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Neurobiology of Disease

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Neurobiology of Disease 27 (2007) 99–101

Normal platelet mitochondrial complex I activity in Huntington's Disease

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Received 27 December 2006; revised 5 April 2007; accepted 27 April 2007
Available online 5 May 2007

Two small case series of platelet mitochondrial complex I activity assays in Huntington's Disease (HD) report discrepant results. We measured platelet complex I and complex I/III activity in 21 subjects with early gene-positive HD and 14 age-matched controls. The 21 participants with HD that we studied are greater than the total of 16 in the two previously published of platelet ETS activity in HD. Reductions >10% were excluded with 80% confidence. A systemic defect in complex I activity is not present in early HD when striatal neuronal degeneration is already present.

Keywords: Huntington's Disease; Mitochondria; Electron transport system; Platelets; Human

Introduction

Abnormalities in mitochondrial function have been described in Huntington's Disease (HD) and implicated in the pathogenesis of the progressive neuronal degeneration (Browne and Beal, 2004; Zeevalk et al., 2005; Orth and Schapira, 2001). In two previous studies of mitochondrial electron transport system (ETS) activity in platelets from patients with HD, one reported a reduction of complex I activity of 72% in five subjects whereas the other reported no significant difference in complex I or complex II/III activity in 11 subjects (Gu et al., 1996; Parker et al., 1990). To clarify this reported discrepancy, we undertook a further study of platelet complex I activity in HD in a larger sample.

Materials and methods

Participants

Huntington's Disease

Participants with HD were recruited from the Washington University Movement Disorders Center and through the Huntington's Disease Society of America.

Inclusion criteria were:

1. Previous gene testing with greater than 38 CAG repeats of one allele for IT15.
2. Self-reported symptoms either absent or present for duration of less than 4 years.

Exclusion criteria were:

1. Less than 18 years old.
2. Major neurological or psychiatric disease other than HD or clinically significant lesions on brain imaging.
3. Regular treatment or exposure in the last 6 months to neuroleptics, metoclopramide, alphamethyldopa, clozapine, olanzapine, quetiapine, flunarizine, cinnarizine, reserpine, amphetamines, MAO inhibitors or other medications that might interfere with mitochondrial metabolism.
4. Currently taking chloramphenicol or valproic acid.
5. Ever having taken dopaminergic medications.

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2007.04.008

6. Anticholinergics, amantadine, CoQ10, selegiline and vitamins E and C must be discontinued for 30 days prior to entry into the study.
7. Diabetes mellitus that is controlled by medications.
8. Pregnancy.

All underwent clinical neurological evaluation by Movement Disorder specialists and were assigned a duration of symptoms.

Normal controls

Normal controls were recruited contemporaneously by public advertisement and from friends and spouses of patients. All underwent clinical neurological evaluation by a neurologist.

Inclusion criteria were:

1. Disease free by subject's own history including no history of migraine, childhood febrile seizures and head trauma with loss of consciousness.
2. Taking no medication by subject's own history.
3. No signs or symptoms of neurological disease other than mild distal sensory loss in the legs consistent with age.
4. No pathological lesions on MR scan done for this study. Mild atrophy and punctate asymptomatic white matter abnormalities were not considered pathological.

Exclusion criteria were the same as for the participants with HD.

Normal controls were recruited as part of a larger study including patients with Parkinson Disease and then retrospectively age-matched to the patients with Huntington's Disease without reference to the platelet ETS activity or striatal volume measurements.

In vitro measurements of platelet electron transport chain complex activity

One hundred milliliters of blood anticoagulated with 14% citrate–dextrose solution was collected, coded and shipped overnight to the University of California, San Diego (UCSD) Mitochondrial Disease Laboratory for blinded analysis.

A platelet pellet was produced from platelet-rich plasma by centrifugation at 1000×*g* at room temperature. The pellet was washed and pelleted twice in modified Tyrode buffer (150 mM NaCl, 5 mM HEPES pH 7.4, 0.55 mM NaH_2PO_4, 7 mM $NaHCO_3$, 2.7 mM KCl, 0.5 mM $MgCl_2$, 5.5 mM D-glucose, 1 mM EDTA-K_2). The pellet was resuspended in 6 ml of a buffer (250 mM sucrose, 0.35% fatfree BSA, 1 mM ATP) and then subjected to pressurization with nitrogen at 800 psi for 30 min at room temperature in a Parr bomb to release mitochondria. The suspension was centrifuged at 800×*g* for 10 min and the supernatant was collected. The pellet was resuspended in the same buffer without BSA and again treated in the Parr bomb. Purified mitochondria were prepared by Percoll separation of the pooled supernatants followed by washing in 250 mM sucrose/1 mM ATP. Purified mitochondria resuspended in 250 mM sucrose were stored in liquid nitrogen until assayed.

Complex I and citrate synthase activities were measured by established techniques (Haas et al., 1995; Shults et al., 1997). Complex I/III activity was measured as rotenone sensitive NADH: cytochrome *c* reductase (NCCR) activity. In the NCCR assay mitochondria are thawed and then subjected to one additional freeze–thaw cycle to disrupt mitochondrial membranes and permit access of substrates. Assay buffer (50 mM K-phosphate pH 7.4, 1.5 mM KCN), 100 μM NADH (added fresh) and 10–20 μg of mitochondrial preparation are added to the cuvette and pre-incubated for 3 min at 30 °C. After addition of 100 μM oxidized cytochrome *c*, the reaction is followed for 3 min at 550 nm. The reduction of oxidized cytochrome *c* is assayed spectrophotometrically by an increase in absorbance at 550 nm. The reaction is followed for 3 more min after the addition of 25 μM rotenone to allow calculation of the rotenone-sensitive complex I/III activity. Activity of NCCR is expressed as an apparent first-order rate constant. Citrate synthase activity measurements were used to determine if there were any differences in mitochondrial mass between the two groups.

Magnetic resonance imaging

A 3-D MPRAGE sequence (TR/TE/TI = 1900/3.93/1100 ms, FA = 8°, 7:07 min, 128 × 256 × 256 matrix 1.25 × 1 × 1 mm voxels) was acquired using a Siemens Magnetom SONATA 1.5 T scanner producing a high-resolution T1-weighted image for outlining the caudate and putamen. The regions were identified by an investigator blinded to subject diagnosis. The putamen was outlined as previously described (Black et al., 1998). The caudate was defined on the coronal view. The medial border was defined by the edge of the lateral ventricle. Where separation from putamen by white matter was not clear, an arbitrary straight line was drawn laterally and inferiorly following the direction of the internal capsule on this section. The volume of each caudate and putamen for each participant was determined from the number of voxels within each structure and the voxel volume size. Since both caudate and putamen show atrophy by MRI, an overall combined striatal volume was computed to use for intergroup comparison.

Statistical analysis

Comparisons between participants with HD and age-matched normal controls were performed by 2-sided unpaired *t*-test with SPSS 12.0 for Windows (SPSS, Inc). The primary comparisons of ETS activities were performed on the measurements of complex I and complex I/III activities in nmol/mg/min since there was no difference in citrate synthase activity between the two groups (see below) and normalization to citrate synthase activity increased the variance of the measurements, thus reducing power to detect differences between the means.

Informed consent

This research received prior approval from the Washington University Human Studies Committee (IRB). Written informed consent was obtained from each participant.

Results

Studies were performed in 21 participants with HD and 14 age-matched controls. In the participants with HD, the number of CAG repeats was 39–52, confirming that all of the participants in this group had genetically defined HD. Neurological evaluation

Table 1
Platelet citrate synthase activity, electron transport system activity and striatal volumes in Huntington's Disease and normal controls

	Huntington's Disease	Normal controls	*p* value
Number of participants	21	14	
Age (years)	42 (27–52)	42 (23–56)	
Gender	14M, 7F	9M, 5F	
Citrate synthase	432±210	398±121	0.550
Complex I	17.14±6.76	16.06±4.32	0.567
Complex I/III	22.48±12.18	19.01±6.31	0.278
Striatal volume (ml)	14.24±3.64	17.89±1.79	<0.001

Citrate synthase, complex I and complex I/III activities are in nmol/mg/min. Age is expressed as mean (range). All other measurements are mean±SD. M—male, F—female.

revealed that 10 were without symptoms and 11 had had symptoms for 12–72 months.

There were no statistically significant differences in platelet citrate synthase, complex I or complex I/III activities (Table 1). The 95% confidence intervals for the difference between the means excluded reductions of complex I activity greater than 17% and reductions of complex I/III activity greater than 16%. The 80% confidence intervals for the difference between the means excluded reductions of complex I activity greater than 9% and reductions of complex I/III activity greater than 4%. Complex I and complex I/III activities normalized for citrate synthase activity also showed no difference (data not shown, $p=0.884$ and $p=0.657$, respectively).

There was a highly statistically significant reduction in striatal volume in the participants with HD compared to normal controls (Table 1).

Discussion

The 21 participants with HD that we studied are greater than the total of 16 in the two previously published of platelet ETS activity in HD (Gu et al., 1996; Parker et al., 1990). The confidence intervals for the differences between the means exclude with 95% certainty reductions of 17% or more and exclude with 80% certainty reductions of 10% or more. Since cerebral pathology was already manifest by significantly reduced striatal volume, these data do not support the hypothesis that a general systemic defect of complex I mitochondrial oxidative phosphorylation is involved in the mechanism of neuronal loss in early HD. These results do not exclude other systemic defects in ETS or selective cerebral defects of mitochondrial oxidative phosphorylation as the primary cause of neuronal degeneration in HD nor do they exclude a defect in mitochondrial oxidative phosphorylation developing as a secondary consequence of HD. While it is possible that huntingtin protein is not expressed in platelets, it has been reported in other peripheral blood cells and the increased expression of the A2A receptor protein in platelets indicates likely expression in platelets as well (Sawa et al., 2005; Varani et al., 2003).

Acknowledgments

This research was supported by USPHS grants NS41771 and NS35966, the Lillian Strauss Institute for Neuroscience, the Barnes-Jewish Hospital Foundation (the Jack Buck Fund and the Elliot Stein Family Fund), the Huntington's Disease Society of America Center of Excellence at Washington University, the American Parkinson Disease Association (APDA) Advanced Center for Research at Washington University and the Greater St. Louis Chapter of the APDA. We thank Clifford Shults (deceased) for his assistance with organizing the study as well as Lennis Lich, John Hood, Susanne Fritsch and the Washington University Cyclotron Staff for their assistance.

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