CHAPTER 11

THE NEUROPROTECTIVE ROLE OF CREATINE

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Abstract: Significant progress has been made in identifying neuroprotective agents and their translation to patients with neurological disorders. While the direct causative pathways of neurodegeneration remain unclear, they are under great clinical and experimental investigation. There are a number of interrelated pathogenic mechanisms triggering molecular events that lead to neuronal death. One putative mechanism reported to play a prominent role in the pathogenesis of neurological diseases is impaired energy metabolism. If reduced energy stores play a role in neuronal loss, then therapeutic strategies that buffer intracellular energy levels may prevent or impede the neurodegenerative process. Recent studies suggest that impaired energy production promotes neurological disease onset and progression. Sustained ATP levels are critical to cellular homeostasis and may have both direct and indirect influence on pathogenic mechanisms associated with neurological disorders. Creatine is a critical component in maintaining cellular energy homeostasis, and its administration has been reported to be neuroprotective in a wide number of both acute and chronic experimental models of neurological disease. In the context of this chapter, we will review the experimental evidence for creatine supplementation as a neurotherapeutic strategy in patients with neurological disorders, including Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease, as well as in ischemic stroke, brain and spinal cord trauma, and epilepsy.

1. INTRODUCTION

There is substantial evidence to suggest that impaired energy metabolism, in concert with mitochondrial dysfunction, plays a critical role in the pathogenesis and progression of neurological diseases as a primary and/or secondary mechanism in the neuronal death cascade (Beal, 2005; Tarnopolsky and Beal, 2001). This not only includes neurodegenerative disorders, but also acute and chronic conditions.
involving the central and peripheral nervous systems. While other mechanisms may exacerbate bioenergetic dysfunction, such as protein aggregation and altered transcription, impaired energy metabolism may trigger pro-apoptotic signaling, oxidative damage, excitotoxicity, and impede nuclear and mitochondrial DNA repair (Figure 1). These pathologic messages can interact and potentiate one another, resulting in a continued cycle of energy depletion. Energy is critical to the biological and molecular regulation of multiple cellular functions and, as such, reduced energy levels threaten cellular homeostasis and integrity.

While the concept of neuroprotection was first considered by ancient Greek physicians using hypothermia to treat stroke, a modern-day therapeutic strategy

![Figure 1. Mitochondria-mediated neuronal dysfunction in neurological disease. Protein aggregation may disrupt the mitochondrial membrane potential and alter excitotoxin-induced Ca\textsuperscript{2+} influx. In addition, impaired respiratory enzyme activities may result in reduced ATP generation and increased levels of reactive oxygen species (O\textsubscript{2}\textsuperscript{−} and H\textsubscript{2}O\textsubscript{2}). Free radical generation may then result in further damage to cellular macromolecules through nitrosylation, oxidation, and peroxidation, which can directly contribute to neuronal injury. In addition, the release of cytochrome c from damaged/dysfunctional mitochondria triggers the activation of the apoptotic cascade and the release of initiator and executioner caspases, resulting in neuronal cell death. Impaired energy metabolism may trigger pro-apoptotic signaling, impair cellular homeostasis, and impede nuclear and mitochondrial DNA repair. Each of these pathological mechanisms may in turn further exacerbate mitochondrial dysfunction and energy loss. Creatine supplementation improves intracellular high-energy phosphate levels, improving multiple cellular functions and neuronal survival.](image-url)
The Neuroprotective Role of Creatine

inducing sustained ATP levels may have both direct and indirect importance in ameliorating the severity of many of the pathogenic mechanisms associated with neurological disorders. Indeed, if neuronal dysfunction and loss are caused by reduced energy stores, then therapeutic regimens that buffer intracellular energy levels may impede progression or prevent the neurodegenerative process. Creatine, a naturally occurring compound produced endogenously and acquired exogenously through the diet, is important in maintaining cellular energy homeostasis. As such, creatine has been shown to be one of a number of ergogens that may provide a relatively safe and immediately available therapeutic strategy to patients with neurological disease. Creatine, through its metabolite phosphocreatine, provides a cellular reserve of high-energy phosphates. It plays an important role in cellular high-energy phosphate transport. In addition to ameliorating bioenergetic defects, creatine may also indirectly benefit other pathophysiological mechanisms by improving cellular homeostasis. While the investigational use of creatine as a supplement began in the early 20th century, interest in the use of oral creatine supplementation began in earnest in the 1990s, as it became a widely used ergogenic supplement for performance enhancement by professional and amateur athletes (Greenhaff et al., 1993; Harris et al., 1992; Persky and Brazeau, 2001; Tarnopolsky et al., 1992; Wyss and Kaddurah-Daouk, 2000). Creatine (methylguanidino-acetic acid) is a guanidino compound synthesized endogenously from arginine, methionine, and glycine, predominantly in the liver, as well as in the kidneys, pancreas, testes (Bloch and Schoenheimer, 1941; Persky and Brazeau, 2001; Walker, 1979), and the brain (Braissant et al., 2001). Creatine is also derived exogenously through the diet in the consumption of meat and fish (Balsom et al., 1994). In order to maintain sufficient body stores of creatine, approximately 2 g are required daily through both diet and endogenous synthesis (Casey and Greenhaff, 2000). Over 90% of creatine is found in skeletal muscle, mostly as phosphocreatine (PCr), with the remaining stores in the brain and other organs (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). Significant amounts of creatine are present in tissues with high and fluctuating energy demands. Creatine is non-enzymatically converted to creatinine and excreted through the kidneys (Casey and Greenhaff, 2000). Creatine is also an excellent stimulant of mitochondrial respiration, resulting in the generation of PCr (Kernec et al., 1996; O’Gorman et al., 1996). It is a critical component of the creatine kinase system in maintaining cellular energy needs.

The major source of energy in the brain is ATP, which is tightly coupled to creatine and PCr levels within the cell. Creatine is shuttled across membranes via a sodium-dependent creatine transporter protein, CreaT (Schloss et al., 1994; Snow and Murphy, 2001; Willott et al., 1999). CreaT regulates tissue levels in response to low dietary intake or high endogenous creatine levels (Guerrero-Ontiveros and Wallimann, 1998; Loike et al., 1988). Creatine kinase catalyzes the reversible transfer of a phosphoryl group from PCr to adenosine diphosphate (ADP), forming adenosine triphosphate (ATP). As such, creatine offsets energy depletion by forming PCr, providing a spatial energy buffer to re-phosphorylate ADP to ATP at cellular sites of energy consumption and, in the reversible reaction, forming PCr and ADP
from creatine and ATP at cellular sites of high-energy phosphate production (PCr shuttle hypothesis) (Bessman and Carpenter, 1985; Meyer et al., 1984; Tombes and Shapiro, 1985; Van Brussel et al., 1983). Increasing creatine levels may, therefore, help to prevent reduced energy stores and improve neuronal function. Creatine is also involved in regulating glycolysis, stabilizing the mitochondrial, octameric form of creatine kinase, and inhibiting the mitochondrial permeability transition pore (O’Gorman et al., 1996, 1997). The opening of the mitochondrial permeability transition pore is associated with both apoptotic and necrotic cell death mechanisms (Bernardi et al., 1998). Another potential neuroprotective mechanism of creatine supplementation is the ability of PCr to stimulate synaptic glutamate uptake and thereby reduce the accumulation of extracellular glutamate and the potential for excitotoxicity (Xu et al., 1996). Creatine has also been reported to act as a direct anti-oxidant, scavenging reactive oxygen species that may further potentiate mitochondrial dysfunction if left unchecked (Lawler et al., 2002). While creatine did not significantly reduce levels of hydrogen peroxide or lipid peroxidation in these studies, creatine was effective in reducing superoxide anions and peroxinitrite. Interestingly, the neuroprotective effects of creatine may be independent of mitochondrial creatine kinase (Brustovetsky et al., 2001; Klivenyi et al., 2004). Brustovetsky and colleagues reported that creatine had no effect on the mitochondrial permeability transition pore in isolated brain mitochondria (Brustovetsky et al., 2001). In addition, Beal and colleagues showed that creatine administration in mice deficient in ubiquitous mitochondrial creatine kinase increased brain levels of creatine and PCr, suggesting that the neuroprotective effects of creatine are the result of maintaining PCr and ATP levels and are not due to inhibition of the mitochondrial permeability transition pore (Klivenyi et al., 2004). Of great interest is the fact that oral supplementation of creatine monohydrate (20 g/d over 4 wk) in healthy human volunteers significantly increases the levels of total creatine in the brain (Dechent et al., 1999). Quantitative localized proton magnetic resonance spectroscopy in vivo yielded a statistically significant increase (8.7%) of the mean concentration of total creatine in the brain with region-dependent increases, least in white matter and greatest in the thalamus.

2. CREATINE SUPPLEMENTATION IN NEURODEGENERATIVE DISORDERS

2.1. Huntington’s Disease

Huntington’s disease (HD) is an autosomal dominant inherited neurodegenerative disorder that is characterized by progressive motor dysfunction, emotional disturbances, dementia, and weight loss. HD occurs worldwide, in all races and ethnic groups (Kremer et al., 1994). Its prevalence is 5–10 per 100,000, with a new mutation rate as high as 1–3% (Myers et al., 1993). There are about 30,000 affected individuals in the United States. Another 150,000 Americans have a genetic risk for developing the disease. The average age of onset is approximately 37
years, however the range is from infancy into the 9th decade. There is increasing reason to believe that pathologic alterations occur in the brain for years before symptoms manifest themselves (Paulsen et al., 2001). Once symptomatic, affected individuals are rapidly disabled by early functional decline, and require increasing care and supervision for another 15–25 years before succumbing to the effects of severe physical and mental deterioration. Because of the chronic and increasingly intensive multidisciplinary care it requires, and its genetic nature, HD disproportionately consumes medical, social, and family resources (Helder et al., 2001). The neuropathological hallmark of HD is selective neuronal degeneration, particularly within the neostriatum, in which medium-sized spiny striatal projection neurons are disproportionately affected early and most severely, while large and medium-sized aspiny interneurons are relatively spared (Ferrante et al., 1985; Hersch et al., 2004). While the earliest and most striking neuropathological changes are found in the neostriatum, neuronal loss has also been identified in other regions of the brain (Hersch et al., 2004). Proliferative and degenerative changes in vulnerable neurons suggest that the presence of mutant huntingtin leads to both compensatory and degenerative genetic programs in a prolonged process leading to neuronal dysfunction and death (Ferrante et al., 1991; Graveland et al., 1985). HD is caused by an expanded trinucleotide CAG repeat in the gene coding for the large, highly conserved protein, huntingtin. The huntingtin gene was first cloned in 1993 (Huntington’s Disease Collaborative Research Group, 1993). Emerging evidence suggests it is involved in fast axonal transport (Szebenyi et al., 2003; Trushina et al., 2003), specifically enhancing vesicular transport of brain-derived neurotrophic factor along microtubules (Gauthier et al., 2004; Ross, 2004). In individuals with HD, the polymorphic CAG repeat region near the 5’ end of the gene is expanded beyond the normal range, leading to translation of an expanded polyglutamine stretch in the protein (Huntington’s Disease Collaborative Research Group, 1993). In the normal population the number of CAG repeats varies from 17 to 29. In individuals with HD there are more than 38 repeats. Once expanded into the pathogenic range, there is an inverse relationship between the CAG repeat number and the age of disease onset, with higher repeat numbers associated with younger age. HD is one member of the family of neurodegenerative triplet repeat disorders with anticipation and a gain of function mutation (Robitaille et al., 1997). These include spinocerebellar ataxias, dentato-rubro-pallido-luysian atrophy, Machado-Joseph disease, and spinal bulbar muscular atrophy. As in HD, selective loss of neurons underlies these diseases, and misfolding and abnormal aggregation of the mutant protein occurs. It has thus been hypothesized that neurodegeneration in these disorders may have similar molecular bases (Ross, 1997). As such, therapeutic strategies that are effective in HD will likely be highly relevant for these other disorders as well.

While mitochondrial dysfunction and impaired energy metabolism have not been definitively determined in neurodegenerative disorders, there is strong evidence, particularly in HD, that an energy disturbance plays a prominent role in the pathogenesis of these diseases (Beal, 2005; Ryu et al., 2005; Ryu and Ferrante, 2005). The
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concept of defective cellular energy metabolism in neurological diseases, particularly HD, was suggested by Beal and Albin in separate publications as an alternative excitotoxic hypothesis and has been coined ‘slow onset excitotoxicity’ (Albin and Greenamyer, 1992; Beal, 1992). The relevant observations were first made by Olney, showing that partial membrane depolarization produced NMDA receptor-mediated excitotoxicity in which the voltage-dependent magnesium block was released from the NMDA calcium channel (Olney and de Gubareff, 1978a,b; Novelli et al., 1998; Zeevalk and Nicklas, 1991). Strong evidence exists for early metabolic dysfunction, energy depletion, and a role of increased oxidative signaling in patients with HD. Weight loss occurs early, frequently prior to the onset of the movement disorder (Djousse et al., 2002). Positron emission tomography studies have demonstrated reduced glucose utilization in both presymptomatic and symptomatic HD patients (Kuhl et al., 1985; Kuwert et al., 1990; Mazziotta et al., 1987). Glucose hypometabolism appears early, prior to striatal atrophy (Kuhl et al., 1982, 1985). Magnetic resonance spectroscopy (MRS) has demonstrated a significant decrease in the phosphocreatine to inorganic phosphate ratio in resting muscle in patients with HD and increased lactate concentrations in the cerebral cortex (Koroshetz et al., 1997). In another study, increased lactate levels were reported in both the basal ganglia and occipital cortex in symptomatic HD patients; however, they were not found in HD patients asymptomatic or at risk for the disease (Jenkins et al., 1993, 1998). These findings suggest that there is a progression of impaired energy metabolism.

Energy defects may also result from mitochondrial damage caused by oxidative stress as a consequence of free radical generation. Ultrastructural studies on brain biopsies of HD patients have provided evidence of mitochondrial abnormalities and increased levels of lipofuscin, a pigment that accumulates as a consequence of free radical mediated membrane damage (Tellez-Nagel et al., 1974). Evidence for oxidative damage in HD also includes DNA fragmentation, increased oxidative damage products of protein nitration, lipid peroxidation, and DNA oxidation, and inducible markers for oxidative stress (Browne et al., 1999). Consistent with the findings of mitochondrial damage, we have preliminary data that shows a significant reduction in mitochondrial size and number in striatal caudate neurons in presymptomatic HD patients, with greater loss of mitochondria and further reduced mitochondrial size in moderate to severe grades of HD (Ryu et al., 2005). Impaired energy metabolism may be the result of altered electron transport activities. Indeed, while significant reductions in complex I, II-III, and IV activities are present in the neostriatum from HD patients, they were not found in other brain regions (Brennan et al., 1985; Browne and Beal, 1994; Gu et al., 1996; Mann et al., 1990; Parker et al., 1990). Electron transport chain complex subunits have been reported to be involved in selective degeneration of the basal ganglia in Leber’s optic neuropathy (Howell et al., 1991; Jun et al., 1994) and, as such, it is not unreasonable to suggest that the genetic HD mutation may alter nuclear encoded components of electron transport complexes, resulting in a primary bioenergetic defect. It is of interest to note that in patients with other trinucleotide repeat diseases, such as spinocerebellar
ataxias, mitochondrial abnormalities and metabolic defects are present, linking a common mechanism of energy deficiency to the polyglutamine gene mutation (Mastrogiacomo et al., 1996; Matsuishi et al., 1996).

While the role of huntingtin aggregates continues to be debated, the evidence points to a proximal toxicity residing in mutant huntingtin, its proteolytic fragments, and their interactions with other proteins (Ryu and Ferrante, 2005). A secondary consequence of the gene defect may be impaired energy metabolism via mitochondrial dysfunction (Beal, 2005). N-terminal huntingtin fragments may directly impair mitochondrial function, leading to increased oxidative damage, as mitochondria are a major source of free radicals in the cell. Indeed, it appears clear that such factors as the mass effect of cytosolic and nuclear huntingtin aggregate burden, the sequestration by huntingtin aggregates of transcription factors and neuronal proteins that are essential for neuronal survival and their subsequent reduced activity (Cha, 2000; Sugars and Rubinsztein, 2003), altered proteasomal function (Bence et al., 2001), and the localization of mutant huntingtin aggregates to cellular organelles such as mitochondria (Panov et al., 2002) have a deleterious effect upon neuronal function and survival. Therefore, therapeutic strategies that buffer intracellular energy levels may play an important role in the treatment of HD, and polyglutamine diseases in general.

There is also substantial evidence from experimental models of HD that suggests an important interplay between energy metabolism defects, aberrant mitochondrial function, and excitotoxicity in the pathogenesis of HD (Beal, 1996, 2000; Beal et al., 1993; Browne and Beal, 2004; Browne et al., 1999; Grunewald and Beal, 1999; Palfi et al., 1996; Schulz et al., 1995; Tarnopolsky and Beal, 2001). Both necrotic and apoptotic cell death may be triggered by reduced cellular energy states (Desagher and Martinou, 2000; Green and Reed, 1998; Roy and Nicholson, 2000). Other possible sequelae of energetic deficiency and mitochondrial dysfunction include reduced redox potentials of cellular membranes, dysfunction of the mitochondrial permeability transition pore, and activation of initiator and executioner caspases, each one of which may further contribute to the cell death cascade (Beal, 2000; Green and Reed, 1998; Kiechle et al., 2002). In vitro studies have shown that N-terminal huntingtin fragments may directly impair mitochondrial function, resulting in calcium abnormalities and subsequent energy deficiency (Panov et al., 2002). Consistent with a link between energy depletion and the pathological phenotype of HD, there are a number of mitochondrial inhibitors that act at complexes of the electron transport chain, resulting in high-energy phosphate deficiency and reduced cellular levels of ATP, and mimicking the behavioral and neuropathological phenotype of HD in both primates and rodents (Alston et al., 1977; Beal et al., 1993; Brouillet et al., 1993; Brouillet et al., 1995; Henshaw et al., 1994; Palfi et al., 1996; Schulz et al., 1995). One such naturally occurring plant toxin, 3-nitropropionic acid (3-NP), is an irreversible inhibitor of succinate dehydrogenase and, thus, both the Krebs cycle and complex II activity of the electron transport chain (Candlish et al., 1969; Ludolph et al., 1991). 3-NP exposure is associated with HD-like symptoms in both humans and animals and, as such, has
been used as an experimental model for HD (Alston et al., 1977; Beal et al., 1993; Brouillet et al., 1995; Ludolph et al., 1991, 1992; Palfi et al., 1996). Accidental ingestion of 3-NP in humans results in dystonia with jerk-like movements and bilateral damage to the basal ganglia, as determined by brain imaging (Ludolph et al., 1991). In animals, 3-NP-induced experimental striatal lesions are associated with energetic deficiency, showing marked reductions in cellular levels of ATP (Hamilton and Gould, 1987; Ludolph et al., 1992). Sodium azide, a complex IV (cytochrome oxidase) inhibitor, produces striatal damage and a hyperkinetic movement disorder in primates (Mettler, 1972).

As noted above, creatine administration has several potential neuroprotective effects including buffering of intracellular energy reserves, stabilizing intracellular calcium, reducing extracellular glutamate, inhibiting activation of the mitochondrial permeability transition, and acting as an anti-oxidant. The salubrious neuroprotective effects of creatine have been widely reported in experimental models of neurological diseases, particularly in neurotoxin and transgenic models of HD (Andreassen et al., 2001a; Balestrino et al., 1999; Brewer and Wallimann, 2000; Brustovetsky et al., 2001; Carter et al., 1995; Dedeoglu et al., 2003; Ferrante et al., 2000; Hausmann et al., 2002; Ikeda et al., 2000; Klivenyi et al. 2003, 2004a,b; Malcon et al., 2000; Matthews et al., 1998, 1999; Royes et al., 2003; Ryu et al., 2005; Shear et al., 2000; Sullivan et al., 2000; Zhu et al., 2004). Both creatine and phosphocreatine prevented death of cultured striatal and hippocampal neurons when exposed to 3-NP (Brustovetsky et al., 2001). These studies also showed that creatine decreased mitochondrial swelling induced by inhibitors of creatine kinase octamer-dimer transition. Creatine administration in rodents provided significant protection against 3-NP-induced behavioral and neuropathological phenotype (Shear et al., 2000). Using the mitochondrial toxins 3-NP and malonate to mimic the energy deficiency found in HD, creatine supplementation significantly reduced striatal lesion volumes (Matthews et al., 1998). The neuroprotection by creatine was associated with higher levels of PCR and creatine and reduced lactate levels in the brain, consistent with improved energy homeostasis.

Transgenic animal models have greatly advanced the study of human neurological diseases, providing experimental systems to study molecular pathogenesis and to test potential therapeutic strategies for translation to humans experiencing these diseases. Transgenic mouse models of HD, which have been used extensively to assess potential neuroprotective therapies, have energetic deficits in the brain and are ideal for examining the therapeutic potential of creatine (Beal and Ferrante, 2004; Hersch and Ferrante, 2004). While there are a number of bioenergetic therapeutic agents, including creatine, coenzyme Q\textsubscript{10}, and lipoic acid, that augment energy levels and improve the behavioral and neuropathological phenotype in transgenic R6/2 HD mice (Andreassen et al., 2001b,c; Dedeoglu et al., 2003; Ferrante et al., 2000, 2002), dietary creatine supplementation has had the greatest efficacy (Ferrante et al., 2000). Creatine supplementation formulated at 1%, 2% and 3% in the diet (chow), starting at three weeks of age, significantly improved survival, reduced gross brain atrophy, delayed atrophy of striatal neurons, and
reduced the formation of mutant huntingtin protein aggregates in both the striatum and neocortex. In addition, motor performance was improved and body weight loss was reduced in the creatine-treated R6/2 mice. The brain levels of creatine were significantly increased in the treated mice, as determined using nuclear magnetic resonance spectroscopy, while decreases in N-acetylaspartate (NAA) concentrations were delayed (Ferrante et al., 2000). There was an inverted ‘U’ shaped efficacy curve according to dose, such that 2% creatine in the diet resulted in an approximately 18% extension of survival in the R6/2 mice, while both 1% and 3% creatine chow formulations resulted in approximately 9% and 5% in survival extension, respectively. The reduced efficacy with 3% creatine may reflect down-regulation of the creatine transporter, leading to a relative reduction in brain creatine levels. Creatine treatment in another transgenic model of HD, the N171-82Q HD mouse model, confirmed the initial results (Andreassen et al., 2001a).

The effectiveness of creatine at different stages of the R6/2 HD phenotype have also been examined by initiating creatine administration (2% in the chow) after clinical symptoms appear in the R6/2 HD mice at 6, 8, and 10 weeks of age (Dedeoglu et al., 2003). These time points are analogous to early, middle, and late stage disease in human HD. There was a significant extension in survival in the 6- and 8-week start groups, as well as improved motor performance, body weight, and neuropathology. There is marked bioenergetic impairment in the R6/2 HD mice (Dedeoglu et al., 2003; Smith et al., 2006). While there was a significant reduction in creatine and ATP in the striatum of untreated R6/2 HD mice, creatine and ATP levels were markedly improved by 39% and 65%, respectively, in the creatine-treated R6/2 mice. We recently performed a dose-ranging and efficacy study of high-dose creatine (Foran et al., 2006). The preliminary data shows a dose response effect using 2%, 4%, 6%, and 10% creatine, with greatest survival (22% extended survival) from 6% creatine in the diet. Creatine at the optimal dose (6% in the diet) improved gross brain atrophy, and normalized brain weight and striatal neuronal size to wild-type levels in littermate control mice at 91 days, in comparison to untreated R6/2 mice. These improvements are significantly greater than those previously published (Ferrante et al., 2000). In addition, administration of 6% creatine significantly improved brain ATP and creatine levels, as well as urine 8-hydroxy-2’-deoxyguanosine levels. This is the first instance of parallel efficacy with HD patients (Hersch et al., 2006). These findings are consistent with a role for energy deficiency in HD pathogenesis and suggest that creatine therapy may benefit HD patients if started before or after clinical symptoms are present. Because there have been few phase III studies in HD patients at this time, it has not yet been confirmed that experiments demonstrating improved phenotypes in transgenic mice are predictive of benefits in humans. Similarly, it is unknown whether the magnitude of benefit in mice predicts the magnitude of benefit in humans. Nevertheless, preclinical studies using creatine in HD mouse models have provided the therapeutic rationale for the use of creatine in HD patients. Results from human clinical trials will illuminate the value of mouse clinical trials.
There is strong evidence to suggest that a bioenergetic defect exists in HD, as discussed above. Creatine supplementation is intended to augment cerebral energy reserves and thereby reduce neuronal metabolic and oxidative stress, and slow neurodegeneration. While there have been several clinical trials of creatine supplementation in HD, none of these trials have been powered to detect significant slowing of progression, although some have revealed improvement in clinical outcome measures. These trials have largely demonstrated safety and tolerability of creatine in HD patients. In addition, the biomarkers used in these studies have provided information that supports further trials. Creatine, 3–5 g/d, has been shown to be safe and well tolerated by early-stage HD patients, with blood serum creatine levels increasing over two-fold (Kieburtz, 2001). Verbessem et al. (2003) treated 26 HD patients with 5 g/day creatine and 15 patients with placebo for one year and found no differences in measures of strength, neurological status, or cognitive status. In a one-year open-label pilot study, Tabrizi et al. (2003) treated 13 individuals with HD (3 were presymptomatic, 10 were symptomatic) with creatine (10 g/d) for 12 months. Creatine administration in this study was safe and well tolerated and resulted in increased brain concentrations of creatine as demonstrated by MRS. The United Huntington Disease Rating Scale (UHDRS) scores were unchanged after 12 months. For this reason, the authors suggest that creatine supplementation at 10 g/d may be effective in stabilizing disease progression. In a multi-center double-blind placebo-controlled study of 8 g/day of creatine in 32 HD patients compared to 32 patients on placebo for four months, creatine was safe and well tolerated; however, no effects on the UHDRS were observed due to the short study period. Serum levels of creatine were increased up to 15-fold. Brain levels of creatine were significantly increased by 7.2% and NAA levels (a biomarker of neuroprotection) were increased by 16% (NAA/tCr) as measured by MRS (Hersch et al., 2006). Bender and colleagues used MRS to examine another biomarker of creatine’s activity in HD patients treated with 20 g/day for 5 days, followed by 6 g/day for 8–10 weeks (Bender et al., 2005). They demonstrated a significant reduction in glutamate levels in the parieto-occipital cortex. This is very interesting because glutamate release and excitotoxicity are enhanced by energetic deficiency and are considered to play a significant role in the pathogenesis of HD. While none of these studies were sufficiently powered to be informative about whether or not creatine slows the clinical progression of HD, they do attest to its safety and tolerability, and to its favorable effects on serum and brain levels of creatine and on biomarkers of HD pathology. It remains unclear whether optimal creatine dosing has been determined. It may well be that higher doses of creatine are necessary to significantly slow the disease process. The most efficacious neuroprotective dose of creatine in transgenic mouse studies was 2% of the diet, corresponding to 30–35 g/d in HD patients weighing 70 kg, suggesting that the dose of creatine supplementation in HD patients may have been underestimated. While mouse and human bioavailability may not correspond well, such a dose is at least feasible for humans. In addition, creatine is taken up into the brain by a sodium- and chloride-dependent transporter (Schloss et al., 1994; Snow and Murphy, 2001; Willott et al., 1999). Down-regulation of the
creatinine transporter may occur with extended constant consumption at high doses and may alter the efficacy of creatine supplementation. Improved efficacy may be accomplished using an intermittent dosing regimen of creatine. Consequently, a dose escalation study has been initiated to determine whether there is a maximally tolerated dose in HD, as well as whether there are doses at which serum and brain levels of creatine are maximized.

2.2. Parkinson’s Disease

Parkinson’s disease (PD) is characterized by chronic progressive neurodegeneration of brainstem neurons, particularly dopaminergic neurons in the substantia nigra pars compacta, and the loss of their projection axon terminals in the neostriatum. This loss of dopaminergic neurons is associated with a slow onset of clinical symptoms associated with motor disability. There is a direct correlation between disease progression, neuronal loss, and motor dysfunction (Bernheimer et al., 1973; Damier et al., 1999). Abnormal movements are characterized by both akINESIA and bradykINESIA, resting tremor, rigidity, and gait abnormalities with postural instability and difficulty in initiating movement. The presence of symptoms in PD can be correlated with the loss of dopaminergic neurons when depletion reaches 60%, and with dopamine depletion in the striatum by 60% to 80% (Bernheimer et al., 1973). Although, recent imaging studies show that much less dopamine depletion may be required for detection of early symptoms (Hilker et al., 2005). PD affects approximately 1% of the population over 65 years of age. It is second to Alzheimer’s disease as the most common neurodegenerative disorder. The neuropathological phenotype has been replicated in rodents, using a number of selective toxins, or through genetic manipulation. The toxin-induced models, however, result in acute loss of dopamine, while genetic models have produced more exact parkinsonian pathology and may be more relevant to human PD (Hwang et al., 2003; Jiang et al., 2005; Nunes et al., 2003; Simon et al., 2001; van den Munckhof et al., 2003). Nevertheless, both toxin-induced and genetic models have been important in translational studies for therapeutic clinical trials in PD patients.

While the exact cause of PD is unknown, there is significant evidence to suggest that impaired energy metabolism, as a result of mitochondrial dysfunction, may play a role in the pathogenesis of Parkinson’s disease (Beal, 2005). There is evidence that mitochondrial complex I defects occur in parkinsonism. In idiopathic PD, complex I activity in the electron transport chain is reduced by 30–40% in the substantia nigra (Bindoff et al., 1989; Nicklas et al., 1985). In addition, studies show reduced complex I activity in PD muscle and platelets (Hass et al., 1995; Parker et al., 1989). These findings have been strengthened by recent studies in animal models using selective inhibitors of complex I of the electron transport chain. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a Parkinson syndrome in both humans and in experimental animals that produces motor abnormalities consisting of bradykinesia, increased muscle tone and a characteristic resting tremor. Furthermore it induces marked depletion of dopamine stores.
and results in progressive degeneration of dopaminergic nigrostriatal neurons by a mechanism involving impaired high-energy phosphate production (Beal, 2001). The pathogenesis of neuronal degeneration following MPTP administration has been intensively investigated (Bove et al., 2005). The neurotoxic effects of MPTP are thought to be mediated by its metabolite 1-methyl-4-phenylpyridinium (MPP\(^+\)). MPTP is converted by monoamine oxidase B to MPP\(^+\), and MPP\(^+\) is selectively taken up by the high-affinity dopamine and noradrenaline uptake systems, and is subsequently accumulated within mitochondria of dopaminergic neurons. There MPP\(^+\) disrupts oxidative phosphorylation by inhibiting complex I of the electron transport chain. This can lead to a number of deleterious effects on cellular function. These include impaired intracellular calcium buffering, generation of free radicals in mitochondria, and activation of neuronal nitric oxide synthase, a calmodulin-dependent enzyme.

The effects of creatine supplementation on MPTP-induced parkinsonism have been examined. It has been reported that there was a dose-dependent neuroprotection against dopaminergic neuronal loss in the substantia nigra of mice treated with MPTP (Matthews et al., 1999). The effects of creatine administration on the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) paralleled those seen with dopamine. Creatine produced marked protection against MPTP-induced depletions of dopamine, DOPAC, and HVA. There was no significant loss of nigral neurons in creatine/MPTP-treated animals, as compared to control non-MPTP lesioned mice. Creatine supplementation has also been shown to be protective in organotypic cell cultures of mesencephalic neurons. Creatine provided neuroprotection against MPP\(^+\)-induced tyrosine hydroxylase immunoreactive cell loss. The decrease of TH-immunoreactive neurons in the MPP\(^+\)-treated cells correlated with an increase in immunoreactivity for active caspase-3, which was not seen in cells receiving creatine supplementation (Andres et al., 2005). Recent combination therapeutic studies using creatine and rofecoxib resulted in an additive neuroprotective effect of the two compounds in MPTP-treated mice (Klivenyi et al., 2003).

These findings set the stage for a recent randomized, double-blind, futility clinical trial in PD patients using creatine (NINDS NET-PD Investigators, 2006). Creatine supplementation (10 g/d creatine monohydrate) in PD patients was not rejected as futile and met the criteria for further clinical testing. Creatine was considered well tolerated in this patient population, and 91% of the test population continued throughout the full course of the study. In the 12-month evaluation of the clinical progression of PD, there were no significant differences in the Unified Parkinson’s Disease Rating Scale (UPDRS) scores in comparison to the futility threshold values. While the study was not designed to determine whether creatine was effective in slowing clinical disease progression, the magnitude of the effects of creatine on the UPDRS score progression was comparable to that observed in a recent PD clinical trial with 1,200 mg/d coenzyme Q10 in which the rate of deterioration in the UPDRS was markedly slowed. These findings suggest that interventions, such
as creatine, that improve bioenergetic dysfunction may hold therapeutic promise in PD. A randomized, placebo-controlled Phase III clinical trial is being planned.

2.3. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a clinically severe and fatal neurological disorder characterized by a range of progressive motor disabilities. Degeneration of motor neurons throughout the central nervous system results in progressive muscle wasting and paralysis that leads inexorably to death (Rowland and Shneider, 2001). The cause of selective motor neuron death in ALS is not known. Generally fatal within 2–5 years of onset, ALS has a prevalence of 2–3 per 100,000 people. The incidence of ALS is 1–2/100,000/year and may be rising. It has recently been reported that there is a significantly increased risk of developing ALS in Gulf War veterans and within the military service outside of the Gulf War (Haley, 2003; Horner et al., 2003; Weisskopf et al., 2005). The vast majority of ALS cases occur sporadically, but about 5–10% of ALS cases are familial. The genetic linkage of several mutations in the gene for Cu/Zn superoxide dismutase (SOD1) with some cases of familial ALS provided the first indication of a potential causal factor in the disease process (Rosen et al., 1993). The similarity in the clinical course and pathological features of familial (FALS) and sporadic ALS (SALS) has led a number of investigators to search for genetic mutations associated with FALS as a strategy for elucidating disease pathogenesis and defining novel treatments in both sporadic and inherited forms of the disease. Current medical care focuses on symptom management. Supportive care ameliorates symptoms and makes ALS more manageable for patients and their families, but does not significantly affect the primary disease process. Riluzole, the only FDA-approved ALS therapy, is associated with a 2–3 month prolongation of survival (Bensimon et al., 1994; Miller et al., 2003). To date, no other drug therapies slow or abrogate the disease process in ALS.

Mitochondrial abnormalities have been reported in sporadic ALS (Hirano et al., 1984; Okamoto et al., 1990; Sasaki and Iwata, 1996). Decreased mitochondrial respiratory chain activities have also been found in patients with ALS (Borthwick et al., 1999; Wiedemann et al., 2002). Missense mutations in the enzyme copper/zinc superoxide dismutase (SOD1) cause about 25% of FALS cases (Rosen et al., 1993), whose clinical and pathological features are indistinguishable from those in sporadic ALS. This has prompted the view that all forms of the disease may be better understood and ultimately treated by studying pathogenesis and therapy in rodent models of ALS transgenic mice and rats expressing mutant forms of SOD1 (Andersen et al., 2003; Brown and Robberecht, 2001). Transgenic mice with high expression of FALS mutant SOD genes develop clinical and pathological features similar to those seen in the human disease, including hindlimb weakness and loss of motor neurons (Bruijn et al., 1997; Gurney et al., 1994; Newbery and Abbott, 2002). Beneficial therapeutic trials in transgenic ALS mice have generated clinical trials
in humans with ALS (Choudry and Cudkowicz, 2005; Festoff et al., 2003; McGeer and McGeer, 2005; Rothstein, 2003).

While a number of pathogenic mechanisms of neuronal damage have been implicated (Bruijn et al., 2004; Cleveland and Rothstein, 2001), there is experimental evidence to suggest that they are interdependent and may converge with mitochondrial physiology. One putative mechanism reported to play a role in the pathogenesis of ALS is mitochondrial dysfunction and subsequent impairment of cellular energy metabolism. Mitochondria play a critical role in associated metabolic and apoptotic pathways that regulate neuronal survival. Their altered function may be relevant to motor neuron disease. Evidence that impaired energy metabolism may contribute to the death of motor neurons in ALS comes primarily from model systems. Indeed, both reduced cellular energy and mitochondrial dysfunction have been reported to be early and significant features of motor neuron disease in ALS mice (Kong and Xu, 1998). Cytochrome c release is a marker for mitochondrial dysfunction in ALS models (Zhu et al., 2002). Neuropathological studies of motor neurons in SOD1 transgenic mice revealed mitochondrial vacuolation prior to cell loss (Dal Canto and Gurney, 1994; Wong et al., 1995). SOD1 accumulates in vacuolated mitochondria (Jaarsma, 2006) and mutant SOD1 aberrantly binds to mitochondria where it forms high-molecular weight, SDS-insoluble species (Pasinelli et al., 2004). Mutated SOD1 in mitochondria may cause mitochondrial defects, which contribute to precipitating the neurodegenerative process in motor neurons (Mattiazzi et al., 2002). It has been reported that chronic mitochondrial inhibition leads to selective motor neuron death in vitro (Kaal et al., 2000). Observations in sporadic ALS have found altered mitochondrial morphology in liver, suggesting a role for mitochondrial dysfunction (Masui et al., 1985; Nakano et al., 1987). There is also a high frequency of mitochondrial DNA mutations in cortical ALS motor neurons (Dhaliwal and Grewal, 2000); however, other studies question whether these mutations in fact impair mitochondrial respiration (Gajewski et al., 2003; Swerdlow et al., 1998). Finally, PCr has been shown to be a direct energy source for glutamate uptake into synaptic vesicles. Impaired glutamate uptake has been demonstrated in a transgenic animal model of ALS and is implicated in the pathogenesis of human ALS, linked to loss of the EAAT2 glutamate transporter (Howland et al., 2002; Rothstein et al., 1992, 1995). As such, compounds with beneficial effects on bioenergetics are therefore rational therapeutic candidates for ALS.

Several preclinical studies have found that creatine is beneficial in the SOD1 G93A ALS mouse model (Dupuis et al., 2004; Klivvenyi et al., 1999, 2004). In addition, creatine in combination with minocycline demonstrated additive neuroprotective effects in ALS mice (Zhang et al., 2003). Oral administration of creatine has also been shown to reduce the progression of motor neuron disease in the wobbler mouse (Ikeda et al., 2000). It has been reported that in the G93A transgenic SOD1 mouse model of ALS, creatine supplementation delays onset and slows progression of the clinical and pathological phenotypes at doses of 1–2% in the diet (Klivenyi et al., 1999, 2004). Creatine treatment improved the mean survival for animals by 18% and showed a significant increase in motor performance. There
was a marked amelioration of ventral horn neuron loss in comparison to untreated G93A mice. Cortical concentrations of glutamate as measured by in vivo microdialysis and nuclear magnetic resonance (NMR) spectroscopy were significantly higher in G93A mice compared to littermate wild-type mice at 115 days of age, with attenuated increases in glutamate measured in creatine-treated G93A mice. These results are consistent with impaired glutamate transport in G93A transgenic SOD1 mice. In addition, significant depletions in cortical ATP content in presymptomatic G93A mice have been reported. Reduced ATP was partially ameliorated by creatine administration in these studies, suggesting that bioenergetic defects are involved in the initial stages of mutant SOD1-induced toxicity in G93A mice (Browne et al., 2006).

These translational therapeutic trials in ALS mice suggest that creatine supplementation might be neuroprotective in ALS patients. On this basis, there have been several clinical trials. A double-blind, placebo-controlled, sequential clinical trial was performed to assess the efficacy of 10 g/d creatine monohydrate in 175 ALS patients (Groeneveld et al., 2003). Creatine supplementation, while being well tolerated, showed no benefit on survival or disease progression. In another randomized double-blind, placebo-controlled trial in 104 patients with ALS treated with 5 g/d creatine over six months, there was also no clinical benefit observed (Shefner et al., 2004). It has been suggested, however, that the target dose using bioenergetic nutraceutical compounds may need to be much greater in treating neurodegenerative disorders, particularly ALS, given the fulminant progression of disease (Ferrante et al., 2005). To that end, an open-label dose-escalation trial was recently performed to assess the safety and tolerability of high doses of coenzyme Q_{10} in ALS. In this study, coenzyme Q_{10} was considered safe and well tolerated in 31 subjects treated with doses as high as 3 g/d for 8 months. Based upon these findings and the proposed high-dose (30–35 g/d) clinical trial using creatine in HD, a safety and tolerability trial using high-dose creatine (30 g/d) is being considered for further clinical testing in ALS patients.

## 2.4. Alzheimer’s Disease

Alzheimer’s disease (AD) is a devastating neurodegenerative disease with associated manifest dementia that affects roughly 4.5 million aged adults in the United States today (Bossy-Wetzel et al., 2004). Shifts that will occur in population demographics anticipate the number of affected individuals to exceed 13 million by 2050 (Hebert et al., 2003). With age being a major risk factor, most AD patients become symptomatic in the seventh decade (Mesulam, 2000). AD is the fourth leading cause of death in the United States. The most common feature of the early stages of AD, and perhaps the hallmark symptom of the disease, is the impairment of episodic memory. However, there has been some debate as to whether alterations in sensory and information processing precede these episodic memory impairments (Dudkin et al., 2005). Once manifest, the disease progresses relentlessly. Currently there is no effective treatment to significantly alter the course of AD. The etiology of AD
has been well documented in pathological specimens as concentrated collections of neuritic plaques and neurofibrillary tangles that accumulate in the brain. AD is a complex, multifactorial disease in which several genes act independently or in concert with each other or with environmental agents, resulting in beta-amyloid (Aβ) deposition in the brain, neurofibrillary tangle formation and cell death.

AD has a number of fundamental etiologies that lead to the neuropathological manifestations of the disease. AD patients that are at high risk for developing the disease have impaired cerebral metabolic rate *in vivo* before clinical evidence of disease is present, suggesting that impaired energy metabolism is not solely attributed to the loss of brain substance, and may be a harbinger of disease onset (Blass *et al.*, 2000). Previous data has demonstrated that mitochondrial dysfunction and subsequent impaired energy metabolism are central features of the neuropathological findings that occur in AD brain (Beal, 2005), although this has been somewhat controversial. While alterations in ATP production and concentration have been reported in human AD (Aksenov *et al.*, 2000; Hoyer, 1993; Shoffner, 1997), a spectrophotometric analysis in AD brain homogenates did not find a primary dysfunction in mitochondrial respiratory chain complexes (Casademont *et al.*, 2005). The cytoplasmic BB-CK isoenzyme has been reported to be reduced by 80% in AD brain homogenates (Aksenov *et al.*, 1997). Impaired energy metabolism in AD is also supported by decreases in PCR levels in AD brain as revealed by magnetic resonance spectroscopy (Pettegrew *et al.*, 1994). The loss of ATP is an early event in Aβ neurotoxicity (Mark *et al.*, 1997). Aβ has been reported to cause mitochondrial dysfunction in neurons and, conversely, impaired energy metabolism increases amyloidogenic amyloid precursor protein (APP) processing (Gabuzda *et al.*, 1994; Gasparini *et al.*, 1997, 1999; Webster *et al.*, 1998). Increased mitochondrial dysfunction is demonstrated by a reduction in cytochrome oxidase activity in AD cortex (Mutisya *et al.*, 1994; Parker and Parks, 1995) and an increase in mitochondrial DNA damage in postmortem human AD brain (Beal, 1995; Mecocci *et al.*, 1994). Similarly, mitochondrial mass is reduced in AD cortex. Reductions in ATP formation and an increase in reactive oxygen species, as a consequence of Aβ exposure, have also been observed in cytoplasmic hybrid NT2 cells containing mitochondrial DNA from AD and control platelets (Cardoso *et al.*, 2004; Swerdlow *et al.*, 1997).

Creatine administration has been shown to be neuroprotective against Aβ toxicity in embryonic hippocampal neurons isolated from rat brains (Brewer and Wallimann, 2000). Treatment with creatine increased the molar ratio of PCr/ATP in such neurons by 61%, with the reserve energy ratio rising 4-fold. Neurons were also partially protected from exposure to Aβ using creatine in adult rats, but not in older rats. Many of the mitochondrial abnormalities, such as an age-dependent increase in anti-oxidant enzymes and oxidative protein modifications (Pappolla *et al.*, 1998; Praticò *et al.*, 2001; Rodrigo *et al.*, 2004; Smith *et al.*, 1998), are also observed in transgenic murine models of AD. As such, we have performed preclinical trials in APP Tg2576 mice. By 6 months of age, ATP levels are significantly reduced in Tg2576 mice relative to wild-type littermate control mice. Creatine supplementation
in chow (2% in the diet) starting at weaning resulted in significant neuroprotection in the Tg2576 mice. Creatine treatment protected against reductions in brain weight in APP Tg2576 mice and reduced Aβ plaque burden by 6 months. The fact that there is convincing evidence of impaired bioenergetics in AD and that creatine supplementation ameliorates the biochemical and neuropathological phenotype in APP Tg2576 mice may well be sufficient rationale for supporting clinical trials using creatine supplementation in AD patients.

3. MITOCHONDRIAL ENCEPHALOPATHIES

Metabolic activity within the central nervous system is relatively high, making brain tissue especially vulnerable to mitochondrial respiratory chain abnormalities. Mitochondrial encephalopathies are multi-system diseases characterized by biochemical and genetic mitochondrial defects with differing clinical symptomatologies and brain area involvements. Mitochondrial encephalopathies are age-related with respect to disease onset, occurring mostly in infants and young children, but also in adulthood in some patients (Barisic et al., 2002; Komura et al., 2003; Kremer et al., 1993; Lofberg et al., 2001; Scaglia and Northrop, 2006; Tarnopolsky et al., 1998; Walter et al., 2002). Included in this group of mitochondrial dysfunction disorders are lactic acidosis with stroke-like episodes (MELAS); Leigh syndrome; myoclonus epilepsy and ragged-red-fibers (MERRF); Kearns-Sayre syndrome (KSS); neuropathy, ataxia, and retinitis pigmentosa (NARP); mitochondrial neurogastrointestinal encephalopathy (MNGIE); Alpers syndrome; and coenzyme Q10 deficiency. In these diseases, specific and/or multiple mitochondrial DNA mutations have been detected (Hirano et al., 2006). The exact pathophysiology of these diseases is not completely understood. While there has been little or no consensus in treatment, therapeutic strategies have been adopted as the result of single case studies and are based on the use of anti-oxidants and respiratory chain substrates and cofactors such as creatine and coenzyme Q10. Adult onset encephalopathy involving mitochondrial dysfunction can also occur, as in Wernicke’s encephalopathy (Desjardins and Butterworth, 2005). The latter disorder is more commonly encountered in chronic alcoholism, as well as in AIDS. Chronic alcoholism results in thiamine deficiency with a corresponding decrease in activity of the rate-limiting enzyme of the tricarboxylic acid cycle, which is thiamine diphosphate-dependent. Thiamine deficiency results in selective neurodegeneration, particularly in the thalamus, pons, and cerebellum. While the exact cause of specific neuronal death is unknown, mechanisms associated with neuronal energetic deficiency and subsequent lactic acidosis, and NMDA receptor-mediated excitotoxicity have been implicated.

As such, it has been suggested that creatine supplementation may improve the clinical symptoms of mitochondrial encephalopathies associated with energetic dysfunction (Baker and Tarnopolsky, 2003; Barisic et al., 2002; Hagenfeldt et al., 1994; Komura et al., 2003, 2006; Kremer et al., 1993). In a single case study of a patient with MELAS treated with creatine monohydrate, clinical symptoms were...
ameliorated after four weeks of treatment (Barisic et al., 2002). Proton magnetic resonance spectroscopy performed at 6 and 12 months of treatment showed high creatine levels in the brain. Urinary creatinine excretion increased upon short-term (12 days) high-dosage creatine supplementation (20 g per day), most likely because creatinine is a metabolic product of creatine. In a small study of 5 patients with either KSS or MELAS, creatine administration (0.08–0.35 g/kg body weight/day) resulted in improved measures of skeletal muscle function, with an increase in maximum performance by 12% on average (Komura et al., 2003). In addition, these investigators have shown that in a single study of Leigh syndrome, creatine monohydrate supplementation improved gross and fine motor skills and respiratory and cardiac functions in their patients (Komura et al., 2006). Creatine has also been shown to be effective in patients with mitochondrial cytopathies (Tarnopolsky et al., 1997). A randomized, controlled trial of creatine monohydrate administration in seven patients with mitochondrial cytopathies, primarily MELAS, showed an increased high-intensity anaerobic and aerobic exercise performance. Creatine supplementation resulted in a 19% increase in ischemic, isometric grip strength (P < 0.01) and an overall 11% increase (P < 0.01) in non-ischemic dorsiflexion torque. There is also strong evidence that coenzyme Q$_{10}$ is effective in mitochondrial encephalopathies, especially in MELAS (Abe et al., 1999; Berbel-Garcia et al., 2004; Chen et al., 1997; Ihara et al., 1989; Shinkai et al., 2000). As the therapeutic mechanisms of action of coenzyme Q$_{10}$ and creatine are similar, these studies may be further evidence of the potential efficacy of creatine in mitochondrial encephalopathies.

4. GENETIC AND SECONDARY DISORDERS OF CREATINE METABOLISM

Inborn errors of creatine metabolism in humans may provide better insight into the pathogenic mechanisms associated with these diseases. Mutations in two enzymes responsible for creatine biosynthesis, L-arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT), as well as mutations in a sodium- and chloride-dependent creatine transporter (SLC6A8), have been identified. In patients with GAMT and AGAT mutations, clinical symptoms include mental and motor retardation, extra-pyramidal symptoms, and seizures (see chapter 8; Stockler et al., 2007; Item et al., 2001; Stromberger et al., 2003). The gene encoding SLC6A8 is located on the X-chromosome, thereby usually affecting males, who show more severe mental retardation and absence of speech than affected females (Mancini et al., 2004). While not always consistent, plasma and CSF levels of creatine are usually low in these patients with variable, but usually elevated creatinine levels in urine (Verhoeven et al., 2005). There have been some successes in the use of creatine supplementation in these disorders. Both AGAT and GAMT disorders, which are associated with a lack of creatine and PCr in the brain and other bodily tissues, are improved clinically by creatine supplementation (Mercimek-Mahmutoglu et al., 2006; Stöckler et al., 1996a,b; Sykut-Cegielska et al., 2004;
The Neuroprotective Role of Creatine (Wyss and Schulze, 2002). On the other hand, creatine supplementation has shown no benefit in patients with mutations in SLC6A8 (Anselm et al., 2006). Secondary disorders of creatine metabolism also respond to creatine supplementation. In gyrate atrophy, an autosomal recessive disorder causing hyperornithinemia and resulting in chorioretinal degeneration and type 2 muscle fiber atrophy, creatine therapy increases muscle area, augments PCr/P_i ratios by 1.5-fold, normalizes PCr/ATP ratios, and reduces tubular aggregate abnormalities in skeletal muscle (Heinanan et al., 1999a,b). These patients were treated with creatine for 8–15 years, using 1.5–2.0 g/d, thereby providing evidence that prolonged creatine treatment may be possible in other disorders of creatine and PCr deficiency or dysregulation.

5. ISCHEMIC STROKE

Stroke remains one of the major causes of death and a leading cause of functional impairment, resulting in long-term disability. The latter is manifest by neurological dysfunction and significant reduction in the ability to perform activities of daily living. There are greater than 20 million incident strokes worldwide each year, resulting in more than 5.5 million annual deaths (World Health Report, 2002). Ischemic stroke is by far the most prevalent, accounting for about 88% of all strokes. Despite the advent of treatment using intravenous tissue-type plasminogen activator and the promise of additional acute therapies, effective pre- and post-stroke prevention are paramount for reducing the burden of stroke (Adams et al., 2005; Sacco et al., 1997).

The maintenance of ion gradients across the neuronal cell membrane involves a significant degree of metabolic energy provided by ATP. Within minutes of reduced or lack of blood flow, a cascade of events occur resulting in failure of sodium/potassium pumps, influx of extracellular calcium ions, and subsequent excitotoxicity that ultimately results in neuronal death. Neurons most severely affected by hypoxic injury die rapidly by necrosis, while those neurons that are exposed to a lesser degree of hypoxia in the penumbral zone succumb via apoptosis (Tatsumi et al., 2003). Creatine supplementation may result in improved ability of the neuron to withstand ischemia-mediated energetic deficiency.

Creatine supplementation has been reported to be neuroprotective in an experimental model of anoxia in neonatal mice (Wilken et al., 2000). After 30 minutes of anoxia, both ATP and PCr concentrations were significantly higher in creatine-treated pups than unsupplemented controls, suggesting that hypoxic energy failure in neonatal mice can be prevented by creatine applied before hypoxic events. In a model of transient hypoxic ischemia, six-day-old rats received creatine (3 g/kg/d) for 3 days prior to unilateral common carotid artery ligation followed by hypoxia. The creatine-treated rats showed a significant reduction in volume of brain edema and an increased ‘energy potential’ as reflected by the ratio of PCr to inorganic phosphate that was measured by 31P-magnetic resonance spectroscopy (Adcock et al., 2002). Neuronal cell injury was significantly lower in the cortex of the animals that had received creatine. In a separate report, oral creatine administration resulted in a
marked reduction in ischemic brain infarction and neuroprotection after cerebral ischemia in mice, with a direct correlation between the preservation of bioenergetic cellular status and the inhibition of activation of caspase cell-death pathways \textit{in vivo} (Zhu \textit{et al.}, 2004). Post-ischemic caspase-3 activation and cytochrome c release were significantly reduced in creatine-treated mice. In addition, creatine administration buffered ischemia-mediated cerebral ATP depletion, suggesting that creatine may be neuroprotective in this experimental paradigm through mechanisms independent of mitochondrial cell-death pathways. With respect to the latter, it has been shown that creatine-mediated neuroprotection may involve improved cerebrovascular function (Prass \textit{et al.}, 2007). The authors found a 40\% reduction in infarct volume from transient focal cerebral ischemia after 3 weeks of dietary creatine administration without any changes in bioenergetic status as reflected by brain creatine, PCr, and ATP concentrations. There were, however, increased cerebral blood flow and vasodilatory responses after stroke in creatine-treated mice, suggesting that creatine-mediated neuroprotection may be associated with improved cerebrovascular function. This experimental data demonstrates that there is increased ability to resist ischemic injury by creatine supplementation and that these findings correlate with improved maintenance of energy metabolism and cellular homeostasis. In ischemic stroke, creatine may be multimodal by also inhibiting hypoxia-mediated release of cytochrome c and downstream activation of caspase-3, and may improve cerebrovascular flow. Prophylactic creatine supplementation may be beneficial in patients at high risk for stroke in preventing neuronal damage and loss.

\section{TRAUMATIC BRAIN AND SPINAL CORD INJURY}

Traumatic brain (TBI) and spinal cord injuries (SCI) remain a major health and social problem. These types of injuries often occur in early adulthood and have a major impact for society. Traumatic brain and spinal cord insults result in both immediate mechanical damage and subsequent tissue damage and loss. The frequency, complexity, severity, and diversity of head and spinal cord injury are myriad with extensive long-term disabilities. Approximately 15 million people sustain a TBI or SCI each year in North America. According to the Centers for Disease Control and Prevention, TBI and SCI cost the nation an estimated tens of billions of dollars each year, and treatment of secondary conditions comes at still much higher costs. The leading causes of TBI and SCI are motor vehicle accidents, falls, and being struck by a foreign object. TBI can be quite diverse, depending upon the areas of the brain that are involved. Trauma to the vertebral column alters the spinal cord’s ability to send and receive messages from the brain, resulting in loss of sensory, autonomic, and motor function below the level of injury. The incidence of SCI is highest among young individuals, age 16–30, with males representing approximately 80\%. While there is no current cure for TBI and SCI, ongoing studies in drug testing, transplantation, and surgical therapies continue to make progress. Education and prevention may have the greatest impact in reducing these injuries. Injury can arise immediately after trauma or within days of injury. While
the primary cause of neuronal death is the physical damage of impact, the secondary effects of trauma are less apparent and are associated with edema, ischemia, inflammation, altered calcium homeostasis, and impaired energy metabolism related to mitochondrial dysfunction and ATP loss (Sullivan et al., 1998, 1999; Taoka and Okajima, 1998; Xiong et al., 1997, 1998). ATP loss may also be the result of vascular damage and consequent hypoxia. As such, enhanced neuronal survival in traumatic injuries may be dependent upon adequate ATP supplies. As discussed above, creatine may provide enhanced neuroprotection after these cellular events.

There have been efforts to demonstrate the efficacy of creatine in experimental models of TBI and SCI (Hausmann et al., 2002; Rabchevsky et al., 2003; Scheff and Dhillon, 2004). Surgically-induced cortical contusions representing the sequelae of human closed-head injury were performed in mice. These resulted in severe behavioral deficits, cortical tissue loss, neuronal loss in the hippocampus, and blood brain barrier damage. Intraperitoneal injection of creatine, 3 mg/kg/day up to five days before injury, significantly reduced the TBI tissue damage. Neuroprotection was also observed in TBI rats fed creatine. Mitochondrial membrane potential was significantly greater in creatine-treated rats than untreated animals, with reduced levels of reactive oxygen species in treated rats. Induction of mitochondrial permeability transition was significantly inhibited in creatine-treated TBI animals. In a subsequent study, creatine administration significantly lowered free fatty acid and lactate levels in comparison to untreated rats. Both are markers of cellular injury following TBI (Scheff and Dhillon, 2004). In addition, the neuroprotective effects of creatine have been reported in SCI (Hausmann et al., 2002). Following hindlimb paralysis by surgical laminectomy in adult rats, creatine-treated animals recovered better with greater scores on an open field locomotor test. Creatine-treated rats had significantly smaller volumes of scar tissue. These experimental studies support the concept that creatine supplementation may provide neuroprotection after CNS injury and that a prophylactic dose may be efficacious when given prior to elective brain and spinal cord surgery.

7. EPILEPSY

It is estimated that epilepsy affects 0.5–1% of the population worldwide, with higher rates in developing countries (Hauser and Kurland, 1975). In the United States, there are approximately 2.5–3 million people with epilepsy, with a slightly higher prevalence in men. There are numerous types of epilepsy, primarily because the etiology of epilepsy is related to various risk factors at different ages. In Western developed countries, the risk of epilepsy increases with age, due to tumors, strokes, and neurodegenerative diseases. Epilepsy in children and young adults is usually due to congenital brain disease or one of the idiopathic generalized epilepsies, such as absence epilepsy or juvenile myoclonic epilepsy. Overall, most epilepsies are localization related, with a small percentage being due to idiopathic generalized epilepsies (Loiseau et al., 1990). Localization-related, or focal, epilepsies begin in a small area of dysfunctional cortex and may spread to adjacent areas of the brain or,
eventually, throughout the entire brain. These are usually due to trauma, infection, tumor, stroke, or rare cortical malformations. Idiopathic generalized epilepsies are thought to originate from the thalamus and cause the entire cortex to seize nearly simultaneously.

A seizure is an abnormal, synchronized discharge of a large population of neurons, while epilepsy is defined as recurrent unprovoked seizures. The pathophysiology of seizures is simply an abnormal excitation of neurons with a lack of normal neuronal inhibition. The basic principles of seizures arise from dysfunction of the neuronal membrane resting potential. Intracellularly, sodium is low and potassium is high, while the opposite is true in the extracellular space. Energy, in the form of ATP, is required to maintain this gradient, thereby creating a negative neuronal membrane potential. When there are sufficient excitatory action potentials, voltage-gated sodium channels open and sodium rapidly enters the cell, thereby depolarizing the membrane and creating an action potential. This action potential is propagated along the axon, resulting in neurotransmitter release, which in turn excites an adjacent neuron. When the resting membrane of the cell reaches a certain level, potassium channels are activated, thereby hyperpolarizing the cell and creating a refractory period where the neuron cannot be excited. Sodium-calcium ion channels are believed to be involved in seizure termination. Ion channel mutations have been identified in several nervous system diseases, some of which are inherited epilepsies (Dichter and Wilcox, 1997).

There is substantial evidence for increased metabolism, oxidative damage, and cell death in epilepsy. During a seizure, there is a sudden increase in metabolic demand as evidenced by increased glucose uptake and blood flow to the involved tissue with a subsequent enhanced production of lactate (Theodore, 1999). In animal models, repeated seizures have also been shown to be associated with oxidative damage to cellular components (Liang and Patel, 2004). Neuronal loss and cell death in epilepsy occur through excitatory amino acids, such as glutamate, leading to a sustained depolarization and activation of voltage-dependent sodium channels, influx of chloride and water, and necrotic cell death. In parallel, activation of NMDA receptors by glutamate causes calcium influx into neurons, leading to mitochondrial dysfunction, inhibition of ATP production, increased production of free radicals, and eventually apoptotic cell death.

Mitochondria have been shown to be the major site of oxidative damage in animal models of chemically-induced seizures. Kainate, an excitotoxin resembling glutamate, induces status epilepticus and has been shown to lead to free radical production in mitochondria by inhibition of aconitase with eventual death of hippocampal neurons (Liang et al., 2000). Oxidative damage to mitochondrial lipids (Baran et al., 1987) and DNA (Liang et al., 2000) has also been shown in kainate models of epilepsy. Oxidative damage is found in areas more susceptible to kainate injury, such as in hippocampal CA1 and CA3 fields. There is also a strong correlation between seizure-induced mitochondrial oxidative stress and age (Patel, 2004). Conversely, known mitochondrial mutations are associated with seizures. In MERRF, as discussed above, there are defects in mitochondrial
complexes I and IV that lead to increased oxidative changes and disease pathology (Shoffner et al., 1990). Finally, increased oxidative damage, whether through neurodegenerative conditions or normal aging, may also lead to increased seizure susceptibility. Mice heterozygous for superoxide dismutase 2 (SOD2) have an increased risk of kainate-induced seizures and develop more seizures with age, correlating with mitochondrial dysfunction (Liang and Patel, 2004).

Therapeutic antioxidant compounds have been demonstrated to decrease free radical generation during seizures. In trimethyltin-induced seizures in rats, hippocampi showed an increase in oxidative damage that was ameliorated, as were seizures, with ascorbic acid administration (Shin et al., 2005). Melatonin, an oxygen free radical scavenger and a GABA receptor regulator, when administered to kainate-injected mice, significantly reduced clinical seizure activity and almost completely abolished lipoperoxidation products in the brain (Tan et al., 1998). A ketogenic diet, which decreases blood glucose, has been shown to decrease seizure frequency. In mice fed a ketogenic diet, it has been shown that there is an increase in mitochondrial uncoupling proteins with an associated significant reduction in reactive oxygen species (Sullivan et al., 2004). Finally, some anti-epileptic drugs have been shown to have anti-oxidant properties. Zonisamide has been shown to decrease free radical production associated with seizures in kainate animal models (Ueda et al., 2005; Masumizu et al., 2005).

Creatine, as an intracellular facilitator of high-energy phosphate transport and as an anti-oxidant, makes it a likely therapeutic candidate in epilepsy. There have been many recent spectroscopy studies examining alterations in creatine levels in vitro as well as in vivo in animal models of epilepsy and in humans. In vitro studies showed that stimulated hippocampal neurons fired faster and had faster recovery rates when phosphocreatine (PCr)/NAA levels were higher (Williamson et al., 2005). In in vivo trials, creatine administration has been shown to ameliorate hypoxic seizures in both rabbit pups and rats (Holtzman et al., 1998, 1999). Rabbit pups were given subcutaneous creatine injections three days before inducing hypoxia. Early seizures were prevented and late seizures were significantly reduced by 60%. Additionally, brain levels of creatine, as detected by NMR spectroscopy, initially doubled as compared to controls and eventually reached adult levels (Holtzman et al., 1999).

More recently, it has been shown that seizures and increased lactate production after intrastratial methylmalonate (MMA) injections are significantly decreased by creatine pre-administration (Royes et al., 2003). Brain levels of phosphocreatine, as assayed by HPLC of tissue homogenates, were higher in animals supplemented with creatine, suggesting that creatine may protect against seizure-induced metabolic changes by inhibition of secondary glutamate release. In a subsequent study using the same MMA model, creatine offered protection against protein carbonylation in animals pre-fed creatine (Royes et al., 2006). There has been only one report documenting no protective effect of creatine against seizures (Mikati et al., 2004). Pre-pubescent rats were injected with kainate, leading to status epilepticus, and they then received creatine chow for the subsequent 50 days. Creatine-fed rats showed selective learning impairments, but no difference in spontaneous recurrent seizures.
or hippocampal pathology. These findings might, in fact, be attributable to creatine supplementation occurring after instead of before kainate-induced status epilepticus. R6/2 HD mice also have increased frequency of seizures early on in the disease pathogenesis. As stated above, there is significantly impaired energy metabolism in these mice, consistent with reduced creatine, PCr, and ATP levels (Dedeoglu et al., 2003; Smith et al., 2006). Creatine treatment blocks seizure activity in these mice.

While there are no documented therapeutic trials of creatine supplementation in patients with epilepsy, there is strong evidence that this patient population has decreased intracellular creatine concentrations in the epileptogenic zones. In patients who were candidates for epilepsy surgery, Pan and colleagues found that there was a significant decrease in the PCr/ATP ratio in the ipsilateral amygdala (Pan et al., 2005). It has also been shown that the periphery of cortical malformations is associated with a decrease in NAA/Cr in about 15% of the patients (Mueller et al., 2005). Interestingly, Cohen-Gadol et al. (2004) showed that in mesial temporal lobectomy patients who had undergone pre-operative NMR spectroscopy and post-operative hippocampal pathological analysis, NAA/Cr ratios correlated with neuronal cell loss in most, but not all, hippocampal regions. Surprisingly, the NAA/Cr ratio did not correlate with the duration of seizures. Similar findings have been reported by other investigators (Burneo et al., 2004), suggesting that these changes may be primary and not secondary to seizure activity.

8. CONCLUSION

Neuroprotective compounds targeting identified pathologic mechanisms of disease have the potential to delay the onset and slow the progression of neurological diseases. A large number of studies in both patients and experimental model systems have validated high-energy phosphate deficiency as a promising therapeutic target in neurological diseases. Compounds such as creatine buffer neuronal energy demands and are attractive candidates for targeting this important disease mechanism. Creatine has some advantages over other similar-acting compounds, which include more straightforward bioavailability and the potential to serve as an in vivo biomarker of premanifest and manifest disease. Creatine is available for human use and represents an immediate candidate as a neuroprotective agent for large-scale clinical trials in neurological diseases. Given the safety and tolerability of creatine, it may be especially well suited for long-term use in neurological disorders, providing prophylactic treatment in patients at high risk for recurrent events. Ongoing early phase studies will soon determine an optimal dose range for patients and provide useful biomarkers. An important implication of the multiple levels of molecular pathology and treatment is that it will most likely be necessary to combine neuroprotective therapies to maximize neuroprotection in order to reach the greatest efficacy. As such, creatine may well be suited for use in combination with neuroprotective agents targeting other pathologic mechanisms of disease. Preclinical studies in animal models of neurological diseases testing creatine in
combination with other neuroprotective agents have reported additive efficacy in both clinical and neuropathological outcome measures. It has yet to be determined which combinations might have the most promise for translation to human clinical trials.

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