Biochemistry Research Trends

Creatine

Biosynthesis, Health Effects and Clinical Perspectives

Lorraine Hogan
Editor

NOVA

Nova Biomedical

BIOCHEMISTRY RESEARCH TRENDS

CREATINE

BIOSYNTHESIS, HEALTH EFFECTS AND CLINICAL PERSPECTIVES

BIOCHEMISTRY RESEARCH TRENDS

Additional books in this series can be found on Nova's website under the Series tab.

Additional e-books in this series can be found on Nova's website under the e-book tab.

BIOCHEMISTRY RESEARCH TRENDS

CREATINE

BIOSYNTHESIS, HEALTH EFFECTS AND CLINICAL PERSPECTIVES

LORRAINE HOGAN
EDITOR

nova biomedical
New York

Library of Congress Cataloging-in-Publication Data

ISBN: 978-1-53612-420-0 (eBook)

Published by Nova Science Publishers, Inc. † New York

CONTENTS

PREFACE

Creatine (α-N-methylguanidino acetic acid) is a naturally occurring nitrogenous organic acid synthesized in the kidneys, liver, pancreas and brain (in less quantity), or obtained from a regular diet or as ergogenic nutritional supplement. Creatine is a popular and widely used form of protein supplementation due to its efficacy in improving performance in healthy athletic populations via increased muscle mass and enhanced adenosine triphosphate (ATP) energy regeneration. Chapter One reviews the available findings at animal and cellular studies as well as clinical trials investigating the neuroprotective effect of creatine and the possible mechanisms underlying its effect, which may represent important advances in the management of central nervous system diseases. Chapter Two considers the potential efficacy of creatine as an anabolic and ergonomic therapy for RA patients. In Chapter Three, a low-dimensional antimony-doped tin oxide aggregated nanoparticles (ATO NPs) were synthesized using hydrothermal method in alkaline phase. In Chapter Four, data obtained from –omics technologies and dynamical modeling are executed and analyzed, given the exponential growth of these methodologies in biological scientific research. The relevance of both creatine kinase system and creatine metabolism itself, as part of the molecular system bioenergetics in several components of cell (i.e., cytoskeleton, mitochondria, nucleus, endoplasmic reticulum, etc.), deserve to be

examined from a systemic view that covers ascending and descending causality.

Chapter 1 - Creatine (α-N-methylguanidino acetic acid) is a naturally occurring nitrogenous organic acid synthesized in the kidneys, liver, pancreas and brain (in less quantity), or obtained from a regular diet or as ergogenic nutritional supplement. Creatine supplementation has been reported to exert beneficial effects in a broad range of central nervous system diseases in which mitochondrial dysfunction, energetic imbalance, oxidative and nitrosative stress, glutamate excitotoxicity, calcium dyshomeostasis, inflammation and apoptosis play an etiological role. Since the management of neuropathologies remains unchanged over the last decades, creatine supplementation can be a promising therapeutic approach to these conditions. In line with this, the use of creatine as a neuroprotective strategy has received a lot of attention with several beneficial effects in models of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), ischemic brain injury and psychiatry diseases, such as major depressive disorder (MDD). The mechanisms underlying the neurobiological properties of creatine have been unveiled in recent years, including the neuroenergetic shuttle modulation, antioxidant, neuromodulatory, activation of several intracellular signaling pathways, antiexcitotoxicity and antiapoptotic with a consequent stimulation of neuroprotective and regenerative processes in the central nervous system. This chapter critically reviews the available findings at animal and cellular studies as well as clinical trials investigating the neuroprotective effect of creatine and the possible mechanisms underlying its effect, which may represent important advances in the management of central nervous system diseases.

Chapter 2 - Creatine is a popular and widely used form of protein supplementation due to its efficacy in improving performance in healthy athletic populations via increased muscle mass and enhanced adenosine triphosphate (ATP) energy regeneration. If these effects of creatine supplementation were to be replicated in patients with rheumatoid arthritis (RA), then considerable clinical and patient benefit would ensue, as RA is

a condition characterised by generalised muscle loss and substantially impaired physical function. The muscle loss inherent in RA is termed ‘rheumatoid cachexia,’ and its adverse consequences include reduced strength and physical function and, consequently, diminished quality of life. Whilst regular high-intensity exercise training has been shown to increase muscle mass and restore function in RA patients, this form of therapy has very low uptake amongst RA patients. Thus, acceptable alternatives are required. The aim of this review is to consider the potential efficacy of creatine as an anabolic and ergonomic therapy for RA patients. To date, only two studies have supplemented RA patients with creatine, and the findings from these investigations are inconsistent and inconclusive. However, trials in populations with similar losses of muscle mass and function as RA, including older adults and those with other muscle wasting conditions, indicate that creatine could be an efficacious way of improving muscle mass, strength and physical function in RA patients, and may offer an easy, safe and cheap means of treating rheumatoid cachexia and its consequences.

Chapter 3 - Nanostructure materials or nanomaterials research takes a materials science-based approach to nanotechnology, leveraging advances in dope or un-doped materials metrology and facile synthesis which have been developed in support of various chemical and nano or micro fabrication research. Nanomaterials (NMs) have unique properties, such as optical, structural, electronic, chemical, or mechanical in every dimensions with nanoscale range. In this chapter, a low-dimensional antimony-doped tin oxide aggregated nanoparticles (ATO NPs) were synthesized using hydrothermal method in alkaline phase. The optical, morphological, and structural properties of ATO NPs were characterized in details using FTIR, UV-Vis, FESEM, XEDS, XPS, TEM and XRD techniques. Flat glassy carbon electrode (GCE) was fabricated with a thin-layer of aggregated ATO NPs by conducting coating binders (5% Nafion) for the development of selective and sensitive enzyme-less creatine (Crt) sensor. Electrochemical responses along with higher sensitivity, large-dynamic range and long-term stability towards Crt were performed by electrochemical I-V approach. The calibration curve was found linear over

a wide linear dynamic range of Crt concentration. It is an organized route for the development of non-enzymatic Crt biosensor based on ATO NPs embedded GCE using electrochemical reduction phenomena and significantly applied for the real analysis. As far as the authors know, this report is the maiden publication on highly sensitive Crt biosensor based on the ATO NPs/GCE. This method could be a pioneer development in Crt sensitive biosensor development using ATO NPs in reliable I-V method for the important biosensor applications with useful doped materials coupled nano-technological system.

Chapter 4 - Systems biology represents a powerful interdisciplinary approach which aims to study a phenomenon as a whole by looking at the interconnected network of components that are mutually dependent, hierarchically organized and dynamic. In order to accomplish this goal, data obtained from –omics technologies and dynamical modeling are executed and analyzed, given the exponential growth of these methodologies in biological scientific research. In this sense, the relevance of both creatine kinase system and creatine metabolism itself, as part of the molecular system bioenergetics in several components of cell (i.e., cytoskeleton, mitochondria, nucleus, endoplasmic reticulum, etc.), deserve to be examined from a systemic view that covers ascending and descending causality. Creatine is mainly present in cells with fluctuant and high-energy demands, such as skeletal and cardiac muscle, brain and testes; nonetheless, it should be taken from the bloodstream through a specific Na+-Cl--dependent symporter called SLC6A8 to act as one of the most important ATP buffers via creatine-phosphocreatine shuttle. This tissue-specific feature turns the creatine kinase system into a noteworthy mechanism of regulation of energy fluxes, especially because of its coordination with proteins of the glycolytic linear phsophotransfer system (hexokinase, phosphofructokinase, pyruvate kinase), adenylate kinase (AK), adenosine nucleotide translocase (ANT), voltage-dependent anion channel (VDAC), myosin- and sarcoplasmic-ATPases, nucleoside diphosphate kinase (NDPK), Na+/K+-ATPase, among others. Besides cytoplasmic streaming, cytoskeleton rearrangement, and mitochondrial position, these interactions encompass one of the most complex and

dynamic systems of human body. In fact, effects of creatine supplementation in human show a marked proteomic, transcriptomic and genomic response that determine activation or repression of certain signaling pathways through mechanisms involving positive and negative feedback or feedforward motifs. New organ energy interconnections and microbiome influence are discussed to contemplate a whole representation of creatine interactions.

In: Creatine
Editor: Lorraine Hogan
ISBN: 978-1-53612-414-9

Chapter 1

AN UPDATE ON THE NEUROPROTECTIVE EFFECTS OF CREATINE IN CENTRAL NERVOUS SYSTEM DISEASES

Mauricio P. Cunha*, PhD
Department of Biochemistry, Center of Biological Sciences,
Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

ABSTRACT

Creatine (α-N-methylguanidino acetic acid) is a naturally occurring nitrogenous organic acid synthesized in the kidneys, liver, pancreas and brain (in less quantity), or obtained from a regular diet or as ergogenic nutritional supplement. Creatine supplementation has been reported to exert beneficial effects in a broad range of central nervous system diseases in which mitochondrial dysfunction, energetic imbalance, oxidative and nitrosative stress, glutamate excitotoxicity, calcium dyshomeostasis, inflammation and apoptosis play an etiological role.

* Corresponding Author address: Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina (UFSC), Campus Universitário, Trindade, 88040-900, Florianópolis, SC, Brazil. Phone: +55 (48) 3721-2817; Email: mauricio.personal@gmail.com.

Since the management of neuropathologies remains unchanged over the last decades, creatine supplementation can be a promising therapeutic approach to these conditions. In line with this, the use of creatine as a neuroprotective strategy has received a lot of attention with several beneficial effects in models of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), ischemic brain injury and psychiatry diseases, such as major depressive disorder (MDD). The mechanisms underlying the neurobiological properties of creatine have been unveiled in recent years, including the neuroenergetic shuttle modulation, antioxidant, neuromodulatory, activation of several intracellular signaling pathways, antiexcitotoxicity and antiapoptotic with a consequent stimulation of neuroprotective and regenerative processes in the central nervous system. This chapter critically reviews the available findings at animal and cellular studies as well as clinical trials investigating the neuroprotective effect of creatine and the possible mechanisms underlying its effect, which may represent important advances in the management of central nervous system diseases.

Keywords: antiexcitotoxicity, antioxidant, creatine, neurological and mental diseases, neurotrophic

LIST OF ABBREVIATIONS

5-HT_{1A} Receptor	5-Hydroxytryptamine 1_A Receptor
6-OHDA	6-Hydroxidopamine
8-OHdG	8-Hydroxy-2′-Deoxyguanosine
Aβ	Amyloid β
ADP	Adenosine Diphosphate
AGAT	L-arginine Glycine Amidine Transferase
AMP	Adenosine Monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine Triphosphate
AD	Alzheimer's Disease
HD	Huntington's Disease
ALS	Amyotrophic Lateral Sclerosis

TBI	Traumatic Brain Injury
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CA	*Cornu Ammonis*
COX-2	Cyclooxygenase 2
DCF	Dichlorofluorescein
ERK	Extracellular Signal-Regulated Kinase
GABA	γ-Aminobutyric Acid
GAMT	Guanidineacetate-Methyltransferase
GFAP	Glial Fibrillary Acid Protein
GSK3β	Glycogen Synthase Kinase 3β
H_2DCFDA	2',7' – Dichlorofluorescein Diacetate
IkBα	Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-cells Inhibitor, Alpha
IGF-1	Insulin-like Growth Factor 1
MDD	Major Depressive Disorder
MEK	Mitogen-Activated Protein Kinase Kinase/
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
NFkB	Nuclear Factor kappa B
NINDS	National Institute of Neurological Disorders and Stroke
NMDA	N-Methyl-D-aspartate
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
PD	Parkinson's Disease
PI3K	Phosphatidylinositol-3 Kinase
PKA	Protein Kinase A
PKC	Protein Kinase C
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

There are more than 1,000 diseases, disorders and injuries affecting the central nervous system, such as neurological and psychiatry diseases. Central nervous system diseases associated with progressive loss of structure

and functions of neurons have a high prevalence worldwide. In line with this, approximately 700 million new cases of mental, neurological and behavioral diseases are reported annually, accounting for 13% of the overall global disease burden [1]. These disorders are an important and growing cause of morbidity and disability [2].

Through the years, the pieces of evidence have suggested that impairment of energy production in the brain may play a pivotal role in the pathogenesis of certain neurologic and mental diseases [3]. Moreover, creatine is a guanidine-like compound that plays an important role in the central nervous system's metabolism [4, 5]; it is hypothesized to then protect against brain damage associated with mental and neurological diseases. This chapter summarizes and discusses the therapeutic potential of the energetic molecule creatine in the central nervous system diseases, since *in vitro* and animal studies reported that neurochemical alterations induced by neurologic and psychiatry diseases can be counteracted by creatine. Nowadays, these preclinical evidences of the neuroprotective effect of creatine encourage researchers to investigate whether it can be translated to cure human diseases.

THE CREATINE/PHOSPHOCREATINE SYSTEM IN THE BRAIN

Creatine is widely found in the mammalian brain tissue. Creatine concentrations in the brain are several fold higher than in plasma, so there is active accumulation of this compound in the brain coming from peripheral tissues or synthesized in the central nervous system [6]. Moreover, this guanidine-like compound could be synthesized in the brain, although the majority of total body synthesis takes place in kidney, pancreas and liver [6]. In the central nervous system, some neuronal, astrocytic and oligodendrocyte subpopulations contain both enzymes of creatine synthesis: AGAT (arginine–glycine amidino transferase) and GAMT (guanidinoacetate methyltransferase) and can make their own creatine supply [7, 8]. However, other central nervous system cells possess

only AGAT and are, therefore, net producers of guanidinoacetate, which is released into the extracellular medium. Some oligodendrocytes and astrocytes cells, but not neurons, possess only GAMT and therefore need the creatine transporter SLC6A8 in order to take up the precursor guanidinoacetate [7–9]. Furthermore, some neurons, but not astrocyte, possess none of the enzymes of creatine synthesis and are absolutely dependent of SLC6A8 to take up creatine produced by other cells [8, 10, 11]. Still, other cells of central nervous system do not possess any enzymes of creatine synthesis or transporters and does not use creatine in its metabolism [7, 8].

Creatine obtained from diet or synthesized in peripheral tissues can contribute to brain creatine levels supply. However, the oral creatine supplementation only promotes modest increases on creatine levels in the brain, suggesting that the creatine transporter is near saturated under physiological conditions limiting creatine uptake [12, 13]. Furthermore, transfer of creatine from periphery to brain across the blood–brain barrier or blood-cerebrospinal fluid appears to be limited [14].

The energy demand in the adult brain is extremely high (around 20% of resting metabolism) [15] and the creatine kinase/phosphocreatine system is available in the brain connecting sites of adenosine triphosphate (ATP) production (glycolysis and mitochondrial oxidative phosphorylation) with subcellular sites of ATP utilization (ATPases) [6]. For this purpose, brain contains the cytosolic brain-type creatine kinase [16, 17] and the ubiquitous mitochondrial creatine kinase [6]. The cytosolic creatine kinase generates significant amounts of ATP upon brain activation, when mitochondrial creatine kinase generates phosphocreatine from freshly synthesized ATP [6, 8]. Interestingly, cytosolic brain-type creatine kinase is more expressed in astrocytes and oligodendrocytes than neurons, when ubiquitous creatine kinase is widely expressed in neurons with high oxidative demand [8].

In addition to its role in modulating bioenergetics status in the central nervous system, creatine could be also released in an action-potential dependent (exocytotic) manner, providing strong evidence for its role as a neuromodulator in the brain [18]. Moreover, creatine seems to exert action

on postsynaptic receptors in neurotransmission systems and to modulate memory, mood and neurodegenerative process [8]. Furthermore, creatine produces also pleiotropic effects, such as antioxidant, antiexcitotoxicity and antiapoptotic [6].

ANTIOXIDANT EFFECT OF CREATINE IN THE CENTRAL NERVOUS SYSTEM

Some studies suggest that creatine counteracts to oxidative and nitrosative stress associated with neurological and mental diseases. The first evidence for an antioxidant property of creatine in the central nervous system was reported by Matthews et al. (1998) [19]. In this study, creatine supplementation by 2 weeks was capable of protecting rats from malonate-induced striatal hydroxyl radical production and 3-nitropropionic acid-induced increases in 3-nitrotyrosine concentrations, a marker of peroxynitrite-mediated oxidative injury [19]. Subsequently, a study performed by Klyvenyi et al. (1999) reported that creatine supplementation for 50 days in G93A mice (a amyotrophic lateral sclerosis model) prevented the 3-nytrotyrosine/tyrosine ratio increased in the lower and upper spinal cord, an indicative of nitrosative stress [20]. In the same study the authors reported through the microdialysis approach that the administration of mitochondrial toxin 3-nitropropionic acid caused a significant increase in the conversion of salicylate to 2,3-dihydroxybenzoic acid in the striatum of G93A mice (an indicative of hydroxyl radical production) and creatine supplementation prevented this conversion [20].

From this evidence, several studies indicated that creatine could decrease reactive oxygen and nitrogen species levels induced by several neurotoxins, using 2',7' dichlorofluorescein diacetate (H_2DCFDA) probe, a dye that is used to measure hydroxyl, peroxyl and other reactive oxygen and nitrogen species within the cell. In line with this, creatine decreases dichlorofluorescein (DCF) fluorescence induced by multiple neurotoxins

such as 6-hydroxidopamine (6-OHDA) [21], glutamate [22], high glucose concentrations [23], HIV-1 transactivator of transcription protein [24], and staurosporine [25]. Furthermore, the activation of mitochondrial creatine kinase by creatine prevents H_2O_2 production obeyed the steady-state kinetics of the enzyme to phosphorylate creatine in the cerebral cortex neurons [23].

The superoxide is a natural reactive oxygen anion implicated in oxidative stress at several mammalian body tissues. Interestingly, a study demonstrated that creatine administration is able to prevent the increased xanthine oxidase activity (an enzyme implicated in the superoxide anion generation) induced by pentylenetetrazol in the cerebral cortex of rats [26]. Controversially, Juravleva et al. (2003, 2005) demonstrated that neuron-glia primary cells incubated with creatine increased superoxide production and creatine when combined with glutamate produced adjunctive effect on the superoxide generation [27, 28].

Lipid peroxidation is the oxidative degradation of lipids, resulting in cell damage. Evidences suggest that creatine prevents the increase of the tiobarbituric acid reactive species (a biomarker of lipoperoxidation) in experimental models of PD [21, 29, 30], epilepsy [26], severe traumatic brain injury (TBI) [31], methylmalonic acidemia [32], inborn errors [33, 34], intrapartum hypoxia in the precocial spiny mouse (Acomys cahirinus) [35], but did not alter the lipoperoxidation in rats exposed to chronic cerebral hypoperfusion [36].

Protein carbonylation is a type of protein oxidation that can be promoted by reactive oxygen species. Creatine supplementation decreased the levels of protein carbonyl in the brain of rats submitted to severe TBI [31]. Moreover, acute creatine treatment prevented pentylenetetrazol-induced protein carbonylation in the cerebral cortex of rats.

The level of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative damage to DNA, tended to be lower in the brain of aging mice (2 years) supplemented with creatine in fed, even though this difference failed to reach significance [37]. Furthermore, creatine administered for 16 weeks to Huntington's disease (HD) patients decreased serum 8-OHdG [38].

The oxidation of lipid, protein and DNA could generate neuronal death. Sartini et al. (2012) demonstrated at the first time that creatine protected against H_2O_2-induced spinal cord cell death and glutathione decrease [39]. Cunha et al. (2016b) also demonstrated that SH-SY5Y cells (a neuron mimetic model) incubated with creatine prevented cellular death, NOx production and reactive oxygen and nitrogen species generation induced by H_2O_2 [22]. Additionally, creatine also increased cellular viability and prevented reactive oxygen and nitrogen species formation in striatal cells expressing mutated huntingtin (a HD cell model) subjected to H_2O_2 [25]. Unexpectedly, a study performed by Genius et al. (2012) demonstrated that hippocampal primary cells challenged with creatine did not prevent H_2O_2-induced toxicity and decreased intracellular ATP/PCr ratio [40].

The mechanism like creatine acts as an antioxidant in the central nervous system is still unknown. One hypothesis to explain the antioxidant effects in the central nervous system is that creatine is able to exert antioxidant properties acting as a radical scavenger. Lawler et al. (2002) used a well-controlled acellular experimental setting and the authors reported that creatine was capable of scavenging charged radicals such as the 2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical, superoxide anion and peroxynitrite; by contrast, no significant quenching effect was observed on the radical oxidants H_2O_2 and lipid peroxide tert-butyl-hydroperoxide [41]. Recently, Cunha et al. (2016b) also demonstrated at acellular set of experiments that creatine (concentration-dependent) decreased the amount of nitrite formed in the reaction of oxygen with nitric oxide produced from sodium nitroprusside solution, suggesting also direct scavenger activity of creatine [22]. Controversially, at a cell-free approach employing xanthine oxidase/xanthine as an enzymatic generator of superoxide anions, a rise of chemiluminescence was seen after adding creatine to solution, indicating increased superoxide generation or enhanced life-time of these species [40]. Thus, more evidences are needed to verify the importance of the direct antioxidant properties of creatine for its neuroprotective effect in neurological and mental diseases.

Therefore, another putative explanation for the antioxidant effect of creatine in the neurological and mental diseases is that creatine can activate the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and subsequently translation of antioxidant enzymes such as glutathione peroxidase and hemeoxygenase-1 in the cerebral hippocampus [42].

THE ROLE OF CREATINE ON GLUTAMATE NEUROTRANSMISSION AND EXCITOTOXICITY

Glutamate is the most abundant neurotransmitter in the vertebrate nervous system mediating cognitive functions such as learning and memory, as well as long-term potentiation associated to neuroplasticity [43, 44]. However, the glutamate excess could cause calcium ions to enter in neurons via N-Methyl-D-aspartate (NMDA) receptor channels, leading to neuronal damage and eventual cell death at process known as glutamate excitotoxicity [45].

Of note, several molecular actions of creatine, such as increased Na^+, K^+-ATPase activity, neuronal excitability, reduction of intracellular calcium ions, and antiexcitotoxicity property, appear to be mediated by glutamate receptors. Moreover, Rambo et al. (2012) showed that the incubation of rat hippocampal slices with creatine for 30 min increased Na^+,K^+-ATPase activity and the preincubation of hippocampal slices with NMDA (MK-801) and NMDA receptor subunit 2B (NR2B; ifenprodil) antagonists, but not with the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist (DNQX), blunted the effect of creatine [46].

Furthermore, the electrophysiological actions of creatine are dependent at least in part of glutamate receptors. In line with this, a study demonstrated that creatine gradually increases the amplitude of first population spike and elicits secondary population spike in stratum radiatum of the CA1 region of hippocampal rat slices [32]. Creatine also decreased the intensity of the stimulus to induce population spike, when

compared with control slices [32]. The competitive NMDA receptor antagonist, 2-amino-5-phosphonopentanoic acid (AP5) attenuated creatine-induced increase of amplitude of population spike and appearance of secondary population spike, providing pharmacological evidence of NMDA receptors involvement in the electrophysiological effects of creatine [32]. In addition, creatine also increased [^{3}H] MK-801 binding to hippocampal membranes; further indicating that this compound may produces NMDA receptor activation [32]. However, Genius et al. (2012) showed that creatine produces a direct inhibitory action on the NMDA receptor-mediated calcium response, which initiates the excitotoxic cascade [40]. These studies demonstrate differences in the direct effect of creatine on the NMDA receptor and more evidence with controlled conditions of concentration and time of exposure of creatine are needed to elucidate the effect of creatine on this receptor.

In addition, several *in vitro* reports have indicated that creatine inhibits the glutamate neurotransmission and excitotoxicity. For example, Brewer and Wallimann (2000) demonstrated at first time that hippocampal neurons incubated with creatine greatly reduced glutamate toxicity and protected against glutamate-induced dendritic pruning. In the same study, creatine incubation at hippocampal neurons exposed to glutamate also increased the reserve energy ratio [47]. Subsequently, Juravleva et al. (2003, 2005) showed that creatine when incubated in the neuron/glia cells protected against the toxicity of glutamate [27, 28]. The neuroprotective effect of creatine is dependent at least in part of nitrite, farnesylated Ras and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkBα) immunocontent reduction, suggesting that factor nuclear kappa B (NFkB) activation is an important step in the protective effect of creatine against glutamate [27, 28]. Genius et al. (2012) also demonstrated that embryonal hippocampal and cortical cells challenged with glutamate decreased the cell viability and ATP levels and creatine mediated a direct effect on the bioenergetic balance, thereby acting as a neuroprotectant in the excitotoxicity induced by glutamate [40]. Cunha et al. (2016b) demonstrated that creatine prevented the reduction of SH-SY5Y cell viability induced by glutamate incubation and this effect is dependent at

least in part of antioxidant properties of creatine, since creatine was able to decrease glutamate-induced nitrite and reactive oxygen and nitrogen species production [22].

Studies using animal models also indicated that creatine modulates glutamate receptors, metabolism and excitotoxicity. For example, the glutamate receptors involvement is reported in the cognitive and mood enhancer effect of creatine [48, 49]. Oliveira et al. (2008) demonstrated that the cognitive enhancer effect of intrahippocampal administration of creatine in the Barnes maze test (latency for scape and mean number of errors) was reverted by co-administration of arcaine (polyamine site NMDA receptor antagonist) and intensified by spermidine (polyamine site NMDA receptor agonist), suggesting that the activation of polyamines binding site at NMDA receptor play an important role in the spatial memory induced by creatine [49].

Furthermore, Cunha et al. (2015a) also demonstrated that the antidepressant-like effect of creatine in mice is mediated by NMDA receptor, but not AMPA receptor, since the antidepressant-like effect of creatine in the tail suspension test is antagonized by NMDA, D-serine (glycine-site NMDA receptor agonist) and arcaine administrations, but not by DNQX (AMPA receptor antagonist) [48].

In relation to glutamate metabolism, creatine altered the glutamate levels and uptake in the rodent brain [50, 51]. In line with this, Andreassen et al. (2001b) stated that creatine supplementation significantly increased longevity and motor performance of the G93A mice (an animal model of amyotrophic lateral sclerosis) and attenuated the increases in cortical glutamate levels measured by spectroscopy at 75 days of age [50].

Magni et al. (2007) showed also that creatine administration prevented the glutaric acid-induced inhibition of l-[^{3}H] glutamate uptake in striatal synaptosomes of rats, suggesting that creatine may reduce the deleterious effects of glutaric acid by maintaining glutamate uptake in the synaptic cleft [51].

Of note, the first evidence indicating that creatine could prevent the glutamate excitotoxicity in animals was performed by Malcon et al. (2000) [52]. In this study, oral administration of creatine significantly attenuated

striatal lesions produced by infusion of NMDA, but not AMPA or kainic acid [52]. Moreover, Cunha et al. (2016b) demonstrated that the subchronic administration of creatine in mice for 21 days (but not single administration) prevented the reduction of cell viability in hippocampal slices submitted to glutamate exposition [22].

Clinical trials also reported the effect of creatine supplementation on the glutamate levels in the central nervous system. For example, upon creatine supplementation healthy controls and amyotrophic lateral sclerosis patient had a decline in the unresolved glutamate ([glutamate+ glutamine]/creatine or [glutamate+glutamine]/choline) in the motor cortices [53]. In addition, twenty HD patients showed a 15.6% decrease of unresolved glutamate and a 7.8% decrease of glutamate after creatine supplementation by 8-10 weeks [54]. Therefore, the action of creatine on the glutamate metabolism and it signaling plays a pivotal role in the neuroprotective effect of creatine against mental and neurological diseases.

THE NEUROTROPHIC AND NEUROGENIC PROPERTIES OF CREATINE

Neurogenesis is a complex processes in the mammalian brain involving proliferation, fate specification, differentiation, maturation, migration, and functional integration of newborn cells into the existing neuronal circuitry [55]. Neurons are generated from early embryonic development, with only a few neurogenic zones remaining active in the adult [56–58]. Specifically, the main neurogenic regions in the adult brain are the subventricular zone and the subgranular zone of the dentate gyrus in the hippocampus [57, 59–61]. Moreover, adult hippocampal neurogenesis is extremely regulated by growth factors including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and Insulin-like Growth Factor 1 (IGF-1) [62].

The neurotrophic and neurogenic effects of creatine have been reported in hippocampal, fetal rat spinal cord neurons and progenitor neuronal cells,

where this molecule was shown to induce proliferation, differentiation, neurite arborization and outgrowth, as well as exert antiapoptotic effects. Of note, creatine supplementation in aged mice up-regulated the brain expression of genes correlated with dendrite morphogenesis and cell motility/axon guidance, including the gene expression of several neurotrophic factors such as Bdnf, Ndn, Hgf, and Tgfb2 [37]. Furthermore, acute administration of creatine, similar to fast-acting antidepressant ketamine, increased BDNF levels in the corticosterone-treated mice, but not in the vehicle-treated mice, as compared to respective control groups [63].

The first neuroanatomic evidence investigating the neurotrophic properties of creatine was performed by Ipsiroglu et al. (2001) [64]. In this study, creatine maternal supplementation produced alterations on neuronal morphology of hippocampus of pups, since confocal micrographs of hippocampal CA1 neurons immunostained with biocityn (neuroanatomical tracer taken up by axons and dendrites) increased the numbers of dendritic crossings along the Scholl rings as a function of distance from soma ([64] and reviewed by [65]).

Furthermore, a study performed by Pazini et al. (in press) demonstrated the neurogenic properties of creatine treatment in a depression model induced by chronic treatment with corticosterone in mice [66]. Firstly, this study showed that chronic corticosterone administration decreased hippocampal Ki-67 immunostaining, an indicative of cell proliferation impairment, and creatine treatment by 21 days prevented this effect [66]. Since cellular differentiation is an important event in neurogenesis, Pazini et al. (in press) also demonstrated that chronic corticosterone treatment decreased hippocampal neuronal differentiation (Neuro-D immunostaining) and chronic creatine treatment was able to prevent this effect [66]. Finally, this study also demonstrated that depressive-like model induced by corticosterone increased glial fibrillary acid protein (GFAP) immunostaining (suggestive of astrogliosis) in dentate gyrus of the hippocampus and treatment with creatine for 21 days prevented this effect [66]. Of note, the effect of creatine treatment in the

hippocampal neurogenesis is similar to fluoxetine (a conventional antidepressant) treatment [66].

Di Santo et al. (2013) demonstrated that the cumulative total number, but not size, of floating spheres containing early postnatal rat spiral ganglia, utricle, and organ of Corti-derived progenitors cells over all passages was significantly higher after creatine incubation, suggesting that creatine could increase the cell proliferation [67]. Furthermore, creatine during differentiation of spiral ganglia cells resulted in a significantly higher density of β-III-tubulin-positive cells (an indicative of neuronal differentiation) [67]. Importantly, a combination of creatine with the neurotrophin BDNF resulted in further significantly increased densities of β-III-tubulin-positive cells in cultures of spiral ganglia cells as compared with each drug alone [67].

GABA is the main inhibitory neurotransmitter within the CNS and appears to be involved in a wide variety of physiological functions and mental and neurological diseases [68]. Strategies that increase the differentiation of neural progenitors in GABAergic neurons can be consolidated as important neuroprotective approach [69]. In line with this, cellular studies strongly indicated that creatine increases the differentiation of progenitor cells to GABAergic neuron-like cells. Andres et al. (2005a) showed at first time that chronic creatine incubation (7 days) increased the densities of γ-Aminobutyric acid (GABA)-immunoreactive neurons in the striatal cells, although total neuronal cell number and general viability were not affected [70]. Similar effects were seen after short-term treatment, suggesting that creatine is a differentiation inductor. Interestingly, inhibitors of transcription or translation did not abolish the creatine-mediated differentiation effects, whereas inhibition of mitogen-activated protein kinase (MAPK), also known as extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3-kinase (PI3K) significantly attenuated the creatine induced increase in GABA-immunoreactive cell densities [70]. Accordingly, chronic creatine incubation (7 days) and short-term creatine incubation in rat striatal neural progenitor cells at early (E14) and late (E18) developmental stages significantly increased the percentage of GABA-immunoreactive neurons as compared to control group [71].

Creatine exposure promoted morphological differentiation of GABA-ergic neurons, including an increase of neurite length and the number of branching points in rat striatal neural progenitor cells [71, 72, 73] also demonstrated that although cell survival and total neuronal cell density were not altered by chronic creatine incubation, chronic creatine and short-term creatine exposure produced a significantly higher density of GABA-immunoreactive neurons hinting to a differentiation-inducing mechanism of creatine in the fetal rat spinal cord neurons. In addition, a significant higher content of glutamate decarboxylase (an enzyme that catalyzes the decarboxylation of glutamate to GABA and CO_2) was demonstrated in the rat spinal cord incubated with creatine [73]. In the same study, creatine also affords neuroprotection for GABA-immunoreactive neurons against 3-nitropropionic acid induced toxicity [73].

Moreover, chronic creatine or neurotrophin-4/5 incubation, or a combination of both factors significantly increased numbers of neuronal nitric oxide synthase-immunoreactive neurons in the E14 rat ganglionic eminences [74]. The authors also showed in the double-immunostaining experiments that all neuronal nitric oxide synthase-immunoreactive cells co-localized with GABA-immunoreactive cells, suggesting a nitrergic modulation in GABA interneurons by creatine [74].

In addition, a study also demonstrated that creatine incubation induced the bone marrow stromal cells differentiation into GABAergic neuron-like cells with low yield [75, 76]. Subsequently, the same research group showed also that the combination of all-trans-retinoic acid, the ciliary neurotrophic factor, and creatine substantially induced differentiation of neurosphere-derived neural stem cells provided from bone marrow stromal cells into GABAergic neuron-like cells within 1 week [77]. Darabi et al. (2017) also reported that the mechanism of differentiation was clearly associated with induction of several differential pluripotent genes [77].

Studies also indicated that creatine is able to produce neuronal survival. For example, rat embryonic day 14 (E14) ventral mesencephalic neurons grown as organotypic free-floating roller tube and incubated with creatine by 14 days increased tyrosine hydroxylase-immunoreactive cell density [78]. In line with this, fetal ventral mesencephalic neurons

incubated with creatine increased survival and soma size of dopaminergic neurons [79]. The ability of creatine in the modulation of cell proliferation, differentiation and survival could contribute to neuroprotective effect exerted by creatine against several toxic insults and central nervous system diseases.

THE EFFECT OF CREATINE SUPPLEMENTATION IN THE CENTRAL NERVOUS SYSTEM DISEASES

Creatine seems to exert neuroenergetic and pleiotropic effects (antioxidant, antiexcitotoxicity and neurotrophic) on the brain that can counteract the neurodegeneration associated with several diseases affecting the central nervous system [6, 8, 80]. This chapter has reviewed the main findings of the effects of creatine on several neurological and psychiatric disorders.

THE THERAPEUTIC EFFECT OF CREATINE IN THE PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive movement neurologic disorder associated to motor symptoms, such as: resting tremor, bradykinesia, muscular rigidity, and postural instability, degeneration of dopaminergic neurons in the substantia nigra pars compacta and a reduction on dopamine levels in the striatum of the adult brain [81]. PD is the second most common neurodegenerative disease after Alzheimer's disease (AD) and affects 2% of the population aged over 65 years [82, 83]. One of the major neurobiological characteristics of genetic and sporadic forms of PD is the presence of Lewy bodies composed of the misfolding and fibrillar aggregation of α-synuclein [84]. Recently, other mechanisms are also associated to PD, including reactive oxygen and nitrogen species, neuroinflammation, glutamate excitotoxicity, apoptosis, and a reduction of

trophic factors. There is no cure for PD at the present moment and the goals of neuroprotective treatment are only to alleviate the symptoms of disease for the comfort of patients and to minimize the dyskinesia.

The first evaluation of neuroprotective effect of creatine in PD started in 1999 when it was shown to be strongly protective in a toxic mouse PD model induced by peripheral administration of neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), targeting mitochondrial function [85]. Creatine in the chow for 2 weeks completely prevented the deleterious effects of MPTP on dopaminergic neurons (striatal dopamine depletion and substantial neuronal loss in the substantia nigra pars compacta) [85]. Klivenyi et al. (2004a) reported that creatine administration to the ubiquitous creatine kinase-deficient mice exerted significant neuroprotective effects against MPTP toxicity (tyrosine hydroxylase and nissl immunostaining reduction in the substantia nigra pars compacta and dopamine, DOPAC and homovanillic acid levels decreased in the striatum) that were comparable in magnitude to those seen in wild-type mice, suggesting that the neuroprotective effects of creatine in animal PD model are not mediated by an effect on energetic shuttle mediated by ubiquitous creatine kinase [86].

Since PD is a disease with inflammatory features, the same research group reported also a synergistic neuroprotective effect of combination of creatine and the cyclooxygenase 2 (COX-2) inhibitor rofecoxib in the MPTP toxicity animal model [87]. Additionally, a study showed that creatine when combined with coenzyme Q10 yielded even more neuroprotection in MPTP-lesioned mice, including robust improvements on the dopamine depletion in the striatum, loss of tyrosine hydroxylase immunostaining, lipid peroxidation damage and alpha-synuclein accumulation within dopaminergic substantia nigra neurons [30].

The neuroprotective effect of creatine is also reported at non rodents PD model. For example, Hosamani et al. (2010) demonstrated that creatine supplementation resulted in reduced mortality, better motor performance in a negative geotaxis assay, improvement in the mitochondrial oxidative stress, and restored the glutathione and nitric oxide levels, manganese-dependent superoxide dismutase activity impairment and dopamine levels

depletion in flies exposed to classical dopaminergic neurotoxin rotenone, suggesting an important protective effect of creatine in the dopaminergic degeneration [29]. Interestingly, drosophila model express arginine kinase instead creatine kinase and are not capable to synthesize phosphocreatine, suggesting that neuroprotective effect of creatine is not dependent of energetic shuttle and phosphocreatine synthesis.

Since degeneration of dopaminergic neurons in the substantia nigra and dopamine levels depletion in the striatum are molecular hallmarks of PD, efforts were made to verify the neuroprotective effect of creatine for dopaminergic neurons against neurotoxic insults exposure [21, 78, 79, 88, 89]. Creatine significantly protected dopaminergic cells facing MPP^+-induced deterioration of neuronal morphology including overall process length/neuron, number of branching points/neuron and area of influence per individual neuron in cultured fetal ventral mesencephalic tissue [78]. Also, the neurotoxin rotenone decreases SH-SY5Y cellular viability and creatine is able to prevent this toxicity [89]. Furthermore, the protective effect afforded by creatine against 6-OHDA-induced SH-SY5Y cell death was reversed by inhibitors of different protein kinases, i.e., PI3K, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase dependent of cAMP (PKA), mitogen-activated protein kinase kinase 1/2 (MEK1/2) and protein kinase C (PKC), suggesting that the activation of these kinases may be an important step toward the neuroprotective effect of creatine [88]. Exposure of striatal slices to 6-OHDA caused a significant disruption of the cellular homeostasis increasing the levels of reactive oxygen and nitrogen species and thiobarbituric acid reactive substances production and decreasing mitochondrial membrane potential and the phosphorylation of Akt (Serine473) and glycogen synthase kinase 3 β (GSK3β, Serine9) and creatine incubation abolished these effects [21].

The neuroprotective effect of creatine reported at cell and animal models of PD encouraged significant number of clinical trials, reviews and meta-analysis on this topic. The first clinical trial investigating the neuroprotective effect of creatine was performed by Bender et al. (2006) over 2 years in 60 PD patients [90]. This trial was negative for creatine treatment in the primary outcomes (no effect on single-photon emission

computed tomography variables of disease progression and overall scores of the Unified PD Rating Scale), but slightly positive on measures for mood and behavior and the PD patients had to increase their dopamine dose for symptomatic treatment less than the control group [90]. In 2007, the National Institute of Neurological Disorders and Stroke (NINDS) announced a multi-center, randomized, double-blind, placebo-controlled, phase III trial with 1741 early PD patients receiving dopaminergic therapy and creatine supplementation [91, 92]. Unfortunately, this clinical trial was prematurely terminated due to futility [93, 93].

The conclusion of a meta-analysis using 194 patients and two pilot or Phase II trials revealed no effect on either motor function or activities of daily living, but the authors cautioned that the data was insufficient to exclude the neuroprotective effects of creatine in PD [94]. Recently, another meta-analysis using 1935 patients and three randomized controlled trials phase III robustly point in that current evidence does not support the use of creatine for neuroprotection against PD and future well-designed randomized controlled trials are needed [95].

Klein and Ferrante (2007) and Adhihetty and Beal (2008) pioneering reviewed and discussed the contribution of mitochondria and bioenergetics to the progression of PD, as well as the potential neuroprotective value of creatine supplementation in this neurological disease [96, 97]. Subsequently, Beal (2011) elegantly reviewed the neuroprotective effects of creatine and described the beneficial actions of creatine in PD [98]. In addition, Bender and Klopstock (2016) reviewed critically the beneficial actions of creatine supplementation in the PD at preclinical levels (animal and cell models), as well as initial phase II randomized clinical trials, which however turned out to be negative for all outcome measures at well-designed phase III trial, suggesting a low translation of basic experimental studies to clinical trials in the analysis of neuroprotective effects of creatine in the PD [99]. The neuroprotective effect of creatine in PD is still very speculative and many research groups continue to add new findings about this thematic.

Currently, prospective studies tend to analyze the effect of creatine supplementation in non-motor symptoms of PD, as well as in the

prevention of toxicity and adverse effects of current treatment for the disease. Since standard treatment for PD is levodopa, but prolonged treatment with levodopa generates several motor adverse effects such as dyskinesia, a study investigated the effect of creatine supplementation in the levodopa-induced dyskinesia in an animal PD model [100]. In line with this, rats administered with 6-OHDA (a PD rat model) and treated chronically with levodopa presented abnormal involuntary movements and creatine-supplemented group prevented levodopa-induced dyskinesia [100]. In the same study, creatine supplementation diet reduced mRNA prodynorphin and FosB/DeltaFosB-immunopositive cells in the striatum, an effect associated to anti-dyskinesia behavior elicited by creatine [100].

FINDINGS OF THE NEUROPROTECTIVE PROPERTY OF CREATINE ON ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a severe, chronic and progressive neurologic disease associated with learning/memory and cognition impairment, and degeneration of specific population of neurons with a prevalence of approximately 1.5% of people until 65 years old and 30% until 80 years old (about 48 million people worldwide living with AD) [1, 101]. The main pathological hallmarks of AD are the presence in the brain of senile plaques mainly composed of aggregated amyloid β (Aβ) and neurofibrillary tangles composed of aggregated tau proteins hyperphosphorylated that may initially cause dysfunctions in biochemical communication between neurons and at a later stage produce neuronal death [102–107]. Currently, studies demonstrated abnormalities in neuronal metabolism in AD patients [108–110]. Drugs capable to halt or even stabilizing disease progression are not available and new pharmacological compounds can represent important advances for prevention and neuroprotection against AD.

Since mouse model of creatine transporter deficiency reveals early onset cognitive impairment [111, 112], some studies have been delineated to verify the importance of creatine in memory and learning, as well as in dementias diseases. The literature data widely reported the cognitive enhancer effect of

creatine supplementation at several tasks. Furthermore, creatine administration in the hippocampus of rats decreased the latency for escape and the mean number of errors on Barnes maze test [49, 113]. Oliveira et al. (2008) demonstrated that creatine-induced spatial learning enhancement was reverted by co-administration of arcaine (an antagonist of the polyamine binding site at the NMDA receptor) and intensified by spermidine (an agonist of the polyamine binding site at the NMDA receptor spermidine), suggesting that cognitive-enhancer effect of creatine may be mediated at least in part of NMDA receptor activation at polyamine binding site [49]. Additionally, creatine-induced spatial retention was reverted by co-administration of the CaMKII inhibitor STO-6095, suggesting the involvement of this kinase activation in the facilitated learning and memory induced by creatine administration [113]. Moreover, mice supplemented with creatine had significantly more entries in platform area in Morris water maze test, indicating improved spatial memory [114]. A study demonstrated also that a single administration of creatine improves working memory efficiency at adapted Morris water maze test and mice co-administered with creatine and l-arginine improves reference memory retention in rats, a phenomenon that is possibly associated with increased creatine/phosphocreatine levels and nitric oxide synthesis [115].

The human trials also revealed that creatine supplementation could be a cognitive enhancer compound. A creatine mimetic, namely creatine ethyl ester, led to an improvement over the placebo regimen on several cognitive tasks in young adults [116]. In vegetarians rather than in those who consume meat, creatine supplementation for 5 days resulted in better performance in memory tasks [117]. Another study demonstrated that vegetarians supplemented with creatine for 6 weeks had a significant positive effect on both working memory (backward digit span) and intelligence (Raven's Advanced Progressive Matrices), both memory tasks that require speed of processing [118]. Elderly individuals supplemented with creatine had better performance on random number generation, forward and backward number and spatial recall, and long-term memory tasks as compared to placebo group [119]. However, studies also showed that creatine supplementation did not alter any parameter of cognitive performance in young adults [120] and older people [121].

The propositions that creatine improved memory and learning in both animals and humans studies has led some researches to investigate the effect of creatine supplementation on AD, the major condition associated to dementia. The first study to investigate the neurpoprotective effect of creatine in a cell model of AD induced by Aβ was performed by [47]. In this study, embryonic neurons exposed to $A\beta_{25-35}$ toxicity for 48 hours were partially prevented by creatine incubation as well.

However, the promising results found in AD cell model were not replicated in animal studies. Interestingly, the distance traveled to the platform in the Morris water maze test was significantly higher in the rats administered with Aβ in the subregion CA1 of hippocampus, as compared to control group, suggesting spatial memory impairment [122, 123]. In this model, creatine supplementation before Aβ administration had no effect on learning, memory retrieval, or neuron apoptosis [122]. The same research group used another creatine supplementation paradigm (before and after Aββ administration) [123]. Unexpectedly, an adjunctive effect in the impairment on Morris water maze test was observed when animals were administered with Aβ and supplemented with creatine, suggesting that creatine supplementation before the amyloid beta-induced toxicity could exacerbate the learning and memory impairment of rats submitted to AD animal model [123]. The frustrating results in the investigations of the neuroprotective effects of creatine in animal models of AD have led researchers not to require efforts in clinical trials with AD patients.

ELUCIDATING THE EFFECTS OF CREATINE ON HUNTINGTON'S DISEASE

Huntington's disease (HD) is a progressive autosomal dominantly inherited disorder with worldwide incidence of approximately 2.7 cases per 100,000 people [124]. The symptoms of HD are manifestations of involuntary movements (chorea), cognitive dysfunction and behavioral disturbance [125]. HD is caused by an expansion of copies of CAG in the first exon of the gene encoding huntingtin generating mutated huntingtin

protein synthesis, which in turn is associated to early striatal atrophy and subsequent degeneration of other brain structures, including the cerebral cortex [126, 127]. Among mechanisms of neurodegeneration associated to HD, bioenergetic abnormalities and the unbalance in the Ca^{2+} handling have been suggested as potential contributors to neuronal dysfunction in HD [128, 129]. Currently, besides symptomatic treatments, no therapy modifies the fatal history of HD.

The first evidence reporting the neuroprotective effect of energetic compound creatine on HD was performed by Matthews et al. (1998) at experimental pharmacological models of HD induced by 3-nitropropionic acid and malonate [19]. In this study, oral supplementation with creatine produced significant protection against malonate-induced striatal lesions and hydroxyl radical generation [19]. Furthermore, Matthews et al. (1998) also demonstrated that creatine supplementation produces neuroprotective effect against 3-nitropropionic acid neurotoxicity (phosphocreatine and ATP depletion, increases in striatal lactate and 3-nitrotyrosine concentrations) [19]. Subsequently, Shear et al. (2000) reported that creatine supplementation for 2 weeks can attenuate 3-nitropropionic acid-induced striatal lesions, striatal atrophy, ventricular enlargement, cognitive deficits, and motor abnormalities on a balance beam task [130]. Interestingly, creatine when combined with coenzyme Q10 produced additive neuroprotective effects in reducing striatal lesion volumes, impairment of glutathione homeostasis, lipid peroxidation and DNA oxidative damage produced by chronic subcutaneous administration of 3-nitropropionic acid to rats, an animal model of HD [30]. In the same study, the combination of coenzyme Q10 and creatine produced additive neuroprotective effects on improving motor performance and extending survival in the transgenic R6/2 HD mouse model [30].

One set of studies investigated the neuroprotective effect of creatine supplementation in genetic animal and cell models of the HD. For example, creatine increased cell viability of striatal cells expressing mutant huntingtin with 111 glutamines (HD cell model) and prevented reactive oxygen species formation in HD cells subjected to H_2O_2 and staurosporine [25]. In addition, studies reported that creatine significantly extends

survival and improves clinical and neuropathological phenotype in transgenic R6/2 HD mice strain, which the creatine supplementation starting at weaning (before clinical symptoms appear) [131] or starting at 6, 8, and 10 weeks of age (analogous to early, middle, and late stages of human HD) [132]. Furthermore, creatine supplementation for 24 prevented the Purkinje cell numbers lost in transgenic R6/2 HD mice, but did not improve or delay the ataxic phenotype [133]. Creatine supplementation during 8 weeks increased brain-type creatine kinase expression and reduced Huntingtin aggregate formation in the striatum of R6/2 mice (genetic model of HD) [134]. In other genetic mouse model of HD produced by 82 polyglutamine repeats in a 171 amino acid N-terminal fragment of huntingtin (N171-82Q mice), creatine supplementation significantly improved survival, slowed the development of motor symptoms, and delayed the onset of weight loss [135]. In the same study, creatine lessened brain atrophy and the formation of intranuclear inclusions, as well as attenuated reductions in striatal N-acetylaspartate and delayed the development of hyperglycemia [135].

Creatine administered for 16 weeks was well tolerated, safe and reduced serum 8-OHdG levels, an indicator of oxidative injury to DNA in HD patients [38]. Neuroimaging approaches demonstrated treatment-related slowing cortical and striatal atrophy in premanifest and at-risk HD subjects at 6 and 18 months of high dose of creatine supplementation [136]. In addition, Tuckfield (2015) reported the use of creatine hydrochloride in two premanifest HD patients, with excellent tolerability over more than 2 years of use demonstrating safe and tolerance to creatine supplementation [137].

Although high-dose creatine supplementation for 12 or 24 months was generally well tolerated for HD patients, a study demonstrated that there was no significant change in the mean of total motor score, functional capacity scores, and neuropsychological testing before creatine regimen [138, 139]. Another study showed also that one year of creatine intake did not improve functional, neuromuscular, and cognitive status in HD patients with stage I to III [140]. Although creatine supplementation did not alter the clinical rating scales of HD, a study using proton magnetic resonance

spectroscopy approach showed a 15.6% decrease of glutamate brain levels after creatine treatment for 8-10 weeks [54].

Ryu et al. (2005) reviewed the neuroprotective effect of creatine in the HD patients and concluded that creatine may provide a relatively safe and immediately available therapeutic strategy to HD patients [141]. The authors also suggested that creatine may be the cornerstone of a combined treatment necessary to delay the relentless HD progression. Naia et al. (2011) reviewed the role of creatine that target mitochondrial dysfunction and impaired bioenergetics and concluded that although creatine results in HD are promising, more clinical studies should be performed and analyzed [142].

CREATINE PROPERTIES ON AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a fatal syndrome characterized by a progressive loss of motor neurons on corticospinal tract, brainstem and anterior horn cells of the spinal cord, but also astrocytes, microglia and oligodendrocytes dysfunction, leading to muscle wasting, weakness and spasticity [143–147]. The worldwide ALS incidence is 2–3 new cases/year per 100,000 individuals [148]. Multiple cellular events, including oxidative stress, mitochondrial dysfunction, protein aggregation, impaired axonal transport, neuroinflammation, immunological imbalance and glutamate-mediated excitotoxicity, contributed to ALS [149–155]. Therefore, it is imperative to search for new alternatives to treat ALS patients.

Creatine significantly increased longevity and motor performance of the G93A mice (a mutant mouse model of familial ALS) and significantly attenuated the increases in glutamate levels at 75 days of age, but had no effect at 115 days of age [50]. Interestingly, the first study reported the neuroprotective effect of creatine in G93A mice was performed by Klivenyi et al. (1999) [20]. In this study the authors reported that oral administration of creatine produced a dose-dependent improvement in motor performance and extended survival in G93A transgenic mice, as well as it protected mice from

loss of both lumbar spinal cord motor neurons (reduction of Nissl immunostaining) and substantia nigra neurons (reduction of tyrosine hydroxylase, dopamine transporter and Nissl immunostaining) [20]. In addition, the authors also reported that creatine administration protected G93A transgenic mice from increases in biochemical indices of nitrosative and oxidative damage (3-nitrotyrosine levels increased in the lower and upper spinal cord and conversion ratio of salicylate to 2,3-DHBA increased) [20]. Subsequently, Klivenyi et al. (2004) reported that G93A mice supplemented with creatine started at 30 day of age increased the survival and motor performance and attenuated weight loss [156]. In the same study, the authors showed that creatine when combined with cyclooxygenase 2 inhibitors, such as rofecoxib or celecoxib, produced additive neuroprotective effects and extended survival of mice by approximately 30% [156]. Creatine supplementation in the diet increased creatine levels in cerebellum (25%) medulla (11%) and cerebral cortex (4%) of G93A strain mice, reflecting the ordering of creatine kinase activity [157]. Choi et al. (2009) reported also that creatine prevented N-acetylaspartate levels reduction in the medulla at late stages and produced weight retention in a genetic mouse model of ALS [157].

A clinical study by Mazzini et al. (2001) demonstrated the promising beneficial effect of creatine supplementation for ALS, since maximal voluntary isometric muscular contraction and the slope of fatigue test improved after 7 days of supplementation with creatine in knee extensors and also in elbow flexors [158]. However, during the 6-month follow-up period of clinical protocol all the examined parameters showed a linear progressive decline [158]. In another study, although creatine supplementation for 6 months was well tolerated by ALS patients, no benefit effect of creatine could be demonstrated in any outcome measure (maximum voluntary isometric contraction of eight upper extremity muscles, grip strength, ALS functional rating scale-revised, and motor unit number estimates) [159]. Furthermore, a study also demonstrated that creatine supplementation for 16 months did not affect survival or the rate of decline of functional measurements in ALS patients [160]. Rosenfeld et al. (2008) also showed that creatine monohydrate supplementation did not significantly improve motor, respiratory or functional capacity in the ALS patient population, but there was a trend toward improved survival in

patients taking daily creatine monohydrate [161]. In relation to respiratory function, creatine supplementation for 1-4 months did not alter the pulmonary capacity (forced vital capacity, forced expiratory volume, peak expiratory flow rate and maximum voluntary ventilation) in patients with definite advanced ALS [162].

While the beneficial effects of creatine supplementation on ALS are still not consensual in clinical trials, one study has pointed out that creatine regimen at higher dosages for three week increased about 8% brain creatine levels and decreased 17% glutamate + glutamine brain signals in ALS patients, suggesting that creatine modulates glutamate metabolism and it may be important for neurodegenerative conditions such as ALS [163]. Confirming these results Vielhaber et al. (2001) reported that the ALS patients upon creatine supplementation decrease the glutamate + glutamine/creatine and glutamate + glutamine/choline ratios in motor cortex. In this same study, creatine supplementation is not able to alter the N-acetylaspartate/creatine or the N-acetylaspartate//choline ratios in the motor cortex, as compared to control non-supplemented group [53].

A systematic review, including three clinical trials and 386 participants randomized, examined the efficacy of creatine in prolonging the survival of ALS patients and disease slowing [164, 165]. In this study, no statistical difference in survival and ALS functional rating revised scores between the placebo and creatine groups were observed [164, 165]. There was a trend towards slightly worsened forced vital capacity in the creatine group as compared to placebo [164, 165]. Furthermore, Strong and Pattee (2000) reviewed the theoretical propositions of the neuroprotective effect of creatine and the authors concluded that there is considerable theoretical evidence supporting the use of creatine in the treatment of ALS patients [166]. Ellis and Rosenfeld (2004) also reviewed the pharmacokinetics and rationale for the use of creatine at available evidence from animal models and clinical trials for ALS and related neurodegenerative or neuromuscular diseases [167].

UNDERSTANDING THE EFFECTS OF CREATINE ON TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) is a major cause of mortality and morbidity, which constitutes a considerable health problem throughout the world. At least 1.4 million cases occur each year in the United States (50,000 cases are fatal, 235,000 persons are admitted to hospitals and 1.1 million of persons are treated and released from hospital) [168]. Approximately 5.3 million people live suffering from long-term disabilities, impairment or handicap as a result of TBI [169]. The primary injury associated to TBI is caused as a direct result of the mechanical impact and the secondary injury is consequence from the effects of primary injuries [170, 171]. During the second stage, damage is primarily due to neurotransmitters release, calcium overload, free radical-mediated damage, inflammation, proapoptotic gene activation, mitochondrial dysfunction and energetic status impairment [172].

Studies have indicated that concussion decreases the cerebral creatine levels in athletes [173] and in rats exposed to mild TBI experimental model [174], suggesting that this energetic compound may generate neuroprotective effects when administered post traumatic brain injury. Of note, chronic administration of creatine improved the extent of cortical damage induced by TBI (about 36% in mice and 50% in rats), as well induced maintenance of mitochondrial bioenergetics (increased mitochondrial membrane potential and ATP levels and decreased intramitochondrial levels of reactive oxygen species and calcium) and inhibited the induction of mitochondrial permeability transition pore [175]. Adult rats supplemented with creatine for 2 weeks before TBI presented a significant reduction on both lactate and free fatty acid in hippocampus and cerebral cortex ipsilateral to the injury, suggesting that a creatine-enriched diet can provide substantial neuroprotection in part by suppressing secondary brain injury [176]. Although creatine reduces oxidative stress markers (protein carbonylation, thiobarbituric acid reactive species) in the

ipsilateral cortex to injury, creatine administration does not protect against seizure susceptibility after severe TBI [31].

The administration of creatine for 6 months to children with TBI improved several parameters, including duration of post-traumatic amnesia, duration of intubation, intensive care unit stay, disability, good recovery, self-care, communication, locomotion, sociability, personality/behavior and neurophysical, and cognitive function [177]. Furthermore, the administration of creatine to children and adolescents with TBI improved duration of post traumatic amnesia, duration of intubation, intensive care unit stay, headache, dizziness and fatigue in all patients analyzed [178]. However, further clinical studies should be performed to determine the neuroprotective effects of creatine supplementation after traumatic brain injury. Currently, Royes and Cassol (2016) reviewed the role of creatine in secondary damage induced by TBI in a translational perspective of animal studies to clinical studies [179].

In addition, similar beneficial effects were observed in another central nervous system injury, such as spinal cord injury model, which creatine regimen significantly spared gray matter in rats, suggesting that creatine could be also a promising strategy to promote neuroprotection after spinal cord injury [180]. Furthermore, rats submitted to spinal cord injury and supplemented with creatine scored better than the non creatine supplemented rats in the locomotion in the open-field test and significantly smaller amount of scar tissue surrounding the traumatic cavity was found, suggesting that creatine treatment seems to reduce the spread of secondary injury [181].

PROTECTIVE EFFECTS OF CREATINE AGAINST STROKE AND CEREBRAL HYPOXIA/ISCHEMIA

Stroke (also known as cerebrovascular accident) is the second leading cause of death worldwide [182–184]. Particularly, there are two types of cerebral vascular accidents: ischemic stroke (85% of all cases), occurring

as a result of thrombotic obstruction, and hemorrhagic stroke (15% of cases and high prevalence of mortality), occurring as weakening of blood vessels of the brain generating small ruptures and blood outflow to the brain and surrounding tissues [185]. Reduction in the cerebral blood flow and glucose and oxygen supply to brain is consequence of obstruction of blood vessel in the stroke. Under deprivation of oxygen and glucose, neuronal death occurs initially from anoxia/hypoxia and energy depletion, followed by reperfusion, oxidative stress, glutamate excitotoxicity, and nitric oxide synthesis with energy failure and death [186, 187]. To date, there is no effective treatment to prevent the brain damage in ischemic stroke and new neuroprotective strategies are welcome.

The neuroprotective effect of creatine is widely demonstrated in models of partial or total deprivation of oxygen (hypoxia or anoxia, respectively) at neuronal cell investigation. Kriványek and coworkers were the first to demonstrate the beneficial effect of creatine incubation in the cerebral cortex slices submitted to oxygen deprivation, which the content of phosphocreatine was related to the maintenance of tissue polarization [188]. Subsequently, a study demonstrated that guinea pig hippocampal slice at creatine-fortified buffer markedly elevated tissue phosphocreatine and creatine levels associated with a 300% increase of the evoked response spike in the granule cells of dentate gyrus during anoxia, suggesting that creatine incubation may protect cerebral synaptic transmission in the oxygen deprivation condition [189]. The same research group demonstrated that the guinea pig hippocampal slice submitted to creatine for 3 hours elevated the phosphocreatine levels in molecular layer (fourfold), as well as the time of synaptic transmission during hypoxia (three times as long as it does in the absence of creatine). Additionally, creatine fortified medium produced a delay in the reduction of ATP levels during hypoxia [190]. Corroborating, Yoneda et al. (1983) using also guinea pig hippocampal slices demonstrated that creatine incubation increased the phosphocreatine content of tissue and reduced the rate of ATP exhaustion during anoxia [191]. Furthermore, the concentrations of ATP and phosphocreatine rapidly were recovered in slices preincubated with creatine in the reoxygenation after anoxia period, suggesting that the

elevation of phosphocreatine concentration may therefore prolong the survival time of brain tissue during anoxia and facilitate recovery during reoxygenation [191]. An electrophysiological approach revealed also that the accumulation of phosphocreatine in the hippocampal slice not only prolonged the latency of the loss of the postsynaptic potential during deprivation of oxygen and glucose, but also enhanced the tolerance to the deprivation resulting in the complete recovery of the postsynaptic potential [191]. The same authors demonstrated through the biochemical approaches that phosphocreatine accumulation not only slowed down the rate of decrease of ATP during oxygen and glucose deprivation, but facilitated the recovery of the high-energy phosphates in the reoxygenation period [192]. Furthermore, rat hippocampal slices incubated with creatine (concentration-dependent) increased baseline levels of phosphocreatine, reduced the anoxia-induced decline in phosphocreatine and ATP levels, prevented the impairment of protein synthesis, and reduced neuronal death [193]. Carter et al. (1995) also reported that creatine when combined with the noncompetitive NMDA antagonist MK-801 provided complete adjunctive protection against anoxia condition [193]. In brainstem slices, amplitude and duration of bursts increased in the slices submitted to anoxia and preincubated with creatine, as compared to preanoxic values (59% and 37%, respectively) [194]. In addition, creatine preincubation delayed anoxic depolarization [195], even creatine at low concentration (1 mM) [196]. Creatine is able to delay the disappearance of population spike during anoxia [197].

Creatine-fed rats revealed a statistically significant increase of cerebral creatine concentration and slightly less severe and mildly delayed of brain water diffusion changes during ischemia and similar beneficial trends during early reperfusion although not statistically significance [198]. Furthermore, Zhu et al. (2004) found that rats supplemented with creatine increased phosphocreatine content in the brain and reduced ischemic damage and caspase activation induced by transient focal ischemia [199]. Moreover, after 3 weeks of dietary creatine supplementation mice had 40% reduction in infarct volume after transient focal cerebral ischemia, as compared to control dietary group [200]. However, minor changes in brain

creatine, phosphocreatine, ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) levels were detected following creatine supplementation, suggesting that creatine-mediated neuroprotection can occur independent of changes in the bioenergetic status of brain tissue [200].

In addition, researchers have made great efforts in investigating the neuroprotective effect of a system of continuous release of creatine in the brain by osmotic pump against ischemia experimental models. Creatine affords protection against an in vivo model of global cerebral ischemia when administered directly to the brain by an osmotic pump implanted in the rat lateral ventricle [201–203]. The first study to investigate the effect of creatine administration by osmotic pump on the 10-minutes transient global cerebral ischemia clamping of rat carotid arteries and controlled arterial hypotension was conducted by Payan et al. (1965) [203]. In this study, creatine pretreatment decreases the number of necrotic neurons in hippocampal CA1 area after transient ischemia [203]. Otellin et al. (2003) demonstrated that transient brain ischemia induced glial astrocyte reactivity in the hippocampus of rats and creatine administered by intracerebroventricular route, for 5 days before ischemia and 7 consecutive days after ischemia, prevented this neurotoxicity [202]. Lensman et al. (2006) showed that creatine administered before and continuing with no interruption for 7 days after the cerebral ischemia reduced the damage in all brain regions examined (hippocampus (Cornu Ammonis region 1, 2 and 3 (CA1, CA2 and CA3), and fascia dentate), caudate, putamen, and neocortex) and neurological score [201]. However, Lensman et al. (2006) also showed that creatine administered intracerebroventricularly 30 min after ischemia and continuing without interruption for 7 days did not alter significantly the damage in the brain regions analyzed and neurological scores [201].

Due to low solubility, difficulty in permeability through the blood-brain barrier and low cellular uptake rate, researchers have developed hybrid salts of creatine to attenuate such limitations. Cyclocreatine, an analog of creatine and substrate for creatine kinase, but its phosphorylated form presents low capacity to donor phosphate, as compared to

phosphocreatine, produces both positive [204] and negative [205] effects in brain ischemia. For example, in brains of cyclocreatine-fed mice, the pool of phosphocyclocreatine and ATP is increased during anoxia with a correspondingly increase of inorganic phosphate, suggesting that during ischemic episodes cyclocreatine can prolong the time required to exhaust the available energy stores of ischemic brain [204]. However, a study reported that cyclocreatine did not increase survival time of animals during hypoxia and in the absence of hypoxia caused significant mortality and reduction on the body weight, suggesting high toxicity rate and low efficacy under oxygen deprivation conditions of cyclocreatine supplementation [205]. Another creatine mimetic molecule was phosphocreatine magnesium complex acetate, which reduced the infarct volume by 48% and the Clark's neurological score in mice submitted to transient middle cerebral artery occlusion [206]. Furthermore, a study suggested a significant neuroprotective effect of creatine glycine ethylic ether fumarate, since decreased brain lesion volume and clinical score in Garcia scale produced in rats exposed to focal ischemic and reperfusion experimental model [207]. Recently, hippocampal slices incubated with creatine gluconate (a hybrid salt) was superior to creatine in increasing tissue content of creatine after transporter block and produced slowed down population spike disappearance during anoxia, an effect that creatine *per se* did not have [208], suggesting that creatine when uptake by hippocampal cells promotes neuroprotection against anoxia condition.

Although the experimental results at cellular and animal models are not conclusive about neuroprotective effect of creatine in hypoxia/ischemia and stroke conditions, these studies encouraged analysis of neuroprotective effect on healthy individuals and individuals who had stroke. Hypoxia (gas mixture with 10% oxygen)-induced decrements in cognitive performance (specifically attentional capacity) and corticomotor excitability, wich were restored when healthy young adults participants were supplemented with creatine for seven days [209]. Skrivánek et al. (1995) showed that 119 ischemic stroke patients administered with phosphocreatine by oral route within 8 hours after the onset of symptoms did not have significant improvement [210, 211].

Besides the effects of creatine supplementation on experimental models of stroke and generalized ischemia, administration of creatine given to mother before hypoxia/ischemia of the pups or neonates has demonstrated to improve survival and neurological outcome of mice [212, 213]. Ellery et al. (2016) reviewed the neuroprotective effect of creatine in the perinatal brain from hypoxic-ischemic encephalopathy and concluded that dietary creatine supplementation during pregnancy may be an effective prophylaxis strategy protecting the fetus from the multi-organ consequences of severe hypoxia at birth [214].

Creatine may provide also neuroprotection of neonates even when administered after hypoxia/ischemic insult [215, 216]. In neonatal animals, amplitudes and duration of hypoglossal nerve bursts increased during anoxia (41% and 18%, respectively) in creatine supplemented animals when compared with preanoxic values [194]. Iqbal et al. (2015a) demonstrated that creatine supplementation for 8 weeks following neonatal brain damage resulted in enhanced muscular strength, neuromuscular coordination, body weight, mean swimming speed in the Morris water maze test, but the spatial memory was not improved significantly following hypoxia ischemia encephalopathy [217]. In addition, the ambulation parameters and percentage of infarct volume remained also unaffected following creatine supplementation [217]. Furthermore, a study of the same research group demonstrated that 10 weeks of creatine supplementation significantly improved locomotor and exploratory behavior in the open field test, as well as neuromuscular coordination in the rota rod test and spatial memory in the Morris water maze tests following neonatal brain ischemia [215]. In this study, the authors reported also that creatine supplementation reduced the infarct size induced by neonate brain ischemia [215]. Iqbal et al. (2015b) also demonstrated that long term creatine monohydrate supplementation (15 weeks) following neonatal hypoxic ischemic insult improves neuromuscular coordination in the rota-rod test and spatial learning in Morris water maze test in male albino mouse, but not the brain infarct volume (although it tended to be significantly different, as compared to control group) [218].

Iqbal et al. (2013) point in the involvement of hematological and biochemical parameters in the creatine supplementation effect against neonate ischemia [216]. Alanine aminotransferase activity, low density lipoprotein and total cholesterol levels were higher in mice feeding on normal diet for 8-12 weeks following asphyxia, when compared with the creatine supplemented mice [216]. Glycemic rate was significantly lower in creatine supplemented mice as compared to untreated group following ischemic insult [216]. Subsequently, the same authors investigated the involvement of neuroinflammatory pathways in the neuroprotective effect of long term creatine supplementation against cerebral ischemia [219]. Mouse serum concentration of cytokines interleukin 6, as well as interleukin 18 (cytokines), were significantly higher in mice supplemented with creatine monohydrate for 15 weeks following hypoxic ischemic insult, indicating that long term creatine monohydrate supplementation up regulates cytokines concentrations triggering the neuroinflammatory and neuroprotective responses [219].

THE EFFECT OF CREATINE IN THE SEIZURE DISORDERS

Epilepsy affects approximately 65 million people worldwide [220]. Classically, epilepsy is characterized by excessive or uncontrolled discharges of neurons leading to epileptic seizures, cognitive and mood impairment, and injuries [221–223]. Current antiepileptic treatments have several drawbacks, since they are ineffective for about 22-30% of patients and produce frequent and persistent side effects, such as allergies, neurological and systemic toxicity, depression, memory loss, and osteoporosis [220, 224]. Considering these limitations in the epilepsy management, there is significant clinical interest in finding alternative therapies for epilepsy.

Specifically, epileptic seizures are associated with decreased phosphocreatine/ATP ratio in the brain [225], implicating the metabolism of creatine in the convulsions. Interestingly, the first study to investigate the neuroprotective effect of creatine in convulsions was performed by

Holtzman et al. (1998) using rats pups submitted to hypoxia protocol, since the incidence of clinical seizures is highest in the newborn period [226]. In this study, rat pups injected with creatine for 3 days before postnatal hypoxia decreased hypoxia-induced seizures and deaths associated to an increase of brain phosphocreatine/nucleoside triphosphate ratio and recovery of brain phosphocreatine and ATP depletion after hypoxia [226].

Royes et al. (2003) demonstrated that peripheral acute creatine administration by intraperitoneal route prevented the number and duration of convulsions and the lactate increase in the striatum of rats submitted to intrastriatal injection of methylmalonate and NMDA [227]. Subsequently, the same research group also reported that acute creatine treatment, similar to NMDA receptor antagonist MK-801, protected againstmethylmalonate-induced increase of number and time spent convulsioning, seizure evidenced by electrographic recording in the ipsilateral striatum and cerebral cortex, protein carbonylation and tiobarbituric acid reactive species production in the striatum [32]. The acute creatine administration prevented also methylmalonate-induced creatine and phosphocreatine depletion [32].

Magni et al. (2007) showed that although acute creatine administration by oral route did not prevent convulsions induced by administration intrastriatal of glutaric acid (an experimental rat model of glutaric acidemia type I characterized by striatal degeneration and seizures), creatine treatment decreased seizures (evidenced by electrographic changes), protein carbonylation and Na^+,K^+-ATPase inhibition [51]. Magni et al. (2007) demonstrated also that creatine also protected against the glutaric acid-induced inhibition of glutamate uptake in striatal synaptosomes [51].

Rambo et al. (2013) added several issues on the anticonvulsant effect of creatine [26]. Firstly, the authors demonstrated that acute creatine treatment by oral route in rats prevented the increase in electroencephalographic wave amplitude typically elicited by pentylenetetrazol, a proconvulsant agent [26]. Secondly, the authors reported that creatine treatment increased the latency periods of first myoclonic jerks, lengthened the latency periods of the generalized tonic-clonic seizures and reduced the time spent in the generalized tonic-clonic

seizures induced by pentylenetetrazol [26]. Additionally, the acute creatine treatment prevented Na^+, K^+-ATPase activity inhibition, as well as ATP and ADP levels reduction, xanthine oxidase activity increased, and AMP, adenosine, inosine and uric acid levels increased induced by pentylenetetrazol treatment [26]. Finally, the authors also showed that creatine prevented pentylenetetrazol-induced mitochondrial dysfunction characterized by decreasing of mitochondrial membrane potential and increasing of thiobarbituric acid-reactive substance and protein carbonylation levels in the mitochondria of cerebral cortex [26].

Importantly, the combination between physical training and creatine administration by 6 weeks caused additive anticonvulsant effects, since it increased the onset latency for pentylenetetrazol-induced seizures (number and time of convulsions and electroencephalography records in the cerebral cortex and hippocampus) [228]. Physical training, creatine administration or its combination abrogated the pentylenetetrazol-elicited increased thiobarbituric acid-reactive substances and protein carbonylation, as well as decreased non-protein-thiols content, and the activity of antioxidant enzymes such as catalase and superoxide dismutase [228].

A study demonstrated also that acute creatine treatment by oral route reduces oxidative stress markers (thiobarbituric acid-reactive substance and protein carbonylation levels), but does not protect against seizure susceptibility after severe TBI [31]. In this study, electroencephalography analysis revealed that the injection of a subconvulsant dose of pentylenetetrazol 8 days after neuronal injury, decreased latency for the first clonic seizures and increased the time of spent generalized tonic-clonic seizures compared with the sham group and creatine acute treatment had no effect on convulsive parameters induced in this seizure model [31].

In another animal model of temporal lobe epilepsy induced by peripheral administration of pilocarpine creatine produced beneficial effect. Two hours after pilocarpine-seizure induction, brain-type cytoplasm creatine kinase and creatine transporter immunoreactivity were decreased to 70% and 60% of control level and creatine prevented the brain creatine kinase reduction [229]. Corroborating with these findings, Vielhaber et al. (2003) reported also that creatine feeding in epileptic rats increases

hippocampal N-acetylaspartate concentrations, suggesting improved neuronal survival [230]. However, in the same study the authors showed that hippocampal slices from creatine-treated epileptic rats revealed a more pronounced loss of pyramidal neurons and decrease in activity of mitochondrial enzymes in hippocampal subfields, suggesting deleterious effects of creatine supplementation [230]. The authors also suggested that further studies are necessary to understand the effects of creatine supplementation on temporal lobe epilepsy.

THE EFFECT OF CREATINE ON PSYCHIATRY DISEASES

Although mental disorders are generally classified separately to neurological disorders, there are sometimes unclear boundaries in the distinction between neurological and mental disorders, since psychiatry disorders can now be diagnosed as reliably and accurately as most of the common physical disorders [231, 232]. Of note, 150 million of persons live with depression, 25 million live with schizophrenia, and 90 million live with a substance abuse disorder.

Since psychiatry diseases are associated to impaired brain energy metabolism, studies suggest that interventions modulating energetic status, such as creatine, would improve these conditions [80]. This chapter also summarized the main findings of neuroprotective effect of creatine in the psychiatry disorders, such as major depressive disorder (MDD) and schizophrenia.

MAJOR DEPRESSIVE DISORDER

Among mental disorders, major depressive disorder (MDD) is the most prevalent psychiatric disorder in the population, with a lifetime prevalence of approximately 20% [233, 234]. Depression is a disorder characterized by a broad range of symptoms, including altered mood and cognitive functions, loss of interest or pleasure in daily activities, feelings of guilt or

low self-worth, disturbed sleep or appetite, low energy and recurrent thoughts of death or suicide [234]. The etiology of depression is associated to genetic and environmental factors, including stress, medical conditions, and pharmacologic agents exposition [234–239]. Of note, the neurobiology of depression is associated to monoamine dysfunction, inflammation, oxidative and nitrosative stress, glutamate excitotoxicity, apoptosis and neuronal bioenergetics impairment [240–246]. The antidepressant treatment available has limited success mainly due to the delayed onset of therapeutic effect, partial or no responses attained by the patients, as well as poor tolerability, high cost, and stigma associated with its use [247, 248]. These limitations in the antidepressant therapies have encouraged the search for more effective agents [237, 246, 249].

Initially, the effect of creatine administration was investigated in the predictive tests of antidepressant action, such as forced swimming test and tail suspension test. In these tests the immobility time is measured in rodents submitted to acute inescapable stress of forced swimming or tail suspension, respectively. These tests are sensitive to all major classes of antidepressant drugs, since effective antidepressant treatments decrease immobility time of rodents [250]. Similar to antidepressants, creatine supplementation for 5 weeks produces an antidepressant-like effect in female rats submitted to forced swimming test, since it is able to decrease the immobility time of rats, as compared to control group [251]. Interestingly, male rats maintained on creatine supplementation regimen displayed increased immobility in the rats submitted to forced swimming test [251]. Subsequently, the same research group did not reproduce the previous results obtained with male rats supplemented with creatine and submitted to forced swimming test [252, 253]. Furthermore, Cunha et al. (2012) demonstrated also that acute oral administration of creatine elicited a robust antidepressant like effect in another behavior despair paradigm, namely tail suspension test [254]. Moreover, a intracerebroventricular administration of creatine also produced antidepressant-like effect in the tail suspension test in mice, suggesting a central effect of this guanidine compound [255].

The overall clinical aim of combining antidepressants with various pharmacological groups or substances is to increase the efficacy for depression management whilst minimizing the side effects. Allen et al. (2012) demonstrated that creatine supplementation when combined with subeffective acute dose of classic antidepressant fluoxetine produced the synergistic antidepressant-like effect in the forced swimming test [253]. Cunha et al., (2013) also reported that creatine when combined with antidepressants selective serotonin reuptake inhibitors, such as fluoxetine, paroxetine or citalopram produces synergistic antidepressant-like effect in the tail suspension test [256]. Besides the ability of creatine to produce an antidepressant-like effect when combined with selective serotonin reuptake inhibitors, another study also pointed out that creatine was able to cause a synergistic antidepressant-like effect when combined with the tricyclic antidepressants imipramine and desipramine, as well as with the selective noradrenaline reuptake inhibitor reboxetine [255]. Furthermore, bupropion, a dopamine reuptake inhibitor with subtle activity on noradrenergic and serotonergic metabolism, produced a synergistic antidepressant-like effect when combined with creatine [254].

In addition, Allen et al. (2015) reported that female rats submitted to ovariectomy and ovarian hormone replacement (progesterone + estradiol) supplemented with creatine by 5 weeks reduced the immobility time and increased the swimming time in the forced swimming test as compared to ovariectomy + ovarian hormone replacement without creatine regimen group [252]. A similar trend in testosterone-treated castrates fed creatine rats to reduce the immobility time in the forced swimming test was observed [252], suggesting that creatine when combined with sexual hormones produced the synergistic antidepressant-like effect.

Some studies have pointed out the mechanisms of action in which creatine acts as an antidepressant. Cunha et al. (2013a/b) demonstrated the involvement of monoamine bioavailability in the synaptic cleft in the antidepressant-like effect of acute creatine administration [255, 256]. Studies reported the involvement of monoamine receptors in the antidepressant-like effect of creatine in the tail suspension test through the pharmacological agonists and antagonists utilization. Specifically, the

dopamine D_1 and D_2 receptors, 5-HT_{1A} receptor and α1-adrenoceptor were implicated in the antidepressant-like effect of creatine in the tail suspension test [254–256]. Subsequently, Cunha et al. (2015a) demonstrated the involvement of NMDA receptor inhibition in the antidepressant like effect of creatine [48]. Furthermore, the activation of adenosine A_1 and A_{2A} receptors also were implicated in the antidepressant-like effect of creatine [257].

The activation of intracellular kinases, such as PKA, PKC, CaMKII, MEK 1/2, ERK 1/2, protein kinase B or Akt were also implicated in the antidepressant-like effect of creatine [42, 258]. Classically these kinases coordinate the transcription of genes related to neuroplasticity. Allen et al. (2015) also reported that combined creatine supplementation and testosterone in castrates male rats prevented downregulation of BDNF, doublecortin, and calretinin mRNAs and trended to decrease the immobility time [252]. Similarly, creatine supplementation combined with ovarian hormone replacement in ovariectomized females attenuated downregulation of BDNF and calbindin mRNA levels associated to an antidepressant profile in the forced swimming test, suggesting that combined treatment with creatine and sex steroids is neuroprotective to gonadectomized rats, potentially by reducing metabolic dysfunctions associated with castration or ovariectomy [252]. However, the same study showed that creatine supplemented rats *per se* decreased the expression of BDNF, tyrosine kinase B, doublecortin, calretinin, and calbindin (hippocampal plasticity-related genes) in the hippocampus of male rats and BDNF, doublecortin, and calbindin mRNAs in the hippocampus of females rats, suggesting that creatine supplementation negatively impacted hippocampal neuronal integrity in otherwise healthy brains, possibly through negative compensatory changes in energy metabolism [252].

The effect of creatine administration also was tested in the animal models of depression, such as chronic mild stress and chronic administration of corticosterone. Pazini et al. (2016) recently demonstrated that acute creatine treatment, similar to the fast acting antidepressant ketamine, affords antidepressant-like effect in the tail suspension test in mice administered chronically by 21 days with corticosterone [63].

Furthermore, in the same study the authors suggested that the antidepressant-like effect of creatine could be dependent at least in part of activation of PI3K and mammalian target of rapamycin, since the pretreatment of mice with wortmannin (a PI3K inhibitor) or rapamycin (a mammalian target of rapamycin inhibitor) abolished the anti-immobility effect of creatine in animals submitted to chronic corticosterone administration [63]. Subsequently, Pazini et al. (in press) also showed that the treatment with creatine for 21 days, similar to fluoxetine (a conventional antidepressant), abolished corticosterone-induced increases on the immobility time in the tail suspension test and forced swimming test and decreases on the sucrose consumption in the sucrose preference test associated to decreases on the hippocampal cell proliferation and neuronal differentiation and increases of glial fibrillary acid protein immunostaining (an indicative of astrogliosis) in dentate gyrus of the hippocampus [66].

Another animal model of depression induced by chronic mild stress for 4 weeks significantly produced depressive-like behavior in the forced swimming test and tail suspension test and decreased the serotonin immunostaining in the dorsal raphe nucleus of mice and creatine treatment abolished this effect [259]. Furthermore, creatine supplementation when combined with experimental exercise protocol significantly decreased the immobility time of mice submitted to chronic mild stress in both the forced swimming test and tail suspension test, as compared to animals only supplemented with creatine [259]. In addition, the combination of creatine supplementation and exercise produced synergistic beneficial effect in the chronic mild stress-induced serotonin depletion in the dorsal raphe nucleus and median raphe nucleus [259].

The clinical trials also reported extensively the antidepressant properties of creatine supplementation. The first evidence of antidepressant properties in clinical depression was conducted by [260]. In this study, creatine monohydrate added to ongoing psychotropic treatment in posttraumatic stress disorder patients by 4 weeks improved the scores in the clinician administered posttraumatic stress disorder scale, the Hamilton Rating Scale for Depression, the Clinical Global Impressions scale, and the Sleep Quality Scale, suggesting that creatine supplementation might

represent a pivotal strategy for the management of posttraumatic stress disorder-induced mood dysfunction [260]. The same research group also demonstrated that a patient with posttraumatic stress disorder and comorbid depression and fibromyalgia demonstrated improvement in both conditions following supplementation with creatine [261].

An important study conducted by Roitman et al. (2007) demonstrated that adult seven unipolar depression patients following creatine supplementation by 4 weeks improved significantly the Hamilton Depression Rating Scale, Hamilton Anxiety Scale, and Clinical Global Impression scores, suggesting that creatine may have antidepressant properties [262]. Although adverse reactions were mild and transitory following creatine treatment, bipolar patients treated with creatine for 4 weeks developed hypomania/mania [262]. Interestingly, eighteen patients with bipolar disorder according to DSM-IV criteria supplemented with creatine as adjunctive therapy had a reduction on the total scores in the verbal fluency test, indicative of cognitive improvement in these patients [263]. These studies with bipolar patients demonstrate the need for more well-controlled and well-designed studies to conclude the effects of creatine supplementation in these patients.

Lyoo et al. (2012) also demonstrated that female adult MDD patients receiving creatine augmentation (escitalopram treatment + creatine supplementation) showed significantly greater improvements in Hamilton depression rating scale score, as early as week 2 of treatment, maintaining at weeks 4 and 8 [264]. The same research group also demonstrated at randomized, double-blind, placebo-controlled trial, that 52 women with escitalopram-resistant MDD improved the depressive symptoms as compared to placebo augmentation group [265]. Yoon et al. (2016) also demonstrated that after 8 weeks of creatine supplementation, prefrontal N-acetylaspartate levels increased significantly in the creatine augmentation group compared with the placebo augmentation group [265]. Increment in rich club hub connections was also greater in the depressive patients supplemented with creatine group than in the placebo augmentation group, suggesting that creatine may improve the intricate neural connection [265].

The effect of creatine augmentation also was demonstrated in the elderly and adolescents with treatment-resistant MDD. The mean Children's Depression Rating Scale-Revised score declined in 5 female adolescents with fluoxetine-resistant MDD following creatine supplementation for 2-4 weeks [266]. Subsequently, as part of the National Institute of Mental Health's experimental medicine initiative, Kondo et al. (2016) conducted an elegant placebo-controlled dose-ranging study of adjunctive creatine effect for adolescent females with selective serotonin reuptake inhibitors-resistant MDD [267]. In this study, the mean frontal lobe phosphocreatine levels increased in the depressive patients supplemented with 2, 4 and 10 g of creatine, as compared to placebo group [267]. Furthermore, regression analysis of phosphocreatine levels and depression scores across the entire sample showed that frontal lobe phosphocreatine was inversely correlated with depression scores [267]. In addition, Alves et al. (2013) showed that creatine supplementation for 24 weeks did not alter the Geriatric Depression Scale scores in the elderly sedentary or submitted to strength training [121].

A pilot dose-finding clinical trial of creatine monohydrate augmentation to selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors and noradrenergic and specific serotonergic antidepressant treatment demonstrated that creatine supplementation produced no difference on Hamilton rating scale for depression scores as compared to placebo group [268]. However, two female depression patients supplemented with creatine, but no placebo-supplemented patients, showed early improvement of more than 50% reduction in depression scores after 2 weeks of creatine supplementation, suggesting that creatine augmentation may induce a more rapid antidepressant response [268].

Eleven females with MDD and comorbid methamphetamine dependence supplemented with creatine for 8 weeks reduced Hamilton rating scale and Beck Anxiety Inventory scores as early as week 2 when compared to baseline scores [269]. Also, a reduction in methamphetamine positive urine drug screens (about 50%) was observed in the patients supplemented with creatine [269].

This set of studies led Allen (2012) to review the creatine metabolism in the psychiatric disorders and the therapeutic value of creatine supplementation on these disorders [80].

SCHIZOPHRENIA

Schizophrenia is a complex mental illness with a significant negative impact on individual's and their families. This disorder is characterized by positive symptoms (such as delusions and hallucinations), negative symptoms (such as social withdrawal and reduced motivation) and cognitive impairment [270–272]. The lifetime risk of schizophrenia is about 1% and typically manifests in early adulthood [273].

A randomized, double-blind crossover study showed that ten schizophrenia patients (DSM-IV criteria) supplemented with creatine for 3 months did not alter the positive and negative syndrome scale, the clinical global impressions scale, as compared to placebo group [274]. However, seven inpatients with chronic schizophrenia supplemented with creatine for 6 months mildly improved the schizophrenia symptomatology, but there were no significant changes in cognitive functions [275]. Furthermore, several ward behaviors were also improved in the schizophrenia inpatients supplemented with creatine [275]. In the same study, Levental et al. (2015) also showed that the late onset of Parkinsonism was significantly improved in patients with schizophrenia and supplemented with creatine [275]. Since few studies have been conducted to verify the neuroprotective effects of creatine in schizophrenic patients, more robust evidence with a larger sample size are necessary.

CONCLUSION

Oxidative and nitrosative stress, glutamate excitotoxicity, neurotrophic support dysfunctions and bioenergetic impairment are common features in several neurological conditions and compounds capable of counteracting

these events are valuable candidates for putative new promising interventions. In particular, the guanidine-like compound creatine has recently demonstrated its therapeutic potential in both *in vitro* and *in vivo* models of several neurological pathologies with low toxicity and minimal side effects. The preclinical evidence has encouraged a number of clinical studies investigating the neuroprotective effect of creatine.

Clinical trials demonstrated that creatine supplementation produces beneficial results to many mental and neurological conditions, such as HD, several ischemic conditions, TBI and MDD, causing great enthusiasm in the scientific community. However, further controlled clinical studies are required to better clarify the neuroprotective actions of creatine.

Despite the increasing number of studies supporting the therapeutic potential of creatine, the neurobiological mechanisms underlying its neuroprotective properties are still not fully understood. In particular, creatine seems to couple sites of ATP production (glycolysis and mitochondrial oxidative phosphorylation) with subcellular sites of ATP utilization (ATPases) and increases neuroenergetic efficiency. However, the pleiotropic effects (antioxidant, antiexcitotoxicity and neurotrophic effects) of creatine in the central nervous system also emerge as important aspects in the neuroprotective effect of this guanidine-like compound. However, animal or cellular models, as well as clinical trials, are still necessary to understand the mechanism of action of creatine in central nervous system diseases.

REFERENCES

[1] World Health Organization, editor. Neurological disorders: public health challenges. Geneva: World Health Organization; 2006.

[2] Chandra V, Pandav R, Laxminarayan R, Tanner C, Manyam B, Rajkumar S, et al. Neurological Disorders. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Disease Control Priorities in Developing Countries.

2nd ed. Washington (DC): World Bank; 2006. Available from: http://www.ncbi.nlm.nih.gov/books/NBK11793/.

[3] Tyler FH, Armstron MD. Diseases of the Nervous System Metabolic Aspects of Some Neurological and Muscular Disorders. *Annu Rev Med.* 1954; 5(1):207–22.

[4] Hemmer W, Wallimann T. Functional aspects of creatine kinase in brain. *Dev Neurosci*. 1993; 15(3–5):249–60.

[5] Wallimann T, Tokarska-Schlattner M, Schlattner U. The creatine kinase system and pleiotropic effects of creatine. *Amino Acids.* 2011; 40(5):1271–96.

[6] Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. *Brain Res Bull.* 2008; 76(4):329–43.

[7] Rae CD. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*. 2014; 39(1):1–36.

[8] Rae CD, Bröer S. Creatine as a booster for human brain function. How might it work? *Neurochem Int.* 2015; 89:249–59.

[9] Tachikawa M, Fukaya M, Terasaki T, Ohtsuki S, Watanabe M. Distinct cellular expressions of creatine synthetic enzyme GAMT and creatine kinases uCK-Mi and CK-B suggest a novel neuron-glial relationship for brain energy homeostasis. *Eur J Neurosci*. 2004; 20(1):144–60.

[10] Lowe MTJ, Faull RLM, Christie DL, Waldvogel HJ. Distribution of the creatine transporter throughout the human brain reveals a spectrum of creatine transporter immunoreactivity. *J Comp Neurol.* 2015; 523(5):699–725.

[11] Mak CSW, Waldvogel HJ, Dodd JR, Gilbert RT, Lowe MTJ, Birch NP, et al. Immunohistochemical localisation of the creatine transporter in the rat brain. *Neuroscience.* 2009; 163(2):571–85.

[12] Lyoo IK, Kong SW, Sung SM, Hirashima F, Parow A, Hennen J, et al. Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral

supplementation of creatine-monohydrate. *Psychiatry Res*. 2003; 123(2):87–100.

[13] Rawson ES, Venezia AC. Use of creatine in the elderly and evidence for effects on cognitive function in young and old. *Amino Acids*. 2011; 40(5):1349–62.

[14] Braissant O. Creatine and guanidinoacetate transport at blood-brain and blood-cerebrospinal fluid barriers. *J Inherit Metab Dis*. 2012; 35(4):655–64.

[15] Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab*. 2001; 21(10):1133–45.

[16] Dawson DM, Eppenberger HM, Kaplan NO. The comparative enzymology of creatine kinases. II. Physical and chemical properties. *J Biol Chem*. 1967; 242(2):210–7.

[17] Eppenberger ME, Eppenberger HM, Kaplan NO. Evolution of creatine kinase. *Nature*. 1967; 214(5085):239–41.

[18] Almeida LS, Salomons GS, Hogenboom F, Jakobs C, Schoffelmeer ANM. Exocytotic release of creatine in rat brain. *Synap N Y N*. 2006; 60(2):118–23.

[19] Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, et al. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci*. 1998; 18(1):156–63.

[20] Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, et al. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med*. 1999; 5(3):347–50.

[21] Cunha MP, Martín-de-Saavedra MD, Romero A, Egea J, Ludka FK, Tasca CI, et al. Both Creatine and Its Product Phosphocreatine Reduce Oxidative Stress and Afford Neuroprotection in an *In Vitro* Parkinson's Model. *ASN Neuro*. 2014; 6(6):175909141455494.

[22] Cunha MP, Lieberknecht V, Ramos-Hryb AB, Olescowicz G, Ludka FK, Tasca CI, et al. Creatine affords protection against

glutamate-induced nitrosative and oxidative stress. *Neurochem Int.* 2016; 95:4–14.

[23] Meyer LE, Machado LB, Santiago APSA, da-Silva WS, De Felice FG, Holub O, et al. Mitochondrial creatine kinase activity prevents reactive oxygen species generation: antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity. *J Biol Chem.* 2006; 281(49):37361–71.

[24] Stevens P, Gawryluk J, Hui L, Chen X, Geiger J. Creatine Protects Against Mitochondrial Dysfunction Associated with HIV-1 Tat-Induced Neuronal Injury. *Curr HIV Res*. 2015; 12(6):378–87.

[25] Ribeiro M, Silva AC, Rodrigues J, Naia L, Rego AC. Oxidizing Effects of Exogenous Stressors in Huntington's Disease Knock-in Striatal Cells-Protective Effect of Cystamine and Creatine. *Toxicol Sci*. 2013; 136(2):487–99.

[26] Rambo LM, Ribeiro LR, Della-Pace ID, Stamm DN, da Rosa Gerbatin R, Prigol M, et al. Acute creatine administration improves mitochondrial membrane potential and protects against pentylenetetrazol-induced seizures. *Amino Acids.* 2013; 44(3):857–68.

[27] Juravleva E, Barbakadze T, Mikeladze D, Kekelidze T. Creatine enhances survival of glutamate-treated neuronal/glial cells, modulates Ras/NF-kappaB signaling, and increases the generation of reactive oxygen species. *J Neurosci Res.* 2005; 79(1–2):224–30.

[28] Juravleva E, Barbakadze T, Natsvlishvili N, Kekelidze T, Mikeladze D. Creatine prevents the cytotoxicity of haloperidol by alteration of NO/Ras/NF-κB system. In: Kekelidze T, Holtzman D, editors. Creatine kinase and brain energy metabolism: function and disease. NATO science series: life and behavioral science, *IOS Press*, Amsterdam, 2003, 343:113–119.

[29] Hosamani R, Ramesh SR, Muralidhara null. Attenuation of rotenone-induced mitochondrial oxidative damage and neurotoxicty in Drosophila melanogaster supplemented with creatine. *Neurochem Res.* 2010; 35(9):1402–12.

[30] Yang L, Calingasan NY, Wille EJ, Cormier K, Smith K, Ferrante RJ, et al. Combination therapy with Coenzyme Q $_{10}$ and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's Diseases. *J Neurochem*. 2009; 109(5):1427–39.

[31] Saraiva ALL, Ferreira APO, Silva LFA, Hoffmann MS, Dutra FD, Furian AF, et al. Creatine reduces oxidative stress markers but does not protect against seizure susceptibility after severe traumatic brain injury. *Brain Res Bull*. 2012; 87(2–3):180–6.

[32] Royes LFF, Fighera MR, Furian AF, Oliveira MS, Myskiw J de C, Fiorenza NG, et al. Effectiveness of creatine monohydrate on seizures and oxidative damage induced by methylmalonate. *Pharmacol Biochem Behav*. 2006; 83(1):136–44.

[33] de Andrade RB, Gemelli T, Rojas DB, Bonorino NF, Costa BML, Funchal C, et al. Creatine and Pyruvate Prevent the Alterations Caused by Tyrosine on Parameters of Oxidative Stress and Enzyme Activities of Phosphoryltransfer Network in Cerebral Cortex of Wistar Rats. *Mol Neurobiol*. 2015; 51(3):1184–94.

[34] Kolling J, Wyse ATS. Creatine prevents the inhibition of energy metabolism and lipid peroxidation in rats subjected to GAA administration. *Metab Brain Dis*. 2010; 25(3):331–8.

[35] Ireland Z, Castillo-Melendez M, Dickinson H, Snow R, Walker DW. A maternal diet supplemented with creatine from mid-pregnancy protects the newborn spiny mouse brain from birth hypoxia. *Neuroscience*. 2011; 194:372–9.

[36] Horecký J, Gvozdjáková A, Kucharská J, Obrenovich ME, Palacios HH, Li Y, et al. Effects of coenzyme Q and creatine supplementation on brain energy metabolism in rats exposed to chronic cerebral hypoperfusion. *Curr Alzheimer Res*. 2011; 8(8):868–75.

[37] Bender A, Beckers J, Schneider I, Hölter SM, Haack T, Ruthsatz T, et al. Creatine improves health and survival of mice. *Neurobiol Aging*. 2008; 29(9):1404–11.

[38] Hersch SM, Gevorkian S, Marder K, Moskowitz C, Feigin A, Cox M, et al. Creatine in Huntington disease is safe, tolerable,

bioavailable in brain and reduces serum 8OH2'dG. *Neurology.* 2006; 66(2):250–2.

[39] Sartini S, Sestili P, Colombo E, Martinelli C, Bartolini F, Ciuffoli S, et al. Creatine affects in vitro electrophysiological maturation of neuroblasts and protects them from oxidative stress. *J Neurosci Res.* 2012; 90(2):435–46.

[40] Genius J, Geiger J, Bender A, Möller H-J, Klopstock T, Rujescu D. Creatine Protects against Excitoxicity in an In Vitro Model of Neurodegeneration. Blagosklonny MV, editor. *PLoS ONE.* 2012; 7(2):e30554.

[41] Lawler JM, Barnes WS, Wu G, Song W, Demaree S. Direct Antioxidant Properties of Creatine. *Biochem Biophys Res Commun*. 2002; 290(1):47–52.

[42] Cunha MP, Budni J, Ludka FK, Pazini FL, Rosa JM, Oliveira Á, et al. Involvement of PI3K/Akt Signaling Pathway and Its Downstream Intracellular Targets in the Antidepressant-Like Effect of Creatine. *Mol Neurobiol.* 2016; 53(5):2954–68.

[43] Mattson MP. Glutamate and neurotrophic factors in neuronal plasticity and disease. *Ann N Y Acad Sci.* 2008; 1144:97–112.

[44] Verkhratsky A, Kirchhoff F. Glutamate-mediated neuronal-glial transmission. *J Anat.* 2007; 210(6):651–60.

[45] Lau A, Tymianski M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch.* 2010 Jul;460(2):525–42.

[46] Rambo LM, Ribeiro LR, Schramm VG, Berch AM, Stamm DN, Della-Pace ID, et al. Creatine increases hippocampal Na+,K+-ATPase activity via NMDA-calcineurin pathway. *Brain Res Bull.* 2012; 88(6):553–9.

[47] Brewer GJ, Wallimann TW. Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J Neurochem.* 2000; 74(5):1968–78.

[48] Cunha MP, Pazini FL, Ludka FK, Rosa JM, Oliveira Á, Budni J, et al. The modulation of NMDA receptors and l-arginine/nitric oxide pathway is implicated in the anti-immobility effect of creatine in the tail suspension test. *Amino Acids.* 2015; 47(4):795–811.

[49] Oliveira MS, Furian AF, Fighera MR, Fiorenza NG, Ferreira J, Rubin MA, et al. The involvement of the polyamines binding sites at the NMDA receptor in creatine-induced spatial learning enhancement. *Behav Brain Res.* 2008; 187(1):200-4.

[50] Andreassen OA, Jenkins BG, Dedeoglu A, Ferrante KL, Bogdanov MB, Kaddurah-Daouk R, et al. Increases in cortical glutamate concentrations in transgenic amyotrophic lateral sclerosis mice are attenuated by creatine supplementation. *J Neurochem*. 2001; 77(2):383–90.

[51] Magni DV, Oliveira MS, Furian AF, Fiorenza NG, Fighera MR, Ferreira J, et al. Creatine decreases convulsions and neurochemical alterations induced by glutaric acid in rats. *Brain Res*. 2007; 1185:336–45.

[52] Malcon C, Kaddurah-Daouk R, Beal MF. Neuroprotective effects of creatine administration against NMDA and malonate toxicity. *Brain Res.* 2000; 860(1–2):195–8.

[53] Vielhaber S, Kaufmann J, Kanowski M, Sailer M, Feistner H, Tempelmann C, et al. Effect of creatine supplementation on metabolite levels in ALS motor cortices. *Exp Neurol*. 2001; 172(2):377–82.

[54] Bender A, Auer DP, Merl T, Reilmann R, Saemann P, Yassouridis A, et al. Creatine supplementation lowers brain glutamate levels in Huntington's disease. *J Neurol*. 2005; 252(1):36–41.

[55] Gage FH. Mammalian neural stem cells. *Science.* 2000; 287(5457):1433–8.

[56] Götz M, Huttner WB. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol.* 2005; 6(10):777–88.

[57] Ming G-L, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron.* 2011; 70(4):687–702.

[58] Paridaen JT, Huttner WB. Neurogenesis during development of the vertebrate central nervous system. *EMBO Rep.* 2014; 15(4):351–64.

[59] Altman J, Das GD. Post-natal origin of microneurones in the rat brain. *Nature.* 1965; 207(5000):953–6.

[60] Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell.* 1999; 97(6):703–16.

[61] Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult Neural Stem Cells Bridge Their Niche. *Cell Stem Cell.* 2012; 10(6):698–708.

[62] Lee E, Son H. Adult hippocampal neurogenesis and related neurotrophic factors. *BMB Rep.* 2009; 42(5):239–44.

[63] Pazini FL, Cunha MP, Rosa JM, Colla ARS, Lieberknecht V, Oliveira Á, et al. Creatine, Similar to Ketamine, Counteracts Depressive-Like Behavior Induced by Corticosterone via PI3K/Akt/mTOR Pathway. *Mol Neurobiol.* 2016; 53(10):6818–34.

[64] Ipsiroglu OS, Stromberger C, Ilas J, Höger H, Mühl A, Stöckler-Ipsiroglu S. Changes of tissue creatine concentrations upon oral supplementation of creatine-monohydrate in various animal species. *Life Sci.* 2001; 69(15):1805–15.

[65] Sartini S, Lattanzi D, Ambrogini P, Di Palma M, Galati C, Savelli D, et al. Maternal creatine supplementation affects the morpho-functional development of hippocampal neurons in rat offspring. *Neuroscience.* 2016; 312:120–9.

[66] Pazini FL, Cunha MP, Azevedo D, Rosa JM, Colla A, de Oliveira J, et al. Creatine Prevents Corticosterone-Induced Reduction in Hippocampal Proliferation and Differentiation: Possible Implication for Its Antidepressant Effect. *Mol Neurobiol.* (in press); doi: 10.1007/s12035-016-0148-0.

[67] Di Santo S, Mina A, Ducray A, Widmer HR, Senn P. Creatine supports propagation and promotes neuronal differentiation of inner ear progenitor cells: *NeuroReport.* 2013; 25(7):446-51.

[68] Błaszczyk JW. Parkinson's Disease and Neurodegeneration: GABA-Collapse Hypothesis. *Front Neurosci.* 2016; 10:269.

[69] Kleppner SR, Tobin AJ. GABA signalling: therapeutic targets for epilepsy, Parkinson's disease and Huntington's disease. *Expert Opin Ther Targets.* 2001; 5(2):219–39.

[70] Andres RH, Ducray AD, Huber AW, Pérez-Bouza A, Krebs SH, Schlattner U, et al. Effects of creatine treatment on survival and differentiation of GABA-ergic neurons in cultured striatal tissue. *J Neurochem.* 2005; 95(1):33–45.

[71] Andres RH, Ducray AD, Andereggen L, Hohl T, Schlattner U, Wallimann T, et al. The effects of creatine supplementation on striatal neural progenitor cells depend on developmental stage. *Amino Acids.* 2016; 48(8):1913–27.

[72] Ducray AD, Qualls R, Schlattner U, Andres RH, Dreher E, Seiler RW, et al. Creatine promotes the GABAergic phenotype in human fetal spinal cord cultures. *Brain Res.* 2007; 1137(1):50–7.

[73] Ducray AD, Schläppi J-A, Qualls R, Andres RH, Seiler RW, Schlattner U, et al. Creatine treatment promotes differentiation of GABA-ergic neuronal precursors in cultured fetal rat spinal cord. *J Neurosci Res.* 2007; 85(9):1863–75.

[74] Ducray A, Kipfer S, Huber AW, Andres RH, Seiler RW, Schlattner U, et al. Creatine and neurotrophin-4/5 promote survival of nitric oxide synthase-expressing interneurons in striatal cultures. *Neurosci Lett.* 2006; 395(1):57–62.

[75] Darabi S, Tiraihi T, Delshad A, Sadeghizadeh M. A new multistep induction protocol for the transdifferentiation of bone marrow stromal stem cells into GABAergic neuron-like cells. *Iran Biomed J.* 2013; 17(1):8–14.

[76] Mohammad-Gharibani P, Tiraihi T, Mesbah-Namin SA, Arabkheradmand J, Kazemi H. Induction of bone marrow stromal cells into GABAergic neuronal phenotype using creatine as inducer. *Restor Neurol Neurosci.* 2012; (6):511–525.

[77] Darabi S, Tiraihi T, Delshad A, Sadeghizadeh M, Taheri T, Hassoun HK. Creatine Enhances Transdifferentiation of Bone Marrow Stromal Cell-Derived Neural Stem Cell Into GABAergic Neuron-Like Cells Characterized With Differential Gene Expression. *Mol Neurobiol.* 2017; 54(3):1978–91.

[78] Andres RH, Ducray AD, Pérez-Bouza A, Schlattner U, Huber AW, Krebs SH, et al. Creatine supplementation improves dopaminergic

cell survival and protects against MPP+ toxicity in an organotypic tissue culture system. *Cell Transplant.* 2005; 14(8):537–50.

[79] Andres RH, Huber AW, Schlattner U, Pérez-Bouza A, Krebs SH, Seiler RW, et al. Effects of creatine treatment on the survival of dopaminergic neurons in cultured fetal ventral mesencephalic tissue. *Neuroscience.* 2005; 133(3):701–13.

[80] Allen PJ. Creatine metabolism and psychiatric disorders: Does creatine supplementation have therapeutic value? *Neurosci Biobehav Rev.* 2012; 36(5):1442–62.

[81] Olanow CW, Kordower JH, Lang AE, Obeso JA. Dopaminergic transplantation for parkinson's disease: Current status and future prospects. *Ann Neurol.* 2009; 66(5):591–6.

[82] Lang AE, Lozano AM. Parkinson's disease. First of two parts. *N Engl J Med.* 1998; 339(15):1044–53.

[83] Lang AE, Lozano AM. Parkinson's disease. Second of two parts. *N Engl J Med.* 1998; 339(16):1130–43.

[84] Goedert M. Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci.* 2001; 2(7):492–501.

[85] Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, et al. Creatine and cyclocreatine attenuate MPTP neurotoxicity. *Exp Neurol.* 1999; 157(1):142–9.

[86] Klivenyi P, Calingasan NY, Starkov A, Stavrovskaya IG, Kristal BS, Yang L, et al. Neuroprotective mechanisms of creatine occur in the absence of mitochondrial creatine kinase. *Neurobiol Dis.* 2004; 15(3):610–7.

[87] Klivenyi P, Gardian G, Calingasan NY, Yang L, Beal MF. Additive neuroprotective effects of creatine and a cyclooxygenase 2 inhibitor against dopamine depletion in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. *J Mol Neurosci*. 2003; 21(3):191–8.

[88] Cunha MP, Martín-de-Saavedra MD, Romero A, Parada E, Egea J, del Barrio L, et al. Protective effect of creatine against 6-hydroxydopamine-induced cell death in human neuroblastoma SH-

SY5Y cells: Involvement of intracellular signaling pathways. *Neuroscience.* 2013; 238:185–94.

[89] Yong-Kee CJ, Salomonczyk D, Nash JE. Development and Validation of a Screening Assay for the Evaluation of Putative Neuroprotective Agents in the Treatment of Parkinson's Disease. *Neurotox Res.* 2011; 19(4):519–26.

[90] Bender A, Koch W, Elstner M, Schombacher Y, Bender J, Moeschl M, et al. Creatine supplementation in Parkinson disease: a placebo-controlled randomized pilot trial. *Neurology.* 2006; 67(7):1262–4.

[91] Bloom MZ. NIH announces phase III clinical trial of creatine for Parkinson's disease. *Consult Pharm.* 2007; 22(5):378.

[92] Couzin J. Clinical research. Testing a novel strategy against Parkinson's disease. *Science.* 2007; 315(5820):1778.

[93] NINDS Exploratory Trials in Parkinson Disease (NET-PD) Investigators, Kieburtz K, Tilley BC, Elm JJ, Babcock D, Hauser R, et al. Effect of creatine monohydrate on clinical progression in patients with Parkinson disease: a randomized clinical trial. *JAMA.* 2015; 313(6):584–93.

[94] Xiao Y, Luo M, Luo H, Wang J. Creatine for Parkinson's disease. *Cochrane Database Syst Rev.* 2014; (6):CD009646.

[95] Attia A, Ahmed H, Gadelkarim M, Morsi M, Awad K, Elnenny M, et al. Meta-analysis of Creatine for neuroprotection against Parkinson's disease. *CNS Neurol Disord Drug Targets.* (in press); doi: 10.2174/1871527315666161104161855.

[96] Adhihetty PJ, Beal MF. Creatine and its potential therapeutic value for targeting cellular energy impairment in neurodegenerative diseases. *Neuromolecular Med.* 2008; 10(4):275–90.

[97] Klein AM, Ferrante RJ. The neuroprotective role of creatine. *Subcell Biochem.* 2007; 46:205–43.

[98] Beal MF. Neuroprotective effects of creatine. *Amino Acids.* 2011; 40(5):1305–13.

[99] Bender A, Klopstock T. Creatine for neuroprotection in neurodegenerative disease: end of story? *Amino Acids.* 2016; 48(8):1929–40.

[100] Valastro B, Dekundy A, Danysz W, Quack G. Oral creatine supplementation attenuates L-DOPA-induced dyskinesia in 6-hydroxydopamine-lesioned rats. *Behav Brain Res*. 2009; 197(1):90–6.

[101] Ritchie K, Lovestone S. The dementias. *Lancet Lond Engl.* 2002; 360(9347):1759–66.

[102] Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Hum Mol Genet.* 2010; 19(R1):R12-20.

[103] Iqbal K, Grundke-Iqbal I. Neurofibrillary pathology leads to synaptic loss and not the other way around in Alzheimer disease. *J Alzheimers Dis*. 2002; 4(3):235–8.

[104] Mandelkow EM, Mandelkow E. Tau in Alzheimer's disease. *Trend*s *Cell Biol.* 1998; 8(11):425–7.

[105] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological Alterations in Alzheimer Disease. *Cold Spring Harb Perspect Med.* 2011; 1(1):a006189–a006189.

[106] Terry RD. Neuropathological changes in Alzheimer disease. *Prog Brain Res.* 1994; 101:383–90.

[107] Trojanowski JQ, Lee VM. "Fatal attractions" of proteins. A comprehensive hypothetical mechanism underlying Alzheimer's disease and other neurodegenerative disorders. *Ann N Y Acad Sci.* 2000; 924:62–7.

[108] Cai H, Cong W, Ji S, Rothman S, Maudsley S, Martin B. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr Alzheimer Res.* 2012; 9(1):5–17.

[109] Heiss WD, Szelies B, Kessler J, Herholz K. Abnormalities of energy metabolism in Alzheimer's disease studied with PET. *Ann N Y Acad Sci.* 1991; 640:65–71.

[110] Kessler J, Herholz K, Grond M, Heiss WD. Impaired metabolic activation in Alzheimer's disease: a PET study during continuous visual recognition. *Neuropsychologia.* 1991; 29(3):229–43.

[111] Baroncelli L, Molinaro A, Cacciante F, Alessandrì MG, Napoli D, Putignano E, et al. A mouse model for creatine transporter deficiency reveals early onset cognitive impairment and neuropathology associated with brain aging. *Hum Mol Genet.* 2016; 25(19):4186–200.

[112] Hautman ER, Kokenge AN, Udobi KC, Williams MT, Vorhees CV, Skelton MR. Female mice heterozygous for creatine transporter deficiency show moderate cognitive deficits. *J Inherit Metab Dis*. 2014; 37(1):63–8.

[113] Souza MA, Magni DV, Guerra GP, Oliveira MS, Furian AF, Pereira L, et al. Involvement of hippocampal CAMKII/CREB signaling in the spatial memory retention induced by creatine. *Amino Acids.* 2012; 43(6):2491–503.

[114] Allahyar R, Akbar A, Iqbal F. Effect of creatine monohydrate supplementation on learning, memory and neuromuscular coordination in female albino mice. *Acta Neuropsychiatr*. 2017; 29(01):27–34.

[115] dos Santos FS, da Silva LA, Pochapski JA, Raczenski A, da Silva WC, Grassiolli S, et al. Effects of l-arginine and creatine administration on spatial memory in rats subjected to a chronic variable stress model. *Pharm Biol*. 2014; 52(8):1033–8.

[116] Ling J, Kritikos M, Tiplady B. Cognitive effects of creatine ethyl ester supplementation: *Behav Pharmacol*. 2009; 20(8):673–9.

[117] Benton D, Donohoe R. The influence of creatine supplementation on the cognitive functioning of vegetarians and omnivores. *Br J Nutr*. 2011; 105(07):1100–5.

[118] Rae C, Digney AL, McEwan SR, Bates TC. Oral creatine monohydrate supplementation improves brain performance: a double-blind, placebo-controlled, cross-over trial. *Proc R Soc B Biol Sci*. 2003; 270(1529):2147–50.

[119] McMorris T, Mielcarz G, Harris RC, Swain JP, Howard A. Creatine supplementation and cognitive performance in elderly individuals. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn.* 2007; 14(5):517–28.

[120] Rawson ES, Lieberman HR, Walsh TM, Zuber SM, Harhart JM, Matthews TC. Creatine supplementation does not improve cognitive function in young adults. *Physiol Behav.* 2008; 95(1–2):130–4.

[121] Alves CRR, Merege Filho CAA, Benatti FB, Brucki S, Pereira RMR, de Sá Pinto AL, et al. Creatine Supplementation Associated or Not with Strength Training upon Emotional and Cognitive Measures in Older Women: A Randomized Double-Blind Study. *PLoS ONE.* 2013; 8(10):e76301.

[122] AliMohammadi M, Eshraghian M, Zarindast M-R, Aliaghaei A, Pishva H. Effects of creatine supplementation on learning, memory retrieval, and apoptosis in an experimental animal model of Alzheimer disease. *Med J Islam Repub Iran.* 2015; 29:273.

[123] Alimohammadi-Kamalabadi M, Eshraghian M, Zarindast M-R, Aliaghaei A, Pishva H. Effect of creatine supplementation on cognitive performance and apoptosis in a rat model of amyloid-beta-induced Alzheimer's disease. *Iran J Basic Med Sci.* 2016; 19(11):1159–65.

[124] Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N. The incidence and prevalence of Huntington's disease: A systematic review and meta-analysis. *Mov Disord.* 2012; 27(9):1083–91.

[125] Roze E, Bonnet C, Betuing S, Caboche J. Huntington's disease. *Adv Exp Med Biol.* 2010; 685:45–63.

[126] Martin JB, Gusella JF. Huntington's disease. Pathogenesis and management. *N Engl J Med.* 1986; 315(20):1267–76.

[127] McNeil SM, Novelletto A, Srinidhi J, Barnes G, Kornbluth I, Altherr MR, et al. Reduced penetrance of the Huntington's disease mutation. *Hum Mol Genet.* 1997; 6(5):775–9.

[128] Giacomello M, Hudec R, Lopreiato R. Huntington's disease, calcium, and mitochondria. *BioFactors*. 2011; 37(3):206–18.

[129] Polyzos AA, McMurray CT. The chicken or the egg: mitochondrial dysfunction as a cause or consequence of toxicity in Huntington's disease. *Mech Ageing Dev.* 2017; 161:181–97.

[130] Shear DA, Haik KL, Dunbar GL. Creatine reduces 3-nitropropionic-acid-induced cognitive and motor abnormalities in rats. *Neuroreport.* 2000; 11(9):1833–7.

[131] Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci*. 2000; 20(12):4389–97.

[132] Dedeoglu A, Kubilus JK, Yang L, Ferrante KL, Hersch SM, Beal MF, et al. Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. *J Neurochem.* 2003; 85(6):1359–67.

[133] Kaemmerer WF, Rodrigues CM, Steer CJ, Low WC. Creatine-supplemented diet extends Purkinje cell survival in spinocerebellar ataxia type 1 transgenic mice but does not prevent the ataxic phenotype. *Neuroscience.* 2001; 103(3):713–24.

[134] Lin Y-S, Cheng T-H, Chang C-P, Chen H-M, Chern Y. Enhancement of brain-type creatine kinase activity ameliorates neuronal deficits in Huntington's disease. *Biochim Biophys Acta.* 2013; 1832(6):742–53.

[135] Andreassen OA, Dedeoglu A, Ferrante RJ, Jenkins BG, Ferrante KL, Thomas M, et al. Creatine increase survival and delays motor symptoms in a transgenic animal model of Huntington's disease. *Neurobiol Dis*. 2001; 8(3):479–91.

[136] Rosas HD, Doros G, Gevorkian S, Malarick K, Reuter M, Coutu J-P, et al. PRECREST: A phase II prevention and biomarker trial of creatine in at-risk Huntington disease. *Neurology.* 2014; 82(10):850–7.

[137] Tuckfield C. First use of creatine hydrochloride in premanifest Huntington disease. *Med J Aust.* 2015; 202(7):378–80.

[138] Tabrizi SJ, Blamire AM, Manners DN, Rajagopalan B, Styles P, Schapira AHV, et al. High-dose creatine therapy for Huntington disease: a 2-year clinical and MRS study. *Neurology*. 2005; 64(9):1655–6.

[139] Tabrizi SJ, Blamire AM, Manners DN, Rajagopalan B, Styles P, Schapira AHV, et al. Creatine therapy for Huntington's disease: clinical and MRS findings in a 1-year pilot study. *Neurology*. 2003; 61(1):141–2.

[140] Verbessem P, Lemiere J, Eijnde BO, Swinnen S, Vanhees L, Van Leemputte M, et al. Creatine supplementation in Huntington's disease: a placebo-controlled pilot trial. *Neurology*. 2003; 61(7):925–30.

[141] Ryu H, Rosas HD, Hersch SM, Ferrante RJ. The therapeutic role of creatine in Huntington's disease. *Pharmacol Ther*. 2005; 108(2):193–207.

[142] Naia L, Ribeiro MJ, Rego AC. Mitochondrial and metabolic-based protective strategies in Huntington's disease: the case of creatine and coenzyme Q. *Rev Neurosci*. 2011; 23(1):13-28.

[143] Brown RH. Amyotrophic lateral sclerosis: recent insights from genetics and transgenic mice. *Cell*. 1995; 80(5):687–92.

[144] Eisen A. Amyotrophic lateral sclerosis-Evolutionary and other perspectives. *Muscle Nerve*. 2009; 40(2):297–304.

[145] Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, et al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nat Biotechnol*. 2011; 29(9):824–8.

[146] Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol*. 2009; 187(6):761–72.

[147] Boillée S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, et al. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science*. 2006; 312(5778):1389–92.

[148] Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. *Neuroepidemiology*. 2013; 41(2):118–30.

[149] Cleveland DW, Bruijn LI, Wong PC, Marszalek JR, Vechio JD, Lee MK, et al. Mechanisms of selective motor neuron death in transgenic mouse models of motor neuron disease. *Neurology*. 1996; 47(4 Suppl 2):S54-61; discussion S61-62.

[150] King AE, Woodhouse A, Kirkcaldie MTK, Vickers JC. Excitotoxicity in ALS: Overstimulation, or overreaction? *Exp Neurol*. 2016; 275:162–71.

[151] Morrison BM, Morrison JH. Amyotrophic lateral sclerosis associated with mutations in superoxide dismutase: a putative mechanism of degeneration. *Brain Res Brain Res Rev*. 1999 Jan; 29(1):121–35.

[152] Peters OM, Ghasemi M, Brown RH. Emerging mechanisms of molecular pathology in ALS. *J Clin Invest.* 2015; 125(5):1767–79.

[153] Tan C-C, Yu J-T, Tan M-S, Jiang T, Zhu X-C, Tan L. Autophagy in aging and neurodegenerative diseases: implications for pathogenesis and therapy. *Neurobiol Aging*. 2014; 35(5):941–57.

[154] Van den Bosch L. [The causes and mechanism of selective motor neuron death in amyotrophic lateral sclerosis]. *Verh - K Acad Voor Geneeskd Van Belg*. 2006; 68(4):249–69.

[155] Van Den Bosch L, Van Damme P, Bogaert E, Robberecht W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim Biophys Acta.* 2006; 1762(11–12):1068–82.

[156] Klivenyi P, Kiaei M, Gardian G, Calingasan NY, Beal MF. Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurochem.* 2004; 88(3):576–82.

[157] Choi J-K, Küstermann E, Dedeoglu A, Jenkins BG. Magnetic resonance spectroscopy of regional brain metabolite markers in FALS mice and the effects of dietary creatine supplementation. *Eur J Neurosci.* 2009; 30(11):2143–50.

[158] Mazzini L, Balzarini C, Colombo R, Mora G, Pastore I, De Ambrogio R, et al. Effects of creatine supplementation on exercise performance and muscular strength in amyotrophic lateral sclerosis: preliminary results. *J Neurol Sci*. 2001; 191(1–2):139–44.

[159] Shefner JM, Cudkowicz ME, Schoenfeld D, Conrad T, Taft J, Chilton M, et al. A clinical trial of creatine in ALS. *Neurology*. 2004; 63(9):1656–61.

[160] Groeneveld GJ, Veldink JH, van der Tweel I, Kalmijn S, Beijer C, de Visser M, et al. A randomized sequential trial of creatine in amyotrophic lateral sclerosis. *Ann Neurol*. 2003; 53(4):437–45.

[161] Rosenfeld J, King RM, Jackson CE, Bedlack RS, Barohn RJ, Dick A, et al. Creatine monohydrate in ALS: effects on strength, fatigue, respiratory status and ALSFRS. *Amyotroph Lateral Scler*. 2008; 9(5):266–72.

[162] Drory VE, Gross D. No effect of creatine on respiratory distress in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2002; 3(1):43–6.

[163] Atassi N, Ratai E-M, Greenblatt DJ, Pulley D, Zhao Y, Bombardier J, et al. A phase I, pharmacokinetic, dosage escalation study of creatine monohydrate in subjects with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler*. 2010; 11(6):508–13.

[164] Pastula DM, Moore DH, Bedlack RS. Creatine for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database Syst Rev*. 2012; 12:CD005225.

[165] Pastula DM, Moore DH, Bedlack RS. Creatine for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database Syst Rev*. 2010; (6):CD005225.

[166] Strong MJ, Pattee GL. Creatine and coenzyme Q10 in the treatment of ALS. *Amyotroph Lateral Scler Other Mot Neuron Disord*. 2000; 1 Suppl 4:17–20.

[167] Ellis AC, Rosenfeld J. The role of creatine in the management of amyotrophic lateral sclerosis and other neurodegenerative disorders. *CNS Drugs*. 2004; 18(14):967–80.

[168] Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. 2006; 21(5):375–8.

[169] Schwarzbold M, Diaz A, Martins ET, Rufino A, Amante LN, Thais ME, et al. Psychiatric disorders and traumatic brain injury. *Neuropsychiatr Dis Treat.* 2008; 4(4):797–816.

[170] Maas AIR, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol*. 2008; 7(8):728–41.

[171] Saatman KE, Duhaime A-C, Bullock R, Maas AIR, Valadka A, Manley GT, et al. Classification of traumatic brain injury for targeted therapies. *J Neurotrauma.* 2008; 25(7):719–38.

[172] Zipfel GJ, Babcock DJ, Lee JM, Choi DW. Neuronal apoptosis after CNS injury: the roles of glutamate and calcium. *J Neurotrauma*. 2000; 17(10):857–69.

[173] Vagnozzi R, Signoretti S, Cristofori L, Alessandrini F, Floris R, Isgrò E, et al. Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. *Brain J Neurol.* 2010; 133(11):3232–42.

[174] Signoretti S, Di Pietro V, Vagnozzi R, Lazzarino G, Amorini AM, Belli A, et al. Transient alterations of creatine, creatine phosphate, N-acetylaspartate and high-energy phosphates after mild traumatic brain injury in the rat. *Mol Cell Biochem*. 2010; 333(1–2):269–77.

[175] Sullivan PG, Geiger JD, Mattson MP, Scheff SW. Dietary supplement creatine protects against traumatic brain injury. *Ann Neurol.* 2000; 48(5):723–9.

[176] Scheff SW, Dhillon HS. Creatine-enhanced diet alters levels of lactate and free fatty acids after experimental brain injury. *Neurochem Res.* 2004; 29(2):469–79.

[177] Sakellaris G, Kotsiou M, Tamiolaki M, Kalostos G, Tsapaki E, Spanaki M, et al. Prevention of complications related to traumatic brain injury in children and adolescents with creatine administration: an open label randomized pilot study. *J Trauma*. 2006; 61(2):322–9.

[178] Sakellaris G, Nasis G, Kotsiou M, Tamiolaki M, Charissis G, Evangeliou A. Prevention of traumatic headache, dizziness and fatigue with creatine administration. A pilot study. *Acta Paediatr.* 2008; 97(1):31–4.

[179] Freire Royes LF, Cassol G. The Effects of Creatine Supplementation and Physical Exercise on Traumatic Brain Injury. *Mini Rev Med Chem.* 2016; 16(1):29–39.

[180] Rabchevsky AG, Sullivan PG, Fugaccia I, Scheff SW. Creatine diet supplement for spinal cord injury: influences on functional recovery and tissue sparing in rats. *J Neurotrauma.* 2003; 20(7):659–69.

[181] Hausmann ON, Fouad K, Wallimann T, Schwab ME. Protective effects of oral creatine supplementation on spinal cord injury in rats. *Spinal Cord*. 2002; 40(9):449–56.

[182] Flynn RWV, MacWalter RSM, Doney ASF. The cost of cerebral ischaemia. *Neuropharmacology.* 2008; 55(3):250–6.

[183] Johnston SC, Mendis S, Mathers CD. Global variation in stroke burden and mortality: estimates from monitoring, surveillance, and modelling. *Lancet Neurol.* 2009; 8(4):345–54.

[184] Mathers CD, Boerma T, Ma Fat D. Global and regional causes of death. *Br Med Bull.* 2009; 92(1):7–32.

[185] Ramos E, Patiño P, Reiter RJ, Gil-Martín E, Marco-Contelles J, Parada E, et al. Ischemic brain injury: New insights on the protective role of melatonin. *Free Radic Biol Med.* 2017; 104:32–53.

[186] Choi DW. Ischemia-induced neuronal apoptosis. *Curr Opin Neurobiol.* 1996; 6(5):667–72.

[187] Hossmann KA. Viability thresholds and the penumbra of focal ischemia. *Ann Neurol.* 1994; 36(4):557–65.

[188] Krivanek J, Bures J, Buresova O. Evidence for a relation between creatine phosphate level and polarity of the cerebral cortex. *Nature.* 1958; 182(4652):1799.

[189] Whittingham TS, Lipton P. Cerebral synaptic transmission during anoxia is protected by creatine. *J Neurochem*. 1981; 37(6):1618–21.

[190] Lipton P, Whittingham TS. Reduced ATP concentration as a basis for synaptic transmission failure during hypoxia in the in vitro guinea-pig hippocampus. *J Physiol*. 1982; 325:51–65.

[191] Yoneda K, Arakawa T, Asaoka Y, Fukuoka Y, Kinugasa K, Takimoto K, et al. Effects of accumulation of phosphocreatine on utilization and restoration of high-energy phosphates during anoxia and recovery in thin hippocampal slices from the guinea pig. *Exp Neurol*. 1983; 82(1):215–22.

[192] Okada Y, Yoneda K. Effects of accumulation of phosphocreatine on the survival time of thin hippocampal slices from the guinea pig during deprivation of both oxygen and glucose. *Neurosci Lett*. 1983; 41(1–2):119–24.

[193] Carter AJ, Müller RE, Pschorn U, Stransky W. Preincubation with creatine enhances levels of creatine phosphate and prevents anoxic damage in rat hippocampal slices. *J Neurochem*. 1995; 64(6):2691–9.

[194] Wilken B, Ramirez JM, Probst I, Richter DW, Hanefeld F. Creatine protects the central respiratory network of mammals under anoxic conditions. *Pediatr Res*. 1998; 43(1):8–14.

[195] Balestrino M. Pathophysiology of anoxic depolarization: new findings and a working hypothesis. *J Neurosci Methods*. 1995; 59(1):99–103.

[196] Balestrino M, Rebaudo R, Lunardi G. Exogenous creatine delays anoxic depolarization and protects from hypoxic damage: dose-effect relationship. *Brain Res*. 1999; 816(1):124–30.

[197] Adriano E, Garbati P, Damonte G, Salis A, Armirotti A, Balestrino M. Searching for a therapy of creatine transporter deficiency: some effects of creatine ethyl ester in brain slices in vitro. *Neuroscience*. 2011; 199:386–93.

[198] Wick M, Fujimori H, Michaelis T, Frahm J. Brain water diffusion in normal and creatine-supplemented rats during transient global ischemia. *Magn Reson Med*. 1999; 42(4):798–802.

[199] Zhu S, Li M, Figueroa BE, Liu A, Stavrovskaya IG, Pasinelli P, et al. Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. *J Neurosci*. 2004; 24(26):5909–12.

[200] Prass K, Royl G, Lindauer U, Freyer D, Megow D, Dirnagl U, et al. Improved reperfusion and neuroprotection by creatine in a mouse model of stroke. *J Cereb Blood Flow Metab*. 2007; 27(3):452–9.

[201] Lensman M, Korzhevskii DE, Mourovets VO, Kostkin VB, Izvarina N, Perasso L, et al. Intracerebroventricular administration of creatine protects against damage by global cerebral ischemia in rat. *Brain Res*. 2006; 1114(1):187–94.

[202] Otellin VA, Korzhevskii DE, Kostkin VB, Balestrino M, Lensman MV, Polenov SA. The neuroprotective effect of creatine in rats with cerebral ischemia. *Dokl Biol Sci Proc Acad Sci USSR Biol Sci Sect*. 2003; 390:197–9.

[203] Payan HM, Levine S, Strebel R. Effects of cerebral ischemia in various strains of rats. *Proc Soc Exp Biol Med Soc Exp Biol Med N Y N*. 1965; 120(1):208–9.

[204] Woznicki DT, Walker JB. Utilization of cyclocreatine phosphate, and analogue of creatine phosphate, by mouse brain during ischemia and its sparing action on brain energy reserves. *J Neurochem*. 1980; 34(5):1247–53.

[205] Artru AA, Michenfelder JD. Cyclocreatine phosphate, an analogue of creatine phosphate, does not improve hypoxic tolerance in mice. *J Neurochem*. 1982; 39(4):1198–200.

[206] Perasso L, Adriano E, Ruggeri P, Burov SV, Gandolfo C, Balestrino M. In vivo neuroprotection by a creatine-derived compound: phosphocreatine-Mg-complex acetate. *Brain Res*. 2009; 1285:158–63.

[207] Kratirova NV, Veselkina OS, Kolpakova ME, Chefu SG, Korzhevskiĭ DE, Daĭneko AS, et al. [Effect of intragastric creatine

glycine ethylic ether fumarate administration in a rat model of occlusive ischemia]. *Ross Fiziol Zh Im I M Sechenova.* 2012; 98(10):1258–63.

[208] Adriano E, Garbati P, Salis A, Damonte G, Millo E, Balestrino M. Creatine salts provide neuroprotection even after partial impairment of the creatine transporter. *Neuroscience.* 2017; 340:299–307.

[209] Turner CE, Byblow WD, Gant N. Creatine supplementation enhances corticomotor excitability and cognitive performance during oxygen deprivation. *J Neurosci.* 2015; 35(4):1773–80.

[210] Gaddi AV, Galuppo P, Yang J. Creatine Phosphate Administration in Cell Energy Impairment Conditions: A Summary of Past and Present Research. *Heart Lung Circ.* 2017; pii: S1443-9506(17)30095-1.

[211] Skrivanek, O., Kalvach, P., Benetin, J., Brzak, M., Kukumberg, I., Strnad, P. et al, Creatine phosphate in the treatment of acute ischemic stroke. *Prakticky Lekar*. 1995; 75:7–8.

[212] Adcock KH, Nedelcu J, Loenneker T, Martin E, Wallimann T, Wagner BP. Neuroprotection of creatine supplementation in neonatal rats with transient cerebral hypoxia-ischemia. *Dev Neurosci.* 2002; 24(5):382–8.

[213] Dickinson H, Ellery S, Ireland Z, LaRosa D, Snow R, Walker DW. Creatine supplementation during pregnancy: summary of experimental studies suggesting a treatment to improve fetal and neonatal morbidity and reduce mortality in high-risk human pregnancy. *BMC Pregnancy Childbirth.* 2014; 14:150.

[214] Ellery SJ, Dickinson H, McKenzie M, Walker DW. Dietary interventions designed to protect the perinatal brain from hypoxic-ischemic encephalopathy – Creatine prophylaxis and the need for multi-organ protection. *Neurochem Int.* 2016; 95:15–23.

[215] Allah Yar R, Akbar A, Iqbal F. Creatine monohydrate supplementation for 10 weeks mediates neuroprotection and improves learning/memory following neonatal hypoxia ischemia

encephalopathy in female albino mice. *Brain Res.* 2015; 1595:92–100.

[216] Iqbal S, Nazir N, Gillani Q, Akbar A, Iqbal F. Effect of creatine monohydrate supplementation on various hematological and serum biochemical parameters of male albino mice following neonatal hypoxia-ischemia encephalopathy. *Scientific World Journal.* 2013; 2013:286075.

[217] Iqbal S, Ali M, Akbar A, Iqbal F. Effects of dietary creatine supplementation for 8 weeks on neuromuscular coordination and learning in male albino mouse following neonatal hypoxic ischemic insult. *Neurol Sci.* 2015; 36(5):765–70.

[218] Iqbal S, Ali M, Iqbal F. Long term creatine monohydrate supplementation, following neonatal hypoxic ischemic insult, improves neuromuscular coordination and spatial learning in male albino mouse. *Brain Res.* 2015; 1603:76–83.

[219] Iqbal S, Ali M, Iqbal F. Effect of creatine monohydrate supplementation on relative serum level of IL-6 and IL-18 following neonatal hypoxia ischemia in male albino mouse. *Pak J Pharm Sci.* 2015; 28(6):2141–5.

[220] Kwan P, Brodie MJ. Epilepsy after the first drug fails: substitution or add-on? *Seizure.* 2000; 9(7):464–8.

[221] Hart YM, Sander JW. Epilepsy: questions and answers. *Merit Pub. International;* 2008.

[222] ILAE. Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League against Epilepsy. *Epilepsia.* 1989; 30(4):389–99.

[223] Sander JW, Hart YM, Johnson AL, Shorvon SD. National General Practice Study of Epilepsy: newly diagnosed epileptic seizures in a general population. *Lancet Lond Engl.* 1990; 336(8726):1267–71.

[224] Kwan P, Schachter SC, Brodie MJ. Drug-Resistant Epilepsy. *N Engl J Med*. 2011; 365(10):919–26.

[225] Pan JW, Kim JH, Cohen-Gadol A, Pan C, Spencer DD, Hetherington HP. Regional energetic dysfunction in hippocampal epilepsy. *Acta Neurol Scand.* 2005; 111(4):218–24.

[226] Holtzman D, Togliatti A, Khait I, Jensen F. Creatine increases survival and suppresses seizures in the hypoxic immature rat. *Pediatr Res.* 1998; 44(3):410–4.

[227] Royes LFF, Fighera MR, Furian AF, Oliveira MS, da Silva LGM, Malfatti CRM, et al. Creatine protects against the convulsive behavior and lactate production elicited by the intrastriatal injection of methylmalonate. *Neuroscience.* 2003; 118(4):1079–90.

[228] Rambo LM, Ribeiro LR, Oliveira MS, Furian AF, Lima FD, Souza MA, et al. Additive anticonvulsant effects of creatine supplementation and physical exercise against pentylenetetrazol-induced seizures. *Neurochem Int.* 2009; 55(5):333–40.

[229] Kim DW, Yeo S-I, Ryu HJ, Kim J-E, Song H-K, Kwon O-S, et al. Effects of creatine and β-guanidinopropionic acid and alterations in creatine transporter and creatine kinases expression in acute seizure and chronic epilepsy models. *BMC Neurosci.* 2010; 11:141.

[230] Vielhaber S, Kudin AP, Kudina TA, Stiller D, Scheich H, Schoenfeld A, et al. Hippocampal N-acetyl aspartate levels do not mirror neuronal cell densities in creatine-supplemented epileptic rats. *Eur J Neurosci*. 2003; 18(8):2292–300.

[231] Banner NF. Mental disorders are not brain disorders: Mental disorders are not brain disorders. *J Eval Clin Pract.* 2013; 19(3):509–13.

[232] White PD, Rickards H, Zeman AZJ. Time to end the distinction between mental and neurological illnesses. *BMJ.* 2012; 344:e3454.

[233] Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron.* 2002; 34(1):13–25.

[234] Wong ML, Licinio J. Research and treatment approaches to depression. *Nat Rev Neurosci.* 2001; 2(5):343–51.

[235] Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci.* 2006; 7(2):137–51.

[236] Fava M, Kendler KS. Major depressive disorder. *Neuron.* 2000; 28(2):335–41.

[237] Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature.* 2008; 455(7215):894–902.

[238] Nemeroff CB. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. *J Psychiatr Res*. 2007; 41(3–4):189–206.

[239] Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacol.* 2008; 33(1):88–109.

[240] Filipović D, Todorović N, Bernardi RE, Gass P. Oxidative and nitrosative stress pathways in the brain of socially isolated adult male rats demonstrating depressive- and anxiety-like symptoms. *Brain Struct Funct.* 2017; 222(1):1–20.

[241] Kaster MP, Moretti M, Cunha MP, Rodrigues ALS. Novel approaches for the management of depressive disorders. *Eur J Pharmacol.* 2016; 771:236–40.

[242] Kaufmann FN, Costa AP, Ghisleni G, Diaz AP, Rodrigues ALS, Peluffo H, et al. NLRP3 inflammasome-driven pathways in depression: *Clinical and preclinical findings*. 2017; pii: S0889-1591(17)30064-8.

[243] Morris G, Berk M, Klein H, Walder K, Galecki P, Maes M. Nitrosative Stress, Hypernitrosylation, and Autoimmune Responses to Nitrosylated Proteins: New Pathways in Neuroprogressive Disorders Including Depression and Chronic Fatigue Syndrome. *Mol Neurobiol.* (in press); doi:10.1007/s12035-016-9975-2.

[244] Sanacora G. New understanding of mechanisms of action of bipolar medications. *J Clin Psychiatry*. 2008; 69 Suppl 5:22–7.

[245] Skolnick P. Antidepressants for the new millennium. *Eur J Pharmacol.* 1999; 375(1–3):31–40.

[246] Strakowski SM. Bioenergetics for depression: something different for depression. *Am J Psychiatry*. 2012; 169(9):891–3.

[247] Masand PS. Tolerability and adherence issues in antidepressant therapy. *Clin Ther*. 2003; 25(8):2289–304.

[248] Usala T, Clavenna A, Zuddas A, Bonati M. Randomised controlled trials of selective serotonin reuptake inhibitors in treating depression in children and adolescents: a systematic review and meta-analysis. *Eur Neuropsychopharmacol*. 2008; 18(1):62–73.

[249] Covington HE, Vialou V, Nestler EJ. From synapse to nucleus: Novel targets for treating depression. *Neuropharmacology*. 2010; 58(4–5):683–93.

[250] Cryan JF, Slattery DA. Animal models of mood disorders: Recent developments. *Curr Opin Psychiatry*. 2007; 20(1):1–7.

[251] Allen PJ, D'Anci KE, Kanarek RB, Renshaw PF. Chronic Creatine Supplementation Alters Depression-like Behavior in Rodents in a Sex-Dependent Manner. *Neuropsychopharmacology*. 2010; 35(2):534–46.

[252] Allen PJ, DeBold JF, Rios M, Kanarek RB. Chronic high-dose creatine has opposing effects on depression-related gene expression and behavior in intact and sex hormone-treated gonadectomized male and female rats. *Pharmacol Biochem Behav*. 2015; 130:22–33.

[253] Allen PJ, D'Anci KE, Kanarek RB, Renshaw PF. Sex-specific antidepressant effects of dietary creatine with and without sub-acute fluoxetine in rats. *Pharmacol Biochem Behav*. 2012; 101(4):588–601.

[254] Cunha MP, Machado DG, Capra JC, Jacinto J, Bettio LE, Rodrigues ALS. Antidepressant-like effect of creatine in mice involves dopaminergic activation. *J Psychopharmacol*. 2012; 26(11):1489–501.

[255] Cunha MP, Pazini FL, Oliveira Á, Bettio LEB, Rosa JM, Machado DG, et al. The activation of α1-adrenoceptors is implicated in the antidepressant-like effect of creatine in the tail suspension test. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013; 44:39–50.

[256] Cunha MP, Pazini FL, Oliveira Á, Machado DG, Rodrigues ALS. Evidence for the involvement of 5-HT1A receptor in the acute antidepressant-like effect of creatine in mice. *Brain Res Bull.* 2013; 95:61–9.

[257] Cunha MP, Pazini FL, Rosa JM, Ramos-Hryb AB, Oliveira Á, Kaster MP, et al. Creatine, similarly to ketamine, affords antidepressant-like effects in the tail suspension test via adenosine A1 and A2A receptor activation. *Purinergic Signal.* 2015; 11(2):215–27.

[258] Cunha MP, Budni J, Pazini FL, Oliveira Á, Rosa JM, Lopes MW, et al. Involvement of PKA, PKC, CAMK-II and MEK1/2 in the acute antidepressant-like effect of creatine in mice. *Pharmacol Rep*. 2014; 66(4):653–9.

[259] Ahn N-R, Leem Y-H, Kato M, Chang H. Effects of creatine monohydrate supplementation and exercise on depression-like behaviors and raphe 5-HT neurons in mice. *J Exerc Nutr Biochem.* 2016; 20(3):24–31.

[260] Amital D, Vishne T, Rubinow A, Levine J. Observed effects of creatine monohydrate in a patient with depression and fibromyalgia. *Am J Psychiatry.* 2006; 163(10):1840–1.

[261] Amital D, Vishne T, Roitman S, Kotler M, Levine J. Open study of creatine monohydrate in treatment-resistant posttraumatic stress disorder. *J Clin Psychiatry*. 2006; 67(5):836–7.

[262] Roitman S, Green T, Osher Y, Karni N, Levine J. Creatine monohydrate in resistant depression: a preliminary study. *Bipolar Disord.* 2007; 9(7):754–8.

[263] Toniolo RA, Fernandes F de BF, Silva M, Dias R da S, Lafer B. Cognitive effects of creatine monohydrate adjunctive therapy in patients with bipolar depression: Results from a randomized, double-blind, placebo-controlled trial. *J Affect Disord.* 2016; pii: S0165-0327(16)32114-0.

[264] Lyoo IK, Yoon S, Kim T-S, Hwang J, Kim JE, Won W, et al. A randomized, double-blind placebo-controlled trial of oral creatine monohydrate augmentation for enhanced response to a selective

serotonin reuptake inhibitor in women with major depressive disorder. *Am J Psychiatry*. 2012; 169(9):937–45.

[265] Yoon S, Kim JE, Hwang J, Kim T-S, Kang HJ, Namgung E, et al. Effects of Creatine Monohydrate Augmentation on Brain Metabolic and Network Outcome Measures in Women With Major Depressive Disorder. *Biol Psychiatry*. 2016; 80(6):439–47.

[266] Kondo DG, Sung Y-H, Hellem TL, Fiedler KK, Shi X, Jeong E-K, et al. Open-label adjunctive creatine for female adolescents with SSRI-resistant major depressive disorder: A 31-phosphorus magnetic resonance spectroscopy study. *J Affect Disord*. 2011; 135(1–3):354–61.

[267] Kondo DG, Forrest LN, Shi X, Sung Y-H, Hellem TL, Huber RS, et al. Creatine target engagement with brain bioenergetics: a dose-ranging phosphorus-31 magnetic resonance spectroscopy study of adolescent females with SSRI-resistant depression. *Amino Acids.* 2016; 48(8):1941–54.

[268] Nemets B, Levine J. A pilot dose-finding clinical trial of creatine monohydrate augmentation to SSRIs/SNRIs/NASA antidepressant treatment in major depression. *Int Clin Psychopharmacol*. 2013; 28(3):127–33.

[269] Hellem TL, Sung Y-H, Shi X-F, Pett MA, Latendresse G, Morgan J, et al. Creatine as a Novel Treatment for Depression in Females Using Methamphetamine: A Pilot Study. *J Dual Diagn*. 2015; 11(3–4):189–202.

[270] Bachmann S, Degen C, Geider FJ, Schröder J. Neurological Soft Signs in the Clinical Course of Schizophrenia: Results of a Meta-Analysis. *Front Psychiatry*. 2014; 5:185.

[271] Green MF. Impact of Cognitive and Social Cognitive Impairment on Functional Outcomes in Patients with Schizophrenia. *J Clin Psychiatry*. 2016; 8–11.

[272] Miret S, Fatjó-Vilas M, Peralta V, Fañanás L. Síntomas básicos en la esquizofrenia, su estudio clínico y relevancia en investigación. *Rev Psiquiatr Salud Ment*. 2016; 9(2):111–22.

[273] Owen MJ, Sawa A, Mortensen PB. Schizophrenia. *The Lancet.* 2016; 388(10039):86–97.

[274] Kaptsan A, Odessky A, Osher Y, Levine J. Lack of efficacy of 5 grams daily of creatine in schizophrenia: a randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry*. 2007; 68(6):881–4.

[275] Levental U, Bersudsky Y, Dwalatzky T, Lerner V, Medina S, Levine J. A pilot open study of long term high dose creatine augmentation in patients with treatment resistant negative symptoms schizophrenia. *Isr J Psychiatry Relat Sci*. 2015; 52(1):6–10.

BIOGRAPHICAL SKETCH

Mauricio Peña Cunha

Affiliation: Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, Brazil

Education: *Dr. Cunha* was trained at the Universidade de Brasília (Brasília, Brazil) undertaking his B.Sc. in physical education (2001-2005) and subsequently conducted his master's (2007-2009) and Ph.D.'s (2009-2013) degree, as well as post-doctoral fellowship in Neuroscience (2013-2015) at the Universidade Federal de Santa Catarina (UFSC, Florianópolis, Brazil). Dr. Cunha also received research training as a visiting scholar research at the Universidad Nacional de Cordoba (Cordoba, Argentina, 2009) and Universidad Autonoma de Madrid (Madrid, Spain, 2010).

Business Address: Laboratory of Cell Defense, Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina (UFSC), Campus Universitário, Trindade, 88040-900, Florianópolis, SC, Brazil. Phone: +55 (48) 3721-2817. Email: mauricio.personal@gmail.com.

Research and Professional Experience:

Dr. Cunha's research primarily involves examining the involvement of creatine/phosphocreatine in health, exercise as well as in mental and neurological diseases. The focus of this research has been directed at neuroplasticity, oxidative stress, inflammation, bioenergetic, mood, cognition, pain, development, aging, senescence and mental and neurological diseases. Most recently, his specific interest has involved examining the role of myokine irisin as a driver of neuroprotective effect of exercise and/or creatine supplementation. In the past he has used a variety of tools such as animal behavior (emotional, cognitive, motor and synesthesia) analysis, tissue slices, culture of neuronal cells, cellular viability measurement, oxidative stress biomarkers, mitochondrial function measurement, metabolic enzymes activity, cell defenses enzymes activity, western, dot, and slot blotting, real-time PCR, high performance liquid chromatography, fluorescent, electronic and confocal microscopy, calcium analysis and nitric oxide measurement. Dr. Cunha is also chair of a variety of scientific advisory boards, on the editorial board for a number of journals, and has published numerous papers in the areas of behavior, Anxiety, Mood, Pain, Neurochemistry, Neurochemical, Neurotoxicity, Neurodegenerative diseases, Neuroprotection, Neuropharmacology, Neurobiology, and Oxidative stress.

Professional Appointments:

- Worked as a Contributory Lecturer in Biochemistry Department, Universidade Federal de Santa Catarina, Florianópolis, Brazil (2009-2017).
- Reviewed an expressive number of manuscripts in the prestigious scientific journals.
- Member of editorial board of EC Psychology and Psychiatry and EC Neurology Journals.
- Co-supervisor of Ph.D. students and supervisor of scientific initiation students

Honors:

- CAPES PRODOC Posdoctoral scholar award (2013)
- Juarez Aranha Ricardo award, Sociedade Brasileira de Neurociências (2013).
- CNPq PDJ Posdoctoral scholar award (2014)
- IBRO International Travel Grants Program, IBRO (2014)
- CAPES PNPD Posdoctoral scholar award (2015)
- Young Scientist Program Felowship Award, Sociedade Brasileira de Bioquímica e Biologia Molecular and International Union of Biochemistry and Molecular Biology (2015).

Publications from the Last 3 Years:

Research papers:

1. Manosso Lm, Moretti M, Rosa Jm, Cunha Mp, Rodrigues Al. Evidence for the involvement of heme oxygenase-1 in the antidepressant-like effect of zinc. Pharmacol Rep. 69(3):497-503, 2017.
2. Lieberknecht V, Cunha Mp, Junqueira Sc, Coelho Id, De Souza Lf, Dos Santos Ar, Rodrigues Al, Dutra Rc, Dafre Al. Antidepressant-like effect of pramipexole in an inflammatory model of depression. Behav Brain Res. 320:365-373, 2017.
3. Thomaz Dt, Dal-Cim Ta, Martins Wc, Cunha Mp, Lanznaster D, De Bem Af, Tasca Ci. Guanosine prevents nitroxidative stress and recovers mitochondrial membrane potential disruption in hippocampal slices subjected to oxygen/glucose deprivation. Purinergic Signal. 12(4):707-718, 2016.
4. Cunha, A. S.; Matheus, F. C.; Moretti, M.; Sampaio, T. B.; Poli, A.; Santos, D. B.; Colle, D.; Cunha, M. P.; Blum-Silva, C. H.; Sandjo, L. P.; Reginatto, F. H.; Rodrigues, A. L. S.; Farina, M.; Prediger, R. D. S. Agmatine attenuates reserpine-induced oral dyskinesia in mice: Role of oxidative stress, nitric oxide and glutamate NMDA receptors. Behavioural Brain Research., v.312, p.64 - 76, 2016.

5. Cunha, M. P.; Lieberknecht, Vicente; Ramos-Hryb, Ana Belém; Olescowicz, Gislaine; Ludka, Fabiana K.; Tasca, Carla I.; GABILAN, NELSON H.; Rodrigues, Ana Lúcia S.
Creatine affords protection against glutamate-induced nitrosative and oxidative stress. Neurochemistry International., v.95, p.4 - 14, 2016.
6. Cunha, M. P.; Budni, Josiane; Ludka, Fabiana K.; Pazini, Francis L.; Rosa, Julia Macedo; Oliveira, Ágatha; Lopes, Mark W.; Tasca, Carla I.; Leal, Rodrigo B.; Rodrigues, Ana Lúcia S.
Involvement of PI3K/Akt Signaling Pathway and Its Downstream Intracellular Targets in the Antidepressant-Like Effect of Creatine. Molecular Neurobiology., v.53, p.2954 - 2968, 2016.
7. Colla, A.; Rosa, J. M.; Cunha, M. P.; Rodrigues, A. L. S.
Anxiolytic-like effects of ursolic acid in mice. European Journal of Pharmacology., v.758, p.171 - 176, 2015.
8. Cunha, M. P.; Pazini, F. L.; Rosa, J. M.; Ramos-Hryb, A. B.; Oliveira, A.; Kaster, M. P.; Rodrigues, A. L. S.
Creatine, similarly to ketamine, affords antidepressant-like effects in the tail suspension test via adenosine A1 and A2A receptor activation. Purinergic Signalling., v.11, p.215 - 227, 2015.
9. Moretti, M.; Neis, V.B.; Matheus, F. C.; Cunha, M. P.; Rosa, P. B.; Ribeiro, C.M.; Rodrigues, A. L. S.; Prediger, R. D. S.
Effects of Agmatine on Depressive-Like Behavior Induced by Intracerebroventricular Administration of 1-Methyl-4-phenylpyridinium (MPP+). Neurotoxicity Research., v.28, p.222 - 231, 2015.
10. Kaster, Manuella P.; Moretti, Morgana; Cunha, M. P.; Rodrigues, Ana Lúcia S. Novel approaches for the management of depressive disorders. European Journal of Pharmacology., v.771, p.236 - 240, 2015.
11. Cunha, M. P.; Pazini, F. L.; Ludka, F. K.; Rosa, J. M.; Oliveira, A.; Budni, J.; Ramos-Hryb, A. B.; Lieberknecht, V.; Bettio, L. E.; Martin-De-Saavedra, M. D.; Lopez, M. G.; Tasca, C. I.; Rodrigues, A. L. S.
The modulation of NMDA receptors and l-arginine/nitric oxide pathway is implicated in the anti-immobility effect of creatine in the tail suspension test. Amino Acids (Wien. Print)., v.47, p.795 - 811, 2015.

12. Ludka, F. K.; Constantino, L. C.; Kuminek, G.; Binder, L.; Zomkowski, A. D. E.; Cunha, M. P.; Dal-Cim, T.; Rodrigues, A. L. S.; Tasca, C. I.
Atorvastatin evokes a serotonergic system-dependent antidepressant-like effect in mice. Pharmacology, Biochemistry and Behavior., v.122, p.253 - 260, 2014.
13. Cunha, M. P.; Saavedra, M. D. M.; Romero, A.; Egea, J.; Ludka, F. K.; Tasca, C. I.; Farina, M.; Rodrigues, A. L. S.; Lopez, M. G.
Both Creatine and Its Product Phosphocreatine Reduce Oxidative Stress and Afford Neuroprotection in an In Vitro Parkinson's Model. ASN NEURO., v.6, p.1 - 16, 2014.
14. Cunha, M. P.; Budni, J.; Pazini, F. L.; Oliveira, A.; Rosa, J. M.; Lopes, M. W.; Leal, R. B.; Rodrigues, A. L. S.
Involvement of PKA, PKC, CAMK-II and MEK1/2 in the acute antidepressant-like effect of creatine in mice. Pharmacological Reports., v.66, p.653 - 659, 2014.
15. Colla, A. R.; Oliveira, A.; Pazini, F. L.; Rosa, J. M.; Manosso, L. M.; Cunha, M. P.; Rodrigues, A. L. S.
Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. Pharmacology, Biochemistry and Behavior., v.124, p.108 - 116, 2014.
16. Ludka, Fabiana K.; Zomkowski, Andréa D.E.; CUNHA, M. P.; Dal-Cim, Tharine; Zeni, Ana Lúcia B.; Rodrigues, Ana Lúcia S.; Tasca, Carla I.
Acute atorvastatin treatment exerts antidepressant-like effect in mice via the l-arginine¿nitric oxide/cyclic guanosine monophosphate pathway and increases BDNF levels. European Neuropsychopharmacology., v.23, p.400 - 412, 2013.
17. Machado, Daniele G.; Cunha, M. P.; Neis, Vivian B.; Balen, Grasiela O.; Colla, André; Bettio, Luis E.B.; Oliveira, Ágatha; Pazini, Francis Leonardo; Dalmarco, Juliana B.; Simionatto, Edésio Luiz; Pizollatti Mg; Pizzolatti, Moacir G.; Rodrigues, Ana Lúcia S.
Antidepressant-like effects of fractions, essential oil, carnosol and betulinic acid isolated from Rosmarinus officinalis L., Food Chemistry., v.136, p.999 - 1005, 2013.

18. Cunha, M. P.; Pazini, F. L.; Oliveira, A.; Machado, D. G.; Rodrigues, A. L. S.
Evidence for the involvement of 5-HT1A receptor in the acute antidepressant-like effect of creatine in mice. Brain Research Bulletin., v.95, p.61 - 69, 2013.
19. Saavedra, M. D. M.; Budni, J.; Cunha, M. P.; Gomez-Rangel, V.; Lorrio, S.; Barrio, L.; Lastres-Becker, I.; Parada, E.; Rodrigues, A. L. S.; Lopez, M. G.
Nrf2 participates in depressive disorders through an anti-inflammatory mechanism. Psychoneuroendocrinology., v.38, p.2010 - 2022, 2013.
20. Cunha, M. P.; Saavedra, M. D. M.; Romero, A.; Parada, E.; Egea, J.; Barrio, L.; Rodrigues, A. L. S.; Lopez, M. G.
Protective Effect of Creatine against 6-Hydroxydopamine-Induced Cell Death In Human Neuroblastoma Sh-Sy5y Cells: Involvement of Intracellular Signaling Pathways. Neuroscience., v.238, p.185 - 194, 2013.
21. Cunha, M. P.; Pazini, F. L.; Oliveira, A.; Bettio, L. E.; Rosa, J. M.; Machado, D. G.; Rodrigues, A. L. S.
The activation of α1-adrenoceptors is implicated in the antidepressant-like effect of creatine in the tail suspension test. Progress in Neuro-Psychopharmacology & Biological Psychiatry., v.44, p.39 - 50, 2013.
22. Kaster, M. P.; Budni, J.; Gazal, M.; Cunha, M. P.; Santos, A. R. S. Ou Santos, A.R.; Rodrigues, A. L. S.
The antidepressant-like effect of inosine in the FST is associated with both adenosine A1 and A2A receptors. Purinergic Signalling (Print)., v.9, p.481 - 486, 2013.
23. Cunha, M. P.; Oliveira, A.; Pazini, F. L.; Machado, D. G.; Budni, J.; Bettio, L. E.; Aguiar-Jr, A.S.; Martins, D.F.; Santos, A.R.S.; Rodrigues, A. L. S. The Antidepressant-like Effect of Physical Activity in the Voluntary Running Wheel. Medicine and Science in Sports and Exercise., v.45, p.851 - 859, 2013.

Manuscripts accepted for publication:

1. Ludka, F. K.; Cunha, M. P.; Dal-Cim, T.; Binder, L.; Constantino, L. C.; Massari, C. M.; Martins, W. C.; Rodrigues, A. L. S.; Tasca, C. I. Atorvastatin Protects from Aβ1-40-induced Cell Damage and Depressive-like Behavior via ProBDNF Cleavage. Molecular Neurobiology, 2017.
2. Cunha, M. P.; Pazini, F. L.; Lieberknecht, V.; Budni, J.; Oliveira, A.; Rosa, J. M.; Mancini, G.; Mazzardo, L.; Colla, A. R.; Leite, M. C.; Santos, A. R. S. Ou Santos, A.R.; Martins, D.F.; Bem, A. F.; Goncalves, C. A.; Farina, M.; Rodrigues, A. L. S. MPP+-lesioned mice: an experimental model of mo tor, emotional, memory/learning and striatal neurochemical dysfunctions. Molecular Neurobiology, 2017.
3. Pazini, F. L.; Cunha, M. P.; Padilha, D. A.; Rosa, J. M.; Colla, A. R.; Oliveira, J.; Ramos-Hryb, A. B.; Brocardo, P. S.; Gil-Mohapel, J.; Rodrigues, A. L. S. Creatine Prevents Corticosterone-Induced Reduction in Hippocampal Proliferation and Differentiation: Possible Implication for its Antidepressant Effect. Molecular Neurobiology, 2017.
4. Junqueira, S. C.; Coelho, I. S.; Lieberknecht, V.; Cunha, M. P.; Calixto, J. B.; Rodrigues, A. L. S.; Santos, A. R. S. Ou Santos, A.R.; Dutra, R. C. Inosine, an Endogenous Purine Nucleoside, Suppresses Immune Responses and Protects Mice from Experimental Autoimmune Encephalomyelitis: a Role for A2A Adenosine Receptor. Molecular Neurobiology, 2016.