REVIEW ARTICLE

Creatine as a Neuroprotector: an Actor that Can Play Many Parts

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Abstract

Creatine is a nitrogenous organic acid that plays a central role as an energy buffer in high energy demanding systems, including the muscular and the central nervous system. It can be acquired from diet or synthesized endogenously, and its main destination is the system creatine/phosphocreatine that strengthens cellular energetics via a temporal and spatial energy buffer that can restore cellular ATP without a reliance on oxygen. This compound has been proposed to possess secondary roles, such as direct and indirect antioxidant, immunomodulatory agent, and possible neuromodulator. However, these effects may be associated with its bioenergetic role in the mitochondria. Given the fundamental roles that creatine plays in the CNS, several preclinical and clinical studies have tested the potential that creatine has to treat degenerative disorders. However, although in vitro and in vivo animal models are highly encouraging, most clinical trials fail to reproduce positive results suggesting that the prophylactic use for neuroprotection in at-risk populations or patients is the most promising field. Nonetheless, the only clearly positive data of the creatine supplementation in human beings are related to the (rare) creatine deficiency syndromes. It seems critical that future studies must establish the best dosage regime to increase brain creatine in a way that can relate to animal studies, provide new ways for creatine to reach the brain, and seek larger experimental groups with biomarkers for prediction of efficacy.

Keywords Creatine · Neuroprotection · Bioenergetics · Neurodegenerative disease · Inborn errors of metabolism · Creatine supplementation

Introduction

Creatine is a nitrogenous organic acid firstly described in the early nineteenth century. It plays a central role as an energy buffer for systems throughout the body, particularly high energy demanding systems, like the muscular and the central nervous system (CNS) (Wyss and Kaddurah-Daouk 2000). An average adult has approximately 120 g of creatine in his body. Since creatine is subject to spontaneous removal via a nonenzymatic chemical dehydration to creatinine, 2 g must be acquired or synthesized every day in order to maintain this

value constant (Casey and Greenhaff 2000). There is no main source of creatine in human metabolism since it is provided equally by dietary consumption and endogenous synthesis. The most relevant dietary sources of creatine are found in red meat, fish, and to a lesser extent dairy products (Brosnan and Brosnan 2016). It is known that the small intestine expresses the Na⁺/Cl⁻ creatine transporter (Peral et al. 2002).

Nonetheless, biosynthesis of creatine is a relative simple process that occurs mainly in the kidney and liver, involving a two-step pathway with two enzymes: L-arginine: glycine amidinotransferase (AGAT) and N-guanidinoacetate methyltransferase (GAMT), along with one specific plasma membrane transporter, SLC6A8 (Barcelos et al. 2016). AGAT is present in the kidney, brain, and pancreas (to a very smaller extent) of mammals (Braissant et al. 2001; da Silva et al. 2014). This enzyme is responsible for condensing the amino acids arginine and glycine, generating guanidinoacetate (GAA) and L-ornithine. This first reaction takes place predominantly in the mitochondria intermembrane space and in lower levels in the cytoplasm (Magri et al. 1975). The second enzyme, GAMT, is mostly present in the liver and brain of mammals, being the responsible for the transfer of a methyl

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group from S-adenosylmethionine (SAM) to GAA in order to produce creatine (Ogawa et al. 1988; da Silva et al. 2014). This reaction is responsible for the consumption of roughly 70% of available methyl groups in human bodies (Stead et al. 2006). Most of creatine that arises from this second step is produced in the liver and then secreted in the bloodstream by an unknown mechanism and distributed throughout the body, where it actively enters the cells using the specific creatine transporter, SLC6A8, a Na^+- and Cl^--dependent symporter (Magri et al. 1975; Brosnan and Brosnan 2007; da Silva et al. 2014). Once inside the cells, approximately two-thirds of the available creatine undergo a reversible phosphorylation catalyzed by the enzyme creatine kinase (CK), giving rise to phosphocreatine (PCr). CK has three cytosolic and two mitochondrial isoforms, but in most tissues, a single cytosolic CK isoform is co-expressed with a single mitochondrial CK isoform (Wallimann et al. 2011; Koch et al. 2014).

As previously pointed out, although most of endogenous creatine is produced in the liver, it has been shown that the brain has its own pathway for the synthesis and maintenance of creatine levels since SLC6A8 is present in low levels in the micro capillaries of the blood-brain barrier (BBB), and it is not expressed by most perivascular astrocytes (Braissant 2012; Saunders et al. 2015). As a result, AGAT and GAMT are expressed in all CNS cell types (Braissant et al. 2001). Nonetheless, the expression of these two enzymes appears to occur in a dissociated fashion, with less than 20% of brain cells expressing both AGAT and GAMT. This means that for creatine synthesis to occur, GAA must be transported through SLC6A8 from AGAT to GAMT-expressing cells (Braissant and Henry 2008; Braissant et al. 2010).

After non-enzymatic dehydration and cyclization of creatine, creatinine is produced and it freely diffuses to the bloodstream where it will be eliminated in urine. Creatinine is frequently used as a marker of the renal function (Wyss and Schulze 2002). In order to compensate this loss, creatine synthesis must be regulated physiologically, being that the major regulator is the activity of AGAT, which is the biosynthesis-initiating and rate-limiting step of creatine formation. There is down-regulation of AGAT in the kidney and in the developing brain caused by high levels of its product ornithine and the downstream end product creatine (Hanna-El-Daher et al. 2015). On the other hand, lower levels of creatine are able to stimulate a sustained increase in AGAT activity (Wyss and Kaddurah-Daouk 2000). SLC6A8 is regulated by creatine levels in the bloodstream in a time-dependent manner, being that high levels of creatine cause a faster inhibition response when compared with the stimulation provoked by lower creatine levels. GAA is also able to inhibit this transporter (Wyss and Kaddurah-Daouk 2000).

Creatine Roles

Creatine has been used as a supplement and a potential adjuvant treatment for several disorders. The solid evidence available in the literature considers that the main function of creatine is by far energy buffer and transfer. In addition, studies raise a range of possible secondary creatine functions and effects. However, molecular mechanisms remain a matter of debate, and they may be related to bioenergetic role that creatine has in the mitochondria. The pleiotropic effects of creatine are summarized in Fig. 1.

Creatine as an Energy Reservoir

The main destination of creatine is the system creatine/PCr which strengthens cellular energetics via a temporal and spatial energy buffer that can restore cellular ATP without a reliance on oxygen (Wallimann et al. 2011). Since the rate of ATP diffusion is insufficient to maintain the energy requirements within cells (de Graaf et al. 2000), PCr offers a solution for this problem because it has a higher diffusion capacity and can reversibly transfer its N-phosphoryl group to adenosine diphosphate (ADP). This happens when the concentration of this nucleotide rises inside the cell (Sauer and Schlattner 2004). Therefore, this temporal chemical energy pool in the cytosol is an efficient way to store energy not only in skeletal muscle (destiny of 90% of the body's creatine) but also in other organs and systems, like the CNS, which are highly dependent upon a fair amount of energy in order to properly perform its functions in the human body (Schlattner et al. 2006).

Studies have shown that creatine supplementation protects against ATP depletion and delayed membrane depolarization in in vitro models using hippocampal slices and culture of cortical axons (Balestrino et al. 1999; Shen and Goldberg 2012). Association of CK with ATP-providing or ATP-consuming processes may occur in order to make this buffer more efficient, in a process called metabolite channeling (Schlattner et al. 2006). This dynamic helps to prevent overload of the mitochondrial respiratory chain and accumulation of intracellular Ca^{2+} in rat brain, reducing generation of reactive species that have the power to cause oxidative stress and cytochrome C dissociation from the inner mitochondrial membrane thereby initiating early apoptotic triggering events (Meyer et al. 2006). It has been shown that creatine prevents or delays mitochondrial permeability transition pore opening in mitochondria from transgenic mice, an early event in apoptosis (Dolder et al. 2003). Chronic energy disruption also deteriorates cellular structure, in a level that may damage energy production processes as observed in several neurodegenerative disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD) (van den Bogaard et al. 2011; Martin 2012). In the CNS,
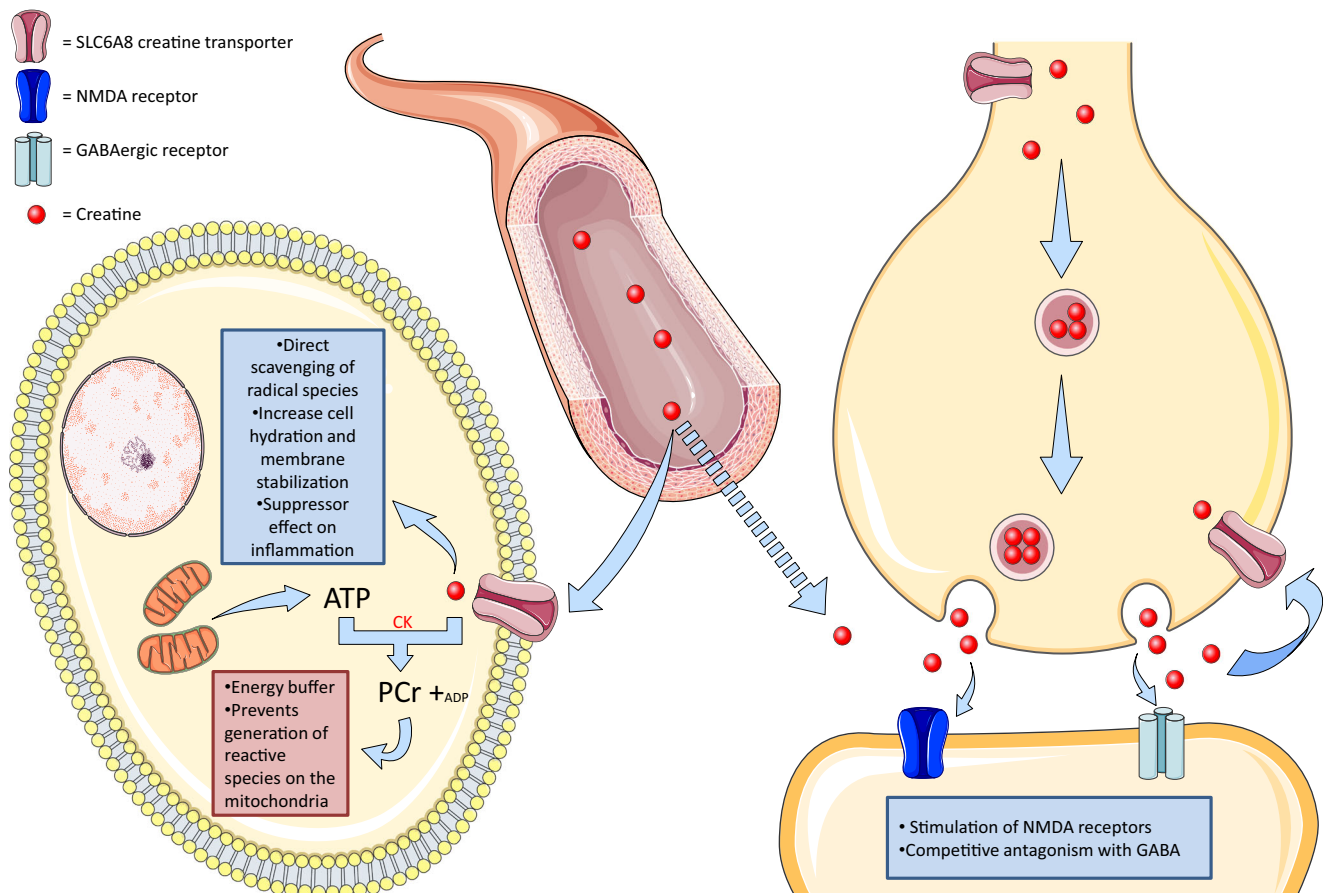
Fig. 1 Creatine produced in the liver travels in the bloodstream, where it actively enters the cells using the specific creatine transporter, SLC6A8. These transporters are in low levels in the micro capillaries of the BBB and are not expressed by the majority of perivascular astrocytes. Therefore, although the passage of creatine from the bloodstream to the CNS does occur, the CNS cells have their own pathway for the synthesis and maintenance of creatine levels. Once inside the cells, approximately two-thirds of the available creatine undergo a reversible phosphorylation catalyzed by CK, giving rise to PCr, a temporal and spatial energy buffer that can restore cellular ATP without a reliance on oxygen, preventing overload of the mitochondrial respiratory chain, and generation of reactive species. In addition, studies raise a range of possible secondary creatine functions and effects that are presented in the figure inside blue boxes: Creatine appears to increase cell hydration and membrane stabilization, and may have direct antioxidant activity, and a possible suppressor effect on inflammation (except in the airways, where it appears to exacerbate the response). Regarding the CNS, in addition to all previous effects, creatine may play a part as a neuromodulator. However, molecular mechanisms for all of these secondary functions remain a matter of debate, and they may be related to creatine's bioenergetic role in mitochondria

creatine plays essential functions in regenerating ATP for glutamate clearance during excitatory synaptic transmission (Oliet et al. 2001). In this context, knock-out mice for CK isoforms showed behavioral abnormalities, including spatial learning impairment and defects in the formation and maintenance of hippocampal mossy fiber connections (Jost et al. 2002; Streijger et al. 2005). Nonetheless, creatine supplementation restored the corticomotor excitability and cognitive decline associated with hypoxia-induced oxygen deprivation in humans (Turner et al. 2015) and in experimental models of brain injury (Scheff and Dhillon 2004; Sakellaris et al. 2006). Therefore, the creatine/PCr system appears to be of great value in disease states or situations where there is disruption of the cellular energy metabolism.

Secondary Creatine Functions and Effects

In addition to the well-known effects of creatine as an enhancer of cellular energetics, there are several other properties that only in recent years have come to light, as it will be presented. It is important to highlight that the mechanistic basis of these properties is still unclear and much of the effects observed may be derived from the energy reservoir provided by creatine. Therefore, studies investigating molecular mechanisms of how creatine exert such secondary effects must be highly encouraged.

Creatine is in constant interaction with reactive species generated in higher levels by tissues with great energy production and consumption. On that account, creatine appears to be both a direct and an indirect antioxidant (Sakellaris et al.

2006) that does not necessarily act by increasing or preventing a drop of the activities of antioxidant enzymes (Guimarães-Ferreira et al. 2012). As one of the major sites of reactive species formation, the mitochondria must have defensive mechanisms to maintain its activity, and creatine may play a crucial role as a defender against such insults. In this context, studies have showed that creatine significantly protects rat mitochondrial DNA from oxidative damage in a dose-dependent manner through an ADP-recycling mechanism (Meyer et al. 2006; Guidi et al. 2008). Therefore, creatine supplementation may play an important role in mitochondrial genome stability and cell viability, which can partially explain the enhancement in health and increased life expectancy found in rats supplemented with creatine (Bender et al. 2008). Direct antioxidant activity was first considered by Lawler and co-workers (Lawler et al. 2002), using a cellular experimental setting that showed that creatine protects against radicals such as superoxide anion ($O2^-$), peroxinitrite ($ONOO^-$), and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical. From there, several experimental protocols tested the ability of creatine in preventing oxidative imbalance induced damage by UV in human epidermal cells (Lenz et al. 2005), by respiratory chain inhibitor rotenone in whole body homogenates of flies (Hosamani et al. 2010), by acute exercise in muscle and plasma of rats (Deminice and Jordao 2012), high levels of GAA in striatum of rats (Kolling and Wyse 2010), and sedentary routine in different organs of rats (Stefani et al. 2014). Furthermore, Sestili and co-workers (Sestili et al. 2006) demonstrated that living cells in the presence of oxidants, creatine and Fe^{2+}, produced a molecule detected via mass spectrometry experiments with different molecular weight when compared with creatine. Based on this, it was possible to speculate that this finding represented a by-product of creatine oxidation derived from direct scavenging of radical species, rather than from spontaneous oxidation. In addition, there is an indirect antioxidant effect provided by creatine on differentiated mouse myotube cultures (C2C12) via up-regulation of peroxiredoxin-4 and thioredoxin-dependent peroxide reductase, two important antioxidant enzymes located in the cytoplasm and mitochondria, respectively (Young et al. 2010). This result opens the possibility to investigate whether this effect is reproducible in other cell types and highlights the superposition of multiple mechanisms that may explain the creatine's antioxidant properties.

Besides creatine antioxidant effects, since the 1970s, studies have shown that creatine has an effect on the inflammatory response, which was observed in various experimentally induced inflammatory models (Khanna and Madan 1978; Madan and Khanna 1979). Although the mechanisms involved are not yet fully understood, in recent years, the action of creatine as an immunomodulatory agent has gained more attention. The study reported by Nomura and co-workers (Nomura et al. 2003) examined the effect of creatine

supplementation on a number of potent inflammatory mediators induced both locally and systemically in endothelial cell in vitro. The results showed that creatine inhibited endothelial permeability and neutrophil adhesion to endothelial cells by suppressing both ICAM-1 and E-selectin expressions on endothelial cells and adhesion molecule expression. Posterior studies showed that creatine has a positive effect on the inflammatory response triggered by micro trauma in skeletal muscles after acute anaerobic sprint and strenuous exercise in human, diminishing levels of C-reactive protein, TNFα, INFα, IL-1β, and PGE2 (Santos et al. 2004; Bassit et al. 2008; Deminice et al. 2013). Interestingly, creatine supplementation combined with these exercise protocols also inhibited the increase in CK and lactate dehydrogenase activity (Santos et al. 2004; Bassit et al. 2010), which indicates less muscle soreness and a decrease in skeletal cell injury. This effect on muscle integrity is one of the possible explanations for the effect of creatine on inflammatory markers since reducing muscle cell death might stop the inflammatory process as a whole (Bassit et al. 2008). A recent study also provided initial evidence that creatine supplementation prevents skeletal muscle atrophy provoked by tumors via attenuation of tumor-induced pro-inflammatory environment in rats (Cella et al. 2019). Based in in vivo studies, it was suggested that the decrease in experimentally induced inflammation was the result of creatine reducing the expression of Toll-Like Receptor (TLR) 2, a plasma membrane-bound protein that recognizes acylated bacterial lipoproteins on the macrophages, key cells involved in the early phases of the immune response (Leland et al. 2011). Additionally, a recent work with arginine (a precursor of creatine) suggest that much of the mechanism of how creatine acts upon inflammation may be evolutionarily conserved (Azeredo et al. 2015).

On the other hand, studies have surprisingly shown that creatine has an opposite effect on the airways, increasing collagen and elastic fibers deposition in airway walls, eosinophil infiltration, and smooth muscle thickness in mice (Vieira et al. 2007, 2009; Ferreira et al. 2010). These changes are probably mediated via the increase in the release of IL-4, IL-5, and IGF-1 by inflammatory cells, as discussed by Riesberg and co-workers (Riesberg et al. 2016). Recent study showed that creatine increases IL-5 levels and the expression of P2 × 7 receptor in peribronchial leukocytes and epithelial cells in rats, worsening asthma pathology via purinergic signaling (Garcia et al. 2019). These results may also be partially explained by an increased availability of L-arginine after supplementation with creatine, leading to an increase in the synthesis of nitric oxide that may act either as a protective or a stimulatory factor regarding inflammation (De Gouw et al. 2001). Collectively, the data lead to the intriguing possibility that creatine boosts T helper cell type 2 (Th2) response after creatine supplementation (Vieira et al. 2007).

Regarding the CNS, creatine supplementation may increase cell hydration and membrane stabilization in mice (Wyss and Schulze 2002), both essential features for neuronal function and signal transduction. Moreover, researchers proposed that exocytotic creatine release would be electrically evoked in an action potential-dependent process, being dependent from Ca^{2+}, inhibited by the Na^+-channel blocker tetrodotoxin and enhanced by the K^+-channel blocker 4-amino-pyridine, consistent with neurotransmitter behavior (Almeida et al. 2006). Creatine seems to affect GABAa receptors via competitive antagonism at the same time that stimulates glutamatergic transmission via NMDA receptors in the hippocampus (Koga et al. 2005; Royes et al. 2008; Joncquel-Chevalier Curt et al. 2015), which may be related to the spatial memory improvement observed in rats after intrahippocampal administration of creatine (Oliveira et al. 2008). Hot spots of expression of CK isoenzymes have been reported in hippocampal pyramidal cells, which are involved in learning and memory (Kaldis et al. 1996), suggesting that the CK/PCr-system plays an essential role for this group of cells. Such theory is supported by studies that showed that creatine supplementation improves the performance of complex central executive tasks during stress caused by sleep deprivation in humans (McMorris et al. 2007), and intelligence/working memory performance tests even in healthy volunteers (Watanabe et al. 2002; Rae et al. 2003). However, these studies are not unequivocal evidence supporting a particular neurotransmitter function since the highly energy-dependent release and recycling of neurotransmitters at the synapse and the bioenergetic function of creatine may explain these neurological findings. Therefore, more studies are needed to generate consensus towards the potential role of creatine as neurotransmitter, in particular by discovering a so far unknown specific postsynaptic creatine receptor.

Creatine and Neurodegenerative/Neurometabolic Disorders

As previously said, creatine is not only important to the muscular tissue but also to other high energy demanding systems (Schlattner et al. 2006). The brain relies on an abundant and uninterrupted supply of energy substrates in order to allow electrical membrane potentials, action potential propagation, signaling activities, and recycling of neurotransmitters. Disruption or imbalances in the supply of energy for such an important organ compromise its functions, leading to alterations that contribute for the pathogenesis and progression of neurological and neurodegenerative conditions. These disorders are a group of acquired or inherited diseases that present a progressive loss of cells from one or multiple regions of the nervous system. A lot of work have been done to elucidate the

mechanisms surrounding such diseases, but most knowledge still remains equivocal. Nonetheless, there are some biochemical features common in the development and progression of these otherwise different pathological states. Chronic energy disruption accompanied by degradation of mitochondrial/cell structure, apoptosis, and oxidative stress is some of these fundamental biochemical processes, and they are in fact present in several neurodegenerative disorders such as PD, AD, HD, and amyotrophic lateral sclerosis (ALS) (Beal 2005; van den Bogaard et al. 2011; Martin 2012). Therefore, since creatine is a fundamental part of the CK/PCr system, it is expected that strategies to boost creatine levels in the brain have potential therapeutic value as it can help to replenish cellular ATP without a reliance on oxygen (Béard and Braissant 2010; Riesberg et al. 2016). One of the challenges of the supplementation with creatine is its difficulty to cross the BBB as previously cited (Braissant 2012). Nonetheless, studies have demonstrated that it is possible to modify brain creatine concentration with oral creatine monohydrate supplementation in different protocols (Dechent et al. 1999; Lyoo et al. 2003; Turner et al. 2015), thus validating the rational behind the studies cited below.

Inborn Errors of Metabolism

Creatine deficiency syndromes, which can be caused by mutations in AGAT, GAMT and SLC6A8 genes, have been identified in humans (Stöckler et al. 1994; Item et al. 2001; Salomons et al. 2001). The alterations in the CNS caused by these inborn errors of metabolism are responsible for the most severe symptoms presented by these patients, like mental retardation, autism, brain atrophy, delays in speech acquisition, or epilepsy. These symptoms are associated not only with the depletion of creatine but also with the toxic accumulation of creatine precursors: arginine and GAA. Mutations in AGAT and GAMT can be treated efficiently with supplementation of creatine (Battini et al. 2006; Marques and Wyse 2016). However, the SLC6A8 deficiency has a less encouraging prognosis. In this deficiency, creatine appears to be able to cross the jejunum via paracellular movement (Orsenigo et al. 2005), providing some improvements on muscular, but none on neurological symptoms since there is no transport of creatine occurring at the BBB (Arias et al. 2007; Valayannopoulos et al. 2012). Nonetheless, a very recent study has tried to overcome this problem by modifying the creatine molecule, creating di-acetyl creatine ethyl ester, a compound that should cross biological membranes independently of the transporter due to its very high lipophilicity. This compound was able to prevent electrophysiological failure and to increase intracellular creatine in hippocampal slices of mice (Adriano et al. 2018).

Our research group has studied the consequences of accumulation of GAA on rat brain, as well as the effect neuroprotector of creatine on GAA effects. We have observed

that creatine is able to prevent several deleterious effects of GAA on energy metabolism and oxidative status, such as the decrease in the activities of complex II, Na$^+$, K$^+$-ATPase, and creatine kinase, as well as the levels of thiobarbituric acid reactive substances, an index of lipid peroxidation (Kolling and Wyse 2010). In addition, these proprieties presented by creatine appear to be beneficial not only to models of creatine deficiencies but also to other experimental models of innate errors of metabolism, including homocystinuria and hyperprolinemia, where creatine was able to prevent memory impairment, lipid peroxidation, CK activity inhibition, and imbalance of redox homeostasis (Kolling et al. 2014; Kolling et al. 2017; Wyse and Netto 2011).

Alzheimer's Disease

AD is the most common form of progressive dementia, with patients presenting a loss of neurons (particularly of the cholinergic system) in cerebral cortex and specific subcortical regions. This neuronal loss is associated with deposits of extracellular plaques (amyloid-β peptide and cellular material) outside and around neurons, and deposits of intracellular neurofibrillary tangles (aggregation of the microtubule-associated protein tau in a hyperphosphorylated form). Mutations in amyloid precursor protein result in abnormalities in its processing, leading to such lesions (Blennow et al. 2006). At the molecular level, brain isoforms of CK have been shown to be significantly inactivated by oxidation in AD patients, and the depositions appear to be rich in creatine (Bürklen et al. 2006), which may lead to additional deleterious effects in the energetic state of neurons, exacerbating the neurodegenerative process. Creatine may exert neuroprotection by reducing protein aggregation since it was demonstrated that this compound can interfere in transglutaminase-catalyzed protein aggregation in sedimentation experiments (Burguera and Love 2006). Furthermore, creatine supplementation has been shown to be an effective neuroprotector against amyloid-β neurotoxicity in hippocampal neurons cell culture (Brewer and Wallimann 2000), and recent studies demonstrated that amyloid-β addition to cell cultures of cortical neurons leads to internalization of NMDA-receptors (Snyder et al. 2005). In addition, since inflammation is considered a driving force in the pathogenesis of AD, the effects observed in cells may be, at least in part, due to the apparent anti-inflammatory properties presented by creatine (McGeer et al. 2016). However, AliMohammadi and co-workers (AliMohammadi et al. 2015) showed that the supplementation with creatine had no effect on learning, memory retrieval, or neuron apoptosis in male Wistar rats submitted to amyloid-β injection. It is important to highlight that the situation is far more complex than it looks and more research is needed before the neuroprotective role of creatine in AD patients can be inferred. To date and to

the best of our knowledge, no human trial of creatine associated with AD has been published.

Amyotrophic Lateral Sclerosis

ALS is a term used to cover the spectrum of neurodegenerative syndromes characterized by progressive loss of motor neurons in the brain and spinal cord that ultimately leads to muscular paralysis, as these neurons degenerate and are replaced by gliosis (Wijesekera and Leigh 2009). Pathogenic mechanisms seem to include oxidative stress, glutamate excitotoxicity, impaired mitochondrial function, and aberrant protein folding (Turner and Talbot 2008). In addition, lower levels of cerebral ATP long before disease onset were identified in mouse models, and reduced CK activity has been reported in transgenic ALS mice (Wendt et al. 2002; Browne et al. 2006). Therefore, providing the cells with exogenous creatine supplementation might provide neuroprotective effects and constitute an effective treatment for ALS patients. Indeed, oral administration of creatine prevented neuronal loss in both the motor cortex and in substantia nigra, minimizing damage caused by reactive species, and producing a dose-dependent improvement in motor performance, as well as greater survival of transgenic mice for the SOD1G93A gene (Andreassen et al. 2001a; Dupuis et al. 2004). Shortly after these results were published, creatine treatment evolved to clinical trials. Despite its promising experimental animal models results, creatine tests in humans failed to reproduce such effects. Creatine presented no effect on survival, motor function, or respiratory function of patients (Drory and Gross 2002; Shefner et al. 2004; Rosenfeld et al. 2008). A more recent systematic review also did not find any statistically significant effect (Pastula et al. 2012). Nonetheless, the discrepancy presented by these studies may be related to the period when the treatment starts. This period is of 40 days before onset of disease in mice, and an average of 500 days after onset of symptoms in patients (Béard and Braissant 2010). Furthermore, most animal models are based on the mutation in copper zinc superoxide dismutase presented by hereditary ALS, while sporadic ALS accounts for most cases and only a small percentage of the human patients have the same genetic defect found in these transgenic mice (Bender and Klopstock 2016). Given that a recent study showed that creatine enhances mitochondrial-mediated oligodendrocyte survival after demyelinating injury in mice (Chamberlain et al. 2017) and that there is loss of oligodendrocyte in mouse models of ALS (Jones 2013), more work should be encouraged to shed light upon the initial discouraging clinical trials.

Parkinson's Disease

PD is a progressive neurodegenerative disease with motor, nonmotor, and behavioral findings. There is no golden

standard for diagnose, so family history and physical examination remain the most commonly used method. Some of the most common clinical symptoms presented by these patients are tremor, bradykinesia, postural imbalance, rigidity, speech impairments, blurred vision, and constipation. It is histologically characterized by changes to the mesencephalic substantia nigra, which leads to a profound loss of dopaminergic input into the striatum and development of alpha-synuclein containing Lewy bodies in the surviving dopaminergic neurons of the region (Gazewood et al. 2013; Johnson 2015). At the molecular level, mitochondrial electron transport system, in particular complex I, appears to play a part in the pathogenesis of PD (Alam and Schmidt 2002), suggesting mitochondrial imbalance and depletion ATP as one of the onset features leading to the development of the condition. Already in 1999, Matthews and co-workers demonstrated that creatine can prevent the loss of dopaminergic neurons in the substantia nigra caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration, the most widely used mouse model of PD. In addition, co-treatment with cyclooxygenase 2 inhibitor rofecoxib and coenzyme Q10 seemed to enhance neuroprotective effects of creatine in transgenic mouse models (Klivenyi et al. 2004; Yang et al. 2009), suggesting that for better results, a combined approach may be the best option. Creatine also improved dopaminergic cell survival against two in vitro model of PD using 1-methyl-4-phenylpyridinium ion (MPP+) or 6-hydroxydopamine (6-OHDA) on an organotypic tissue culture system (Andres et al. 2005). One of the main side effects of the treatment of PD with L-DOPA is the L-DOPA-induced dyskinesia (LID), characterized by abnormal involuntary movements. In order to study the effect of creatine on this feature, Valastro and co-workers (Valastro et al. 2009) induced PD in rats using 6-OHDA and submitted the animals to a diet with creatine. After 32 days on the diet, L-DOPA treatment began and signs for the development of abnormal involuntary movements were observed. The results showed that abnormal involuntary movements were considerably diminished in the creatine group, as well as biochemical markers associated with LID, indicating that creatine may be useful not only as a prevention strategy, but also to alleviate side effects derived from traditional treatment.

Despite so many promising results, once again, when translated to the clinical trials, creatine results are not encouraging. Creatine was shown to result in improved patient mood, but the Unified PD rating scale remained unaltered (Bender et al. 2006). A 2014 systematic review on the subject concluded that all the clinical studies were made with small sample sizes and short duration, and that future well-designed randomized controlled trials with larger sample size and long-term follow-up were needed to assess creatine for PD (Xiao et al. 2014). However, a double-blinded, multicenter, long-term efficacy trial that recruited 1741 PD patients who were randomly treated with either creatine monohydrate (10 g day^{-1}) or placebo showed no effect. Instead of an 8-year follow-up as intended, the study was interrupted because no differences were noted between creatine- and placebo-treated groups after 5 years (Kieburtz et al. 2015). Recently, meta-analysis studies have not supported the use of creatine for neuroprotection against PD, although the authors suggest that more correlated studies are still needed (Attia et al. 2017; Mo et al. 2017). Nonetheless, an improvement in upper-body strength of creatine supplemented PD patients (Hass et al. 2007) has been reported as well as a decrease in cognitive decline of PD patients treated with coenzyme Q10 associated with creatine (Li et al. 2015). These findings could perhaps be a guide to determine whether an earlier treatment would reproduce the beneficial effects observed in animal trials, since the Phase III trial was evaluated in patients with progressed stages of the disease.

Huntington's Disease

HD is an autosomal dominant disorder caused by a CAG repeat expansion in exon 1 of the huntingtin gene, producing a mutant form of the huntingtin protein (mHtt). It is characterized by some main symptoms that include progressive choreoathetotic movements, cognitive impairment, and neuropsychiatric disturbance, leading ultimately to death after a mean survival time of 15–20 years (Quinn and Schrag 1998). Different from other neurodegenerative disorders that have multiple causes and factors involved, HD is caused by a mutation in a single gene. This makes it easier, at least in theory, to search for an effective treatment (Kim and Fung 2014). The exact mechanism by which mHtt leads to neuronal death (mostly GABAergic projections) is still unclear, but energy metabolism deficit has been observed and proposed as a possible factor. This impairment includes reduced mitochondrial complex II and complex III activities, which leads to increased cerebral lactate levels and a reduced PCr/inorganic phosphate ratio in the muscle (Grünewald and Beal 1999; Calabresi et al. 2001). Therefore, boosting intracellular energy stores may be a useful strategy to alleviate symptoms and neurodegenerative progression. In vitro models have shown that creatine protects GABAergic cells against 3-nitropropionic acid (3-NPA), an irreversible succinate dehydrogenase inhibitor, and induced toxicity in striatal cultures (Andres et al. 2005). In rat models of brain injections of 3-NPA or malonate (a reversible succinate dehydrogenase inhibitor), oral supplementation with creatine resulted in smaller lesion sizes (Matthews et al. 1998), and creatine injected intraperitoneally protected against convulsive behavior and lactate production (Royes et al. 2006). Furthermore, in knock-out mouse models of HD, creatine administration resulted in greater survival, increased body weight, delayed motor symptoms, and considerably reduced brain lesion size with huntingtin-positive aggregates (Ferrante

et al. 2000; Andreassen et al. 2001b). A drawback is that in both studies, creatine supplementation started at a pre-symptomatic stage. However, a later study found similar results in post-symptomatic knock-out mice (Dedeoglu et al. 2003). Although well tolerated by patients, clinical trials have failed to improve Unified HD Rating Scale, an index used to assess cognitive, motor function, and functional ability (Verbessem et al. 2003; Tabrizi et al. 2005). On the other hand, brain glutamate levels and serum 8-hydroxy-2′-deoxyguanosine (a marker of oxidative injury to DNA that is considerably elevated in HD) in patients were consistently decreased after a creatine enhanced diet, suggesting at least some efficacy of creatine treatment (Bender et al. 2005; Hersch et al. 2006). A 2014 study by Rosas and co-workers made use of higher doses of creatine to treat patients likely to develop HD since they had affected first degree relatives, or a pre-symptomatic gene mutation detected (Rosas et al. 2014). At 6 and 18 months of creatine or placebo, neuroimaging was done to measure brain atrophy. Individuals in the creatine group had significantly less cortical and striatal atrophy compared to that observed in control group, suggesting that, if used in an ideal window, creatine may be able to delay disease progression and symptoms. Thus, a multicenter, randomized, double-blind, placebo-controlled study of up to 40 g daily of creatine monohydrate in participants with stage I and II HD treated for up to 4 years enrolled 553 participants but was halted for futility after the first interim analysis, providing contradictory data (Hersch et al. 2017).

Conclusion

Creatine has been widely used as an enhancer of muscular performance since the 1970s. In this review, we addressed its possible effects and functions in human beings, as well as the results that creatine presented as an adjuvant treatment in preclinical models and clinical trials for several diseases. The solid evidence available in the literature considers that the main function of creatine is by far to allow fast regeneration of ATP, in ATP demanding sites, via CK activity. In addition, studies raise a range of possible secondary creatine functions and effects, including direct and indirect antioxidant activity, overall anti-inflammatory effects (with the exception of the airways), and possible neuromodulation of synapses. However, molecular mechanisms remain a matter of debate, and they may be related to the bioenergetic role that creatine has in the mitochondria. It is a consensus that creatine supplementation is safe and has no serious collateral effects, as stated by the official position of the International Society of Sports Nutrition which has also refuted concerns surrounding renal toxicity (Kreider et al. 2017). In this context, and given the fundamental roles that creatine plays in the CNS, several preclinical and clinical studies have observed the potential that creatine has to treat degenerative disorders. However, although in vitro and in vivo experimental models are highly encouraging, most clinical trials fail to reproduce positive results, suggesting that animal models are better to address biological aspects of a possible treatment than to predict clinical efficacy.

Indeed, very few, mainly small pilot studies reported promising functional or neurological improvements by creatine in human beings. Therefore, future studies should first try to establish the best dosage regime to increase brain creatine in a way that can relate to animal studies. In order to avoid the inevitable saturation of SLC6A8 and the poor permeability of the BBB for creatine, di-acetyl creatine ethyl ester, a compound that should cross biological membranes independently of the transporter due to its very high lipophilicity, may represent a promising alternative (Adriano et al. 2018). Furthermore, available data suggests that a prophylactic use for neuroprotection in at-risk populations or patients is the most promising field. Therefore, future studies would benefit from biomarkers predictive of efficacy and determination whether baseline bioenergetics status is a significant variable in whether or not creatine supplementation works. To conclude, to this point, the only clearly positive data on human creatine supplementation in neurodegenerative/metabolic diseases concern the (rare) creatine deficiency syndromes, when the enzymes responsible for creatine biosynthesis are impaired.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations 3-NPA, 3-nitropropionic acid; 6-OHDA, 6-hydroxydopamine; AD, Alzheimer's disease; AGAT, L-arginine: glycine amidinotransferase; ADP, Adenosine diphosphate; AMPK, AMP-activated protein kinase; ALS, amyotrophic lateral sclerosis; ATP, adenosine triphosphate; BBB, blood brain barrier; CK, creatine kinase; CNS, central nervous system; GAMT, *N*-guanidinoacetate methyltransferase; GAA, guanidinoacetate; PCr, phosphocreatine; SAM, S-adenosylmethionine; HD, Huntington's disease; LID, L-DOPA-induced dyskinesia; mHtt, huntingtin protein; MPP+, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; Th2, T helper cell type 2; TLR, Toll-Like Receptor

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