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REVIEW ARTICLE

Neuroprotective effects of creatine

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Abstract There is a substantial body of literature, which has demonstrated that creatine has neuroprotective effects both in vitro and in vivo. Creatine can protect against excitotoxicity as well as against β-amyloid toxicity in vitro. We carried out studies examining the efficacy of creatine as a neuroprotective agent in vivo. We demonstrated that creatine can protect against excitotoxic lesions produced by *N*-methyl-D-aspartate. We also showed that creatine is neuroprotective against lesions produced by the toxins malonate and 3-nitropropionic acid (3-NP) which are reversible and irreversible inhibitors of succinate dehydrogenase, respectively. Creatine produced dose-dependent neuroprotective effects against MPTP toxicity reducing the loss of dopamine within the striatum and the loss of dopaminergic neurons in the substantia nigra. We carried out a number of studies of the neuroprotective effects of creatine in transgenic mouse models of neurodegenerative diseases. We demonstrated that creatine produced an extension of survival, improved motor performance, and a reduction in loss of motor neurons in a transgenic mouse model of amyotrophic lateral sclerosis (ALS). Creatine produced an extension of survival, as well as improved motor function, and a reduction in striatal atrophy in the R6/2 and the N-171-82Q transgenic mouse models of Huntington's disease (HD), even when its administration was delayed until the onset of disease symptoms. We recently examined the neuroprotective effects of a combination of coenzyme Q10 (CoQ10) with creatine against both MPTP and 3-NP toxicity. We found that the combination of CoQ and creatine together produced additive neuroprotective effects in a chronic MPTP model, and it blocked the development of alpha-synuclein aggregates. In the 3-NP model of HD, CoQ and creatine produced additive neuroprotective effects against the size of the striatal lesions. In the R6/2 transgenic mouse model of HD, the combination of CoQ and creatine produced additive effects on improving survival. Creatine may stabilize mitochondrial creatine kinase, and prevent activation of the mitochondrial permeability transition. Creatine, however, was still neuroprotective in mice, which were deficient in mitochondrial creatine kinase. Administration of creatine increases the brain levels of creatine and phosphocreatine. Due to its neuroprotective effects, creatine is now in clinical trials for the treatment of Parkinson's disease (PD) and HD. A phase 2 futility trial in PD showed approximately a 50% improvement in Unified Parkinson's Disease Rating Scale at one year, and the compound was judged to be non futile. Creatine is now in a phase III clinical trial being carried out by the NET PD consortium. Creatine reduced plasma levels of 8-hydroxy-2-deoxyguanosine in HD patients phase II trial and was well-tolerated. Creatine is now being studied in a phase III clinical trial in HD, the CREST trial. Creatine, therefore, shows great promise in the treatment of a variety of neurodegenerative diseases.

Keywords Parkinson's · Huntington's · ALS · Mitochondria

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Introduction

The pathophysiology of degenerative neurological diseases is associated with the loss of distinct populations of neurons, which are frequently interconnected. Neuronal loss or

dysfunction can lead a variety of different neurological diseases whose phenotype is dependent on the location of the neuronal loss as well as the speed of the degeneration of the neurons. A large number of processes have been implicated in neurodegenerative diseases. There has been much interest in protein aggregation and in the toxicity of aggregates such as β-amyloid, α-synuclein, TDP43, and huntingtin. Recent studies, however, suggest that the protein aggregates may not directly lead to the neuronal degeneration. One recent study of a transgenic mouse which develops abnormal phosphorylated inclusions with the microtubule associated protein tau, showed that caspase activation preceded the development of the aggregates, and that the neurons containing the aggregates were relatively preserved (de Calignon et al. 2010). It has also been demonstrated that neurons which contain huntingtin aggregates are resistant to cell death (Arrasate et al. 2004). Other processes which have been implicated in neurodegeneration include transcriptional dysregulation. There is a large body of evidence supporting this in a number of neurodegenerative diseases and in particular, in HD (McGill and Beal 2006; Browne and Beal 2006). Other processes which are implicated are excitotoxicity, oxidative stress, energy depletion and mitochondrial dysfunction. These processes have been implicated in HD, PD, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD) (Lin and Beal 2006).

It is thought that mitochondria may play a central role in the pathogenesis of a number of these diseases due to either direct involvement by mutated proteins or by secondary effects of transcriptional dysregulation, or impairment of axonal transport. Mitochondria are not only fundamental to cellular bioenergetics, but they are also key mediators of apoptosis and they can be linked either directly or indirectly to many of the other deleterious processes involved in neurodegeneration. It, therefore, may be possible to intervene in a number of different neurodegenerative diseases with therapeutic strategies which target mitochondrial dysfunction and impaired bioenergetics. One such approach is to utilize creatine monohydrate to improve overall cellular bioenergetics in various neurological disorders.

Mitochondrial involvement in neurodegeneration

Mitochondria are critical organelles involved in regulating the energy status of the cell through oxidative phosphorylation. Oxidative phosphorylation produces a usable form of energy (ATP) for a variety of cellular processes and it is particularly important in the highly metabolic tissues with large ATP requirements such as heart, skeletal muscle, and brain (Adhihetty et al. 2003). The primary role of mitochondria is to supply and regulate energy for the cell. They, however, have also been shown to be involved in controlling a number of cell death processes such as excitotoxicity and apoptosis (Peng and Greenamyre 1998; Green and Reed 1998). Mitochondria are also the major source of reactive oxygen species (ROS) production within cells. A variety of toxins that specifically target the electron transport chain of mitochondria such as 3-nitropropionic acid, 1-methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP), rotenone, and malonate have been shown to reproduce the disease phenotype of either HD or PD (Beal 1996; Thomas and Beal 2007). The finding that MPTP toxicity was associated with impairment of complex I function of the electron transport chain focused the attention of the PD research community on the potential involvement of mitochondria in the disease pathogenesis.

Mitochondria are involved in apoptosis because they contain several proapoptotic proteins which can contribute to cell death upon release into the cytosol. These include both cytochrome c as well as apoptosis inducing factor, endonuclease G, SMAC/Diablo, and OMI/HTRA2 (Primeau et al. 2002). They are also modulated by proteins in the Bcl2/Bax family. Bcl2 localizes to the outer mitochondrial membrane where it prevents the release of factors involved in apoptotic cell death, whereas, Bax increases the release of proapoptotic factors.

The mitochondria are comprised of outer and an inner membrane separated by an intermembrane space. In order for the mitochondrial proapoptotic proteins to be released into the cytosol, a pore must be formed between the inner and outer mitochondrial membranes. One such pore is termed the mitochondrial permeability transition pore (mt-PTP). The mt-PTP consists of several components, which include the mitochondrial matrix protein cyclophilin D, an inner membrane protein, the adenine nucleotide translocase and the outer membrane protein, the voltage-dependent anion channel (VDAC) (Adhihetty et al. 2003). A large number of stimuli can activate the mt-PTP. A classic means of inducing it is by an accumulation of calcium (Ca^{2+}). An enhanced opening of the mt-PTP is also caused by reduction in membrane potential, an increase in inorganic phosphate, a reduction in adenine nucleotides or elevations of ROS. Once the mt-PTP formation and opening has occurred due to a combination of these factors, pro-apoptotic proteins are released into the cytosol to initiate the cell death pathways. This occurs through a complex known as the apoptosome. Although cyclophilin D, the adenine nucleotide translocase and VDAC have traditionally been considered to be key components necessary for mt-PTP formation, there is recent evidence suggesting that cyclophilin D is the only essential component (Baines et al. 2005; Csukly et al. 2006). It appears that VDAC and ANT may be dispensable for mt-PTP formation, but these proteins may serve as regulatory pore proteins, which interact with the Bcl2 family members.

The Bcl2 family of proteins regulates the conformational status of the mt-PTP. The proapoptotic members include BAC, BAX, Bok and anti-apoptotic family members such as Bcl2, Bcl-Xl, and Bcl-w. These factors can titrate the function of one another by forming heterodimers, which then regulate the opening of the pore (Primeau et al. 2002). Mitochondrial oxidative phosphorylation involves a series of electron transfers within the inner mitochondrial membrane. The inefficient transfer of electrons can result in the production of unstable and potentially damaging ROS. As a result, mitochondria are thought to be the primary source of ROS within the cell. The leakage of electrons is known to occur at both complex I and complex III. In addition, many of the dehydrogenases such as α-ketoglutarate dehydrogenase within the Kreb cycle can also generate ROS. It has been estimated that as much as 2% of all oxygen is converted into ROS. The production of mitochondrial ROS is proposed to initiate early apoptotic triggering events. The ROS can directly induce cytochrome c dissociation from the inner mitochondrial membrane and cause subsequent release from the organelle. In addition, ROS can directly interact to facilitate mt-PTP opening. The accumulation of ROS within the matrix is somewhat limited by the mitochondrial antioxidant enzymes including manganese superoxide dismutase, glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidase. In addition, thioredoxins play a role. The levels of glutathione within the mitochondria are crucial in preventing damage to the organelle. ROS can also indirectly influence the apoptotic pathways by activating mitogen-activated protein kinases (MAPKs) as well as various redox sensitive transcription factors involved in the expression of anti and proapoptotic gene expression. Oxidative stress is a common feature of most neurodegenerative diseases, and mitochondria, therefore are implicated in the production of these ROS.

Creatine is a guanidino compound found primarily in meat products and is produced endogenously by the liver, kidney, and pancreas (Juhn and Tarnopolsky 1998a, b; Tarnopolsky and Beal 2001; Adhihetty and Beal 2008). The production of creatine requires the amino acids arginine and glycine. In addition, the amino acid methionine is required to supply a methyl group to the overall structure. Creatine is initially synthesized by the conjugation of arginine and glycine by the rate limiting enzyme L-arginine-glycine guanidino transferase to produce guanidino-acetate. This product is subsequently methylated by *s*-adenosylmethionine, which is catalyzed by the important guanidino-acetate-methyltransferase (GAMT) to produce the end product of creatine. Patients who have an inborn deficiency of GAMT have an abnormally low synthesis of creatine, and they show developmental delays, extrapyramidal movement disorders and seizures (Stockler et al. 1997). Creatine monohydrate supplementation improves the neurological impairment in patients afflicted by this rare disorder (Stockler and Hanefeld 1997; Van der Knapp et al. 2000). Creatine is also effective in patients who have impairment of creatine transport. These studies clearly illustrate the importance of creatine in the maintenance of normal cellular bioenergetics and function.

Creatine is taken up into brain, heart, and skeletal muscle by a sodium dependent transporter and inward movement is enhanced by the presence of insulin (Sora et al. 1994; Steenge et al. 1998). There has been some concern that chronic exogenous supplementation of creatine could potentially result in a compensatory downregulation of creatine transporters in the cell. However, the data to date has not indicated that there is any down-regulation following 2 months of a high physiologic doses of creatine in humans (Juhn and Tarnopolsky 1998a, b). Creatine supplementation has been used in both healthy individuals and patients with neurological disease. The safety of creatine supplementation has been reviewed extensively, and it has been concluded that creatine supplementation does not have any deleterious effects in humans. There was one report of some kidney dysfunction in a patient who had glomerulonephritis, but that has not been replicated in other patients. There are reports that creatine supplementation can cause an elevation of creatinine levels, but this does not appear to have any adverse effects. It does not indicate renal dysfunction. A number of studies have shown that extended creatine supplementation does not appear to alter renal function filtration (Poortsman et al. 1997; Poortmans and Francaux 2000; Mihic et al. 2000).

Creatine exists in the cell both as free creatine and phosphocreatine which together comprise the total creatine pool. In tissues with high energy requirements such as skeletal muscle and brain, PCR serves as a short term energy buffer in which adenosine diphosphate is phosphorylated to adenosine triphosphate. This phosphorogroup transferase is catalyzed by the important creatine kinase (CK) enzyme (Tarnopolsky and Beal 2001). CK is an important mediator of cellular homeostasis since it can reversibly convert creatine into phosphocreatine and, thereby, create a pool of phosphocreatine for temporal and spatial ATP buffering (Andres et al. 2008; Adhihetty and Beal 2008). There are two different isoenzymes of CK, which exist in most tissues. They are localized to different cellular compartments. Cytosolic CK is found as a dimer and is typically associated with subcellular structures within the cell (Wallimann and Hemmer 1994). Another form is that known as mitochondrial creatine kinase, which can exist as either an octomeric or dimeric form within the intermembrane space of mitochondria (O'Gorman et al. 1997). There are two tissue specific mitochondrial CK isoenzymes, which are termed sarcomeric (mtCK) found in

striated muscle and ubiquitous mtCK which has been found in most other tissues including neural tissue. Both the cytosolic and mitochondrial CK isoenzymes contribute to the overall pool of phosphocreatine within the cell to create an efficient energy buffering system.

The phosphocreatine and CK energy pathway represents an extremely efficient energy buffering system for two reasons. First phosphocreatine has a slightly higher diffusion capacity than ATP making PCR transport a more efficient energy delivery system to different cellular locations. Second, the subcellular localization of cytosolic and mitochondrial CK couples areas of energy generation with energy production. Thus, the CK phosphocreatine essentially serves as a spatial "energy shuttle" or energy circuit within the cell (Wallimann et al. 1992). The PCR buffering system is responsible for half of the energy requirements during short-term muscle contraction. The beneficial impact of creatine supplementation and widespread usage originally occurred as a result of its well-defined functional improvements in muscle tissue to ultimately enhance athletic performance. A number of studies have shown that exogenous supplementation of creatine, using a variety of loading strategies, can prolong the duration of short term muscle contractions by bolstering the cellular energy pool. In addition, supplementation with creatine not only has been shown to enhance bioenergetics but also to increase lean body mass in human subjects (Mihic et al. 2000). Creatine is extremely popular due to its potential effects on athletic performance as well as beneficial effects for resistance exercise seen in elderly subjects, which may be due to a reduction in sarcopenia which occurs with aging. The importance of the phsophocreatine system for brain function has been shown using genetically altered mice that lack the brain isoform of the cytosolic CK which show deficits in open field behavior, slower learning, and a loss of hippocampal mossy fiber connections. Mice that lack both the cytosolic and mitochondrial CK within the brain exhibit a more severe phenotype as compared to a single cytosolic CK knockout. Due to the importance of creatine and cellular bioenergetics and metabolism, the hypothesis that exogenous creatine may be beneficial in the treatment of neurodegenerative disease disorders has been advanced (Tarnopolsy and Safdar 2008; Adhihetty and Beal 2008).

Creatine supplementation has been shown to be efficacious in a variety of animal and cellular models of neurodegenerative diseases including AD, PD, ALS, and HD. The beneficial effects are thought to be a result of improvement in overall bioenergetics and/or the mitochondrial deficits, associated with each particular neurodegenerative disease. These diseases are each reviewed in turn in the subsequent paragraphs.

Alzheimer's disease

Alzheimer's disease (AD) is characterized by loss of neurons as well as deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles (Querfurth and La Ferla 2010). The earliest detectable defects in AD patients have been shown to be caused by impaired energy metabolism and mitochondrial electron transport chain dysfunction. These often precede the amyloid deposition. In addition, there is substantial evidence for increased oxidative damage to both a variety of proteins and DNA, as well as to mitochondrial DNA (Bonda et al. 2010; Mecocci et al. 1994). There is a marked reduction in creatine kinase activity in AD brain homogenates which may be related to oxidative stress (Hensley et al. 1995; David et al. 1998; Aksenov et al. 2000). CK enzyme is very sensitive to oxidative stress due to the presence of highly sensitive cysteine residues that can be easily modified by an oxidative insult. One study has shown that AD patients have reduced levels of brain PCr at the onset of symptoms and decreased oxidative metabolism in later stages (Pettegrew et al. 1994). In addition, creatine deposits have been identified in a transgenic model of AD using Fourier transform infrared micro-spectroscopy (Gallant et al. 2006). A variety of potential explanations for this creatine deposition have been proposed, but the most plausible is that it is due to oxidative stress associated with AD, resulting in impairment of both cytosolic and mitochondrial CK. Due to the impairment of the generation of PCr from Cr, it is thought that an excess of Cr accumulates to ultimately form deposition sites in the cell. In addition, it has been suggested that the amyloid precursor protein directly interacts with and binds to mitochondrial CK (Li et al. 2006). It has not as yet been shown that supplementation with creatine has any benefits in AD, but this, however, has not been studied in any significant detail. Creatine administration has been shown to protect against oxidative mediated conversion of octomeric mtCK to the dysfunctional dimeric mtCK (Brewer and Wallimann 2000). Thus elevating creatine levels by exogenous supplementation might protect the CK system and delay the ROS-induced inactivation of CK, which occurs in AD patients (Aksenov et al. 2000). Creatine supplementation may also activate AMPK signaling, as has been shown in skeletal muscle cells (Ceddia and Sweeney 2004). The activation of the AMPK pathway is important in regulating mitochondrial content and function in a PGC-1α dependent pathway (Zong et al. 2002). If creatine can activate AMPK and improve mitochondrial content, or function, this is potentially beneficial. This is particularly the case since PGC-1α levels have been shown to be reduced in post-mortem brain tissue of AD subjects and this correlates with increasing numbers of neurofibrillary tangles, as well

as pathologic and clinical grade prior to death (Qin et al. 2009).

Parkinson's disease

Parkinson's disease (PD) is a consequence of degeneration of dopaminergic neurons resulting in the clinical phenotype of progressive bradykinesia, rigidity, tremor, and gait abnormalities. A number of molecular mechanisms have been shown to contribute to dopaminergic neuronal loss and dysfunction in PD. There is strong evidence that mitochondrial impairment plays a role in the pathogenesis of this illness (Beal 1995, 2009). Numerous authors have shown that there is reduced complex I activity of the mitochondrial electron transport chain in the substantia nigra of postmortem tissue of PD patients, and there is also reduction of complex I activity in platelets of patients with early PD (Bindoff et al. 1989; Parker et al. 1989; Schapira et al. 1990; Krige et al. 1992). The possibility that impaired energy metabolism plays a role in PD has been strengthened by the observations that a number of toxins, including MPTP and rotenone, will specifically inhibit complex I of the electron transport chain. These toxins result in selective loss of dopaminergic neurons and when administered chronically they result in the development of α-synucelin aggregates, a pathologic hallmark of PD. The study of MPTP originated when Parkinsonism was observed in young individuals in southern California (Beal 2001). It was subsequently shown that this was due to the presence of MPTP as a contaminant in the production of synthetic opiates, which the individuals were using as recreational drugs. MPTP was identified as the toxin by mass spectroscopy studies. Subsequent studies of MPTP treatment of both rodents and primates showed that it produced selective degeneration of nigral neurons. This is due to the metabolism of MPTP by monoamine oxidase B to MPP+. MPP+ is then taken up by the dopamine transporter into neurons where it subsequently accumulates within the mitochondria. It then blocks complex I of the electron transport chain and produces increased generation of free radicals.

Furthermore, a number of genetic causes of PD have been linked to mitochondrial dysfunction. These include nuclear encoded genes, which are defects in α-synuclein, parkin, DJ-1, PINK1, LRRK2, and Omi (Thomas and Beal 2007). The defects in parkin and PINK1 are of particular interest, since they are involved in a critical pathway, which utilizes autophagy to get rid of damaged mitochondria. It has been shown that depolarization of mitochondria which occurs with uncoupling agents, results in expression of PINK1 on the surface of the mitochondria (Narendra et al. 2010). This is then recognized by parkin, which ubiquinates the mitochondria leading to the autophagy of the damaged mitochondria. This appears to be a way to regenerate and get rid of damaged mitochondria.

Due to the impaired bioenergetics which have been shown to occur in PD one would suspect that administration of creatine might be beneficial. We showed that oral supplementation of creatine resulted in significant protection against MPTP induced dopamine depletion in mice (Matthews et al. 1999). In addition, we found that creatine supplementation protected against the loss of nissl and tyrosine hydroxylase immunostained neurons in the substantia nigra. Subsequently, a randomized double-blind futility phase II clinical trial of creatine in early PD patients in 2006 indicated that creatine supplementation was not futile (NINDS NET-PD Investigators 2006). In fact, it was shown to delay the increase in the Unified Parkinson's Disease Scale which normally occurs within one year, by as much as 50%. Due to this finding, creatine is now under further investigation in a phase III clinical trial by the NET-PD investigators. This is double-blind placebo controlled phase III clinical study, which is examining 1,720 patients with early stage PD, at 51 medical centers in the United States and Canada. The patients will then be studied for the next 5 to 7 years. This will be the largest phase III clinical trial in symptomatically treated PD subjects which has been attempted. This has been sponsored by NINDS with the NET-PD investigators.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a progressive usually fatal neurodegenerative disease caused by degeneration of motor neurons in both the spinal cord as well as in the motor cortex (Hervias et al. 2006). The disorder leads to muscle weakness and atrophy throughout the body as well as to spasticity due to involvement of both upper and lower motor neurons. ALS is rapidly progressive and most patients die within three to four years. Patients of all races and ethnic backgrounds are equally affected. The typical age of onset is between age 40 and 60. Familial ALS accounts for ~10% of all ALS cases. One of the genetic abnormalities which has been shown to cause ALS are mutations in copper zinc superoxide dismutase, a cytosolic enzyme responsible for scavenging and reducing free radicals in the cell. After this observation was made, a transgenic mouse model with the G93A mutation in SOD was developed (Gurney et al. 1994; Wong et al. 1995). This mouse shows progressive loss of msotor neurons, and increasing weakness. It has been demonstrated that a portion of SOD1 is localized to the mitochondria, particularly on the outer mitochondrial membrane. In that location, it

may interfere with the function of Bcl2, a neuroprotective antiapoptotic protein (Pasinelli et al. 2004; Pedrini et al. 2010) and VDAC. We showed that oral administration of creatine produced dose-dependent improvement of motor performance, and extended survival in the G93A mice (Klivenyi et al. 1999). In addition, creatine supplementation protected against the loss of neurons in both the substantia nigra and the motor cortex, and reduced the extent of oxidative damage. Due to these neuroprotective observations, creatine supplementation was tried in two independent human clinical trials. These trials, however, failed and did not show any evidence of a beneficial effect of creatine on survival and/or disease progression in patients with ALS (Shefner et al. 2004; Groeneveld et al. 2003). This disappointing outcome is amongst others, which have suggested that the SOD transgenic mouse may not be predictive of therapeutic outcome in patients with sporadic ALS.

Huntington's disease

Huntington's disease (HD) also known as huntingtin chorea, is an inherited autosomal dominant progressive neurologic disorder that affects three to seven per 100,000 individuals and has its onset typically between 40 and 50 years of age (Beal and Ferrante 2004). HD is characterized by abnormal "dancelike" body movements termed chorea. There is also impaired coordination, as well as impairments in cognition and psychiatric abnormalities. HD is caused by trinucleotide repeat (CAG) in the huntingtin gene, which results in an expansion of a polyglutamine stretch in the huntingtin protein. The disease is fully penetrant with a CAG repeat length >40. The repeat length between 36 and 39 shows partially penetrance. The huntingtin protein is ubiquitously expressed in both the nervous system and peripheral tissues. Mutant huntingtin is thought to confer its toxic effects to neural tissue by a number of different mechanisms which include: transcription dysregulation, proapoptotic signaling, oxidative injury, excitotoxicity, inflammation, malfunctioning proteolysis, metabolic dysfunction, and mitochondrial dysfunction.

A significant advance in studying HD was the development of transgenic mouse models. The mouse models can be placed into three broad categories: (1) Mice that contain only a fragment of exon 1 of the human huntingtin gene containing the polyglutamine mutations in addition murine wildtype huntingtin; (2) Mice with pathogenic CAG repeat inserted into the existing CAG expansion in murine htt (Hdh knockin mice); (3) Mice that express the full-length human htt (plus murine Hdh).

There is considerable evidence that there is mitochondrial dysfunction which occurs in these mice. There is also evidence for excitotoxicity, and a recent paper suggested therapeutic effects with memantine, a weak NMDA receptor channel blocker (Okamoto et al. 2009). We and others have shown that (1) lactate levels are elevated in both the cerebral cortex in basal ganglia of patients with HD, (2) there is a reduction in phosphocreatine to inorganic phosphate in the resting muscle of HD patients, and (3) there is a reduction in regeneration of ATP and phosphocreatine after mild exercise in both HD patients and presymptomatic gene carriers, and (4) there are reductions in mitochondria electron transport enzymes in HD postmortem tissue (Jenkins et al. 1993; Brouillet et al. 1995; Browne et al. 1997; Koroshetz et al. 1997). Other evidence in support of the role of energetic defects and mitochondrial dysfunction as contributors to HD pathogenesis, is the evidence utilizing animal models based on mitochondrial neurotoxins, such as malonate and 3-nitropropionic acid (3-NP), which showed that these toxins can produce both a behavioral and neuropathological phenotype similar to that which occurs in HD (Beal et al. 1993; Brouillet et al. 1993). The most convincing of these has been administration of 3-nitropropionic acid, an irreversible inhibitor of succinate dehydrogenase and complex 2 of the electron transport chain. A number of studies using isolated mitochondria have shown that there is impaired ability to retain calcium in HD mitochondria. It has been demonstrated that mutant huntingtin is associated with mitochondria as shown using electron microscopy. A number of studies have shown impaired mitochondrial transport in HD neurons.

More recently, much evidence has focused on impaired mitochondrial biogenesis due to downregulation of the transcriptional co-activator PGC-1α (Cui et al. 2006; Weydt et al. 2006). PGC-1α has been demonstrated to be reduced in its expression in postmortem brain tissue of HD patients as well as animal models of HD. HD transgenic mice have been shown to have impaired temperature regulation, which is due to impaired PGC-1α expression and reduced expression of uncoupling protein 1 (UCP-1), which produces increased body temperature in brown adipose tissue (Weydt et al. 2006). It was shown that an increase in PGC-1α occurs with exposure to cold temperatures in HD transgenic mice. However, there is an impairment of upregulation of UCP1, which contributes to impaired temperature regulations seen in HD mice. We carried out extensive studies of PGC-1α in HD transgenic mice, muscle biopsies and myoblasts from HD patients (Chaturvedi et al. 2009). We treated the mice with guanidinopropionic acid which depletes phosphocreatine levels as well as ATP. This results in an increase in AMP which activates AMP kinase. AMP kinase has been shown to phosphorylate PGC-1α and increase its expression. We found that there was reduced PGC-1α in HD myoblasts. Furthermore, we found that administration of guanadinopropionic acid markedly

increased both AMPK and PGC-1α in muscle and brain of wildtype mice, but that these increases were blocked in the HD mice. This was true in the striatum, muscle, liver, and brown adipose tissue. More recently, we found that there were reduced numbers of mitochondria in HD postmortem brain tissue and that this correlates with reduction in expression of PGC-1α mRNA (Kim et al. 2010). The impaired ability to upregulate PGC-1α in myoblasts could be reversed by utilizing shRNA to mutant huntingtin. This was also efficacious in a striatal cell line which had the mutant HD allele as compared to huntingtin with a wildtype allele. These studies taken together show that there is a marked metabolic dysfunction in HD and that both direct effects on mitochondria by mutant huntingtin protein, as well as effects mediated by impaired transcription of PGC-1α may play a role in the pathogenesis of the energetic defects.

This would suggest that any therapeutic treatment, which will buffer intracellular energy stores might be beneficial in the treatment of HD. We, therefore, hypothesized that creatine supplementation in transgenic mouse models might improve cellular bioenergetics and provide neuroprotection. We found that this was the case in both the R6/2 and the N171-82Q transgenic mouse models in which we saw an improvement in motor performance, extended survival, attenuation of loss of body weight and brain weight and reduced neuronal atrophy (Ferrante et al. 2000; Andreassen et al. 2001). We also showed that creatine supplementation is effective in reducing lesion volume produced by the 3-NP mitochondrial toxin model of HD (Matthews et al. 1998). Due to the beneficial effects of creatine supplementation in our neurotoxin HD and transgenic mouse HD models, we carried out a 16-week, randomized double-blind placebo control phase II clinical trial on the safety and tolerability of 8 g per day of creatine in HD patients (Hersch et al. 2006). This study illustrated that creatine supplementation reduced elevated serum levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) back to baseline levels seen in controls. 8-OHdG is a marker of oxidative damage to DNA which creatine therapy improved. An open-label add on study of creatine showed further benefits in HD patients. In particular, there was a slowing of the ongoing cortical atrophy. It was also shown that higher doses as high as 30 g daily exerted clinical benefits in the HD patients. In view of this, a double blind placebo controlled phase III clinical trial has been approved and is currently ongoing at a large number of centers, which are part of the Huntington Study Group. In this clinical trial, patients will be titrated up to the maximum tolerated dose, and then they will be assessed utilizing the UHDRS scale as well as on quality of life scales, cognition and a variety of other measures.

Conclusion

There is a strong body of evidence, which shows that creatine supplementation can exert beneficial effects by increasing the PCr pool and by improving overall cellular bioenergetics. Creatine supplementation may also enhance mitochondrial function and reduce the susceptibility to mitochondrial-mediated apoptosis. These beneficial effects have been seen in both HD transgenic mouse studies as well as initial clinical studies in HD patients. The evidence to date suggests that creatine supplementation improves bioenergetic deficits and may exert neuroprotective effects in both PD and HD. The evidence to date studying creatine supplementation in ALS, however, has been disappointing. As yet, no clinical trials of creatine supplementation in AD have been attempted. Further clinical studies investigating the role of creatine in PD and HD will occur over the next few years, and will, therefore, definitively determine whether this strategy is efficacious in treating these diseases. It will also be of interest to test creatine in other neurodegenerative diseases such as frontotemporal dementia, progressive supranuclear palsy, and cortical basal degeneration. These studies conducted to date show great promise for potential efficacy of creatine in treating a variety of neurodegenerative diseases.

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