compared with liver, kidney, and brain (factor of 3). However, the molar ratio between complex II, III, and IV was conserved, with boundary values of [0.89 –1.45]:[3]:[6.5–7.1]. We chose the molar ratio between complex II, III, and IV was conserved, with boundary values of [0.89 –1.45]:[3]:[6.5–7.1]. We chose the molar content in enzyme complex. Again, we observe a higher kcat for brain than for the other tissues (factor of 1.4–2.6 for complex IV and 2.1–2.6 for complex III). The kcat values that we obtained are similar to those obtained on pure complex IV (47) and present with comparable variations between the type of tissue.

In the last part of our analysis, we looked at the differences in the integrated function of the respiratory chain between the different tissues. First, we verified the integrity of our mitochondrial preparations, as a prerequisite for the analysis of the respiratory rate. We considered the following indexes: state 3 value of respiration and RCR, as RCR alone is not a good indicator, given its important variability on several parameters, even on intact mitochondria (44). The analysis of the respiratory flux values measured at state 3 revealed an apparent discrepancy. Indeed, although skeletal muscle- and heart-isolated mitochondria present with higher respiratory chain enzyme contents, along with higher Vmax values, the state 3 respiratory rate is not different. This could be explained by the fact that most of the control of mitochondrial respiration is supported, at state 3, by the pyruvate carrier (36). However, the situation must be very different in vivo as the various tissues consume different physiological energy substrates, with important preferences in their utilization (27). The steady-state analysis of mitochondrial respiration also reveals large differences between the maximal respiratory chain complexes activity and the flux value, i.e., their velocity at state 3. This indicates again that, in our conditions, the rate of mitochondrial respiration is not tightly controlled by the sole content in OXPHOS complexes, nor their maximal velocities. For instance, the maximal activity of complex IV exceeds by a factor of 15 the activity measured during state 3 respiration in heart-isolated mitochondria. This factor is equal to 12.6, 12.2, 8.1, and 4.9 in muscle, brain, liver, and kidney, respectively. For complex III, this excess capacity ranges from 1.8 to 4.2 in the different tissues. The observation that complex IV does not function at the maximal velocity during state 3 respiration led to the definition of the “excess capacity” (15). This excess could be utilized, at least in part, to accommodate the flux to an increase in energy demand, so that tissues with a high excess capacity could adapt more easily to large-scale variations in energy demand. Interestingly, our observations allow us to extend this notion of excess capacity to the intermediary substrates of the respiratory chain. Indeed, the comparison of the amount of reduced CoQ9 determined during state 3, compared with the total content,
reveals that only ~2% is reduced during steady-state 3 mitochondrial respiration in heart, muscle, and brain. Conversely, this proportion is equal to ~60% in liver and kidney, using the same substrate combination (pyruvate-malate). For cytochrome c, the fraction used at state 3 is ~65% of the total in heart and muscle, compared with 40% in liver, kidney, and brain. Therefore, our results indicate that not all the substrate is engaged (reduced) during mitochondrial respiration. This raises again the problem of a possible existence of different physical and functional pools of mitochondrial CoQ and cytochrome c, as well as their possible compartmentation. This could also be explained by the equilibrium constant or bypass reactions, such as superoxide production from the reduced cytochrome c. Hence, our work generalizes the existence of an excess of both enzyme capacities and substrates concentrations in mitochondria from different tissues. This could be of particular importance for the control of mitochondrial energy production, as well as the physiological adaptation to a sudden change in energy demand or substrate delivery. Such differences could also participate to the observed tissue specificity of mitochondrial diseases. In particular, the excess capacity could be utilized for the compensation of pathological defects in respiratory chain activity and determine in part the biochemical threshold value (37).

To conclude, we aimed to compare the different tissues, while taking into account the numerous data obtained in our study. For this, we performed a PCA that revealed three groups of tissues: 1) heart and muscle (slow type), 2) liver and kidney, and 3) brain. They present with important differences at the level of respiratory chain content, composition, activity, and flux response. Interestingly, each group represents organs of the same embryonic origin, which could suggest that OXPHOS features could be set up early in the tissue development period, or closely related to the type of tissue function. The first group, skeletal muscle and heart, presents with the highest OXPHOS capacity and a low resistance against the occurrence of respiratory chain perturbation, as illustrated by lower threshold values and high control coefficients. Conversely, the second group, liver and kidney, is characterized by a lower OXPHOS capacity and a lower sensitivity to OXPHOS defects. The third group, which contains solely the brain, is between the first and second group regarding the OXPHOS capacity and flux response. More generally, our study demonstrates that oxidative phosphorylation capacity is highly variable and diverse in tissues. It appears to be determined by different combinations of the mitochondrial content, the amount of respiratory chain complexes, and their intrinsic activity (see Fig. 6). This underlines the complexity of the regulation of mitochondrial energy production, which can occur at the level of 1) organellar biogenesis, 2) mitochondrial or nuclear DNA transcription, and 3) enzyme regulation. Different signaling pathways have been described for the modulation of each of those determinants, but their overall control and orchestration are presently not understood. The present observations of a different dosage of mitochondrial content, respiratory chain expression levels, and enzyme complex intrinsic activity in the different tissues also suggest that mitochondrial metabolism is tailored to meet organ-specific features. For instance, a larger mitochondrial compartment per surface of tissue could allow better access to oxygen more efficiently and could allow delivery of ATP throughout the cytosolic compartment. The mitochondrial content and architecture could also be related to some tissue-specific adaptive needs, such as mechanosensing in skeletal muscle (33). Moreover, the different options of a higher content in enzyme complexes (i.e., heart) than more active ones (i.e., brain) could determine differences in tissue sensitivity to changes in energy demand, controlled by the concentration of available substrates. Accordingly, determination of $K_m$ for

![Fig. 6. Representation of the respiratory chain in different tissues: For each tissue, we show the respiratory chain system, taking into account the content in mitochondria, the amount of respiratory chain complexes II, III, and IV, their intrinsic activity, and the total content in intermediate substrate. We also figured the excess capacity of these enzyme complexes and the excess concentration of reducible substrate. The amount of mitochondria was represented in the background by a yellow rectangle, the surface of which is proportional to their tissue amounts (as determined by the CS activity). The content in enzyme complexes (gray squares) and substrates (blue squares) is proportional to the catalytic constant ($k_{cat}$) for the enzymes. The for the substrates, we figured the excess that is not utilized at state 3 (light blue) compared with the content utilized at state 3 (dark blue). To illustrate the diversity in the determinism of the OXPHOS capacity in tissues, we can compare the heart and the brain, looking more closely at CIII. The heart contains more mitochondria than the brain (factor of 3) and more complex III (factor of 9), but a less active enzyme (factor of 0.4). Thus the overall capacity of CIII in heart is ~11 times higher (per mg of tissue proteins) than in brain. However, in both tissues, the excess capacity of CIII is comparable (35% in heart and 25% in brain).](image-url)
cytochrome c for complex IV revealed a twice lower value in brain (11.3 ± 0.9 μM) than in heart (28.2 ± 2.7 μM). Hence, the role of energy demand and the type of substrate utilized for energy production could play an important role in defining the mitochondrial compositional features and steady-state functioning characteristics. Accordingly, our group (35) demonstrated previously on living human cells that a change in the type of energy substrate was accompanied by a rapid modulation of the expression of mitochondrial proteins, as well as organellar structural parameters. Our group (7) also showed that physical exercise could modify the content in mitochondria and the amount of respiratory chain complexes in skeletal muscle. Thus, to further detail the diversity in the OXPHOS system between tissues, it will be necessary to consider the numerous regulations that can occur at the level of the respiratory chain or at the level of the mitochondrial network morphofunctional characteristics. For instance, recent evidence suggests that changes in mitochondrial activity can trigger morphological adaptations of the mitochondrial network (23, 35), and clinical studies further indicate that molecular defects affecting its dynamics lead to pathology (38). This suggests that a link between energy status and organellar network configuration must exist. Indeed, mitochondrial overall configuration is controlled by a balance between fusion and fission events, mediated by specific proteins (5, 25) that could participate in the modulation of energy production. Accordingly, the different tissues present with variable expression levels of the fusion and fission proteins, possibly related to specific energy needs. Also, the in-depth study of the other mitochondrial functions related to the OXPHOS system will contribute to understand what regulates mitochondrial structural and functional features in tissues. Our results have implications for the understanding of mitochondrial physiopathology. Indeed, the clinical manifestation of respiratory chain disorders typically present with a tissue specific, characterized by the fact that a given pathological mutation can affect the different tissues to a variable extent (37). Our data suggest that mutations affecting either the amount of active complexes (i.e., Surf 1) or their catalytic activity (i.e., point mutations) will lead to different degrees of energy defect according to the tissue considered.

ACKNOWLEDGMENTS

The authors thank INSERM, Université Victor Segalen Bordeaux 2, Association Française contre les Myopathies (AFM), Association contre les Maladies Mitochondriales (Ammi), Ligue contre le Cancer, and Région Aquitaine for financial support. We are also grateful to Jean-Pierre Mazat for discussion. We also thank Devin Oglesbee for text corrections.

REFERENCES


