Mitochondrial Respiration and ATP Production Are Significantly Impaired in Striatal Cells Expressing Mutant Huntingtin*

Received for publication, April 29, 2005, and in revised form, June 17, 2005
Published, JBC Papers in Press, June 27, 2005, DOI 10.1074/jbc.M504749200

Tamara Milakovic and Gail V. W. Johnson‡
From the Department of Psychiatry, University of Alabama at Birmingham, Birmingham, Alabama 35294-0017

There is significant evidence that energy production impairment and mitochondrial dysfunction play a role in the pathogenesis of Huntington disease. Nonetheless, the specific mitochondrial defects due to the presence of mutant huntingtin have not been fully elucidated. To determine the effects of mutant huntingtin on mitochondrial energy production, a thorough analysis of respiration, ATP production, and functioning of the respiratory complexes was carried out in clonal striatal cells established from Hdh$^{Q7}$ (wild-type) and Hdh$^{Q111}$ (mutant huntingtin knock-in) mouse embryos. Mitochondrial respiration and ATP production were significantly reduced in the mutant striatal cells compared with the wild-type cells when either glutamate/malate or succinate was used as the substrate. However, mitochondrial respiration was similar in the two cell lines when the artificial electron donor TMPD/ascorbate, which feeds into complex IV, was used as the substrate. The attenuation of mitochondrial respiration and ATP production when either glutamate/malate or succinate was used as the substrate was not due to impairment of the respiratory complexes, because their activities were equivalent in both cell lines. Intriguingly, in the striatum of presymptomatic and pathological grade 1 Huntington disease cases there is also no impairment of mitochondrial complexes I–IV (Guidetti, P., Charles, V., Chen, E. Y., Reddy, P. H., Kordower, J. H., Whetsell, W. O., Jr., Schwarz, R., and Tagle, D. A. (2001) Exp. Neurol. 169, 340–350). To our knowledge, this is the first comprehensive analysis of the effects of physiological levels of mutant huntingtin on mitochondrial respiratory function within an appropriate cellular context. These findings demonstrate that the presence of mutant huntingtin impairs mitochondrial ATP production through one or more mechanisms that do not directly affect the function of the respiration complexes.

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by a pathological expansion of CAG repeats in the gene encoding for a protein called huntingtin. Disease symptomatology and progression are due to massive neuronal dysfunction and death in the striatum, and in the cerebral cortex later in the disease. Even though the identification of the gene that contains the disease-causing mutation (1) was a fundamental discovery, it is still unclear how the mutant huntingtin causes pathogenesis, which is of essential importance for the development of successful treatment strategies.

There is significant evidence that energy production is impaired in HD. Positron emission tomography scans of the striatum showed impaired glucose metabolism early in the disease (2–4). Indeed, striatal hypometabolism has been observed in presymptomatic HD cases (5). Further, NMR experiments in symptomatic HD patients found increased lactate levels in the cortex and basal ganglia (6), suggesting the possibility that glycolysis is up-regulated to compensate for impaired ATP production by the oxidative phosphorylation pathway. Several groups have also reported deficits in respiratory complexes II, III, and IV in postmortem brain tissue from HD cases in which neuronal loss was evident (7–9). Additionally, chronic treatment of rodents or nonhuman primates with 3-nitropropionic acid (3-NP) (an irreversible complex II inhibitor) caused selective damage of the striatum resulting in pathology and symptomatology that resembled HD (10, 11).

HD cell models have been extremely useful in delineating pathogenic processes caused by mutant huntingtin (12–15). Cell lines derived from the striatum of HD knock-in mice are particularly useful tools for examining the molecular mechanisms of HD pathogenesis. Trettel and colleagues (14) have established striatal cell lines from HD knock in (Hdh$^{Q111/Q111}$) and wild-type (Hdh$^{Q7/Q7}$) mice. The STHdh$^{Q111/Q111}$ cell line expresses mutant huntingtin at endogenous levels, and therefore is a genetically accurate cell model of HD. As the striatum is the most affected region in HD, the striatal origin of this cell model makes it optimal for HD studies. Previous studies using the STHdh$^{Q111/Q111}$ and STHdh$^{Q7/Q7}$ cell lines have provided indirect evidence that there is mitochondrial dysfunction in the mutant huntingtin-expressing striatal cells. Reduced cAMP levels have been reported in the STHdh$^{Q111/Q111}$ cells when compared with the STHdh$^{Q7/Q7}$ cells (16). Further, the mutant cells were more sensitive than the wild-type cells to 3-NP-induced toxicity, however both cell lines were equally sensitive to rotenone (a complex I inhibitor)-induced cell death (17). Further, treatment of the STHdh$^{Q7/Q7}$ cells with 3-NP resulted in the initiation of apoptotic cell death, whereas cells expressing mutant huntingtin died by a nonapoptotic process when treated with 3-NP (17). Overall these and other studies clearly demonstrate that the STHdh$^{Q111/Q111}$ and STHdh$^{Q7/Q7}$ cell lines are appropriate models for studying HD pathogenesis.

In this study, we used these clonal striatal cell lines expressing mutant (STHdh$^{Q111/Q111}$) or wild-type (STHdh$^{Q7/Q7}$) huntingtin (14) to examine the effects of mutant huntingtin on...
energy production and mitochondrial respiration. A thorough analysis of O2 consumption, ATP production, and respiratory complex activities was carried out to delineate the effects of mutant huntingtin on mitochondrial bioenergetic processes. Further, we performed threshold analyses to compare handling of inhibition of different respiratory complexes in the two cell lines. We found that mutant cells (STHdhQ111/Q111) have significantly reduced O2 consumption and ATP production rates compared with wild-type cells (STHdhQ7/Q7). However, there were no significant differences in the respiratory complex activities. Also, the handling of mitochondrial inhibitors was similar in the two cell lines. Our findings clearly suggest that mutant huntingtin compromises oxidative phosphorylation and mitochondrial energy production, but this is not through the impairment of respiratory complexes. This is in agreement with a previous study that demonstrated in presymptomatic or grade I HD brain there are no changes in the activities of complexes I–IV in the striatum (18).

Materials and Methods

Materials—All chemicals were from Sigma-Aldrich unless otherwise noted.
Permeabilized cells were used to measure mitochondrial enzyme activities as described under "Materials and Methods." All results are presented as mean ± S.E. of at least four independent experiments. The activity of citrate synthase was lower in the mutant cell line when normalized to the number of cells but not when normalized to the protein content. Respiratory complexes enzyme activities were normalized to the units of citrate synthase activity (unit = 1 μmol/min/ml) and were not different between the two cell lines. Data were analyzed using Student’s t test.

**Cell Culture**—Conditionally immortalized striatal neuronal progenitor cell lines, which were obtained as a gift from Dr. M. MacDonald, were used in this study. The STTHdh^{Q111/Q111} cell line expressing endogenous normal huntingtin and the homozygous mutant STTHdh^{Q441/Q441} cell line expressing mutant huntingtin with 111 glutamines were prepared from wild-type mice and homozygous Hdh^{Q441/M} mice, respectively. Both cell lines have been described previously (14). Cells were cultured in Dulbecco’s modified Eagle’s medium (Mediatech Inc.) supplemented with 5% fetal bovine serum (HyClone) and 4% bovine serum albumin, and 150 mM Tris acetate, pH 7.5.

**Cell Permeabilization**—Cells were permeabilized as described previously (19). Briefly, cells were trypsinized, resuspended in media A (20 mM HEPES, 10 mM MgCl₂, 250 mM sucrose, pH 7.3), and counted followed by permeabilization of aliquots of 1 × 10⁷ cells. An aliquot was resuspended in media A containing digitonin at a final concentration of 0.015% (which was found to be the optimal concentration in preliminary studies) and mixed for 1 min. After centrifugation, the digitonin-containing media A was removed, and permeabilized cells were washed with a large volume of media A to remove any remaining digitonin. For these studies commercially available digitonin was purified as described previously (20) and stored at a 10% stock in Me₃SO at −20 °C.

**Polarographic Measurements**—Respiration rates in the permeabilized cells were measured using an oxgraph (Hansatech Instruments). In this instrument a Clark-type electrode is placed at the bottom of the water-jacketed respiratory chamber. During measurements, the chamber was thermostatted at 37 °C and sealed with a platinum. An electro-magnetic stirrer bar was used to mix the contents of the chamber. Permeabilized cells were resuspended in a respiration buffer (media A, 2 mM potassium phosphate (KH₂PO₄·K₂HPO₄, 1:1.78), pH 7.1, 1% bovine serum albumin, and 150 mM Tris acetate, pH 7.5) at 4 °C, and the pellet was diluted in cold water (2–4 × 10⁷ cells/ml). After osmotic shock treatment, samples were centrifuged (16,000 × g for 5 min at 4 °C), and the pellet was diluted in cold water (2–4 × 10⁷ cells/ml). After osmotic shock treatment, samples were centrifuged again, and the pellet was dissolved in media A and sonicated on ice (3 × 20 s) prior to use in the assay.

**Complex IV Activity**—Complex IV activity was determined polarographically as described previously (26). Permeabilized cells were incubated in the reaction buffer (50 mM MOPS, 0.3% Tween 80, 2 mM CCCP, pH 7.5) in the respiratory chamber for 2 min without or with KCN (1–500 μM). Subsequently, ascorbate (3.5 mM) and TMPD (0.35 mM) were added, and O₂ consumption was observed.

**Threshold Curves and Determination of Thresholds and Spare Capacities**—The threshold curves were constructed from the raw data as described previously (27). To determine thresholds and spare capacities, we followed the method described in Refs. 28 and 29. As described previously, the least-square regression lines beyond the inflection point in each threshold curve were extrapolated to zero complex inhibition. The intersection of these lines with the ordinate axis was used to calculate the complex II activity for the substrate by subtracting 100% from the value at which respiration was unaffected by the complex inhibition. Threshold values were determined from the regression line equations, as the percentage of complex inhibition at which respiration was unaffected (100%). Spare capacity and threshold value terms are as described previously (29). In several threshold curves the inflection point was not evident; therefore, data points obtained with every inhibitor concentration were used for linear regression analysis.

**Analysis of ATP Levels and ATP Production**—ATP levels and ATP production were determined using a luciferin/luciferase assay as described previously (30) utilizing a Turner Designs TD 20/20 (Turner Designs) luminometer.

**To determine ATP levels, cells were collected in lysis buffer (100 mM Tris, 4 mM EDTA, pH 7.75) and boiled for 2 min. Samples were then centrifuged (1000 × g for 1 min), and the supernatants were used in the luciferin/luciferase assay. ATP levels were normalized to protein content in the samples. Protein concentrations were determined from cell lysates before boiling using the bicinchoninic acid assay (Pierce) and used to calculate protein content in the amount of samples used for the ATP assay. Reaction buffer for this assay contained 70 μM luciferin, 0.2 mg/ml luciferase, 10 mM potassium acetate, 0.065% bovine serum albumin, and 150 mM Tris acetate, pH 7.5.**

**To determine mitochondrial ATP production, cells were permeabilized and resuspended in media A. The reaction buffer for this assay was respiration buffer supplemented with an adenylate kinase inhibitor (diadenosine/pentaphosphate, 0.15 mM), respiratory substrates (glutamate (10 mM) plus malate (10 mM)), or succinate (5 mM) plus rotenone (10 μM), luciferin (70 μM), and luciferase (0.05 μg/ml).**
the initial luminescence reading, ADP (200 μM) was added to the reaction, and the increase in luminescence was monitored for 2 min in the kinetic mode.

**Western Blot Analysis**—Western blot analysis was performed according to general protocols. For this analysis, permeabilized cells samples were sonicated, and protein concentrations were determined by the bicinchoninic acid assay. Antibodies used were: complex II, a 70-kDa subunit monoclonal antibody (Molecular Probes, 5 μg of protein, 1:10,000), complex II, a 30-kDa subunit monoclonal antibody (Molecular Probes, 20 μg of protein, 1:1,000), and voltage-dependent anion channel monoclonal antibody (Calbiochem) (20 μg of protein, 1:2,000).

**Statistical Analysis**—Results were analyzed using Student’s t test or paired t test as indicated. ATP production data were analyzed using Chauvenet’s Criterion followed by Student’s t test.

**RESULTS**

**Respiratory Properties of STHdhQ7/Q7 (Wild-type) and STHdhQ111/Q111 (Mutant) Cells**—STHdhQ7/Q7 (wild-type) and STHdhQ111/Q111 (mutant) cells were permeabilized with digitonin (0.015%) and used for oxygen consumption measurements. Digitonin binds to cholesterol in the eukaryotic plasma membrane and creates pores through which soluble components of the cytosol can be released and the respiratory substrates, cofactors, and inhibitors used can easily be introduced into the cell. This approach is optimal for the maintenance of coupled, metabolically active mitochondria in isolated or cultured cells (31–34). Although cholesterol is also present in the outer mitochondrial membrane, it is at substantially lower levels than in the plasma membrane (35), thus making it possible to selectively permeabilize the plasma membrane if low enough concentrations of digitonin are used. However, if the concentration of digitonin is not high enough, the cells will be inadequately permeabilized. Therefore in preliminary studies respiration was measured in cells permeabilized with 0.005–0.02% digitonin to determine the optimal concentration for the striatal cells. These studies revealed that respiration was optimal when 0.015% digitonin was used.

State 4 (in the absence of ADP) and state 3 (in the presence of ADP) respiration rates were determined and compared between the two cell lines (Fig. 1A). All rates were expressed per unit of citrate synthase activity to normalize for the mitochondrial content. State 3 respiration was significantly reduced in the mutant cells compared with the wild-type when either succinate (with rotenone) (feeds electrons into complex II) or glutamate plus malate (feed electrons into complex I) were used as the substrates. However, when ascorbate plus TMPD (an artificial electron donor that feeds electrons into complex IV) was used as the respiratory substrate, there were no differences between the cell lines. State 4 respiration rates in the two cell lines were not significantly different when any of the respiratory substrates were used. To observe maximal respiratory capacity of the two cell lines, respiration rates were measured after the addition of the uncoupler FCCP. The uncoupled respiration rate was significantly lower in the mutant cells compared with the wild-type cells (Fig. 1A).

State 4 and State 3 respiration rates were used to calculate Respiratory Control Ratios (RCRs). RCRs were significantly reduced in the mutant cells compared with the wild-type cells when either succinate (with rotenone) or glutamate plus malate were used as the substrates (Fig. 1B). This reduction is
the result of reduced state 3 respiration in the mutant cells as shown in Fig. 1A. When ascorbate plus TMPD was used as the substrate, the RCRs were not different between the two cell lines (Fig. 1B).

ATP Levels and Mitochondrial ATP Production in Permeabilized STHdhQ7Q7 (Wild-type) and STHdhQ111Q111 (Mutant) Cells—To further analyze the energetic status of the STHdhQ7Q7 (wild-type) and STHdhQ111Q111 (mutant) cells, especially of their mitochondria, we measured total ATP levels and mitochondrial ATP production. To determine ATP levels, total cell lysates were prepared and ATP measured as described under “Materials and Methods.” These data demonstrate that there is no significant difference in the total ATP levels between the two cell lines (Fig. 2A). In contrast, measurement of mitochondrial ATP production revealed that the rate of mitochondrial ATP production was significantly lower in the mutant cells compared with the wild-type cells when either succinate (with rotenone) or glutamate plus malate were used as the respiratory substrates (Fig. 2B). This is consistent with the findings from the oxygen consumption experiments (Fig. 1A) where state 3 rates (with succinate plus rotenone or glutamate plus malate) were significantly lower in the mutant cell line compared with the wild-type.

Mitochondrial Enzyme Activities in STHdhQ7Q7 (Wild-type) and STHdhQ111Q111 (Mutant) Cells—Mitochondrial enzyme activities were measured in permeabilized STHdhQ7Q7 (wild-type) and STHdhQ111Q111 (mutant) cells. The activity of citrate synthase, a tricarboxylic acid cycle enzyme, was measured and normalized to the number of cells or protein content. Citrate synthase activity was decreased in the mutant cells (25%) when normalized to the number of cells (Table I). This corresponded to the observation that mutant cells appear smaller in size and have ~25% less protein content than the wild-type cells (data not shown). When normalized to the protein content, citrate synthase activity was not different between the two cell lines (Table I). This suggests similar mitochondrial load per protein mass in the two cell lines.

To understand why the respiration rates are reduced in the mutant cells, the activity of each of the four respiratory complexes was measured and normalized to units of citrate synthase activity (Table I). No significant differences were observed between wild-type and mutant cells in the activity of any of the four respiratory complexes.

Inhibitor Titrations and Threshold Analyses in Permeabilized STHdhQ7Q7 (Wild-type) and STHdhQ111Q111 (Mutant) Cells—To further analyze mitochondrial respiration in the two cell lines the sensitivity of respiration and complex activities to mitochondrial inhibitors was measured and respiratory thresholds analyses were carried out. Complex I was inhibited with increasing concentrations of rotenone, and respiration rates and complex I activities were measured. Rotenone caused a dose-dependent decrease in both respiration and complex I activity (Fig. 3, A and B). When respiration rates (expressed as a percentage of respiration rate with no inhibitor) were plotted against percent inhibition of complex I activity, no significant threshold was observed (Fig. 3C). The threshold curve was further analyzed using the least squares linear regression method. Also, the calculation of thresholds and spare capacities were carried out as described under “Materials and Methods.” As previously described (29, 36), threshold values represent the extent to which complex activity can be inhibited without affecting respiration rate. Spare capacity represents the percentage of the endogenous respiration rate that could be achieved in addition to the measured endogenous respiration rate (taken as 100%) if the complex was fully utilized for respiration and respiration rate is therefore linearly proportional to the complex activity. Hence, spare capacity is a measure of the complex reserve. Both spare capacity and threshold values were low for complex I as shown in Table II. Further, respiration and complex I activity were equally sensitive to rotenone in STHdhQ7Q7 (wild-type) and STHdhQ111Q111 (mutant) cells.

Complex II was inhibited with increasing concentrations of malonate and state 3 respiration rates (Fig. 4A), and complex II activities (Fig. 4B) were measured. Malonate was used as the complex II inhibitor for our studies because 3-NP takes ~20 min to inhibit complex II (37), which would make our respiration measurements more difficult and less reliable. Respiration rates (expressed as percentage of respiration rate with no inhibitor) were plotted against percent inhibition of complex II activity (Fig. 4C), and the least squares linear regression was applied for analysis of respiratory thresholds. As shown in Table II, the spare capacity and threshold values for complex II were low to moderate in the striatal cultured cells, however in both cases the values were greater in the wild-type when compared with the mutant cells (23–25 for the wild-type cells and ~6 for the mutant cells). However, when respiration and complex II activity titration curves were analyzed (Fig. 4A and B), there was only a slight trend toward the mutant cells being more sensitive than the wild type cells, with a statistical difference occurring at only 100 μM malonate in the respiration curve (Fig. 4A). To further examine the differences between wild-type and mutant cells with regards to complex II threshold, we also measured the sensitivity of uncoupled respiration rates to malonate in the two cell lines. Threshold curves (Fig. 4D), spare capacities, and threshold values (Table II) were obtained when uncoupled respiration was measured, and no significant differences between wild-type and mutant cells was observed. These data indicate that respiration rate and complex II activity are not differentially sensitive to malonate in STHdhQ7Q7 (wild-type) and STHdhQ111Q111 (mutant) cells. In addition we examined expression levels of complex II catalytic subunits (30 and 70 kDa) in the STHdhQ7Q7 (wild-type) and STHdhQ111Q111 (mutant) cells. In accordance with the complex II activity data (Table I), expression levels were similar in the two cell lines (Fig. 4E).

Complex III was inhibited with increasing concentrations of antimycin, and respiration rates and complex III activities were measured. Threshold curves were constructed by plotting respiration rates (percentage of respiration rate with no inhibitor) against percentages of complex III inhibition, and spare capacities and threshold values were determined. Respiration (Fig. 5A) and complex III activity (Fig. 5B) were equally sensitive to antimycin in the both cell lines. A low threshold for both cell lines was observed as evident in Fig. 5C and Table II, similar to what was observed for complex I.

Complex IV was inhibited with increasing concentrations of KCN, and respiration rates and complex IV activities were measured. Respiration in wild-type cells showed a trend toward being more sensitive to KCN inhibition compared with
FIG. 4. Analysis of complex II threshold profiles (by malonate titrations) and catalytic subunits expression levels in permeabilized STHdh<sup>Q7/Q7</sup> (wild-type) and STHdh<sup>Q111/Q111</sup> (mutant) cells. A, malonate titration of respiration. Permeabilized cells were incubated with the indicated concentrations of malonate and state 3 respiration rates were determined polarographically as described under “Materials and Methods.” Measured rates were expressed as a percentage of the control rate (no inhibitor present). B, malonate titration of complex II activity. Complex II activity was determined spectrophotometrically as described under “Materials and Methods,” and activities obtained after incubation with each malonate concentration were expressed as a percentage of the control activity (no inhibitor present). C, threshold curves for complex II. Data from panels A and B were used to construct threshold curves. Linear regression was performed for data points at all inhibitor concentrations. R<sup>2</sup> values and regression equations are shown for both wild-type (w.t.) and mutant (m.) cells. Respiration and complex II activity from both cell lines were equally sensitive to inhibition by malonate. All data are means ± S.E. of at least four independent experiments. D, malonate titration of uncoupled respiration and obtained threshold curves. Permeabilized cells were incubated with FCCP, succinate, rotenone, and increasing concentrations of malonate and uncoupled respiration rates were determined. Data from panels B and D (left) were used to construct threshold curves (D, right). Threshold curves were very similar to the curves obtained with state 3 respiration rates. E, expression levels of complex II catalytic subunits (30 and 70 kDa) in STHdh<sup>Q7/Q7</sup> (wild-type) and STHdh<sup>Q111/Q111</sup> (mutant) cells. Cell lysates were blotted for 30- and 70-kDa complex II subunits and voltage-dependent anion channel (VDAC). Representative immunoblots are shown in E (left). Densitometric analysis was used to quantify complex II subunits expression levels normalized to voltage-dependent anion channel from at least five different preparations (right). No significant differences were observed in the expression of either of the complex II catalytic subunits.
the mutant cells, with statistically significant differences occurring at two KCN concentrations (Fig. 6A). However, complex IV activity was equally sensitive to KCN in both cell lines (Fig. 6B). Respiration rates (expressed as a percentage of respiration rate with no inhibitor) were plotted against percentages of inhibition of complex IV activity to obtain threshold values (39.53 in wild-type and 53.82 in mutant) and spare capacities (71.66 in wild-type and 138.32 in mutant) in both cell lines (Table II). These data indicate that the threshold for complex IV is greater in mutant cells compared with wild-type cells.

**DISCUSSION**

To our knowledge this study represents the first complete analyses of how mutant huntingtin when expressed at physiological levels impacts mitochondrial respiratory function. In our study, we demonstrate that striatal cells derived from a precise genetic mouse model of HD show significantly diminished oxidative phosphorylation as indicated by lower respiration and mitochondrial ATP production rates compared with what was observed in wild-type cells. However, the activities of respiratory complexes and sensitivity to mitochondrial inhibitors were not different between the two cell lines. Taken together, our results suggest that mutant huntingtin compromises mitochondrial ATP production, but this effect is not through the impairment of the respiratory complexes. This is in agreement with previous findings that in presymptomatic or grade I HD brain there are no changes in the activities of complexes I–IV in the striatum (18). Also, the rather low respiratory thresholds for complexes I, II, and III in the striatal cells suggest that the striatum may be more sensitive to mitochondrial inhibitors.

Our data suggest that respiration is compromised in the striatal cells expressing endogenous levels of mutant huntingtin. Although this could not be observed during state 4 respiration, there was a significant reduction in respiration in the mutant cells at high respiration rates such as when it was coupled to ATP production (state 3) or during uncoupler-stimulated respiration. Also, there were no differences in the respiration rates (Fig. 1) or mitochondrial ATP production (data not shown) when TMPD/ascorbate was used as the substrate. Ascorbate plus TMPD substrate is an artificial electron donor that feeds electrons directly to complex IV and is therefore more a measure of isolated complex IV activity than of respiration (38). Given the finding that complex IV activity was the same in wild-type and mutant cells, it is therefore not surprising that respiration and ATP production were not different when TMPD/ascorbate was used as the substrate.

Mitochondrial ATP production rates are significantly lower in the mutant huntingtin expressing cells compared with the wild-type cells when either glutamate plus malate or succinate are used as substrates, which is in accordance with the decreased state 3 respiration in the mutant cells. Interestingly, ATP levels measured in the whole lysates were not different between wild-type and mutant cells. The mechanism underlying this observation is not known at present but is likely not due to a compensatory up-regulation of glycolysis. This is because measurement of total ATP levels in the cells after treatment with oligomycin for 2 h (to inhibit mitochondrial ATP production) revealed that the ATP levels were decreased to the same extent in the wild-type and mutant cells. However, if the cells were treated for 2 h with pyruvate and 2-deoxyglucose (to inhibit glycolysis) the total ATP levels were decreased to a greater extent in mutant striatal cells compared with wild-type striatal cells. These data suggest that, although the overall rate of ATP production by mitochondria from the mutant cells is lower, in intact cells under control conditions the mitochondria are likely not functioning at maximal capacity, and therefore the total cellular ATP levels can be maintained despite the reduced maximal capacity. However, in the case where ATP can no longer be derived from glycolysis (pyruvate and 2-deoxyglucose), then the differences in total ATP become evident because of the fact that the capacity for ATP production by the mitochondria is lower in the mutant cells than in the wild type cells. Therefore it can be speculated that in conditions of neuronal stress the presence of mutant huntingtin may result in a reduction in ATP levels in the cell and contribute to the pathogenic processes in HD (39). Our finding that total ATP levels are not significantly different between the wild-type and mutant cells are in contrast with a previously published study (16) that reported decreased total ATP levels in mutant huntingtin-expressing striatal cells. The reason for these differences is unknown but may be due to differences in methodology. Nonetheless it is clear that in the mutant cells the ability of the mitochondria to produce ATP is impaired.

---

2 Z. Mao and M. Lesort, manuscript in preparation.
The mitochondrial respiratory complex activities were not significantly different between mutant and wild-type cells. This supports a previous finding that complex activities were not altered in the striatum of presymptomatic or lower grade HD cases in which the neuronal loss is minimal (18). It is also interesting to note that decreased respiratory complex activities were not evident in several transgenic HD mouse models (18, 40). All these data would indicate that mutant huntingtin does not impair the activity of the respiratory complexes. Decreases in complex II, III, and IV activities in the postmortem brains of higher grade HD cases, where there is substantial neuronal dysfunction and/or neuronal cell loss (7, 8), could be a consequence of changes in the ratios of cell types due to the neuronal loss and gliosis, although other factors such as oxidative stress (as a secondary effect of mitochondrial impairment) may also play role.

Respiration rates and complex activities were equally sensitive to mitochondrial inhibitors in wild-type and mutant cells, except that there was the trend of respiration being less sensitive to complex IV inhibition in the cells expressing mutant huntingtin. The trend was present at all inhibitor concentrations reaching statistical difference at two data points. But the trend was not present in the complex IV activity titration curves. This resulted in higher values for spare capacity and respiratory threshold for the mutant cells. As demonstrated previously (29), an increase of respiratory threshold could be the compensation for the defect present elsewhere in the respiratory network. When complex II was analyzed, threshold and spare capacity were lower in the mutant compared with wild-type cells. The fact that, in the titration curves statistical difference was observed at only one inhibitor concentration, together with the fact that the differences were not significant when the uncoupled respiration was measured, led to the conclusion that the differences between wild-type and mutant cells were not of biological relevance. This is also supported by the findings that complex II activities and the expression of the catalytic subunits were lower in the two cell lines, therefore indicating there was no defect at the level of complex II.

There have been several reports suggesting altered sensitivity of transgenic HD mice to malonate or 3-NP (41–43). Also, a previous study from our laboratory showed that striatal cells expressing mutant huntingtin were more sensitive than wild-type cells to 3-NP induced toxicity, but not to rotenone treatment (17). Interestingly, while wild-type cells die by apoptosis when treated with 3-NP, cell death in the mutant cells was nonapoptotic (17). Even though mutant huntingtin-expressing cells do have compromised mitochondrial energy production, this is not triggered by impairment of complex II. Our thorough analysis of complex II showed that mutant huntingtin does not affect complex II activity or function. However, it has been shown previously that the mitochondrial ATP-sensitive K⁺ channel is composed of several proteins, including the catalytic subunits of complex II (44), and intriguingly 3-NP is known to activate this channel (44). Therefore, we could speculate that alterations in the activity of this channel could contribute to the increased sensitivity of mutant striatal cells to 3-NP. Further, activation of this channel leads to dissipation of mitochondrial membrane potential (Vm) and a decrease in the ability to retain mitochondrial calcium (45). Hence mutant

![Figure 5](http://www.jbc.org/)
huntingtin-induced alterations in the mitochondrial ATP-sensitive K⁺ channel could explain the decreased Ψm and calcium retention capacity reported in several HD models (13), as well as the altered sensitivity of transgenic HD mice to malonate or 3-NP (41–43). Our future studies will be directed toward examining this possibility.

If the respiratory complexes are functioning properly, what could be the reason for compromised respiration in the striatal cells expressing mutant huntingtin? The dependence of oxidative phosphorylation on mitochondrial calcium concentration has been observed for different respiratory substrates, including glutamate plus malate and succinate (46). This previous study demonstrated that both state 3 respiration and ATP synthesis, but not state 4 respiration, were significantly dependent on mitochondrial calcium (46). Also, the mitochondrial calcium concentration affected the rate of uncoupler-stimulated respiration (46). Therefore it can be speculated that the compromised respiration in the striatal cells expressing mutant huntingtin could be the result of altered mitochondrial calcium concentrations. In support of this hypothesis it has been reported that calcium buffering is altered in lymphoblast mitochondria from HD patients as well as brain mitochondria from transgenic mice expressing full-length mutant huntingtin (13). However, additional research is needed to substantiate this hypothesis.

As estimated by respiratory threshold analysis, cultured striatal cells have rather low thresholds for complexes I, II, and III. This is different to what was previously found in nonsynaptic rat brain mitochondria (complex I and III ~ 70%) (47), and in PC12 cells (complex II ~ 70%) (28). However, variations in the complex I threshold in different brain mitochondria have already been demonstrated. It was found that synaptic rat brain mitochondria have complex I threshold of ~25% (48). Also in PC12 cells the threshold for complex I was ~7% (28), which is similar to what we observed in the striatal cells (Table II). In contrast, the complex IV threshold for the striatal cells appears similar to what was previously reported for nonsynaptic brain and hippocampal mitochondria (47, 49). Low thresholds of complexes I, II, and III indicate that respiration would be easily affected by the impairment or inhibition of these complexes in the striatal cells.

In conclusion, these studies show that mutant huntingtin compromises the ability of mitochondria to produce ATP. However, this effect is not through the impairment of any of the respiratory complexes. Even though mutant huntingtin increases sensitivity to 3-NP as shown previously (17), this effect is not through the impairment of complex II respiratory activity. Additional studies fully elucidating the effects of mutant huntingtin on mitochondrial function will be important to understand HD pathogenesis.

Acknowledgments—We are grateful to Dr. M. E. MacDonald for providing us with STHdhQ7/Q7 and STHdhQ111/Q111 cell lines. We are also thankful to Dr. M. J. Kumar for explanations and suggestions regarding threshold analysis and Dr. M. Lesort for his constructive comments on the manuscript and also for sharing his unpublished data with us.

(percentage of control) versus percent inhibition of complex IV activity obtained at each concentration of KCN. Data points below the inflection point in each curve were used for linear regression. Linear equations and R² values are shown. Significant thresholds were observed in each cell line, as shown in Table II. Complex IV activity from both cell lines was equally sensitive to complex IV inhibition by KCN. Respiration in wild-type cells showed a trend toward being more sensitive to KCN inhibition compared with the mutant cells, with statistically significant differences at two KCN concentrations (Student’s t test, * p < 0.05). All data are means ± S.E. of at least three independent experiments.
REFERENCES

1. The Huntington’s Disease Collaborative Research Group (1993) Cell 72, 971–983
2. El Manara, D. E., Phelps, M. E., Markham, C. H., Metter, E. J., Riege, W. H., and Winter, J. (1982) Ann. Neurol. 12, 425–434
3. Kuhl, D. E., Metter, E. J., Riege, W. H., and Markham, C. H. (1994) Ann. Neurol. 35, suppl. S119–S125
4. Kuhl, D. E., Markham, C. H., Metter, E. J., Riege, W. H., Phelps, M. E., and Mazzio, J. C. (1985) Res. Publ. Assoc. Res. Nerv. Ment. Dis. 63, 199–209
5. Feigin, A., Leenders, K. L., Missimer, J. K., Kuenig, G., Spetsieri, P., Antonini, A., and Eidelberg, D. (2001) J. Nucl. Med. 42, 1591–1595
6. Jenkins, B. G., Koroshetz, W. J., Beal, M. F., and Rosen, B. R. (1993) Neurology 43, 2689–2695
7. Gu, M., Gash, M. T., Mann, V. M., Javoy-Agid, F., Cooper, J. M., and Schapira, A. H. (1996) Ann. Neurol. 39, 385–389
8. Browne, S. E., Bowling, A. C., MacGarry, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F. (1997) Ann. Neurol. 42, 646–653
9. Mann, V. M., Cooper, J. M., Javoy-Agid, F., Agid, Y., Jenner, P., and Schapira, A. H. (1990) J. Neurosci. 10, 2634–2643
10. Kuhl, D. E., Metter, E. J., Riege, W. H., and Markham, C. H. (1984) Ann. Neurol. 15, suppl. S119–S125
11. Brouillet, E., Hantraye, P., Ferrante, R. J., Dolan, R., Loew-Willig, A., Kowall, N. W., and Beal, M. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7105–7109
12. Sanchez, I., Mahlke, C., and Yuan, J. (2003) Nature 421, 373–377
13. Panov, A. V., Gutekunst, C. A., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F. (1997) Ann. Neurol. 41, 646–653
14. Mann, V. M., Cooper, J. M., Javoy-Agid, F., Agid, Y., Jenner, P., and Schapira, A. H. (1990) J. Neurosci. 10, 2634–2643
15. Korte, A., Zisch, M., Riederer, P., Utermoehle, H., and Smith, D. E. (2000) J. Neurochem. 75, 3163–3167
16. Gines, S., Seong, I. S., Fossale, E., Ivanova, E., Trettel, F., Gussella, J. F., Wheeler, V. C., Persichetti, F., and MacDonald, M. E. (2000) Hum. Mol. Genet. 9, 2799–2809
17. Li, S. H., Cheng, A. L., Li, H., and Li, X. J. (1999) J. Neurosci. 19, 5159–5172
18. Stene, S., Ding, M. L., Li, Y., and Thorne, S. (2001) Methods Enzymol. 334, 264–284
19. Hofhaus, G., Shakeley, R. M., and Attardi, G. (1996) Methods Enzymol. 264, 476–483
20. Kun, E., Kirsten, E., and Piper, W. N. (1979) Methods Enzymol. 55, 115–118
21. Robinson, J. B., Brent, L. G., Sprengel, R., and Shriver, R. D. (1987) in Mitochondria, a Practical Approach (Darley-Uman M., Vickwood, D., and Wilson, M. T., eds) pp. 160–161, IRL Press, London
22. Hata, Y., and Stigall, D. L. (1978) Methods Enzymol. 52, 21–37
23. Ragan, C. L., Wilson, M. T., Darley-Uman M., and Lowe, P. N. (1987) in Mitochondria, a Practical Approach (Darley-Uman M., Vickwood, D., and Wilson, M. T., eds) pp. 79–112, IRL Press, London
24. Chretien, D., Bourgeron, T., Rotig, A., Mannich, A., and Rustin, P. (1990) Biochem. Biophys. Res. Commun. 173, 26–33
25. Helmsheil, R. J., Murphy, M. P., Tordler, R. F., and Oppenheim, F. G. (2002) Biochem. Biophys. Acta 1556, 73–80
26. Darley-Uman, V. M., Capaldi, R. A., Takamiya, S., Millett, F., Wilson, M. T., Malatesta, P., and Sartor, P. (1987) in Mitochondria, a Practical Approach (Darley-Uman M., Vickwood, D., and Wilson, M. T., eds) pp. 113–152, IRL Press, London
27. Rossignol, R., Malagut, M., Mazat, J. P., and Letellier, T. (1999) J. Biol. Chem. 274, 33426–33432
28. Mallajosyula, J. K., Andersen, J. K., and Nicholls, D. G. (2004) Methods Enzymol. 385, 385–389
29. Drew, B., and Leeuwenburgh, C. (2003) Ann. J. Physiol. 285, R1259–R1267
30. Harris, G. I., Balaban, R. S., Barrett, L., and Mandel, L. J. (1981) J. Biol. Chem. 256, 10319–10328
31. Hatefi, Y., and Stiggall, D. L. (1978) J. Biol. Chem. 253, 519–524
32. Hatefi, Y., and Stiggall, D. L. (1978) Methods Enzymol. 52, 21–37
33. Dubinsky, W. P., and Cockrell, R. S. (1975) Methods Enzymol. 334, 264–284
34. Fiskum, G., Craig, S. W., Decker, G. L., and Lehninger, A. L. (1980) J. Biol. Chem. 255, 669–681
35. Colbeau, A., Nachbaur, J., and Vignais, P. M. (1971) Biochim. Biophys. Acta
36. Rossignol, R., Malagut, M., Mazat, J. P., and Letellier, T. (1999) J. Biol. Chem. 274, 33426–33432
Mitochondrial Respiration and ATP Production Are Significantly Impaired in Striatal Cells Expressing Mutant Huntingtin
Tamara Milakovic and Gail V. W. Johnson

J. Biol. Chem. 2005, 280:30773-30782.
doi: 10.1074/jbc.M504749200 originally published online June 27, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M504749200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 15 of which can be accessed free at
http://www.jbc.org/content/280/35/30773.full.html#ref-list-1