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REVIEW ARTICLE

Creatine deficiency syndromes and the importance of creatine synthesis in the brain

Olivier Braissant · Hugues Henry · Elidie Béard · Joséphine Uldry

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Abstract Creatine deficiency syndromes, due to deficiencies in AGAT, GAMT (creatine synthesis pathway) or SLC6A8 (creatine transporter), lead to complete absence or very strong decrease of creatine in CNS as measured by magnetic resonance spectroscopy. Brain is the main organ affected in creatine-deficient patients, who show severe neurodevelopmental delay and present neurological symptoms in early infancy. AGAT- and GAMT-deficient patients can be treated by oral creatine supplementation which improves their neurological status, while this treatment is inefficient on SLC6A8-deficient patients. While it has long been thought that most, if not all, of brain creatine was of peripheral origin, the past years have brought evidence that creatine can cross blood–brain barrier, however, only with poor efficiency, and that CNS must ensure parts of its creatine needs by its own endogenous synthesis. Moreover, we showed very recently that in many brain structures, including cortex and basal ganglia, AGAT and GAMT, while found in every brain cell types, are not co-expressed but are rather expressed in a dissociated way. This suggests that to allow creatine synthesis in these structures, guanidinoacetate must be transported from AGAT- to GAMT-expressing cells, most probably through SLC6A8. This new understanding of creatine metabolism and transport in CNS will not only allow a better comprehension of brain consequences of creatine deficiency syndromes, but will also contribute to better decipher creatine roles in CNS, not only in energy as ATP regeneration and buffering, but also in its recently suggested functions as neurotransmitter or osmolyte.

Keywords Creatine deficiency syndromes · Creatine · Guanidinoacetate · Brain · AGAT · GAMT · SLC6A8

O. Braissant (✉) · H. Henry · E. Béard · J. Uldry
Inborn Errors of Metabolism, Clinical Chemistry Laboratory, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Avenue Pierre-Decker 2, CI 02/33, 1011 Lausanne, Switzerland
e-mail: Olivier.Braissant@chuv.ch

Introduction

The creatine (Cr)/phosphocreatine (PCr)/creatine kinase (CK) system plays essential roles in maintaining the high energy levels necessary for brain development and functions, through regeneration and buffering of ATP levels (Wallimann et al. 1992, 2007; Wyss and Kaddurah-Daouk 2000; Brosnan and Brosnan 2007; Andres et al. 2008). Recent works suggest that creatine in CNS may also act as true neurotransmitter and one of the main CNS osmolytes (Bothwell et al. 2002; Almeida et al. 2006). In mammals, half of Cr is obtained from diet, the other half being synthesized endogenously by a two-step mechanism involving L-arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT). Cr is distributed by blood to tissues and taken up by cells through a specific Cr transporter, SLC6A8, also abbreviated CT1, CRTR, CTR or CreaT (Wyss and Kaddurah-Daouk 2000).

Cr deficiency syndromes are caused by mutations in AGAT, GAMT and SLC6A8 genes (Stöckler et al. 1994; Salomons et al. 2001; Item et al. 2001). Their common phenotype is an almost complete lack of Cr in CNS, as measured by magnetic resonance spectroscopy (MRS), CNS appearing as the main organ affected in these primary Cr deficiencies. Patients develop severe neurodevelopmental delay and present neurological symptoms in early infancy, such as mental retardation, delays in speech acquisition or epilepsy (Stöckler et al. 2007). Oral Cr

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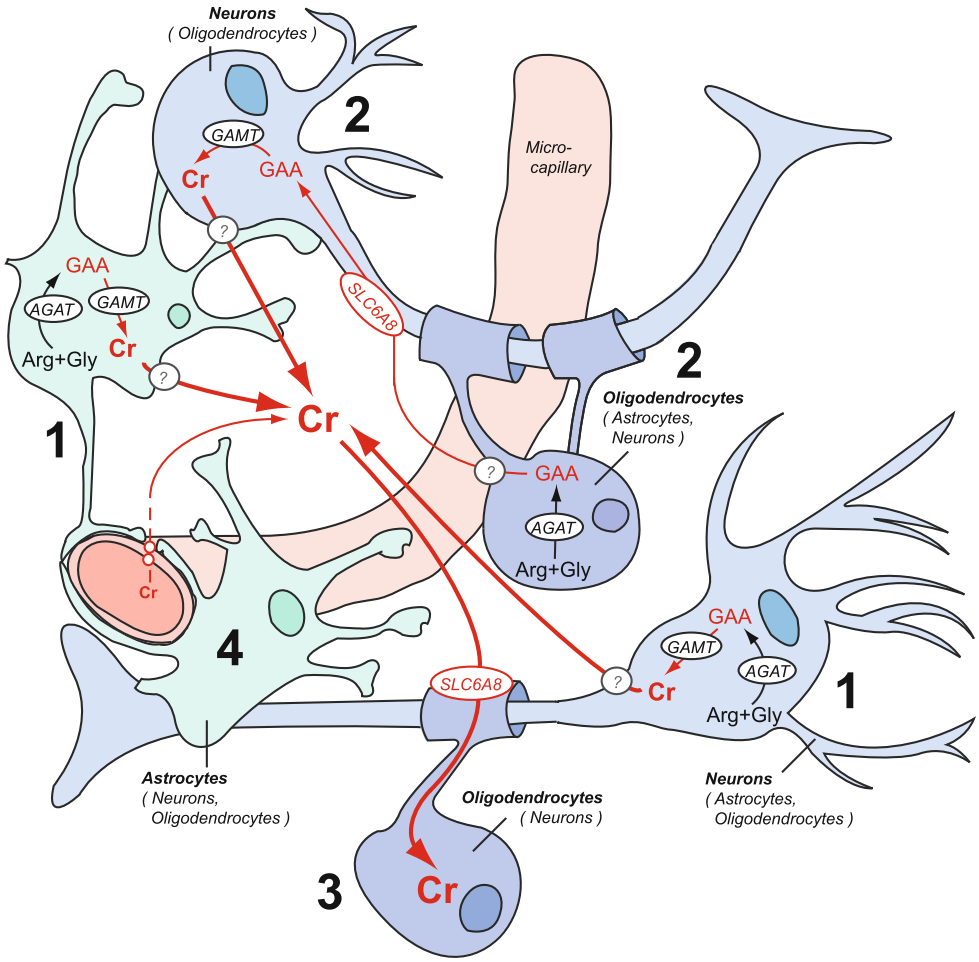

Fig. 1 Creatine synthesis and transport in central nervous system: model illustrating endogenous synthesis versus uptake from periphery, including the various combinations of AGAT, GAMT and SLC6A8 expression within brain cells (Braissant et al. 2010). (*1*) Cr endogenous synthesis within cells co-expressing AGAT and GAMT. (*2*) Cr endogenous synthesis through AGAT-expressing cells synthesizing GAA, and GAA uptake by SLC6A8 in GAMT-expressing cells; this conformation appears the prevalent one in most brain regions. (*3*) Cells expressing only SLC6A8 ("users" of Cr). (*4*) Cells silent for AGAT, GAMT and SLC6A8. While microcapillaries express SLC6A8, astrocytic feet lining them do not. This implies that only low amounts of peripheral Cr can enter the brain through the limited endothelial surface that is free of astrocytic feet and that CNS must also ensure its own endogenous synthesis of Cr. So far, the way Cr (and GAA) can leave cells is poorly known. Cr: creatine; AGAT: L-arginine:glycine amidinotransferase; GAMT: guanidinoacetate methyltransferase; GAA: guanidinoacetate; SLC6A8: Cr transporter

supplementation strongly improves the neurological status of AGAT- and GAMT-deficient patients (Stöckler et al. 1996; Schulze et al. 1998; Battini et al. 2002; Schulze and Battini 2007), while this treatment is inefficient on SLC6A8-deficient patients (Bizzi et al. 2002; Póo-Argüelles et al. 2006; Arias et al. 2007). Secondary Cr deficiencies are also observed in other pathological states of the brain, like stroke, hyperammonemic states or gyrate atrophy of the choroid and retina (GA) (Valle et al. 1981; Braissant et al. 2008; Lei et al. 2009).

It has long been thought that most, if not all, cerebral Cr was of peripheral origin (Wyss and Kaddurah-Daouk 2000). However, AGAT and GAMT are expressed in CNS and brain cells synthesize their own Cr (Braissant et al. 2001b; Braissant et al. 2002). 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, at least in physiological conditions, and particularly from their feet lining MCEC (Fig. 1; Braissant et al. 2001b; Ohtsuki et al. 2002; Tachikawa et al. 2004). This made us suggest 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 an exclusive supply from the blood (Braissant et al. 2001b; Braissant and Henry 2008). Similar to periphery with predominant expression of AGAT in kidney and GAMT in liver (Edison et al. 2007; da Silva et al. 2009), recent data also suggest that the Cr synthesis pathway may be dissociated in CNS, guanidinoacetate (GAA) being transported through SLC6A8 from

AGAT- to GAMT-expressing cells to allow synthesis of Cr in the brain (Braissant et al. 2010).

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

Creatine deficiency syndromes

CNS is the main organ affected in patients suffering from the 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 (Stöckler et al. 1994; Salomons et al. 2001; Item et al. 2001). AGAT and GAMT deficiencies are autosomal recessive diseases, while SLC6A8 deficiency is an X-linked disorder. Cr deficiency syndromes appear among the most frequent inborn errors of metabolism (IEM), the prevalence of SLC6A8 deficiency being estimated at 2% of all X-linked mental retardations (Rosenberg et al. 2004) and at 1% of males with mental retardation of unknown etiology (Clark et al. 2006). AGAT and GAMT deficiencies are rarer, but the prevalence of all combined Cr deficiencies was estimated between 0.3% and 2.7% of all mental retardation (Lion-François et al. 2006; Arias et al. 2007).

Cr-deficient patients present neurological symptoms in infancy (Schulze et al. 1997; Battini et al. 2002; DeGrauw et al. 2002). In particular, mental retardation and delays in speech acquisition can be observed (AGAT, GAMT and SLC6A8 deficiencies), as well as intractable epilepsy (GAMT and SLC6A8 deficiencies), autism, automutilating behavior, extrapyramidal syndrome and hypotonia (GAMT deficiency) (Stöckler et al. 2007). The diverse phenotypic spectrum of neurological symptoms observed in AGAT-, GAMT- and SLC6A8-deficient patients shows the importance of Cr for psychomotor development and cognitive functions and is probably explained by the wide pattern of AGAT, GAMT and SLC6A8 expression in the mammalian brain (see below). The recently proposed roles of Cr as co-transmitter on GABA postsynaptic receptors (Almeida et al. 2006) and of regulator of appetite and weight on specific hypothalamic nuclei (Galbraith et al. 2006) might also contribute to this phenotypic diversity. The more complex phenotype of GAMT deficiency, including intractable epilepsy, extrapyramidal movement syndromes and abnormalities in basal ganglia is probably due to the toxicity, and particularly the epileptogenic action, of brain GAA accumulation characteristic of GAMT deficiency (Schulze et al. 2001), which may occur through activation of $GABA_A$ receptors by GAA (Neu et al. 2002). GAA may also inhibit the complex between Na^+/K^+-ATPase and CK (Zugno et al. 2006). Severe epilepsy may also appear in SLC6A8-deficient patients (Mancardi et al. 2007). This may be due to the observed CNS GAA accumulation in some SLC6A8-deficient patients (Sijens et al. 2005), which could be caused by impairment of GAA transport through deficient SLC6A8, from AGAT- to GAMT-expressing brain cells (see below) (Braissant et al. 2010).

Treatments and outcome of Cr deficiency syndromes

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 (Stöckler et al. 1996; Ganesan et al. 1997; Item et al. 2001; Battini et al. 2002). For GAMT-deficient patients, combined arginine restriction and ornithine substitution coupled with Cr treatment decreases GAA and also improves clinical outcome (Schulze et al. 1998, 2001). Despite improvement of clinical outcome by Cr supplementation, many AGAT- and GAMT-deficient patients remain with CNS developmental problems. However, the pre-symptomatic treatment of AGAT- and GAMT-deficient patients appears to prevent, so far, most of the Cr-deficiency effects on their brain (Battini et al. 2006; Schulze et al. 2006; Schulze and Battini 2007). Oral supplementation of Cr is inefficient in replenishing brain Cr in SLC6A8-deficient patients, who remain with mental retardation, severe speech impairment and progressive brain atrophy (Cecil et al. 2001; Bizzi et al. 2002; DeGrauw et al. 2002; Póo-Argüelles et al. 2006). Attempts to treat SLC6A8-deficient patients with arginine as precursor of Cr also failed to improve their neurological status (Fons et al. 2008). Similarly, the use of a lipophilic Cr-derived compound, creatine ethyl ester, failed to replenish brain Cr concentration in SLC6A8-deficient patients, as well as to improve their neurological status (Fons et al. 2010).

Secondary creatine deficiencies in CNS

Several other CNS pathologies that cause a secondary Cr deficiency in brain cells have been identified. Excess of ammonium (NH_4^+) is toxic for CNS. In pediatric patients, hyperammonemia can be caused by various acquired or inherited disorders, the most frequent being urea cycle diseases, which can cause irreversible damages to the developing brain (Leonard and Morris 2002) by altering

several amino acid pathways and neurotransmitter systems, nitric oxide synthesis, axonal and dendritic growth and signal transduction pathways (Cagnon and Braissant 2007, 2008, 2009) eventually leading to energy deficit, oxidative stress and cell death (Braissant 2010b). In particular, NH_4^+ exposure generates a secondary Cr deficiency in brain cells (Ratnakumari et al. 1996; Choi and Yoo 2001; Braissant et al. 2002). NH_4^+ appears to inhibit AGAT enzymatic activity and differentially alters AGAT, GAMT and SLC6A8 gene expression in a cell type-specific manner, which may alter the energy requirements of brain cells (Braissant et al. 2008; Braissant 2010a). Ischemic stroke in CNS leads to a rapid diminution in brain total Cr (Cr + PCr) (Obrenovitch et al. 1988; Gideon et al. 1992; Mathews et al. 1995; Lei et al. 2009). This lower Cr level in stroke causes a decrease in high-energy phosphate production and leads to a failure in most energy-dependent processes necessary for cell survival. Gyrate atrophy of the choroid and retina (GA) is an inborn error of metabolism leading to blindness in the first 10 years of life and is caused by mutations in ornithine δ-aminotransferase (OAT) (Valle et al. 1981). OAT deficiency generates a secondary Cr deficiency, as it generates an important ornithine accumulation facilitating the reversed AGAT reaction, therefore depleting GAA for Cr synthesis (Sipilä 1980). This is particularly true in CNS, where GA neurological symptoms may be related to this secondary Cr deficiency (Näntö-Salonen et al. 1999; Valayannopoulos et al. 2009).

AGAT, GAMT and SLC6A8 in CNS

Cr is synthesized in the mammalian brain (Pisano et al. 1963; Van Pilsum et al. 1972), in nerve cell lines as well as in primary and organotypic brain cell cultures (Daly 1985; Dringen et al. 1998; Braissant et al. 2002). AGAT and GAMT are expressed in CNS, where they are found in all the main structures of the brain, in every main cell types (neurons, astrocytes and oligodendrocytes, Fig. 1; Braissant et al. 2001b; Tachikawa et al. 2004; Schmidt et al. 2004; Nakashima et al. 2005). Moreover, we have shown very recently that in most regions of the rat brain, AGAT and GAMT are rarely co-expressed within the same cell (see below; Braissant et al. 2010). Organotypic rat cortical cultures, primary brain cell cultures (neuronal, glial or mixed) and neuroblastoma cell lines have a Cr transporter activity (Möller and Hamprecht 1989; Almeida et al. 2006; Braissant et al. 2008). In vivo, mouse and rat CNS can take up Cr from the blood against its concentration gradient (Ohtsuki et al. 2002; Perasso et al. 2003). SLC6A8 is expressed throughout the main regions of adult mammalian brain (Schloss et al. 1994; Happe and Murrin 1995; Braissant et al. 2001b; Tachikawa et al. 2008; Mak et al. 2009). In the first detailed analyses of Cr transporter expression in CNS, it was demonstrated that SLC6A8 is found in neurons and oligodendrocytes but, in contrast to AGAT and GAMT, cannot be detected in astrocytes (Fig. 1; Braissant et al. 2001b), except for very rare ones in cerebellum (Mak et al. 2009). This holds true also for the retina, where SLC6A8 is expressed in retinal neurons, but not in astrocytes (Nakashima et al. 2004; Acosta et al. 2005). In contrast to its absence in astrocytes lining microcapillaries, SLC6A8 is present in MCEC making BBB (Braissant et al. 2001b; Ohtsuki et al. 2002; Tachikawa et al. 2004).

It must be emphasized also that AGAT, GAMT and SLC6A8 are expressed very differently by brain cells, depending on whether the analysis is made in vivo or in vitro, as well as in vitro depending on the type of cultures. 20 years ago, an important paper by Möller and Hamprecht showed a detailed description of the in vitro Cr uptake capacity of numerous types of primary brain cells, as well as immortalized cell lines, all cultured in the presence of serum. They concluded that astrocytes have the highest activity of Cr transporter (Möller and Hamprecht 1989). We showed that in vivo AGAT and GAMT can be found in all brain cell types, while, in contrast to what was demonstrated in primary cultures of astrocytes, SLC6A8 is not expressed by astrocytes (Braissant et al. 2001b). We further demonstrated that organotypic cultures such as brain 3D mixed-cell aggregates cultured in the absence of serum synthesize their own Cr and express AGAT, GAMT and SLC6A8 as the in vivo CNS, including the absence of SLC6A8 from astrocytes (Braissant et al. 2008). In contrast, when we analyzed brain 3D neuron-enriched aggregates also cultured in the absence of serum, from which astrocytes and oligodendrocytes have been eliminated, we showed that in the absence of glial cells, AGAT and GAMT are totally silent from neurons and from the very few remaining astrocytes, while both genes are well expressed in both cell types when glial cells are present (Braissant et al. 2008). It is known that brain cells cultured as 2D monolayers or in the presence of serum develop a pathological reactive state that completely alters their behavior in terms of gene expression and protein activities. This is particularly true for astrocytes, which develop constant reactive gliosis as illustrated by their increase in GFAP and vimentin expression as compared with in vivo conditions (Langan and Slater 1992; F.Tschudi-Monnet and P.Honegger, personal communication; and unpublished results). We have shown that SLC6A8, which is silent in astrocytes in vivo as well as in 3D brain cells aggregates cultured in the absence of serum, is activated in these same astrocytes when they are placed in a reactive state (in that case exposure to ammonium; Cagnon and

Braissant 2007; Braissant et al. 2008; Braissant 2010b). It appears, thus, that most models of cultured brain cells, in particular when cultured in the presence of serum, do not behave as the in vivo CNS for Cr metabolism and transport. Thus, to analyze Cr in brain cells in conditions as near as possible of the in vivo CNS, complex 3D, organotypic and mixed-cell culture systems in the absence of serum should be used (Braissant 2010a).

Creatine in CNS: endogenous synthesis versus uptake from periphery?

The in vivo expression of AGAT and GAMT within mammalian CNS, as well as the in vitro endogenous synthesis of Cr by various types of cultured brain cells, including primary and organotypic cultures, suggest that brain synthesizes Cr (Braissant et al. 2007). However, it was thought for a long time that most, if not all, of the Cr needed by CNS comes from periphery through BBB (Wyss and Kaddurah-Daouk 2000).

The discovery that SLC6A8 cannot be detected in astrocytes, particularly in their feet sheathing MCEC made us suggest, however, that in mature CNS, BBB has a limited permeability for Cr, despite SLC6A8 expression by MCEC and their capacity to import Cr (Braissant et al. 2001b; Ohtsuki et al. 2002; Tachikawa et al. 2004; Nakashima et al. 2004; Acosta et al. 2005). 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 (Ohtsuki et al. 2002; Perasso et al. 2003), 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 (Stöckler et al. 2007; Schulze and Battini 2007). Similarly, GAMT$^{-/-}$ KO mice treated with high doses of Cr replenish their brain Cr, but only slowly (Kan et al. 2007). The effective but limited entry of Cr from blood to CNS through MCEC but without going through astrocytes may occur through the limited surface of CNS microcapillary endothelium that is free of astrocytic feet (Fig. 1; Virgintino et al. 1997; Ohtsuki 2004).

One strong argument in favor of the "brain endogenous Cr synthesis" hypothesis comes from Cr measures in the CSF of Cr-deficient patients (see Braissant and Henry 2008, and references therein). SLC6A8-deficient patients present normal Cr levels in CSF, but are strictly unable to import Cr from periphery (Cecil et al. 2001; DeGrauw et al. 2002; Bizzi et al. 2002; Póo-Argüelles et al. 2006). In contrast, GAMT-deficient patients show strongly decreased levels of Cr in CSF but are able to import Cr from the blood (Stöckler et al. 1994; Schulze et al. 1997). 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 the periphery are the two ways available for the brain to secure Cr homeostasis for its energy and functions. As uptake from the 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) might fulfill these roles in the brain, as CAT1 is expressed in MCEC as well as ubiquitously in neuronal and glial cells, as CAT2(B) is present in neurons and oligodendrocytes, and as CAT3 is restricted to neurons (Braissant et al. 1999; Hosokawa et al. 1999; Braissant et al. 2001a).

The hypothesis of endogenous Cr synthesis in the brain might seem to contradict the in vivo characteristics of SLC6A8 deficiency, which, despite AGAT and GAMT expression in CNS, presents an absence (or a very low level) of brain Cr by MRS (Salomons et al. 2001). This apparent contradiction is probably explained by our very recent data on AGAT, GAMT and SLC6A8 expression patterns in the brain. AGAT and GAMT are found in every CNS cell type (Braissant et al. 2001b), but appear rarely co-expressed within the same cell (Braissant et al. 2010). This suggests that to allow Cr synthesis in the brain, GAA must be transported from AGAT- to GAMT-expressing cells (Fig. 1). 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 (Braissant et al. 2010). These observations may explain the absence of Cr in CNS of SLC6A8-deficient patient, despite normal expression of AGAT and GAMT in their brain (Braissant and Henry 2008), as well as the lack of effect of treatment of SLC6A8-deficient patients with arginine as a precursor of Cr (Fons et al. 2008). Recent studies also demonstrated the potential role of SLC6A8 (and taurine transporter) for GAA transport across BBB and in brain parenchymal cells (Tachikawa et al. 2008, 2009).

While we have shown that AGAT and GAMT can be found in all brain cell types (Braissant et al. 2001b), other studies demonstrated particularly high levels of GAMT in glial cells (Schmidt et al. 2004; Tachikawa et al. 2004; Braissant et al. 2008), suggesting that the final CNS step for Cr synthesis may predominantly be glial. However, this probably depends on the brain region considered, as in cortex only 20% of astrocytes express GAMT in comparison with 48% of neurons (Braissant et al. 2010).

Adult versus developmental 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 the expression of AGAT and GAMT in CNS parenchyma. Fetal and perinatal (or immature) CNS probably behaves differently for its Cr needs.

The Cr/PCr/CK system plays essential roles in energy homeostasis during vertebrate embryonic development (Wallimann et al. 1992), the fetal needs in Cr being partly supported by active transport of Cr from mother to fetus (Davis et al. 1978; Ireland et al. 2008). On the other hand, AGAT, GAMT and SLC6A8 are also well expressed during vertebrate embryogenesis, including in the brain (Schloss et al. 1994; Sandell et al. 2003; Schmidt et al. 2004; Braissant et al. 2005; Wang et al. 2007; Ireland et al. 2009). We have shown that AGAT and GAMT are expressed in the whole developing CNS parenchyma (Braissant et al. 2005). 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 the periventricular zone and choroid plexus, the predominant metabolic exchange zones of fetal CNS before microcapillary angiogenesis and differentiation of BBB (Braissant et al. 2005, 2007).

Creatine and guanidinoacetate within normal versus creatine-deficient CNS

In normal conditions, Cr within human CSF is maintained in the 17–90 μM range, while GAA is maintained at a 1000× lower level (0.015–0.114 μM). By MRS, total Cr is measured between 5.5 and 6.4 mM in the cortical gray matter, while GAA was estimated to 1.6 mM (see Braissant and Henry 2008, and references therein).

With the exception of SLC6A8-deficient heterozygous females where brain Cr deficiency is partial (Cecil et al. 2003), all three Cr deficiencies present the virtual absence of the Cr peak measured by MRS in cortex and basal ganglia (Stöckler et al. 2007). However, despite this lack of Cr detection by MRS, Cr remains present within the brain of Cr-deficient patients. In SLC6A8 deficiency, Cr CSF levels do not differ from age-matched controls (Cecil et al. 2001; Salomons et al. 2001; DeGrauw et al. 2002). In AGAT deficiency, total Cr levels in cortical gray matter are decreased to 12% of age-matched controls (Battini et al. 2002). In GAMT deficiency, CSF Cr levels are strongly decreased (<2 μM) (Schulze et al. 1997, 2003; Ensenauer et al. 2004), while in cortical gray matter total Cr was measured in the 0.2-1.5mM range (Stöckler et al. 1994; Mancini et al. 2005).

GAA accumulation in body fluids is characteristic of GAMT deficiency, where its toxicity is responsible for the more complex and specific phenotype of GAMT-deficiency (see above). GAA CSF levels in GAMT-deficient patients are 60–1000× higher than in age-matched controls, while GAA was estimated to 3.6 mM within cortical gray matter. No precise data are available on GAA levels within AGAT- and SLC6A8-deficient CNS, but it was shown that GAA can also accumulate in the brain of SLC6A8-deficient patients (Sijens et al. 2005). As described above, GAA may appear as a key intermediate player for endogenous Cr synthesis in CNS, as it must most probably be transported from AGAT- to GAMT-expressing cells for Cr synthesis to occur (Braissant et al. 2010), just as it does in periphery between AGAT in kidney and GAMT in liver (Edison et al. 2007; da Silva et al. 2009). It was recently demonstrated that both BBB endothelial cells (Tachikawa et al. 2009) and CNS parenchymal cells (Tachikawa et al. 2008; Braissant et al. 2010) are able to take up GAA by SLC6A8. The Km value of SLC6A8 for GAA appears ten times lower than that for Cr (Tachikawa et al. 2008). Thus, entry of GAA into CNS in normal conditions must be inhibited by blood Cr levels (1–3.5 μM for GAA versus 6–50 μM for Cr). This entry might, however, be facilitated under GAMT deficiency, blood GAA levels becoming higher than Cr levels (12–39 μM for GAA versus 1–5 μM for Cr) (Almeida et al. 2004), therefore contributing to GAA accumulation into the GAMT-deficient brain.

Models for creatine synthesis and trafficking in CNS

Taken together, (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 measures of Cr and GAA within CNS both in normal and Cr-deficient conditions, lead us to propose the following model to understand Cr synthesis and trafficking within the brain (Fig. 1) (Braissant and Henry 2008):

In normal conditions, SLC6A8 is expressed by MCEC, but not by the surrounding astrocytic feet, implying that limited amounts of Cr can enter the brain through BBB. In most brain regions (including cortex and basal ganglia, where most MRS measures are performed), 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. Moreover, the brain of GAMT-deficient patients accumulates GAA. Cr transporter-deficient patients lack functional SLC6A8 on MCEC and thus cannot be treated by oral Cr. Moreover, 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.

Conclusion

Brain is the main organ affected in Cr deficiency syndromes due to deficiencies in AGAT, GAMT or SLC6A8, which lead to a complete absence or a very strong decrease of Cr in CNS. AGAT- and GAMT-deficient patients can be treated by oral creatine supplementation which improves their neurological status, while this treatment is inefficient on SLC6A8-deficient patients. 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. 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. This probably explains why, despite AGAT and GAMT expression in their brain, SLC6A8-deficient patients remain with a Cr-depleted CNS.

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