Respiratory Chain Enzyme Activities in Lymphocytes from Untreated Patients with Parkinson Disease

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Respiratory chain enzyme activities were studied in lymphocytes from patients with Parkinson disease (PD) (n = 16) and age-matched control subjects (n = 15). The patients had received no therapy before the study was conducted. Complex I, III, and IV activities were significantly lower (P<0.05) in patients than in control subjects. A complex I defect was found in one patient, whereas complex IV was defective in another. Two patients had combined defects of both complexes. The use of lymphocytes for investigating the respiratory chain enzymes provides an easy, noninvasive method to assess mitochondrial function in patients with PD. Furthermore, our study supports the hypothesis that a biochemical defect in the respiratory chain may be involved in the pathogenesis of PD.

Indexing Terms: mitochondria • oxidative phosphorylation

Parkinson disease (PD), a common adult-onset neurodegenerative disorder of unknown etiology, is associated with neuronal death in the substantia nigra. Research into the etiology of PD has focused mainly on toxins that inhibit mitochondrial respiration. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces a Parkinson-like syndrome in both human and nonhuman primates (1). MPTP is metabolized to 1-methyl-4-phenylpyridinium (MPP+) by monoamine oxidase B (EC 1.4.3.4) in glia (2); MPP+ is taken up into dopaminergic neurons by the dopamine reuptake pump (3). The finding that MPP+ inhibits mitochondrial complex I suggests that an energy failure resulting from inhibition of the mitochondrial respiratory chain is the most likely mechanism of neuronal death in MPTP-induced parkinsonism.

The mitochondrial respiratory chain consists of five enzymatic complexes located within the inner mitochondrial membrane. Four enzymes (complexes I–IV) transport electrons from NADH or succinate to oxygen, and these complexes pump protons out of the mitochondria to form an electrochemical gradient. The fifth enzyme (complex V) uses that electrochemical gradient to synthesize ATP from ADP (5).

Decreased complex I activity has been reported in platelets (6), substantia nigra (7, 8), and skeletal muscle (9, 10) from patients with PD. Likewise, there have been reports of combined defects of the respiratory chain complexes (9–11).

In the present study we assessed respiratory chain enzymes in lymphocytes from patients with PD. Because the isolation of platelets requires greater blood volumes than does the isolation of lymphocytes, analysis of the latter could represent an easier, less invasive, and less time-consuming method for assessing the role of oxidative metabolism in the pathogenesis of PD.

Material and Methods

Patient and Control Subjects

Age-matched control subjects consisted of six diseased individuals with neurological disorders other than mitochondrial myopathies and nine apparently normal individuals who were free from neurological disorders. Patients with PD (n = 16) were ambulatory and were selected on the basis of classic PD manifestations (12). Tremor, rigidity, bradykinesia, and gait impairment were present in all of them. Dementia was not a prominent feature in any patient. The patients had received no L-dopa or any other medication before the study was conducted.

Isolation of Lymphocytes

Lymphocytes were isolated from patients and control subjects by a standard method (Lymphoprep; Nycomed, Oslo, Norway). Briefly, 10 mL of heparinized blood was layered over Lymphoprep in siliconized glass centrifuge tubes. The tubes were centrifuged (Omnifuge 2.0 RS Heraeus Sepatech) for 15 min at 800 × g at room temperature. The lymphocyte layer was isolated and transferred to clear siliconized tubes and again centrifuged for 15 min at 1000 × g to obtain a pellet. Contaminating erythrocytes were removed by hypotonic lysis with the addition of 1 mL of water. The cells were resuspended in 5 mL of 9.6 g/L NaCl and centrifuged for 15 min at 1000 × g. The supernate was decanted. This procedure was repeated as needed until the pellet was free of erythrocytes. Usually one further wash was sufficient.

Respiratory Chain Enzyme Assay

Fresh lymphocyte pellets were homogenized by sonication in 20 mmol/L potassium phosphate buffer (pH 7.5) for 15 s (three bursts of 5 s each) at 30 W on ice. The homogenate, containing 2–5 g/L protein, was kept on ice and used for assay the same day. Homogenates stored at 4°C for more than 1 day or frozen at −20°C for any period of time lose respiratory chain enzyme activities at these protein concentrations.
Succinate cytochrome c reductase (EC 1.3.2.2, complexes II and III) (13) was measured by monitoring the reduction of cytochrome c at 550 nm in the presence of succinate and enzyme (40–50 μL of lymphocyte homogenate).

Assays for rotenone-sensitive NADH cytochrome c reductase (EC 1.6.2.1, complexes I and III) (13) were measured by monitoring the reduction of cytochrome c at 550 nm in the presence of NADH, rotenone, and the enzyme (40–50 μL of lymphocyte homogenate). The rotenone-resistant activity was subtracted from the total NADH cytochrome c reductase activity to yield the activity of the rotenone-sensitive cytochrome c reductase.

Citrate synthase (EC 4.1.3.7) (14) was measured by monitoring the change in absorbance at 412 nm caused by the reaction of 5,5'-dithiobis(2-nitrobenzoic acid) with the free coenzyme A formed by the condensation of acetyl-CoA with oxalacetate in the presence of the enzyme (5–10 μL of lymphocyte homogenate).

Succinate dehydrogenase (EC 1.3.99.1, complex II) (15) was measured at 600 nm by monitoring the oxidation of succinate in the presence of the artificial electron acceptor, 2,6-dichlorophenol-indophenol, and the enzyme (40–60 μL of lymphocyte homogenate).

Cytochrome c oxidase (EC 1.9.3.1, complex IV) was also determined spectrophotometrically by the decrease in absorbance at 550 nm of reduced cytochrome c in presence of the enzyme (30–60 μL of lymphocyte homogenate) (16). Reduced cytochrome c was freshly prepared before each experiment by adding a few grains of sodium borohydride to a 10 g/L solution of the pigment in 10 mmol/L potassium phosphate buffer (pH 7.0). Addition of 0.1 mol/L HCl stabilized the reduced cytochrome c, and the excess borohydride was removed by centrifugation at 12,000 × g for 4 min. Enzyme assays were performed in triplicate with a DU-68 spectrophotometer (Beckman Instruments, Brea, CA). Incubation temperatures were 30 °C for complexes I and III, II and III, and II and citrate synthase, and 38 °C for complex IV.

Protein content was measured by the method of Lowry et al. (17) with the use of human serum albumin as a standard.

Activities or respiratory chain complexes were reported as nanomoles of substrate per minute per milligram of protein (U/g). The ratio of complexes I and III to II and III was used to estimate defects of complex I (18). Abnormal amounts of activity were defined as being values below the 5% confidence interval of the control group.

All chemicals were from Boehringer Mannheim (Mannheim, Germany) and Sigma Chemicals (St. Louis, MO).

Statistical Analysis of Data

Results were expressed as means ± SD; differences between means were evaluated by a two-tailed Student's t-test for unpaired samples.

Results

The age ranges of patients with PD (52.2 ± 10.5 years; mean ± SD) and control subjects (53.7 ± 12.2) were similar. The influence of aging on the results of the enzyme activities was ruled out. Repeated assays (n = 10) of the same lymphocyte homogenates (control subjects and patients) gave highly reproducible results (CV 3–5%), both within the same day (single samples) and between days (different cell preparations from the same subject). Table 1 summarizes the data for all five enzyme activities in lymphocytes from patients and control subjects. The activities of rotenone-sensitive NADH cytochrome c reductase (complexes I and III) and cytochrome c oxidase (complex IV), as well as the ratio of I and III to II and III complexes, were significantly lower in patients than in control subjects (P <0.05). However, the rest of the enzyme activities did not differ significantly between both groups. Respiratory chain was defective in four patients. One of them had a deficiency of complex I. A defect of complex IV was found in another one, and combined defects of complexes I and IV were found in the other two patients.

Discussion

Our results show that the activities of the respiratory chain complexes I and IV were significantly reduced in lymphocytes from patients with PD compared with age-matched control subjects. Moreover, a defect in complex I was found in one patient, complex IV was defective in another, and two patients showed combined defects of both complexes. In addition, the specific activity of citrate synthase was similar in patients and control subjects, suggesting that equivalent amounts of mitochondria were present in lymphocytes of both groups. Parker et al. (6) found a decrease of complex I activity (45% of mean in control subjects) in platelets of PD patients, indicating that the complex I defect was present in an apparently unaffected tissue. Similarly, Shoffner et al. (10) and Bindoff et al. (9), using skeletal muscle from patients with PD, reported low activities in complexes I and IV and variable findings in complexes II.

Table 1. Respiratory Chain Enzyme Activities in Lymphocytes from Patients with PD and Control Subjects

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control subjects (n = 10)</th>
<th>Patients (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase (complex II)</td>
<td>2.18 ± 0.42</td>
<td>2.35 ± 0.40</td>
</tr>
<tr>
<td>Succinate cytochrome c reductase (complexes I and III)</td>
<td>5.30 ± 0.85</td>
<td>4.0 ± 0.5*</td>
</tr>
<tr>
<td>Succinate cytochrome c reductase (complexes II and III)</td>
<td>2.80 ± 0.55</td>
<td>2.45 ± 0.42</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>53 ± 5</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>Cytochrome c oxidase (complex IV)</td>
<td>11.90 ± 2.55</td>
<td>6.50 ± 1.43*</td>
</tr>
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</table>

* Significantly different from control subjects (P <0.05, unpaired t-test).
and III. Schapira et al. (7) and Mizuno et al. (8) found a marked reduction in complex I activity in substantia nigra obtained from PD patients postmortem. Differences in cell turnover and neuronal dropout or postmortem artifacts might account for the variable respiratory chain defects found. In contrast with the studies reported previously, our patients had received no therapy before the analysis was performed. Therefore, the possible influence of L-dopa or any other drug in our results was ruled out.

The mechanisms causing these defects, and whether the defects are primary or secondary, remain unknown. The lack of a family history of PD in most cases of this disease seems to rule out a primary genetic defect involving a nuclear gene. The combined defects of the respiratory chain found in two of our patients could be consistent with mitochondrial DNA mutations (19). It is possible that respiratory chain defects are secondary to the effects of environmental toxins that inhibit the respiratory chain and lead to increased free radical production (1, 4, 20). Such inhibition could ultimately lead to nonselective damage of respiratory chain components (21, 22) and even to damage of mitochondrial DNA (23).

Functional defects of the respiratory chain were reported by others in platelets, muscle, and brain of PD patients. Here, we report that such defects also occur in lymphocytes of patients with PD. These results, together with the MPTP hypothesis, support the view that a biochemical defect in the respiratory chain may be involved in the pathogenesis of PD. Our results also indicate that lymphocyte analysis provides an easy, noninvasive method for investigating respiratory chain enzymes and assessing mitochondrial function in patients with PD.

In contrast to our results, Yoshino et al. (24) recently showed normal complex I and complex IV activities and a slightly decreased activity of complex II in lymphocytes from PD patients (24). In addition, Mann et al. (25) reported normal platelet and skeletal muscle complex I activities in PD. Thus, further work is needed at the molecular level to determine reasons for these discrepancies.

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