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Cardiac dysfunction in the R6/2 mouse model of Huntington's disease

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Recent evidence suggests that mutant huntingtin protein-induced energetic perturbations contribute to neuronal dysfunction in Huntington's disease (HD). Given the ubiquitous expression of huntingtin, other cell types with high energetic burden may be at risk for HD-related dysfunction. Early-onset cardiovascular disease is the second leading cause of death in HD patients; a direct role for mutant huntingtin in this phenomenon remains unevaluated. Here we tested the hypothesis that expression of mutant huntingtin is sufficient to induce cardiac dysfunction, using a well-described transgenic model of HD (line R6/2). R6/2 mice developed cardiac dysfunction by 8 weeks of age, progressing to severe failure at 12 weeks, assessed by echocardiography. Limited evidence of cardiac remodeling (e.g. hypertrophy, fibrosis, apoptosis, β_1 adrenergic receptor downregulation) was observed. Immunogold electron microscopy demonstrated significant elevations in nuclear and mitochondrial polyglutamine presence in the R6/2 myocyte. Significant alterations in mitochondrial ultrastructure were seen, consistent with metabolic stress. Increased cardiac lysine acetylation and protein nitration were observed and were each significantly associated with impairments in cardiac performance. These data demonstrate that mutant huntingtin expression has potent cardiotoxic effects; cardiac failure may be a significant complication of this important experimental model of HD. Investigation of the potential cardiotropic effects of mutant huntingtin in humans may be warranted.

Keywords: Huntingtin; Huntington's disease; Heart; Mitochondria; Polyglutamine; Cardiovascular; Transgenic; Nitrotyrosine; Acetylation

Introduction

Huntington's disease (HD) is a devastating genetic disease, characterized primarily by a progressive loss of cognitive and motor function, leading to severe patient disability and death (Bates et al., 2002). HD is caused by the mutation of a single ubiquitously expressed gene product of unknown function, the huntingtin protein, which contains an expanded polyglutamine repeat domain in HD patients (Huntington's Disease Collaborative Research Group, 1993). The mechanisms by which mutant huntingtin causes cellular dysfunction and death remain undefined; recent evidence has implicated metabolic and energetic dysfunction in HD neurons (including mitochondrial effects) (Brennan et al., 1985; Browne et al., 1997; Koroshetz et al., 1997; Brouillet et al., 1999; Sanchez-Pernaute et al., 1999; Schapira, 1999; Tabrizi et al., 1999; Ferrante et al., 2000; Jenkins et al., 2000; Tabrizi et al., 2000; Andreassen et al., 2001b; Panov et al., 2002; Panov et al., 2003; Choo et al., 2004; Brustovetsky et al., 2005; Milakovic and Johnson, 2005; Panov et al., 2005; Seong et al., 2005; Benchoua et al., 2006; Squitieri et al., 2006). Biochemical and imaging studies in both HD patients and mouse models demonstrate reduced mitochondrial complex activities and altered metabolic substrate distributions (*N*-acetylaspartate, lactate, phosphocreatine) in the basal ganglia (Brennan et al., 1985; Browne et al., 1997; Sanchez-Pernaute et al., 1999; Schapira, 1999; Tabrizi et al., 1999; Jenkins et al., 2000; Schapiro et al., 2004; Jenkins et al., 2005; Reynolds et al., 2005; Tsang et al., 2006). Despite increased food intake, HD patients can exhibit weight loss (Djousse et al., 2002; Hamilton et al., 2004; Trejo et al., 2004) and weight loss is a characteristic of transgenic mouse models of HD (Bates et al., 1998; Li et al., 2005; Stack et al., 2005). Agents that boost mitochondrial efficiency (e.g. creatine, coenzyme Q) are under investigation as potential therapeutic strategies in HD patients (Koroshetz et al., 1997; Matthews et al., 1998; Ferrante et al., 2000; Huntington's Disease Study Group, 2001; Andreassen et al., 2001b; Schilling et al., 2001; Ferrante et al., 2002; Dedeoglu et al., 2003; Tabrizi et al., 2003; Verbessem et al., 2003; Ryu et al., 2005; Tabrizi et al., 2005; Hersch et al., 2006;

Abbreviations: HD, Huntington's disease; TUNEL, terminal deoxy nucleotidyl fragment end labeling; LV, left ventricle; FS, fractional shortening; LVID, LV internal dimension at diastole; VTI, velocity–time integrals; CO, cardiac output; β_1-AR, β_1-adrenergic receptor; 3NT, 3-nitrotyrosine; RNS, reactive nitrogen species.

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Smith et al., 2006; Stack et al., 2006). While these studies strongly suggest that metabolic dysfunction may contribute to mutant huntingtin-mediated cell death, they fail to explain why the ubiquitous expression of mutant huntingtin has cytotoxic effects primarily in neurons (i.e. mutant huntingtin expression occurs in most cell types, not just neurons at risk). Indeed, levels of huntingtin expression do not directly correlate with neuronal vulnerability, with relatively resistant corticostriatal neurons and striatal cholinergic interneurons expressing high levels of huntingtin and vulnerable striatal projection neurons expressing variable levels of huntingtin (Fusco et al., 1999). Current hypotheses include loss of brain-derived neurotrophic factor synthesis by corticostriatal neurons, the high metabolic demand of neurons relative to other non-excitable cell types, or the slow turnover of neurons, as terminally differentiated cells, allowing for greater accumulation of huntingtin protein relative to other cell types (Bowling and Beal, 1995; Davies et al., 1999; Zuccato et al., 2001). Skeletal muscle cells (another differentiated excitable cell type with high metabolic demand) from HD patients demonstrate many of the same energetic disturbances evident in HD striatal neurons (Arenas et al., 1998; Sathasivam et al., 1999; Lodi et al., 2000; Luthi-Carter et al., 2002; Orth et al., 2003; Ribchester et al., 2004; Schapira and Lodi, 2004; Saft et al., 2005; Strand et al., 2005; Gizatullina et al., 2006). These findings raise the possibility that the ubiquitous expression of mutant huntingtin may place other cell types that are under high metabolic demand at risk.

While HD has been considered primarily a neurological disease state, multiple epidemiological studies have shown that cardiac failure is the second leading cause of death in HD patients (Chiu and Alexander, 1982; Lanska et al., 1988; Sorensen and Fenger, 1992). Cardiac failure is implicated as the cause of death in over 30% of HD patients, compared to less than 2% of age-matched non-HD patients in the general population (Chiu and Alexander, 1982; Lanska et al., 1988; CDC/NCHS and AHA, 1988–94). Energetic dysfunction and metabolic injury are causative factors in both acute and progressive cardiac disease states, and many of the same energetic changes that occur in HD neurons (mitochondrial dysfunction, altered substrate distributions) are typically seen in failing cardiac myocytes (Ingwall, 1993; Paolisso et al., 1994; Francis et al., 1995; Katz, 1998; Vogt and Kubler, 1999). However, the mechanisms of cardiac failure remain largely unstudied in both clinical settings and experimental models of HD. Here we tested the hypothesis that expression of the mutant huntingtin protein leads to cardiac dysfunction in a highly relevant animal model of HD, the R6/2 transgenic mouse. Further mechanistic studies assessed the HD heart for histopathological evidence of cardiac failure, determined the intra-cardiomyocyte distribution of mutant huntingtin, and investigated mechanistic roles for protein acetylation and protein oxidation in the development of HD-related cardiac failure.

Methods

The R6/2 transgenic mouse model of Huntington's disease

Male R6/2 mice (expressing exon 1 of the human huntingtin gene with an expanded CAG repeat, repeat length $n \sim 150$) and wildtype littermate controls were bred in our animal facility (Mangiarini et al., 1996). Breeder pairs were obtained from Jackson Laboratories (Bar Harbor, ME). Genotyping by polymerase chain reaction was conducted at 6 weeks of age to determine study groups. Mice (12–15 per group) were studied at 8, 10, and 12 weeks for the functional experiments, then tissues were collected at 12 weeks for histological experiments. All animal procedures are in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and have been approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

Murine echocardiography

At 8, 10, and 12 weeks of age, *in vivo* cardiovascular performance was assessed in R6/2 mice and littermate controls ($n = 12$–15 for each group) using a Sonos 5500 echocardiography unit (Hewlett-Packard, Andover, MA), as previously described (Weinstein et al., 2000; Mihm et al., 2001a; Mihm et al., 2002). Mice were anesthetized by halothane inhalation (~1% halothane in 95/5% oxygen/CO_2). Parameters were determined using the American Society for Echocardiography leading-edge technique in blinded fashion. These parameters allowed the determination of left ventricular (LV) fractional shortening (FS) by the equation: $FS = [(LVIDd - LVIDs)/LVIDd] \times 100\%$, where LVID refers to the LV internal dimension at diastole (d) and systole (s). Ascending aortic flow waveforms were recorded using a continuous wave Doppler flow probe oriented in a short axis, suprasternal manner. Velocity–time integrals (VTI) were calculated from these waveforms. After sacrifice, aortic root cross-sectional area was measured and cardiac output was calculated by the equation: CO = heart rate × VTI × aortic cross-sectional area. As a measure of ventricular diastolic function, transmitral flow waveforms were also recorded, using continuous wave Doppler oriented in the parasternal, long axis position, collecting E wave (passive ventricular filling) and A wave (component of ventricular filling attributable to atrial contraction) waveforms. Peak transmitral flow, VTI, and acceleration/deceleration slopes were determined for each waveform. Where E and A waveforms were not baseline resolved, E wave slopes were extrapolated from the upper 2/3 of the waveform (from apex toward baseline). Intra- and inter-observer variability for these measurements were 3% and 5% respectively. All systolic and diastolic parameters were analyzed by a blinded observer.

Cardiac immunohistochemistry

Following functional analyses, hearts were rapidly excised and bisected equatorially just distal to the mitral valves then formalin-fixed and paraffin embedded by standard protocols (Weinstein et al., 2000; Mihm et al., 2002). Left ventricular tissues were prepared as 5 μm cross-sections and mounted on slides for histological and immunohistochemical analysis, as we have previously described (Weinstein et al., 2000; Mihm et al., 2002). General cardiac morphology and extent of cardiac fibrosis deposition were assessed using Masson's Trichrome stain with a kit-based approach (Sigma Chemical). Immunohistochemical studies were conducted to measure cardiac levels of β_1-adrenergic receptor, protein ubiquitination, protein 3-nitrotyrosine formation, lysine acetylation, and expanded protein-bound polyglutamine repeat domains (>37 repeats recognized as a indirect measure of mutant huntingtin). Antibodies employed for these studies were anti-β_1-adrenergic receptor (1:800 dilution, Research Diagnostics); anti-protein–ubiquitin (1:8000 dilution, Dako Corp.); anti-protein–3-nitrotyrosine (1:800 dilution, Upstate Biotechnology); anti-acetyl-lysine (pan-antibody for protein-bound acetylated lysine, 1:4000 dilution, Upstate Biotechnology); and anti-polyglutamine