

Review Article

The Creatine Kinase/Creatine Connection to Alzheimer's Disease: CK Inactivation, APP-CK Complexes, and Focal Creatine Deposits

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Cytosolic brain-type creatine kinase (BB-CK), which is coexpressed with ubiquitous mitochondrial uMtCK, is significantly inactivated by oxidation in Alzheimer's disease (AD) patients. Since CK has been shown to play a fundamental role in cellular energetics of the brain, any disturbance of this enzyme may exasperate the AD disease process. Mutations in amyloid precursor protein (APP) are associated with early onset AD and result in abnormal processing of APP, and accumulation of A β peptide, the main constituent of amyloid plaques in AD brain. Recent data on a direct interaction between APP and the precursor of uMtCK support an emerging relationship between AD, cellular energy levels, and mitochondrial function. In addition, recently discovered creatine (Cr) deposits in the brain of transgenic AD mice, as well as in the hippocampus from AD patients, indicate a direct link between perturbed energy state, Cr metabolism, and AD. Here, we review the roles of Cr and Cr-related enzymes and consider the potential value of supplementation with Cr, a potent neuroprotective substance. As a hypothesis, we consider whether Cr, if given at an early time point of the disease, may prevent or delay the course of AD-related neurodegeneration.

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FUNCTION AND SUBCELLULAR LOCALIZATION OF THE CREATINE KINASE ISOENZYME FAMILY MEMBERS IN BRAIN

Large amounts of energy are required to maintain the signalling activities of the cells in the central nervous system (CNS). The dominant share of energy consumption in the brain can be assigned to brain function-related processes, for example, for maintenance of membrane potential by the Na⁺/K⁺-ATPase, Ca²⁺ homeostasis by the Ca²⁺-ATPase, neurotransmitter processing, intracellular signalling, and axonal as well as dendritic transport [1]. Mechanisms to facilitate energy transfer within cells that require fluctuating high energy levels, such as those in skeletal muscle, heart, and brain, include the juxtaposition of intracellular sites of ATP generation with sites of ATP consumption, as well as the transfer of high-energy phosphates between these sites by the creatine kinase (CK)/phosphocreatine (PCr) system [1, 2].

CK is categorized into four isoforms based on its tissue expression (muscle or brain) and subcellular distribution (cytosolic or mitochondrial). In sarcomeric muscle, dimeric cytosolic muscle-type CK (MM-CK) is localized to the M-band [3], the sarcoplasmic reticulum (SR) [4, 5], and the plasma membrane. At these sites, MM-CK is functionally coupled to the myofibrillar actomyosin ATPase [6–8], the SR Ca²⁺-ATPase [4, 5], and the plasma membrane Na⁺/K⁺ ATPase [9], respectively, and utilizes PCr for local in situ regeneration of ATP. In the brain, the dimeric cytosolic form of CK is called brain-type CK (BB-CK). The octameric mitochondrial CK (MtCK) is classified into two forms: sarcomeric muscle form (sMtCK) and brain form called ubiquitous MtCK (uMtCK) [10, 11]. Both MtCKs are located in the mitochondrial intermembrane space [12], along the entire inner membrane and also at peripheral contact sites [13], where inner and outer membranes are in close proximity [14, 15]. There, MtCK can directly transphosphorylate

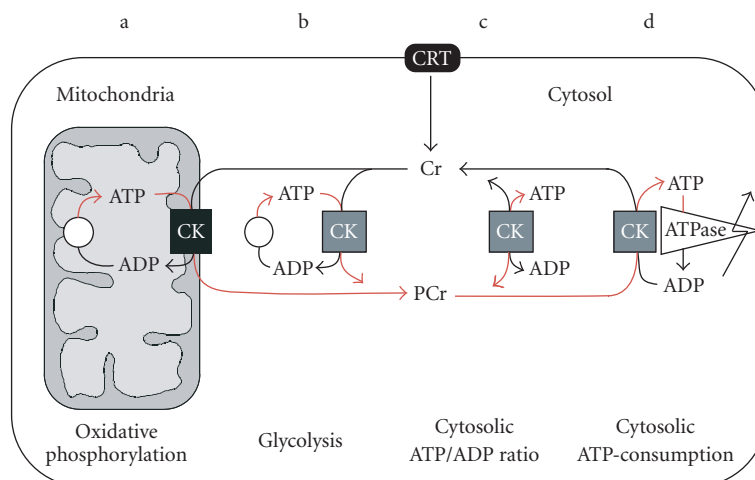


FIGURE 1: The CK/PCr system.

intramitochondrially produced ATP into PCr [16], which is then exported into the cytosol.

uMtCK is always coexpressed with dimeric cytosolic BB-CK [17] at various levels throughout the entire brain. In cerebellum, both of these CK isoforms are found highly concentrated in the glomeruli structures of the cerebellar granular layer. However, the level of BB-CK is much higher than uMtCK in cerebellar Bergmann glial cells. In addition, both isoforms are highly expressed in the choroid plexus and in hippocampal granule and pyramidal cells. The hippocampus is important for learning and memory and is most severely affected in AD [18].

Generation of ATP, hence CK activity, is critical for CNS function. Neurons require a great amount of ATP to maintain membrane polarization, Ca^{2+} influx from organelles, processing of neurotransmitters, intracellular signalling systems, and axonal and dendritic transport [1]. Interestingly, CK is specifically associated with these important processes. On a subcellular level, BB-CK has been found in association with synaptic vesicles [19] and synaptic plasma membranes [20]. On the other hand, supporting glial cells also require ATP for neurotransmitter uptake. In the rat hypothalamus, BB-CK plays an essential role in regenerating ATP for glutamate clearance during excitatory synaptic transmission [21]. Therefore, the number of synapses and synaptic plasticity can be profoundly regulated by ATP levels in neuronal and non-neuronal cells [22].

During brain development, there is a coincidence in the timing of maximal expression of BB-CK and myelin basic protein in the cerebellum which is an indication for a role of BB-CK in myelination [23]. Both BB-CK and uMtCK levels are increased in a coordinated fashion during postnatal brain development [24]. In brain, CK has been shown to be associated with synaptic membranes [25] and to facilitate glutamate uptake into vesicles [26], thus being directly involved in the energetics of neurotransmitter uptake. CK has also been shown to be associated with acetylcholine

receptor-rich membranes [27] and to be involved in quantal release of acetylcholine in synaptosomes [28]. Further, CK together with enolase are part of a complex which is involved in axonal transport [29] and thus support the energetics of these transport events. CK has also been shown to be functionally coupled to the Na^+ - K^+ ATPase [9, 30], as well as to the ATP-gated K^+ -channel [31, 32]. This seems to be important due to the fact that about 50% of total brain energy is used by the Na^+ ion pump [1]. In addition, CK knock-out mice display a significant neurological phenotype [33, 34]. Based on these findings, a functional CK/PCr energy shuttle system has been proposed [35], where BB-CK and uMtCK would constitute an efficient energy buffering and shuttle system in brain [36], similar to that observed in muscle.

THE CREATINE KINASE/PHOSPHOCREATINE SHUTTLE SYSTEM IN THE BRAIN

As mentioned above, the major energy-consuming process in neural cells is the transport of ions by the Na^+/K^+ -ATPase [37]. Even though the cellular pools of ATP are rather small and the movement of ATP within cells by diffusion is slow [1], no significant change in overall ATP levels can be detected during activation of excitable tissues [38]. This is because ATP is continuously and efficiently replenished from the large pools of PCr through the CK reaction, as has been shown in detail in muscle cells [39–42]. The CK isoenzymes catalyze the reversible transfer of the high-energy N-phosphoryl group of phosphocreatine (PCr) to ADP to yield ATP. The concept of the creatine kinase/phosphocreatine (CK/PCr) shuttle system (Figure 1) describes the functional association of CK isoenzymes with discrete intracellular compartments at sites of ATP production and utilization. Thus, PCr and Cr serve as cytosolic energy transducers to connect these intracellular sites and together with precisely localized CK isoenzymes constitute an organizational feature that increases the efficiency of energy metabolism [1, 39, 41].

The CK/PCr system functions as a temporal and spatial energy buffer, as well as a regulator of cellular energetics [39, 42, 43]. It maintains high global ATP/ADP ratios by preventing a rise in intracellular free ADP and thus preserves the thermodynamic efficiency of ATPases even at high cellular ATP turnover [36]. By this mechanism, an inactivation of cellular ATPases by rising [ADP] is avoided and a net loss of adenine nucleotides is prevented [39, 44, 45]. Thus, the CK/PCr system is a rapidly available source for ATP transport and resynthesis not only in muscle but also in the brain. The high activity of CK in the brain, together with high concentrations of its substrates, PCr and Cr, as well as the phenotype of mice deficient in brain-type CK isoforms [34, 35] and the effects of Cr supplementation on brain function (see below) strongly indicate that CK is a key enzyme in brain energy metabolism [46] and that PCr is an important energy reservoir and energy transport molecule [47]. A schematic drawing of the subcellular micro-compartmentation of CK enzymes and their colocalization with ATP-producing and -consuming sites within the cell is depicted in Figure 1 (from Schlattner U and Wallimann T. Metabolite channeling: creatine kinase micro compartments, to (Lennarz WJ, and Lane MD, eds.) *Encyclopedia of Biological Chemistry*. Vol 2. New York, USA: Academic Press; (2004):646–651; with permission by ELSEVIER Publishing Company).

Isoenzymes of CK are found in different compartments such as mitochondria (a) and the cytosol (b)–(d) in a soluble form (c) or associated to a different degree to ATP-delivering processes, for example, mitochondrial oxidative phosphorylation (a) or glycolysis (b) or to ATP-consuming processes, like ATPases or other ATP-requiring or ATP-regulated processes (d). A large cytosolic PCr pool up to 30 mM is built up by CK using ATP generated by oxidative phosphorylation (a) or glycolysis (b). PCr is then used to buffer global (c) and local (d) ATP/ADP ratios. In cells that are polarized and/or have a very high or localized ATP consumption, these CK isoenzymes, together with easily diffusible PCr, also maintain an energy shuttle between ATP-providing and ATP-consuming processes (a), (d). Metabolite channeling occurs where CK is associated with ATP-providing or ATP-consuming transporters, ion pumps, or enzymes that are operating also in brain (a), (d). Cr is synthesized mostly in kidney and liver. Cells can take up Cr from the blood stream by a specific creatine transporter CRT. In brain, CRT is prominently localized at the blood-brain barrier, but is also seen on the plasma membrane of neurons [48–51].

Details on the importance of CK and its substrates for brain function are revealed by recent studies on the neurological and behavior phenotype of CK knockout mice [33, 34]. Mice lacking the expression of one CK isoform, cytosolic BB-CK or uMtCK, display abnormalities in formation and maintenance of hippocampal mossy fibre connections and in behaviors such as habituation, spatial learning, and seizure susceptibility [34]. On the other hand, adult mice lacking both BB-CK and uMtCK, the so-called CK double knockout mice, display reduced body weight and are severely impaired in spatial learning in both dry and wet maze, and display lower nest building activity and acoustic

startle reflex responses [34]. Morphological analysis of CK double knockout brains revealed a reduction of brain weight and hippocampal size, a smaller regio-inferior area, and relatively larger supra-pyramidal and intra-infra-pyramidal mossy fiber area [34]. These results suggest that the lack of both brain-specific CK isoforms renders the synaptic circuitry less efficient in coping with sensory or cognitive activity related challenges in the adult brain and fully support the physiological importance of CK for normal brain function.

CREATINE SYNTHESIS AND UPTAKE IN BRAIN

In vertebrates, Cr is synthesized mostly in the liver and kidney and is then transported through the blood and taken up by target tissues with high energy demands. Cr biosynthesis involves two sequential steps catalyzed by L-arginine: glycine amidinotransferase (AGAT) and S-adenosylmethionine:guanidinoacetate N-methyltransferase (GAMT) [51]. It has been shown that a certain amount of Cr is synthesized endogenously in the developing brain [51–53], and recently both AGAT and GAMT, as well as creatine transporter (CRT) have been identified and localized in distinct cell populations of the developing brain [51, 52]. GAMT immunoreactivity is very strong in oligodendrocytes, moderate in astrocytes, and not detected in embryonic neurons. These observations led to the conclusion that Cr in neurons is derived in part from local glial populations surrounding the neurons, indicating a novel neuron-glial relationship involving Cr trafficking [49]. However, the majority of Cr seems to get taken up continuously through the blood-brain barrier by CRT [48], which works against a huge Cr gradient [54]. Nevertheless, certain brain cells seem to have the capacity for endogenous Cr biosynthesis, especially in the developing brain [51]. Patients with genetic CRT-deficiency lack any detectable Cr in the brain [55] and have severe neurological phenotypes including hypotonia, developmental speech delay, autism, and brain atrophy [55, 56]. These cases emphasize the importance of the substrates of CK, Cr, and PCr, for normal brain function in man.

DISTURBED ENERGY METABOLISM IN NEURODEGENERATIVE DISEASES

A common feature of severe neurodegenerative disorders, such as Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and AD are mutations in nuclear or mitochondrial DNA. This leads to secondary mitochondrial dysfunction accompanied by a more or less severely disturbed energy metabolism as well as a disturbed energetic status of the brain [57]. Cellular energy reserves are important for normal brain function, however, the cellular energy state also appears to play a key role in regulating and initiating apoptosis or necrosis of brain cells, since mitochondria are known to be essential in controlling specific apoptotic pathways [58]. For example, in patients with ALS, a chronically deficient intake of energy [59], increased mitochondrial volume (swelling), oxidative damage, and decreased complex I activity have been observed [60]. Further,

a loss of mitochondrial membrane potential and chronically elevated cytosolic Ca^{2+} -levels accompany these observations [61].

One of the most important hallmarks in the pathogenesis of senile dementia of the Alzheimer type (AD) is the marked decrease of cerebral glucose metabolism [62] caused by disturbed acetyl-CoA synthesis and critically lowered oxidative phosphorylation [63]. Measurement of local cerebral glucose metabolism by positron emission tomography (PET) has become a standard technique to study dementia [64]. By this method, a regional impairment of cerebral glucose metabolism in neocortical association areas in the brains of AD patients could be shown [64]. In addition, cortical acetylcholine esterase activity is significantly lower in patients with AD compared to age-matched normal controls [64]. A decrease of the oxidative energy metabolism in senile dementia and the resulting ATP deficit may thus change protein degradation, synaptic transmission and ion homeostasis [63]. Furthermore, disturbed function and abnormal morphology of mitochondria are also associated with AD and PD [65].

A global decrease in cerebral metabolic rate also occurs in AD and in other dementias, and the AD brain is characterized by a variable, but often marked, loss of neurons, a deposition of extracellular plaques, and intracellular neurofibrillary tangles [66–68]. Impaired energy metabolism [68] and altered cytochrome c oxidase activity are among the earliest detectable defects in AD [69–72]. Most recently, focal deposits of Cr have been discovered in AD [73], suggestive of a perturbed energetic status and deregulated Cr synthesis and/or uptake (see further below).

MITOCHONDRIAL ASPECTS OF NEURODEGENERATION

The involvement of mitochondria in neurological disorders is frequently discussed. It is known that pathological states and mitochondrial dysfunction often lead to the excessive generation of free radicals and subsequent oxidative damage [74]. Studies of AD patients have identified decreased complex IV activity and mitochondrial DNA mutations [75, 76]. Recently, a role for mitochondria has been indicated in $\text{A}\beta$ -induced apoptosis. The $\text{A}\beta$ -binding alcohol dehydrogenase (ABAD) has been reported to interact with $\text{A}\beta$ in the mitochondria of AD patients and transgenic mice [77] and to potentiate $\text{A}\beta$ -induced apoptosis and free-radical generation in neurons. Furthermore, in brains from patients with autopsy-confirmed AD and clinical dementia ratings before death, the activity of tricarboxylic acid cycle (TCA) enzymes (pyruvate dehydrogenase complex, isocitrate dehydrogenase, and the alpha-ketoglutarate dehydrogenase) of mitochondria were significantly decreased. Changes in TCA cycle activities correlated with the clinical state of the disease, suggesting a coordinated mitochondrial alteration [77]. Recently, a structural and functional interplay between dendritic mitochondria and spines/synapses was discovered in in vitro cultured neurons [78]. A small fraction of mitochondria is present within dendritic protrusions of cultured neurons [78]. Interestingly, in these cultured neurons, Cr supplementation enhances mitochondrial activity and causes a higher density

of spines and synapses. Remarkably, the ability of neurons to form new excitatory synapses in response to stimulation is also correlated with increased activity of dendritic mitochondria [78]. Neuronal activity itself affects the motility, fusion/fission balance, and subcellular distribution of mitochondria in dendrites, depending on calcium influx. This seems to be physiologically relevant, because repetitive depolarization that stimulates synapse formation causes the redistribution of mitochondria into dendritic protrusions [78]. These results suggest a local involvement of mitochondria in synapse formation and development. Taken together, these findings are in agreement with the concept that the characteristic loss of synapses in disorders like AD arises in part from mitochondrial dysfunction [79].

PERTURBED CK FUNCTIONS AND LOWER PCR/CR RATIOS ARE LINKED TO NEURODEGENERATIVE DISEASES INCLUDING AD

Oxidative alterations of proteins and lipids have been implicated in the progression of neurodegenerative disorders [80, 81]. Protein carbonyls, considered a marker of protein oxidation, are increased in AD [82]. Using a proteomic approach, BB-CK, glutamine synthase (GS), and ubiquitin carboxy-terminal hydrolase L-1 (UCH L-1) were identified as the three major specifically oxidized proteins in AD brains [83]. Oxidative modification of CK rapidly inactivates the enzyme and results in abnormal partitioning of CK between the soluble and pellet fractions [84]. As a consequence, CK activity in AD brain homogenates is decreased by 86%, as shown by [α 32P]8N3ATP incorporation into the enzyme, but the expression level of CK is decreased by less than 14% [84]. These findings can be explained by the fact that all CK isoforms possess a highly reactive cysteine residue that is specifically modified by sulfhydryl reagents or oxygen or peroxynitrite radicals [85]. Loss of BB-CK activity [82, 84, 86, 87], resulting from its oxidation [87], implies that the maintenance of a healthy cellular energy state is perturbed in the AD brain and that energy supply in glia cells, neurons, and synaptic elements is altered. This is corroborated by one study, where Alzheimer's patients were found to have reduced levels of brain PCr in early stages of the disease and decreased oxidative metabolism in later stages compared to healthy persons, indicating that the AD brain is under energetic stress [88].

FORMATION AND PRESENCE OF FOCAL CREATINE DEPOSITS IN AD BRAIN

The recent discovery of Cr deposits in the brains of transgenic APP mice by Fourier transform infrared microspectroscopy (FTIR) in frozen and desiccated brain slices [73] raises many questions concerning its role in AD. At present, it is not known whether the creatine exists as precipitated microcrystals or as localized, sequestered pools in vivo. In this section, we speculate on some possible origins for these deposits.

Cr is a very prominent compound in both muscle and brain, where total Cr (PCr plus Cr) may reach 50 mM or 20 mM, respectively, the latter being strongly dependent on the region of the brain [36]. Intracellularly, under normal energetic conditions, 2/3 of total Cr is in the form of energy-rich PCr and 1/3 in the form of Cr. During cellular energy stress, the PCr/Cr ratio decreases. Cr is less insoluble than PCr, having a solubility limit of roughly 100 mM in aqueous solution, depending on temperature and pH. Thus, in glycolytic skeletal muscle that is highest in total Cr, this solubility limit is nearly reached if all PCr was converted to Cr. In brain this is less likely, given the lower total Cr concentration. However, it is conceivable that upon massive destruction of neurons in AD accompanied by lysis and cell death, significant amounts of Cr are set free within the region of brain cell destruction. Thus, if concentrated, Cr might precipitate in the extracellular space of the brain, giving rise to focal deposits *in vivo*. However, no such neuronal loss is observed in the transgenic APP mice, nor are there obvious changes in cell morphology that would support such a hypothesis. In fact, the focal Cr deposits are seen in frozen brain sections that have to be desiccated before FTIR microspectroscopy measurements [73]. It is therefore not unreasonable to suppose that under *in vivo* (hydrated) conditions, the elevated Cr exists in solution, for example, inside intact cells or in vacuoles or other subcellular compartments, and that this Cr only solidifies into focal microcrystals when the tissue is dried.

Another explanation is based on a breakdown in the synthesis and/or neuronal uptake pathway of Cr. Some glial cells, especially oligodendrocytes, synthesize Cr, which is then released to be taken up by neurons [49, 51]. AD is accompanied by inflammation and an increase in the number of glial cells, providing an additional source of Cr. Neurons normally take up Cr from the extracellular space by CRT. If, however, neurons were energetically stressed, Cr uptake could be limited, because the uptake of Cr via CRT is accompanied by concomitant Na^+ and Cl^- cotransport into the cell [54]. This Na^+ has to eventually be pumped out of the cell by the ATP-driven Na^+/K^+ -ATPase that also uses a major part of cellular energy [30]. Thus, if Cr uptake into neurons was hampered, the net result would be a slow accumulation of Cr in the extracellular space.

A third possibility is that the oxidation of BB-CK and uMtCK [85] limits the formation of PCr, which is in turn depleted to support ADP to ATP conversion, thus favoring the generation of excess Cr.

Our recent data (see below) raise yet another possibility that uMtCK targeting to the mitochondria may be disrupted by a loss of APP function [89], which in turn would result in a decrease in synthesis of PCr and a concomitant accumulation of Cr within cells.

Since the microdeposits of Cr detected by FTIR microspectroscopy are found distributed focally across large regions of the hippocampus in the transgenic AD model mice [73], occasionally colocalized on the edges of AD plaques, it is likely that at least some of the Cr deposits are not intracellular, but rather in the extracellular space. They could

be generated either by Cr leakage from dying cells or by impaired Cr trafficking between glia and neurons, as outlined above. However, irrespective of primary course, the factor triggering Cr deposits would be a disturbed energy charge of neurons under stress.

INTERACTION AND COMPLEX FORMATION OF APP WITH MITOCHONDRIAL CREATINE KINASE

Recent biochemical data from our laboratories provide a direct link between APP and uMtCK and shed new light on putative molecular mechanisms that lead to energetic abnormalities in AD brain (discussed above) [89]. Using a functional proteomic screen, in which APP interacting proteins were isolated based on their biochemical affinity and identified by peptide mass fingerprinting, we found that the short cytoplasmic tail of APP family proteins interacts with several different mitochondrial targeted proteins (see [89] and Li and Homayouni, unpublished observations). This interaction was of high affinity toward the preprotein forms, containing the N-terminal signal sequence, of the mitochondrial proteins. Importantly, coexpression of APP C-terminal regions dramatically stabilized uMtCK in cultured cells. APP family proteins are type-I transmembrane glycoproteins that undergo sequential N- and O-linked glycosylation in the ER/Golgi pathway. Using co-immunoprecipitation assays, we found that uMtCK associated with the full-length but lower molecular weight APP proteins, suggesting that the interaction occurs prior to maturation of APP proteins. Immunohistochemical analysis indicated that APP and uMtCK colocalize in ER/Golgi and not in mitochondria of cultured primary neurons as well as in transiently transfected nonneuronal cells. These results raise the possibility that APP family proteins may function as cytoplasmic chaperone-like proteins to stabilize mitochondrial proteins such as uMtCK. Indeed, APP is induced and accumulates in the ER/Golgi of cultured cells after treatments that induce oxidative metabolic stress or ER stress via disruption of the ER folding machinery, thus affecting protein maturation and causing accumulation of unfolded proteins within the ER lumen [90, 91]. In turn, some evidence indicates that APP induction can play a protective role against cell stress and axonal injury [91, 92].

Why then only mutations in APP, and not its other family members, have been linked to AD? We propose that mutations in APP result in a dual attack on the mitochondria. First, these mutations enhance the generation of $\text{A}\beta$ peptide [93], which were shown to be directly toxic to mitochondria through an interaction with ABAD [77]. Second, based on our recent data, we speculate that the loss of the normal function of APP in targeting of uMtCK, and perhaps other mitochondrial proteins to the mitochondria, would result in a further compromise of mitochondrial function in the affected neurons. This hypothesis is consistent with a stochastic model proposed by Clarke and colleagues [94], in which the mutated cell exists in an altered steady state and upon a random insult initiates a cascade of events resulting in cell death. It has been shown that mutations in APP

increase the vulnerability of cells to oxidative stress [91, 95]. Although, these findings pertain to APP mutations, which are linked only to early-onset AD, they suggest a more general mechanism for the pathogenesis of AD involving dysregulation of mitochondrial function [65, 96, 97].

NEUROPROTECTIVE EFFECTS OF CR SUPPLEMENTATION FOR NEURODEGENERATIVE DISEASES

Over the past decade, the ergogenic benefits of synthetic Cr monohydrate have made it a popular dietary supplement, particularly among athletes [98]. The anabolic properties of Cr also offer hope for the treatment of diseases characterized by muscle weakness and atrophy, as well as for rehabilitation [99]. In serum-free cultured cells, Cr supplementation has been shown to protect rat hippocampal neurons against glutamate and A β toxicity [100]. In an animal model of traumatic brain injury (TBI), it has been shown that Cr supplementation protects against neuropathology of TBI through mechanisms involving maintenance of mitochondrial bioenergetics and preservation of ATP levels [101]. This is due to the fact that newly entered Cr is phosphorylated inside the cell by the catalytic activity of CK, leading to an increased PCr/ATP ratio and, thus, a higher energy charge in the cell. Brustovetsky et al demonstrated neuroprotective effects of PCr and Cr pretreatments against energetic deprivation caused by 3NP and glutamate excitotoxicity in cultured neurons [102]. There, surprisingly, extracellular PCr was more efficacious than Cr. This could be explained by the fact that PCr is able to bind to and stabilize cell membranes [103].

Recent data using human fetal striatal and mesencephalic tissue identified Cr as a potent natural survival and neuroprotective factor for GABA-ergic neurons in a model for HD [104] and of dopaminergic neurons in a model for PD [105–107]. Cr is also beneficial in animal models of cerebral ischemia [108–110] and spinal cord injury [111, 112]. In the G93A transgenic mouse model for ALS, long-term Cr supplementation extends life span, significantly improves motor coordination [113], and reverts the cholinergic deficit in some forebrain areas at an intermediate stage of ALS [114]. In rat and mouse models of cerebral ischemia, oral Cr administration resulted in neuroprotection and remarkable reduction in ischemic brain infarction [109, 115]. Postischemic caspase-3 activation and cytochrome *c* release were significantly reduced in creatine-treated mice. Cr administration buffered ischemia-mediated cerebral ATP depletion [115]. These authors suggest that a prophylactic Cr supplementation, similar to what is recommended for an agent such as aspirin, may be considered for patients in high stroke-risk categories.

Supplementation with Cr has been used as an adjuvant to a therapeutic scheme in numerous diseases associated with muscle and neuromuscular degeneration. To date, two clinical pilot trials to test the efficacy of Cr monohydrate in ALS have been completed without any measurable improvements in overall survival or in a composite measure of

muscle strength [116, 117]. However, these pilot studies were powered only to detect a 30–50% or greater change in rate of decline of muscle strength. These trials raised new questions about the optimal dosage of Cr and its beneficial effects on muscle fatigue, a measure distinct from muscle strength. A large multicenter clinical trial is currently underway to further investigate the efficacy of Cr monohydrate in ALS and to address these unresolved issues. To date, evidence shows that Cr supplementation at 5–10 grams over a time period of 12 months has a good safety profile and is well tolerated by patients with ALS.

In a trial with HD patients, Cr supplementation lowered brain glutamate levels [118]. Very recent data from a randomized, double-blind, placebo-controlled study with 64 subjects with Huntington's disease (HD), 8 g/day of Cr administered for 16 weeks, show that Cr was well tolerated and safe. Serum and brain Cr concentrations increased in the Cr-treated group and returned to baseline after washout. Intriguingly, serum 8-hydroxy-2'-deoxyguanosine (8OH2'dG) levels, an indicator of oxidative injury to DNA, were markedly elevated in HD, but were reduced significantly by Cr treatment [119]. In patients with a novel cytochrome *b* mutation, Cr supplementation attenuated the production of free radicals and the paracrystalline intramitochondrial inclusions [120] brought about by crystallization of over-expressed MtCK inside mitochondria [121]. The rationale for the use of Cr along with available evidence from animal models and clinical trials for ALS and related neurodegenerative or neuromuscular diseases have been described in [122]. Thus, it is obvious that Cr as a simple nutritional supplement shows a great potential for neuroprotective effects in various neuromuscular and neurodegenerative diseases.

RATIONALE FOR CREATINE SUPPLEMENTATION IN ALZHEIMER'S DISEASE

Very recent data by Snyder et al show that A β addition to cortical neurons in cell cultures leads to internalization of NMDA-receptors with concomitant dephosphorylation of the NMDA receptor subunit NR2B at Tyr 1472 [123]. Since it has been shown earlier that Cr supplementation significantly protects neurons against A β neurotoxicity [100], it can be inferred that Cr may indirectly benefit AD patients by reducing the effects of A β toxicity and NMDA-receptor internalization. It may also alleviate the deterioration of glutamatergic neurotransmission and synaptic plasticity that are vital for learning and memory. For example, treatment of hippocampal neurons with 20 mM Cr significantly increased both basal and activity dependent synaptogenesis [78]. In two studies, Cr supplementation has been shown to improve mental concentration [124], as well as memory and learning [125] in healthy human subjects. It is possible that this will also be true for early stage AD patients.

Given the evidence for metabolic dysfunction in AD, we hypothesize that Cr supplementation at an early time point of the disease might be useful in compensating for the disturbed energy metabolism in subjects with AD by replenishing the energy pools, activating mitochondrial respiration

[126, 127] and protecting cells from apoptosis [127, 128]. Although Cr cannot increase energy charge if CK is damaged, for example, by oxidative damage (see below), very early in the course of AD, CK is still functioning to some extent, so it is reasonable to assume that Cr may be of benefit in those early phases. Further, CK isoenzymes are known to be prime targets of oxidative damage by free radicals [85–87] that are a hallmark of many neuromuscular and neurodegenerative diseases. The substrate Cr, together with MgADP or MgATP, upon forming a transition state complex in the active site of CK, has a protective effect against inactivation of CK isoenzymes by free radicals, such as oxygen radicals and peroxynitrite. In the case of MtCK, Cr in the presence of nucleotide, additionally prevents the dissociation of native octameric MtCK into dimers [85]. Thus, an elevation of the intracellular concentration of Cr by Cr supplementation may confer additional protection to CK and concomitantly delay the free-radical induced inactivation of the CK system in brain that is seen in AD [87].

Cr might be expected to improve energetic conditions for all cells, as well as for “at risk neurons,” in animal models of neurodegenerative diseases, providing temporary protection. Such protection would occur in a vital time period when cell fate is still in balance, or perhaps precritical, before secondary excitotoxicity might threaten weakened neurons. Such protection by Cr, however, could only be expected if the CK system were not compromised in a significant way such that PCr would still be synthesized by CK. An additional mechanism by which Cr may exert neuroprotection, could be through activation of AMPK in the brain, in a manner similar to that recently shown in muscle cells [129]. Since AMPK is a cellular energy sensor and fuel gauge, this would lead to short-term and long-term compensatory reactions to help the cells recruit more energy sources, for example, by up-regulation of glucose transport and elevation of fatty acid oxidation [130].

Lastly, Cr may exert neuroprotection by reducing protein aggregation. For example, Cr was found to reduce transglutaminase-catalyzed protein aggregation, *in vitro*, [131] a process thought to be relevant for the formation of protein aggregate formation in several neurodegenerative diseases, including Alzheimer’s, Parkinson’s, and Huntington’s disease.

Thus, one may postulate that Cr supplementation, in combination with other established clinical interventions, may be a very valuable adjunct therapy for patients at an early stage of the disease progression. However, additional studies are needed first to address the questions of where exactly the microcrystalline Cr deposits are located, for example, intra- or extracellularly, and whether they are associated with specific structures of the brain. In addition, it would be important to be able to quantify Cr in these deposits. Since these focal Cr microdeposits are observed in the brain of transgenic APP mice, as well as AD patients [73], there is a valid concern that supplementation with extra Cr might exacerbate rather than ameliorate this situation. However, as reasoned above, Cr, if given at an early time point of disease, may prevent or delay the formation of Cr deposits that are

a consequence of cellular pathology. In any case, Cr supplementation should be tested first on the transgenic APP mice in which the Cr deposits have been found. In the long term, if warranted by the outcome of such tests, further trials with AD patients could be performed. If successful, this cheap and safe intervention, involving as a nutritional supplement, may extend a huge socioeconomic benefit by improving the quality of life of AD patients and lowering exploding health care costs.

ABBREVIATIONS

| | |
|-----------|--|
| 3NP | 3-nitropropionic acid |
| 8OH2’dG | 8-hydroxy-2’-deoxyguanosine |
| A β | amyloid beta peptide |
| ABAD | A β -binding alcoholdehydrogenase |
| AD | Alzheimer’s disease |
| AGAT | L-arginine:glycine amidinotransferase |
| ALS | amyotrophic lateral sclerosis |
| AMPK | AMP-stimulated protein kinase |
| APP | amyloid precursor protein |
| BB-CK | cytosolic brain-type creatine kinase |
| CK | creatine kinase |
| CNS | central nervous system |
| Cr | creatine |
| CRT | Na ⁺ and Cl [−] dependent creatine transporter |
| FTIR | Fourier transform infrared |
| GAMT | S-adenosylmethionine:guanidinoacetate N-methyltransferase |
| HD | Huntington’s disease |
| MM-CK | cytosolic muscle-type creatinekinase |
| PCr | phosphocreatine |
| PD | Parkinson’s disease |
| PET | positron emission tomography |
| sMtCK | sarcomeric mitochondrial creatine kinase |
| SR | sarcoplasmic reticulum |
| TBI | traumatic brain injury |
| TCA | tricarboxylic acid |
| UCH L-1 | ubiquitin carboxy-terminal hydrolase 1-1 |
| uMtCK | ubiquitous mitochondrial creatine kinase |

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