Creatine, central nervous system and creatine deficiency syndromes

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Abstract: It was long thought that most of brain creatine was of peripheral origin. However, recent works have demonstrated that creatine crosses blood-brain barrier only with poor efficiency, and that CNS must ensure parts of its creatine needs by its own creatine synthesis pathway, thank to the brain expression of AGAT and GAMT (creatine synthesis) and SLC6A8 (creatine transporter). This new understanding of creatine metabolism and transport in CNS allows a better comprehension of creatine deficiency syndromes, which are due to deficiencies in AGAT, GAMT and SLC6A8 and mainly affect the brain of patients who show severe neurodevelopmental delay and present neurological symptoms in early infancy.

Keywords: Creatine, brain, AGAT, GAMT, SLC6A8, guanidinoacetate, creatine deficiency syndromes.

1 Introduction

The creatine (Cr) / phosphocreatine (PCr) / creatine kinase (CK) system plays essential roles to maintain the high energy levels necessary for brain development and functions, through regeneration and buffering of ATP levels [21,72]. The Cr/PCr/CK system also allows the shuttle of high-energy phosphates from mitochondria to their cytoplasmic sites of utilization [70]. Cr was also suggested recently as true neurotransmitter and one of the main central nervous system (CNS) osmolytes [3,9]. In mammals, pools of Cr are maintained through uptake from diet and endogenous synthesis. Cr biosynthesis involves L-arginine:glycine amidinotransferase (AGAT) yielding guanidinoacetate (GAA) from arginine and glycine, and guanidinoacetate methyl-transferase (GAMT), yielding Cr from GAA. Cr is distributed by blood to tissues, where cells take it up by a specific transporter, SLC6A8, also called CRT1, CT1, CreaT or CRT [72].

It has long been thought that most, if not all, cerebral Cr was of peripheral origin [72]. However, AGAT and GAMT are expressed in CNS and brain cells synthesize their own Cr [11,20]. In contrast, while SLC6A8 is expressed by microcapillary endothelial cells (MCEC) at blood-brain barrier (BBB), allowing CNS to import Cr from periphery, it is absent from astrocytes and particularly from their feet lining MCEC [11,47,65]. This suggested that BBB has a limited permeability for peripheral Cr, and that CNS must supply an important part of its Cr needs by endogenous synthesis rather than on exclusive supply from the blood [6,11,18]. Recent data also suggest that in the brain, the Cr synthesis pathway may be dissociated as it is in periphery, the intermediate GAA being transported through SLC6A8 from AGAT- to GAMT-expressing cells for Cr synthesis to occur in CNS [15].

Cr deficiency syndromes are caused by mutations in AGAT, GAMT and SLC6A8 genes [38,53,62]. Their common phenotype is an almost complete lack of Cr in CNS, which appears as the main organ affected in these primary Cr deficiencies. Patients develop severe neurodevelopmental delay and present neurological symptoms in early infancy, like mental retardation, delays in speech acquisition or epilepsy [63]. Oral Cr supplementation strongly improves the neurological status of AGAT- and GAMT-deficient patients [5,55,58,61], while this treatment is inefficient on SLC6A8-deficient patients [4,7,51]. Secondary Cr deficiencies are also observed in other CNS pathological states, like stroke, hyperammonemic states or gyrate atrophy of the choroid and retina (GA) [6].

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This review is focused on the latest data on Cr synthesis and transport in CNS, in order to delineate a comprehensive frame on Cr metabolism and transport in the brain, both in normal and in the Cr-deficient conditions characteristic of Cr deficiency syndromes.

2 Creatine in the brain

2.1 Functions of creatine in CNS

The Cr/PCr/CK system plays essential roles to maintain the high energy phosphates levels necessary for CNS (maintenance of membrane potential and ions gradients, Ca^{++} homeostasis, neurotransmission, intracellular signaling systems as well as axonal and dendritic transport, axonal and dendritic growth) [6]. Apart of its main functions in energy, Cr was recently suggested as neurotransmitter or neuromodulator. Indeed, neurons can release Cr in an action potential-dependent manner [3], and a mechanism of Cr recapture from the synaptic cleft may exist through SLC6A8 [48]. Cr was also suggested as one of the essential CNS osmolytes [2,8], and as one potential appetite and weight regulator through action on specific hypothalamic nuclei [32].

2.2 AGAT, GAMT and SLC6A8 in CNS

It has long been thought that most of brain Cr was of peripheral origin, be it taken from the diet or synthesized endogenously through AGAT and GAMT activities in kidney and liver respectively [21,72]. However, Cr is synthesized in the mammalian brain [50,69], in nerve cell lines as well as in primary and organotypic brain cell cultures [20,27,30]. AGAT and GAMT are expressed in all the main structures of the brain, in every main cell types (neurons, astrocytes and oligodendrocytes; [11,54,65]). Moreover, we have shown that in most region of the rat CNS, AGAT and GAMT rarely appear co-expressed within the same cell [15]. Organotypic rat cortical cultures, primary brain cell cultures (neuronal, glial or mixed) and neuroblastoma cell lines have a Cr transporter activity [3,16,44]. *In vivo*, mouse and rat CNS can take up Cr from the blood against its concentration gradient [47,49]. SLC6A8 is expressed throughout the main regions of the adult mammalian brain [11,42,65]. It was demonstrated that SLC6A8 is found in neurons and oligodendrocytes but, in contrast to AGAT and GAMT, cannot be detected in astrocytes [11], except for very rare ones in cerebellum [42]. In contrast to its absence in astrocytes lining microcapillaries, SLC6A8 is present in MCEC making BBB [11,47,65].

2.3 Brain creatine: endogenous synthesis or uptake from periphery?

The discovery that SLC6A8 cannot be detected in astrocytes, particularly in their feet sheathing MCEC, made us suggest that in mature CNS, BBB has a limited permeability for Cr, despite SLC6A8 expression by MCEC and their capacity to import Cr [1,11,45,47,65]. *In vivo* data confirmed this hypothesis: the blood to brain transport of Cr through BBB is effective in rats and mice but is relatively inefficient [47,49], and long term treatment of AGAT- and GAMT-deficient patients with high doses of Cr allows only a slow and in most cases partial replenishment of their brain Cr pools (see below) [55,63]. One strong argument in favor of the "brain endogenous Cr synthesis" hypothesis comes from Cr measures in the cerebrospinal fluid (CSF) of Cr-deficient patients (see below) [18]. SLC6A8 deficient patients present normal Cr levels in CSF, but are unable to import Cr from periphery [7,23,29,51]. In contrast, GAMT-deficient patients show strongly decreased levels of Cr in CSF but are able to import Cr from the blood [57,62]. This also suggests that Cr synthesis in the brain might still remain operational, although very partially, under SLC6A8 deficiency, while it is completely blocked in AGAT and GAMT deficiencies. Endogenous synthesis, or a very efficient uptake from periphery, are the two ways available for the brain to secure Cr homeostasis for its energy and functions. As uptake from periphery does not appear efficient, CNS might privilege Cr endogenous synthesis. The brain capacity for Cr synthesis would thus depend on the efficient supply of arginine, the limiting substrate for Cr synthesis, from blood to CNS, and then also on local trafficking of arginine between brain cells. We and others have shown that cationic amino acid transporters (CATs) CAT1, CAT2(B) and CAT3 might fulfill these roles in the brain [10,17,35].

The hypothesis of endogenous Cr synthesis in the brain might seem contradictory with the *in vivo* characteristics of SLC6A8 deficiency (see below), which, despite AGAT and GAMT expression in CNS, presents an absence (or a very low level) of brain Cr by magnetic resonance spectroscopy (MRS) [53]. This

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apparent contradiction is probably explained by our recent data on AGAT, GAMT and SLC6A8 expression patterns in the brain. AGAT and GAMT are found in every CNS cell type [11], but appear rarely co-expressed within the same cell [15]. This suggests that to allow Cr synthesis in the brain, GAA must be transported from AGAT- to GAMT-expressing cells. This GAA transfer most probably occurs through SLC6A8, as shown in the same study by Cr and GAA competition studies and the use of stable isotope-labeled GAA, demonstrating its uptake by brain cells followed by its conversion to Cr by GAMT activity [15]. These observations may explain the Cr absence in CNS of SLC6A8-deficient patient, despite normal expression of AGAT and GAMT in their brain [6,18]. Recent studies also demonstrate the potential role of SLC6A8 for GAA transport across BBB and in brain parenchymal cells [64,66].

2.4 Cr in developmental versus adult CNS

As described above, the adult (or mature) brain might privilege Cr endogenous synthesis *versus* uptake from periphery, due to low permeability of BBB for Cr and thank to the expression of AGAT and GAMT in CNS parenchyma. Fetal and perinatal (or immature) CNS probably behaves differently for its Cr needs. Fetal needs in Cr are partly supported by active transport of Cr from mother and embryo [28,36]. AGAT, GAMT and SLC6A8 are also well expressed during vertebrate embryogenesis, including in the brain [19,37,54]. We have shown that AGAT and GAMT are expressed in the whole developing CNS parenchyma [19]. However, their low level (GAMT in particular) at early developmental stages suggests that in contrast to adult brain, embryonic CNS depends predominantly on external Cr supply, be it from embryonic periphery or from maternal origin. This is coherent with SLC6A8 expression in the whole embryonic CNS already at early stages (E12.5 in rat), with particularly high levels in periventricular zone and choroid plexus, the predominant metabolic exchange zones of fetal CNS before microcapillary angiogenesis and differentiation of BBB [14,19].

3 Creatine deficiency syndromes

CNS is the main organ affected in patients suffering from Cr deficiency syndromes, inborn errors of Cr biosynthesis and transport caused by AGAT, GAMT or SLC6A8 deficiency which are characterized by an absence or a severe decrease of Cr in CNS as measured by MRS [38,53,62]. As the prevalence of SLC6A8 deficiency was estimated at 2% of all X-linked mental retardations [52] and at 1% of males with mental retardation of unknown etiology [26], while all combined Cr deficiencies were estimated between 0.3% and 2.7% of all mental retardation [4,41], Cr deficiency syndromes appear as some of the most frequent inborn errors of metabolism (IEM).

Cr-deficient patients present neurological symptoms in infancy, such as mental retardation and delays in speech acquisition; GAMT deficiency exhibits a more complex phenotype, including intractable epilepsy, extrapyramidal movement syndromes and abnormalities in basal ganglia [63]. The diverse phenotypic spectrum of neurological symptoms observed in Cr deficiency syndromes demonstrate the importance of Cr for psychomotor development and cognitive functions. The more complex phenotype of GAMT deficiency is probably due to the toxicity of brain GAA accumulation [56], which may occur through activation of $GABA_A$ receptors by GAA [46] or inhibition of the complex between Na^+/K^+-ATPase and CK [73]. Severe epilepsy is also observed sometimes in SLC6A8-deficient patients [43]. This may be due to the observed CNS GAA accumulation in some SLC6A8-deficient patients [59], that could be caused by impairment of GAA transport through deficient SLC6A8, from AGAT- to GAMT-expressing brain cells (see below) [15].

AGAT- and GAMT-deficient patients can be treated by oral supplementation of Cr. While this strongly improves their neurological status and CNS development, very high doses of Cr must be used, and replenishment of cerebral Cr takes months and only results, in most cases, in partial restoration of cerebral Cr pools [5,33,38,61]. The pre-symptomatic treatment of AGAT- and GAMT-deficient patients appears to improve even more their clinical outcome [55]. For GAMT-deficient patients, combined arginine restriction and ornithine substitution coupled to Cr treatment decrease GAA and also improve clinical outcome [56,58]. However, despite improvement of clinical outcome by Cr supplementation, most AGAT- and GAMT-deficient patients remain with CNS developmental problems. Oral supplementation of Cr is inefficient in replenishing brain Cr in SLC6A8-deficient patients, who remain with

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mental retardation, severe speech impairment and progressive brain atrophy [7,23,29,51]. Attempts to treat SLC6A8-deficient patients with arginine and glycine as precursors of Cr gave encouraging results in two SLC6A8-deficient patients [25,71], while it failed to improve the neurological status of four others [31].

4 Secondary creatine deficiencies in CNS

Several other CNS pathologies can lead to a secondary Cr deficiency in brain cells. Excess of ammonium (NH_4^+) in CNS, as seen in pediatric patients under various acquired or inherited disorders like urea cycle diseases, can cause irreversible damages to the developing brain [13,22,40]. NH_4^+ exposure generates a secondary Cr deficiency in brain cells [16,20], eventually leading to energy deficit, oxidative stress and cell death [12,13]. Ischemic stroke in CNS leads to a rapid diminution in brain total Cr (Cr + PCr), causing a decrease in high energy phosphates production which leads to a failure in most energy-dependent processes necessary for cell survival [39]. Gyrate atrophy of the choroid and retina, an IEM caused by mutations in ornithine δ-aminotransferase (OAT) [68], generates a secondary Cr deficiency [60] which in CNS may contribute to GA neurological symptoms [67].

5 Model for creatine synthesis and trafficking in CNS

Altogether, (i) the absence of Cr within the brain of Cr-deficient patients, (ii) the CNS expression patterns of AGAT, GAMT and SLC6A8, (iii) the low permeability of BBB for Cr, and (iv) the brain levels of Cr and GAA both in normal and Cr-deficient conditions, lead us to propose the following concept for Cr synthesis and trafficking within CNS [6]. In normal conditions, SLC6A8 is expressed by MCEC, but not by the surrounding astrocytic feet, implying that limited amounts of Cr enter the brain through BBB. In most brain regions, brain cells express AGAT and GAMT in a cell-dissociated way, and GAA must be transported from AGAT- to GAMT-expressing cells by SLC6A8 for Cr synthesis to occur. In AGAT and GAMT deficiency, no Cr can be synthesized within CNS, but SLC6A8 expression in MCEC allows the limited entry of Cr within the brain, and thus their treatment by oral Cr and the partial replenishment of the brain Cr pools. The GAMT-deficient brain accumulates GAA. Cr transporter-deficient patients lack functional SLC6A8 on MCEC, and thus cannot be treated by oral Cr. Their endogenous CNS Cr synthesis pathway is also deficient, as in most brain regions, GAA cannot cross from AGAT- to GAMT-expressing cells due to their lack in functional SLC6A8.

6 Creatine as therapeutic potential for brain diseases

Troubles in CNS energy metabolism due to mitochondrial dysfunction, either from oxidative stress, mitochondrial DNA deletions, pathological mutations or altered mitochondria morphology, play critical roles in the progression of neurological diseases as a primary or secondary mechanism in neuronal death cascade [24]. Cr is known to play essential roles in stabilizing mitochondrial function and in decreasing neuronal cell death, and Cr supplementation was shown to improve the bioenergetic deficit associated with several brain pathologies, including Huntington's, Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis, stroke and hyperammonemia [6,34].

7 Conclusions

Cr plays its main role in energy metabolism, allowing ATP regeneration through CK enzymatic activity. In recent years, new roles of Cr have been suggested in CNS, like a function of neuromodulator or even true neurotransmitter. The recent years have brought new knowledge on Cr metabolism and transport in the brain, allowing a better understanding on the pathophysiology of Cr deficiency syndromes in brain cells [6]. In particular, there is evidence that BBB presents a low permeability for Cr, and that CNS must ensure parts of its needs in Cr by endogenous synthesis. Moreover, in many regions of the brain, Cr endogenous synthesis appears to be dissociated, GAA needing to be transported by SLC6A8 from AGAT- to GAMT-expressing cells for Cr synthesis to occur [15,18].

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