Creatine Kinase-Phosphocreatine Shuttle: Can the Energy "Swiss Bank" for Cells Provide Neuroprotection

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Abstract:

Creatine Monophosphate is a well studied supplement used to primarily increase energy stores of muscle cells for enhancement of athletic performance. However, the breadth of research on the cognitive benefits and neuroprotection by creatine is minimal in comparison. The purpose of this paper is to compile the researched cognitive benefits of creatine and highlight its potential to be used as a neuroprotectant in patient populations susceptible to neurodegenerative disorders. The paper explores creatine metabolism, layout of creatine biological machinery in neurons, and studies effects of supplementation on patients with Alzheimer's, Parkinson's, and Huntington's disease. The study found the neurodegenerative disorders of Parkinson's and Huntington's diseases to respond best to creatine supplementation. The study looks at creatine through the lens of mitochondrial dysfunction in these disorders and how creatine supplementation can be used to mitigate the excitotoxicity and reactive oxidative stress caused by dysfunctional mitochondria. This paper urges research to continue exploring the possibility of using creatine to mitigate progression of neurodegenerative disorders.

1. Introduction:

Creatine is a popular supplement used by athletes to aid in high-intensity exercise. The body phosphorylates creatine to create Phosphocreatine. Phosphocreatine is an efficient energy source for the immediate regeneration of ATP. Oral supplementation increases the muscle stores of Phosphocreatine which delays the energy depletion. However, it is important to understand that creatine is produced endogenously. It provides a vital buffering system used by cells for efficient transport of energy and for immediate energy regeneration amongst rapid intense activities. Creatine Kinase (CK) reaction is a fundamental metabolic pathway in vertebrates. Adenosine Triphosphate (ATP) and free creatine (Cr) are synthesized via a reversible transfer of a gamma phosphate group from Phosphocreatine (PCr) to Adenosine Diphosphate (ADP). Creatine is utilized by muscle tissues as a temporal energy buffering system. In fast-twitch skeletal muscles, a large pool of PCr is held for immediate regeneration of ATP as ADP accumulates from myosin-actin powerstrokes. Creatine is an efficient carrier of energy for the cell to transport the high energy phosphate from one area to another distant location intracellularly. Distinct CK isoenzymes work together to deliver energy from production sites to consumption sites. Rather than

storing large excess amounts of ATP, cells store high-energy phosphate as phosphocreatine because PCr can accumulate at higher concentrations and rapidly regenerate ATP through the creatine kinase reaction. The brain is vulnerable to energy failure due to its high and fluctuating energy demands paired with limited reserves of metabolites. The brain relies on constant ATP turnover to maintain physiological processes essential for life. Hence, the brain has its own production of creatine separate from those produced via the kidney-liver axis. The central nervous system (CNS) does not readily take up peripheral creatine due to the inefficiency of transport over the blood brain barrier. However, some do cross over via diffusion patterns explained later in the review. Due to the reliance of the CNS for creatine to maintain its energy intensive properties, there are active studies in the field to deduce how efficacious exogenous supplementation of creatine is for slowing progression of neurodegenerative disorders. This review aims to explain the metabolic pathway of creatine, the structure and function of creatine kinase isozymes, and how supplementation of creatine can be used as a neuroprotectant against neurodegenerative disorders.

2. The Creatine Kinase/Phosphocreatine System:

Creatine (Cr) (α-N-methylguanidino acetic acid) (PubChem CID: 586) is a non-protein amino acid synthesized in the kidneys, liver, pancreas, brain and testes. Most concentrations of this molecule are found in the brain and skeletal muscles. Cr is also found in cardiomyocytes, enterocytes, and spermatozoa etc.[1] Endogenous synthesis of Cr occurs through a ping pong mechanism by L-Arginine-Glycine amidinotransferase (AGAT) which transfers the amidino group of L-arginine to the amine group of L-glycine.[2] This reaction yields L-ornithine and guanidinoacetate (GAA) which is then methylated via Guanidinoacetate N-Methyltransferase (GAMT) from L-methionine. Post synthesis, Cr is shipped out to target tissues via bloodstream and transported into cells via Na^+ and Cl^- dependent creatine transporter (SLC6A8).[3] After Cr enters cells, it is transphosphorylated into phosphocreatine (PCr). Cr primarily exists as a zwitterion. Cr is chemically broken down into creatinine via a non-enzymatic reaction and monomolecular reaction dependent on pH.[4] This byproduct (~1.7% total Cr per day) is excreted via urine through the kidney.[5] PCr is highly valuable for the cell to maintain its ATP concentrations in high energy demand tissues. Many processes such as muscle contraction, vesicle trafficking, ion pumping, and cytoskeletal movement rely on PCr for constant ATP regeneration.[6]

Cr is utilized by cells due to its small molecular size and less negative charge. These factors cause the cell to store much higher concentrations of PCr compared to ATP which is a thermodynamic improvement for energy metabolism.[5] It is important to emphasize that creatine kinase (CK) isozymes are found at various cellular compartments such as the sarcomere, cytosol and mitochondria to connect ATP production with consumption. This sophisticated system is known as the CK/PCr system.[7] The maximal rate of ATP synthesis by oxidative phosphorylation is (2.5 μmoL·s−1·g−1) compared to the rate of CK reaction of (30 μmoL·s-1·g−1).[8]

Cytosolic CK is a dimer made up of muscle or brain isoforms to create various isozymes such as MM-CK, MB-CK and BB-CK.[9] There are also two forms of the mitochondrial isoform, ubiquitous mitochondrial CK (Miu -CK) and sarcomeric mitochondrial CK (Mis-CK), due to the fluctuating environment of the mitochondria.[7,10] The Cr/PCr system shuttles the PCr from the mitochondria to energy intensive cytoplasmic sites for use. The cytosolic isoforms form dimers whereas the mitochondrial isoforms form octamers or readily dissociate into dimers.[11] The mechanism of the enzyme takes place in an ordered biomolecular mechanism with equilibrium pushing towards PCr production. The enzyme produces PCr and MgADP through a reversible transfer of phosphoryl group from MgATP to Cr.

The location of Mi-CK allows for coupling with oxidative phosphorylation processes. Specifically, the adenine nucleotide translocase (ANT) allows the cell to convert mitochondrial energy in highly diffusible form. Mi-CK is found in the intermembrane space. The octamer has a high affinity for acidic phospholipids such as cardiolipin which is abundant in the inner mitochondrial membrane. Mt-CK are located within these cardiolipin patches which allows them to couple with the ANT pathway. The Mt-CK contacts both the inner mitochondrial membrane and outer mitochondrial membrane which allows for stability of the organelle and creates a direct pathway for metabolite exchange.

Metabolite channeling is the process in which ATP generated by oxidative phosphorylation is exported from the matrix via the ANT to the Mt-CK. Mt-CK then catalyzes the transfer of the phosphate group from this ATP to Cr which produces PCr and ADP. This ADP is then taken by ANT and sent back to the matrix to be turned back into ATP. The resulting PCr is exported out the mitochondria via the voltage dependent anion channel (VDAC) to the cytoplasm. This system is helpful as there is a steady flux of ADP sent back to be phosphorylated again and there is a steady flux of PCr created to be used during strenuous exercise.

3. Catalytic mechanism:

Creatine kinase produces PCr and MgADP by catalyzing a reversible transfer of phosphoryl groups between MgATP and creatine. First, the conserved residue E2277 acts as a general base catalyst by accepting a proton from the guanidinium group of creatine. Next, the rate determining step of the reaction is when the gamma phosphate group of ATP is transferred to the Cr molecule.

The active site of the enzyme is located in the deep cleft between the N-terminal domain and C-terminal domain. Upon substrate binding, two flexible loops created by residues 60-70 and 323-3327 in cytosolic CK moves from an open form to a closed form. The creatine binding site has E227 which hydrogen bonds to creatine. S280 then anchors the molecule while it also interacts with Q313, R336 and R1277.

The ATP binding site is made up of five conserved arginine residues (R125, R127, R231, R287, and R315)[7]. Magnesium is required in the reaction to neutralize and stabilize the phosphate groups of ATP in the reaction complex. The transition state is stabilized by arginine rich loops which get protonated prior to phosphate release which allows for the nucleophilic attack. Experimentally it is observed that the energy barrier for the rate determining step is 16.7 kcal/mol[7]. The directional flux of the reaction is controlled by the local ATP:ADP ratio.

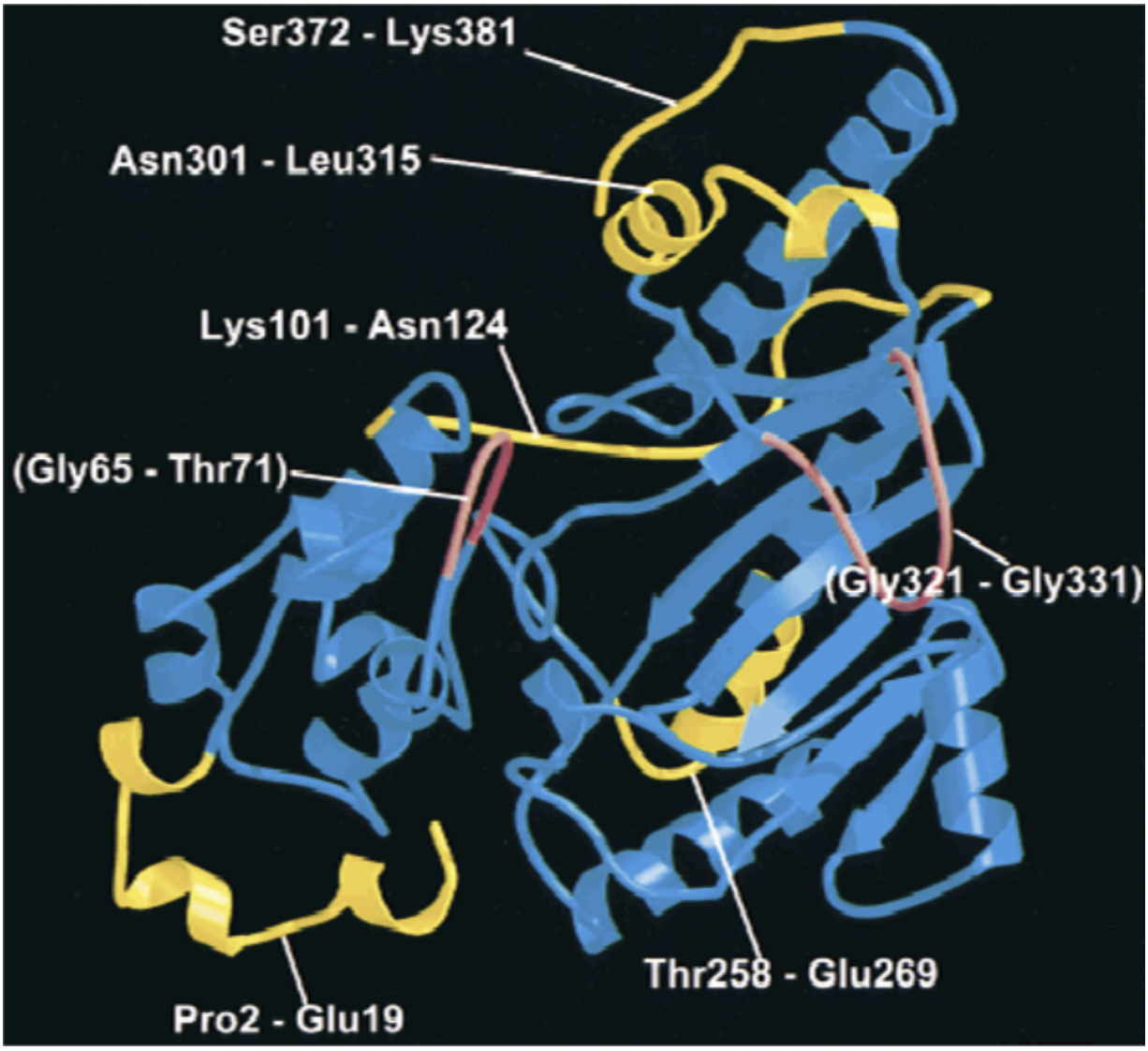
Previously, it was held that creatine kinase was used solely for energy buffering in muscles to upkeep with demands of the powerstroke. However, after the discovery of the various isoforms it was clear that there was a PCr shuttle system in various parts of the cell in which PCr is used as transport in the stead of ATP. Out of the two cytosolic isoforms of CK that are known, only the muscle isoforms interact with the M-line of the sarcomere.[12] The interaction occurs through two specific lysine pairs that are conserved in the MM-CK. This motif is not found in the BB-CK.[12] Mitochondrial CK bind to their respective membranes via the C-terminal region of the protein whereas the N-terminal region is used for octamer formation. It has been elucidated that individual subunits of CK catalyze the transphosphorylation reaction. The reversible reaction occurs via a bimolecular random mechanism with the equilibrium pushing to favor production formation. This equilibrium is controlled by the pH and concentration of magnesium ions in the cellular environment. At pH 8 and above, the enzyme catalyzes in a random biomolecular mechanism whereas when the pH is below 7 the forward reaction is conducted in an ordered mechanism.[12] This means that MgATP must bind before creatine binds. CK conducts a biomolecular nucleophilic substitution to form a quaternary sp^3d transition state called (E-TSAC) which is made up of the CK + Cr + MgADP . The true substrates for

the reaction are MgADP and MgATP. The magnesium ion is needed to stabilize the highly negative charge of the ADP and ATP molecules. Optimal activity occurs when there is an excess concentration of magnesium compared to the ADP and ATP concentrations.[5] Overall the thermodynamic advantage of the PCr shuttle is that the free energy change for PCr hydrolysis is (-45 kJ/mol) vs the (-30.5 kJ/mol) of ATP.[13] The cell utilizes PCr shuttle because ATP molecules are bulky and highly negative which make it hard for the cell to arrange high intracellular concentrations whereas the PCr is much smaller and less negatively charged so it can reach much higher intracellular concentrations.

X-ray crystallography was useful to determine how substrates were positioned in the active site of CK and which residues specifically stabilized the reaction state. Prior to this experimentation, researchers had evidence of CK binding to ATP and Cr through kinetic and spectroscopic experiments.[14] These experiments suggested that CK undergoes a conformational change during catalysis.

X-ray crystallography provided an atomic map of the enzyme. The crystals showed that each monomer has a small N-terminal domain and larger C-terminal domain with the ATP-binding cleft between them.[11] They also identified flexible loops near the active site which suggested that CK was not rigid and could aid in catalytic activity. Figure 1, shows the conserved common core of CK isoenzymes shown in cyan and isoenzyme-specific regions in yellow. The figure highlights in red the two conserved flexible loops of Gly65–Thr71 and Gly321–Gly331. These loops are placed near the catalytic region and help the enzyme close around its substrate during catalysis. Hence, figure 1 supports that CK is not a rigid enzyme, but flexible due to its mobile structural elements.

Figure 1. Ribbon Structure of Creatine Kinase B type monomer

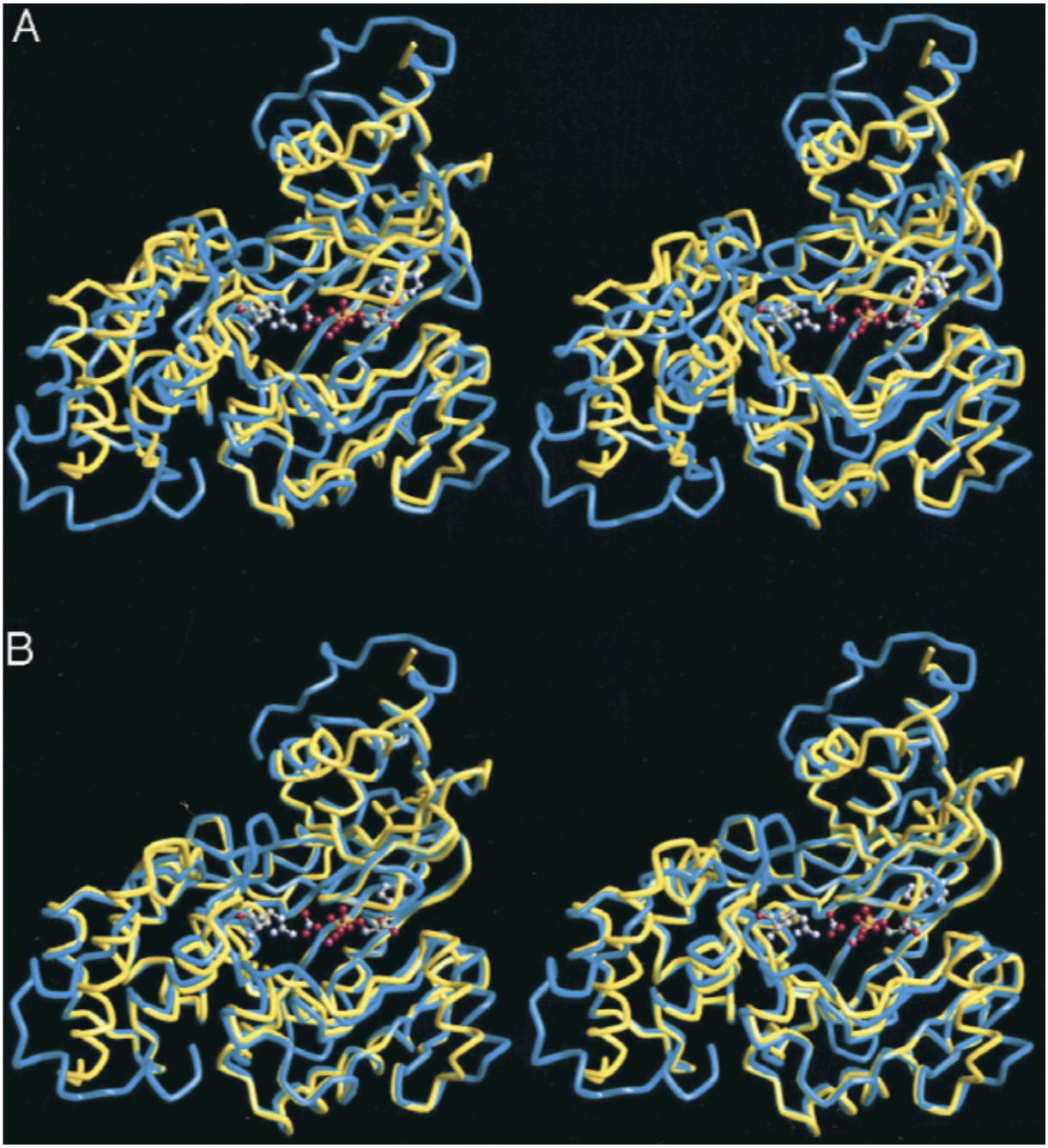


Yellow portions depict isoenzyme-specific regions whereas the blue show the conserved common core shared among CK isoenzymes. The flexible loops are shown in pink and are conserved across CK isoenzymes. These loops are positioned near the catalytic region showing their importance in conformational movement and phosphoryl transfer during CK catalysis.[15]

CK is similar in homology to arginine kinase (AK) which is a part of the phosphagen kinase family. Before the CK crystal structure was available, AK structure was used to deduce CK structure and function due to similar homology. AK and CK share 38-44% sequence identity, have similar kinetic properties, and have a conserved active-site cysteine motif.[16] Hence, it was possible to superimpose the CK structures onto the AK transition state

structure to model what the transition state conformation of CK looks like. This comparison is shown in figure 2. The figure depicts B-CK monomer superimposed with the transition-state analog complex of AK. Part A of the figure highlights how the overall backbone of CK and AK are aligned and the two enzymes share a similar global fold. Part B of the figure shows how CK aligns domain by domain which is useful to compare with AK transition state since CK had not yet been crystallized in a complete transition-state complex. This comparison discovered the flexible CK loops. This is useful because prior experimentations only showed general folding of CK and not the ternary complex with creatine bound. AK was able to be used as a template for CK modeling due to their similar homology and domains.

Figure 2. Superposition of B type creatine kinase with arginine kinase



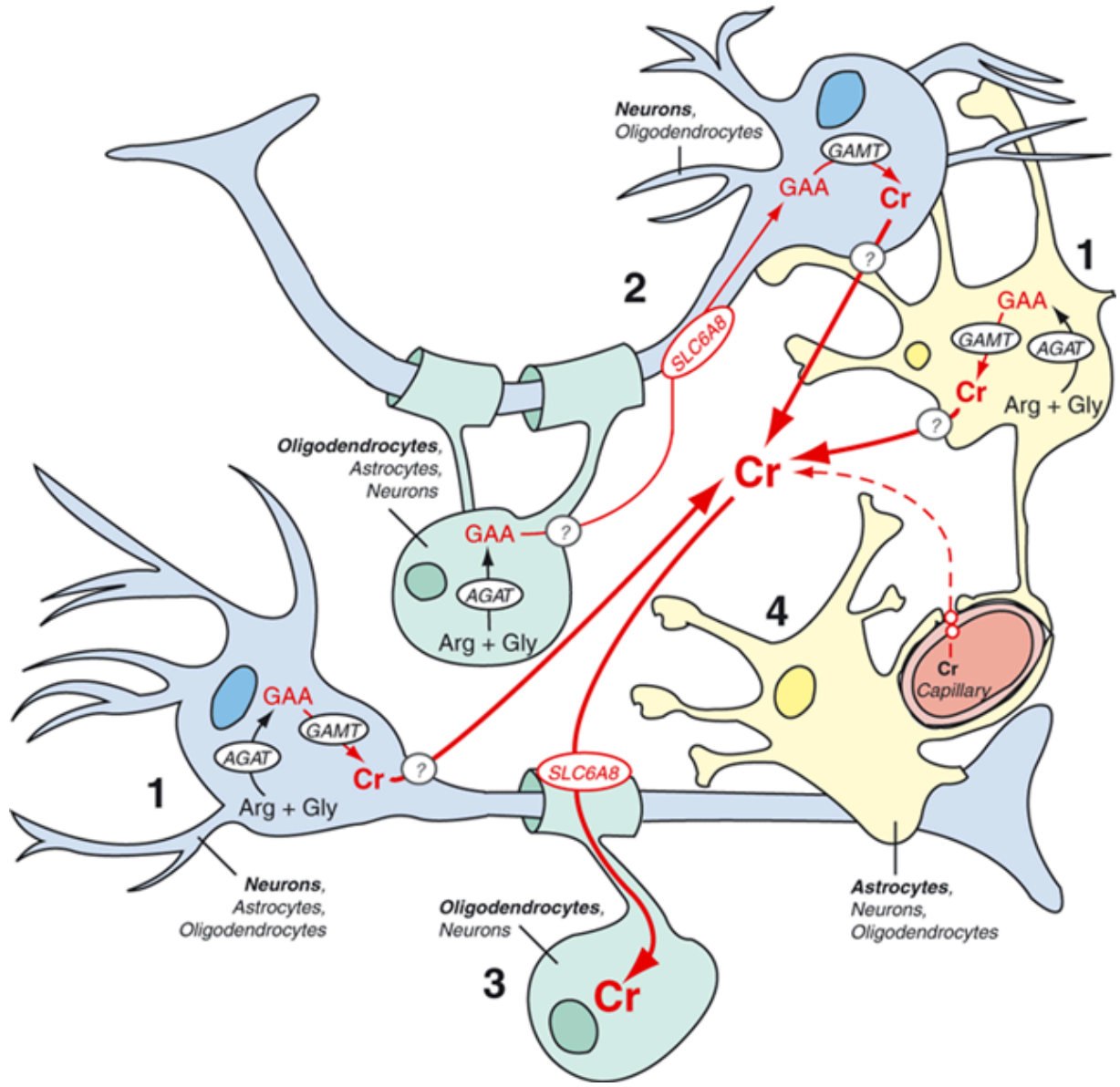
Panel A shows an alignment of the two enzymes to depict their overall structural similarity. Panel B shows domain by domain alignment to model the transition conformation of B-CK. This comparison supports the use of arginine kinase as a structural template for understanding CK catalysis and conformational movement. B type creatine kinase is shown in blue and arginine kinase is shown in yellow. [15]

Evidence of homology between AK and CK is that both of them have Glu225 placed in the same position relative to the guanidino group of AK-TSAC.[17] Conserved regions on CK include the negatively charged NEED box and reactive cysteine motif which is found in phosphagen kinases. The crystal structures of CK elucidated the nucleotide-binding pocket made from conserved arginine residues.[12] These positively charged residues can stabilize the negatively charged phosphate groups of ATP and ADP. This is vital for enzyme activity since precision of orientation determines if the reaction occurs or not.

The crystal structures allowed researchers to understand how AK and CK catalytic cleft chemistry was conserved, but there are structural differences. AK had negatively charged glutamate to interact with its substrate arginine whereas CK uses hydrophobic isoleucine and valine to stabilize creatine. The technique of x-ray crystallography was used to understand that specific residues are not the most important for chemistry, but conserved motifs that create stable electrostatic environments are vital for enzymatic transfers.

4. Creatine Kinase in the Brain: Transport and synthesis

Figure 3. Creatine Transport in the CNS Depicting SLC6A8 Transporters



The figure shows endogenous synthesis of Creatine in the central nervous system through AGAT and GAMT expressed in oligodendrocytes and astrocytes. Creatine readily diffuses through the blood-brain-barrier to accumulate creatine for use in the neurons. However, this diffusion is inefficient. The figure shows how some neurons only express either AGAT or GAMT which leads them to have to transport precursors of creatine to other cells for synthesis (e.g GAA shipped from oligodendrocyte to neuron).[8]

The PCr shuttle plays a vital role regenerating and buffering ATP levels in the brain to maintain high energy levels for development and function[5]. In humans, half of the Cr is gathered from diet and the other half is produced

endogenously via the AGAT and GAMT pathway in the kidney-liver axis which distributes the Cr throughout the body via blood and the Cr transporter, SLC6A8, takes up Cr into tissues[5]. However in the brain, the uptake of Cr is different from other tissues. Due to the blood brain barrier (BBB) utilizing non-fenestrated capillaries via tight junctions to control the flux of metabolites traveling between the brain and blood, peripheral Cr in blood is not readily transported through. A common misconception was that cerebral Cr was mainly from peripheral uptake. However, the fact that AGAT and GAMT are highly expressed in brain cells for the parenchymal cell's own synthesis, proves this wrong[18]. But, it is not completely wrong. Non-fenestrated microcapillary endothelial cells (MCEC) express SLC6A8 to import Cr into the CNS. This means that there is still peripheral uptake of Cr, but the majority is synthesized within the CNS itself. However, SLC6A8 is missing from astrocyte feet which line the BBB which is problematic because astrocytes provide direct connection for metabolites from blood to neurons. However, without the SLC6A8 transporter in astrocytes, peripheral Cr must be taken up via SLC6A8 found in MCEC which is then sent into the extracellular space and must rely on diffusion to reach neurons[19]. Unfortunately, this is a very limited import of Cr. This concept is shown by figure 3 in which limited diffusion of creatine across BBB is depicted as well as the placements of SLC6A8 transporters throughout. The enzymes AGAT, GAMT, and transporter SLC6A8 are expressed in neurons, astrocytes, and oligodendrocytes which are integral to endogenous CNS Cr synthesis.[20]

To further showcase the importance of the PCr system in the CNs, it has been found that AGAT, GAMT and SLC6A8 deficiencies can lead to intellectual disability, delays in speech acquisition, intractable epilepsy, autism and extrapyramidal syndrome.[21] Further studies were conducted on patients deficient of SLC6A8 transporter because they had minimal Cr uptake in cells.[22,23] The patients were found to remain with intellectual disability and progressive brain atrophy after oral supplementation of Cr.

It is also important to note that the SLC6A8 transporters in the parenchymal cells of CNS can take up GAA, a precursor to Cr.[24] The K_M value of SLC6A8 for GAA is (269-412 μM) compared to the K_M of Cr (29-46 μM).[25] It is interesting to note that there is a ten fold increase in the K_M value of GAA compared to Cr. Hence, it is apparent that Cr outcompetes GAA for binding with its higher affinity. However, it is highly beneficial for the SLC6A8 transporter in the CNS to uptake GAA as well because AGAT and GAMT are expressed more in some CNS cells over others. This means that the precursors created via AGAT have to be transported out to cells with GAMT to produce Cr. Hence, transportation of the GAA precursor is vital for the brain to maintain its stores of PCr. This is useful because uptake

of peripheral Cr is minimal in the brain due to the reliance of simple diffusion through the extracellular matrix of the BBB to neurons instead of direct transfer to neurons via astrocytes. This transport via astrocytes does not occur because they do not contain SLC6A8 transporters in their feet connecting to the pericytes on endothelial cells.

5. Creatine's neuroprotective roles in disorders with energy impairment

Due to the role that PCr system plays in energy buffering and bioenergetics, there are plenty of studies which look at the role the system plays in neurodegenerative disorders. Various neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease are associated with bioenergetic problems.[26,27] There is lots of evidence to suggest that mitochondrial deficits are directly linked to neurodegenerative pathology. These deficits can lead to apoptosis of neurons due to the apoptotic factors related with mitochondria. Mitochondria are also involved in excitotoxicity and reactive oxygen species (ROS) production which can lead to neurodegenerative disorders.[28] Hence, mitochondria are an important target for therapeutic intervention for neurological disease.

Mitochondria initiate apoptosis by using pro-apoptotic proteins which can lead to cell death. These organelles can also produce ROS which initiate the release of pro-apoptotic proteins. These proteins include cytochrome c, Smac/Diablo, and apoptosis-inducing factor.[29] These proteins are released through a transition pore called mitochondrial permeability transition pore (mtPTP) that is the hallmark of apoptosis.[30] This transition pore is between the inner and outer mitochondrial membrane. The mtPTP can open due to increases in oxidative stress, reduction in ATP/ADP, and accumulation of Ca^{2+}. Much of the oxidative stress in mitochondria arises from the electron transport chain reactions which transfers electrons complex to complex producing unstable ROS byproducts. These byproducts can then trigger cytochrome c release from the inner mitochondrial membrane which initiates apoptosis. The accumulation of this oxidative stress is a common theme found among neurological disorders and studying oxidative stress in mitochondria may be helpful as treatment for neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease. Hence it is reasonable to study how creatine supplementation can be used to slow the progression of these neurodegenerative disorders since higher creatine stores will upkeep the failing energy metabolism.

Parkinson's Disease (PD) is accompanied by dysfunction of dopaminergic neurons in the substantia nigra and neuronal loss in the motor cortex.[31] These dysfunctions are connected to mitochondrial dysfunction and oxidative stress in the progression of the disease.[32] It is also found that the neurotoxin,

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) disrupts mitochondrial function leading to PD. This neurotoxin disrupts complex 1 of the ETC and actively occurs in the dopaminergic neurons of the substantia nigra. This shows the significant contribution of mitochondria to PD. Hence, Cr supplementation was advocated as efficacious for PD patients since Cr could restore the bioenergetic deficit and improve mitochondrial function. It was found that creatine supplementation protected against loss of Nissl stained neurons in the substantia nigra.[33] A randomized, double-blind, Phase II futility trial conducted in 2006 by the NINDS NET-PD investigators demonstrated that creatine supplementation was not futile and warranted further investigation in Phase III clinical trials.[34] However, the early promise did not translate to clinical efficacy in the larger Phase lll trial. The Phase lll trial was terminated because creatine did not improve clinical outcomes compared to the placebo. This implies that even though creatine can aid in metabolic buffering of the preclinical models, PD progression in humans is too complex to be corrected by simply increasing creatine stores. This complexity is due to the mitochondrial dysfunction, oxidative stress, and dopaminergic neuronal loss present in PD patients.

Huntington's disease (HD) is an autosomal dominant progressive neurological disorder. The disease is characterized by "dance-like" movements without coordination called chorea. The disease is caused by a trinucleotide expansion (CAG) in the Huntington gene which results in a diseased protein with polyglutamine repeat stretches. The mutant protein has been implicated to neural damage via excitotoxicity, oxidative stress and mitochondrial dysfunction.[35] Animal models have also shown that HD can be modeled via ETC inhibition.[36] It has also been shown that inhibiting succinate dehydrogenase (complex II) produces HD like symptoms.[37] These implications show that mitochondrial dysregulation is a vital therapeutic target for HD treatment. These findings suggest that treatments that help maintain cellular energy levels could slow the onset or progression of Huntington's disease. In animal models, creatine improved motor function, increased survival, preserved body and brain weight, reduced neuronal damage, and decreased the buildup of toxic protein aggregates.[38] Based on these results, a 16-week randomized, double-blind, placebo-controlled Phase II trial was conducted in patients with Huntington's disease using 8 g/day of creatine.[39] The study showed that creatine was safe and well tolerated, and it reduced levels of a marker of oxidative DNA damage. Given these positive findings from both animal and early clinical studies, a larger Phase III clinical trial was approved.

Alzheimer's disease (AD) has a popular hallmark for neurofibrillary tangles and deposits of extracellular plaques accompanied by an extensive loss of neurons.[40] It was later found that there were noticeable problems in AD patients with their energy metabolism and their cell's mitochondrial electron transport chain.[41] Due to creatine's efficacy in improving mental concentration and learning, it was seen as a possible treatment for AD patients since they could benefit at earlier stages of the disease onset.[42] As discussed previously in this review, neural uptake of peripheral Cr is poor due to the lack of SLC6A8 transporters found on astrocytes. However, Cr supplementation was given to AD patients hoping that the exogenous supplementation may increase the bioenergetics of early stages of the disease onset when the CK system is still functioning. At later stages of AD, the oxidative stress can reduce CK activity because the cysteine residue of the enzyme is easily oxidized.[43] However, studies have shown it to be futile in using creatine as a neuroprotectant for AD because of the accumulation of creatine already in the neurons of late stage AD patients from CK inhibition of the oxidative stress. To paint a clearer picture, creatine supplementation will give the cells more creatine. However, the CK machinery still has to phosphorylate it, but this cannot be done if the CK are oxidatively damaged. Mitochondria provide the ATP used by CK to make PCr. If the electron transport chain is impaired, then the cell has less ATP to create PCr. So Cr supplementation cannot help energy metabolism if mitochondrial dysfunction is already severe. Late stage AD is present with neuronal loss. Creatine can help buffer energy in living cells, but it cannot reverse cell death or plaque accumulation. Hence, extra supplementation did not bring about significant change in patients with late stage AD compared to the supplementation given to AD patients at an early stage who still had functioning CKs.

All together, studies on PD, HD and AD suggest the Cr has potential biochemical use, but limited clinical translation as a stand alone neuroprotective therapy. These diseases share similarity in mitochondrial dysfunction, oxidative stress and impaired ATP production. The neuronal changes and damages these diseases present provide a reason for targeting the CK/PCr system. In preclinical models, Cr appears to be beneficial in metabolically stressed neurons that are still viable. In these models, the increased Cr store supports PCr formation and mitochondrial stability. However, human trials show that simply increasing Cr stores is not enough to alter disease progression. In later trials of PD and HD, Cr failed to show benefit. This highlights the severity of neuronal loss, excitotoxicity, inflammation and structural damage garnered from the neurodegenerative disorders that are not reversible by Cr alone. AD further shows this limitation because Cr benefits depends on functioning mitochondria and active CK.

Hence, if mitochondrial ATP production is already severely impaired, added Cr cannot be efficiently converted into PCr. Therefore, the varied results from these neurodegenerative disorders suggest that Cr may be beneficial in early disease progression. Hence, the failure of Cr in later stage studies does not brush aside the relevance of CK/PCr system in neurodegenerative disorders. Instead, these studies show that Cr support must occur early in disease progression and must be in the right cell environment for effective neuroprotection.

6. Conclusion:

The creatine kinase-phosphocreatine shuttle is an integral biochemical pathway that allows tissues with high energy demands to rapidly regenerate ATP and transports high energy phosphate efficiently. It is much more than simply an energy reserve system for cells. This regeneration of ATP is vital in tissues such as the brain where energy demands are constant and high stakes. This review discussed endogenous synthesis of creatine in various tissues, transport mediated uptake, and how the various isoforms of CK are coordinated. This literature review focused on the neuroprotective potential of creatine and showed how it is not universally effective for all neurodegenerative disorders. Although creatine supplementation has beneficial effects in certain models of Parkinson's disease and Huntington's disease, its use in the CNS is limited by poor transport across the blood brain barrier and the fact that creatine itself may already be impaired at later stages of disease. This review highlights that simply increasing peripheral concentration of creatine via exogenous supplementation, does not guarantee improvement in CNS metabolism. Future research should continue to investigate how creatine is transported and synthesized in the brain, which stages of neurodegenerative disorders are responsive to intervention, and whether targeting the CK/PCr pathway can provide therapeutic outcomes. Overall, the CK/PCr shuttle is an excellent model for understanding energy buffering and transportation in cells.

Academic Integrity and AI Use: AI tools were not used to generate scientific content. All scientific content, analysis, and conclusions were reviewed and revised by the author.

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