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Synthesis and transport of creatine in the central nervous system: Importance for cerebral functions

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Abbreviations: AD, Alzheimer disease; AGAT, L-arginine:glycine amidinotransferase; ALS, amyotrophic lateral sclerosis; BBB, blood-brain barrier; B-CK, brain creatine kinase; CK, creatine kinase; Cr, creatine; GA, gyrate atrophy of the choroid and retina; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; HD, Huntington disease; IEM, inborn errors of metabolism; MCEC, microcapillary endothelial cells; M-CK, muscle creatine kinase; mPTP, mitochondrial permeability transition pores; MRS, magnetic resonance spectroscopy; NH_4^+, ammonium; OAT, ornithine δ-aminotransferase; PCr, phosphocreatine; PD, Parkinson disease; ROS, reactive oxygen species; SLC6A8, Cr transporter; sMtCK, sarcomeric mitochondrial creatine kinase; uMtCK, ubiquitous mitochondrial creatine kinase.

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Abstract

Apart of its well known function of “energetic buffer” through the creatine / phosphocreatine / creatine kinase system allowing the regeneration of ATP, creatine has been recently suggested as a potential neuromodulator of even true neurotransmitter. Moreover, the recent discovery of primary creatine deficiency syndromes, due to deficiencies in AGAT or GAMT (the two enzymes allowing creatine synthesis) or in the creatine transporter SLC6A8, has shed new light on creatine synthesis, metabolism and transport, in particular in CNS which appears as the main tissue affected by these creatine deficiencies. Recent data suggest that creatine can cross blood-brain barrier but only with a poor efficiency, and that the brain must ensure parts of its needs in creatine by its own endogenous synthesis. Finally, the recent years have demonstrated the interest to use creatine as a neuroprotective agent in a growing number of neurodegenerative diseases, including Parkinson and Huntington diseases. This article aims at reviewing the latest data on creatine metabolism and transport in the brain, in relation to creatine deficiencies and to the potential use of creatine as neuroprotective molecule. Emphasis is also given to the importance of creatine for cerebral function.

Keywords: creatine, guanidinoacetate, brain, creatine deficiency, mitochondria, neuroprotection.

Running title: Creatine in central nervous system.

Introduction

Creatine (Cr) (α-N-methylguanidino acetic acid) is a nitrogenous organic amino acid playing essential roles in energy metabolism by interconversion to its high energy phosphorylated analogue phosphocreatine (PCr). This reaction is catalyzed by the ubiquitous enzyme creatine kinase (CK). CK isoforms are highly expressed in tissues with high and fluctuating energy demands, such as muscle and brain (Wallimann *et al.* 1992; Wyss and Kaddurah-Daouk 2000). PCr dephosphorylation yields energy, as ADP is converted to ATP by the transfer of N-phosphoryl group from PCr to ADP. The Cr/PCr system also allows the shuttle of high-energy phosphates from mitochondria to cytoplasmic sites of utilization (Wallimann *et al.* 2007) (**Figure 1**).

Pools of Cr in vertebrates are maintained through uptake from diet and endogenous synthesis. This biosynthetic pathway involves two enzymes: L-arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT). Cr is distributed by blood to tissues, where cells take it up by a specific transporter, SLC6A8, also called CRT1, CT1, CreaT or CRT (Wyss and Kaddurah-Daouk 2000).

It has long been thought that cerebral Cr was principally of peripheral origin (Wyss and Kaddurah-Daouk 2000). However, AGAT and GAMT are expressed in CNS (Braissant *et al.* 2001; Braissant *et al.* 2005) suggesting that the brain is able of its own Cr synthesis. 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 (Braissant *et al.* 2001; Ohtsuki *et al.* 2002; Tachikawa *et al.* 2004). 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

(Braissant *et al*. 2001; Braissant and Henry 2008). Considering that CNS ensures parts, if not all, of its Cr needs, and thus does not depend only on Cr issued from periphery, is coherent with the essential roles played by the Cr/PCr system in CNS energy homeostasis, and by Cr as potential neuromodulator or neurotransmitter (see below) (Wallimann *et al*. 1992; Wyss and Kaddurah-Daouk 2000; Brosnan and Brosnan 2007; Andres *et al*. 2008).

Cr deficiency syndromes, caused by mutations in AGAT, GAMT and SLC6A8 genes, have been identified in human (Stöckler *et al*. 1994; Salomons *et al*. 2001; Item *et al*. 2001). CNS is the main organ affected in patients suffering from Cr deficiency syndromes. Their common phenotype is an almost complete lack of Cr in the brain and the development of several neurological symptoms like mental retardation, delays in speech acquisition or epilepsy (Stöckler *et al.* 2007). AGAT- and GAMT-deficient patients can be treated by oral Cr supplementation (Stöckler *et al.* 1996a; Schulze *et al.* 1998; Battini *et al.* 2002; Schulze and Battini 2007), while Cr supplementation of SLC6A8-deficient patients is inefficient (Bizzi *et al.* 2002; Póo-Argüelles *et al.* 2006; Arias *et al.* 2007). Several other brain pathological states can also lead to secondary Cr deficiencies in brain cells, 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).

Recent studies have shown that Cr administration has a therapeutic potential for neurodegenerative disorders with bioenergetic deficits like Huntington (HD) or Parkinson (PD) diseases (Gualano *et al.* 2010). Cr supplementation may also play important protective roles in a number of other pathological conditions, including brain ischemia and stroke, brain and spinal cord trauma, epilepsy or hyperammonemia (Balestrino *et al.* 1999; Tarnopolsky and Beal 2001; Braissant *et al.* 2002; Klein and Ferrante 2007).

This review is focused on the latest data on Cr synthesis and transport in CNS and their functions for brain cells. Cr deficiency syndromes will be discussed, as well as other brain pathologies leading to secondary Cr deficiencies in brain cells. Finally, the therapeutic potential of Cr for various brain pathologies will be considered.

Creatine metabolism and transport

The Cr/PCr/CK system is essential to maintain energy levels in most tissues, and is highly active in particular in those with high and/or fluctuating energy demand such as skeletal muscle, heart and brain (Wallimann *et al.* 1992). The Cr/PCr/CK system not only serves as intracellular buffer for ATP, but also as high-energy phosphate shuttle from mitochondrial sites of production to cytoplasmic sites of consumption (**Figure 1**).

Part of intracellular Cr is converted by CK into the high-energy compound PCr. Four CK isoforms have been described, based on tissue expression and subcellular distribution: two cytosolic forms, M-CK (muscle) and B-CK (brain) and two mitochondrial forms, sarcomeric muscle form (sMtCK) and brain form called ubiquitous MtCK (uMtCK) (Wallimann *et al.* 1992; Schlattner *et al.* 2006). Each CK isoform has a specific function, mitochondrial CKs using ATP to convert Cr to PCr for export to cytoplasm, and cytosolic CKs using PCr to convert ADP to ATP at sites of energy demand, and to convert excess ATP to PCr for energy storage (Wallimann *et al.* 1992; Wallimann *et al.* 1998).

Total Cr (Cr + PCr) in 70kg young adults amounts for approximately 120g. Both Cr and PCr are non-enzymatically and irreversibly degraded to creatinine at a rate of about 1.7% of total

body pool per day (Wyss and Kaddurah-Daouk 2000). Creatinine is excreted via kidneys, the amount of creatinine eliminated being proportional to muscle. The amount of Cr provided by diet or by endogenous synthesis depends on creatinine excretion but accounts for about 2g per day (Casey and Greenhaff 2000).

In human, half of Cr stores originate from food, mainly fresh meat, fish and dairy products, while the other half is biosynthesized endogenously through the AGAT/GAMT pathway. From the precursors arginine (limiting factor) and glycine, AGAT catalyzes the formation of guanidinoacetate (GAA) and ornithine. This step occurs mostly in kidney where Cr level exerts a negative feedback loop on AGAT gene regulation at transcriptional level (McGuire *et al.* 1984; Brosnan *et al.* 2009). The second reaction, catalyzed by GAMT and occurring mostly in liver, uses S-adenosylmethionine to methylate GAA, producing Cr and S-adenosylhomocysteine (Brosnan *et al.* 2009). AGAT and GAMT expression are positively regulated by growth hormone, thyroid hormone and sex hormones (Carlson and Van Pilsum 1973; McGuire *et al.* 1984; Guthmiller *et al.* 1994; Lee *et al.* 1994). While AGAT and GAMT highest expression is found in kidney and liver respectively, they are also expressed at lower levels in various other tissues, including CNS (Lee *et al.* 1998; Wyss and Kaddurah-Daouk 2000; Braissant *et al.* 2001).

Cr is transported by blood to Cr-requiring tissues and taken up in cells with high energy demand by a Cr-specific transporter, SLC6A8. SLC6A8 is a member of the solute carrier family 6, a large family of membrane transporters that mediate the transport of various neurotransmitters and/or amino acids across plasma membrane with the co-transport of two Na^+ and one Cl^- (Chen *et al.* 2004). This transport is electrogenic and driven by the sodium gradient established by Na^+/K^+-ATPase (Dai *et al.* 1999). SLC6A8 expression is important in

tissues with high energy demand, such as skeletal muscle, heart, brain, retina, or with important (re)absorptive functions, such as kidney and intestine (Guimbal and Kilimann 1993; Braissant *et al.* 2001; Peral *et al.* 2002; Mak *et al.* 2009). Cr uptake is regulated by different factors, like insulin which activates Na^+/K^+-ATPase and presumably increases the driving force for Cr uptake (Snow and Murphy 2001), or the Na^+ gradient and intracellular Cr concentrations (Brosnan and Brosnan 2007).

Creatine metabolism, transport and functions in the brain

Functions of creatine in CNS

The Cr/PCr/CK system plays essential roles to maintain the high energy levels necessary for CNS (maintenance of membrane potential and ions gradients, Ca^{++} homeostasis, neurotransmission, intracellular signaling systems as well as axonal and dendritic transport) (Wyss and Kaddurah-Daouk 2000). The brain represents only 2% of body mass but may spend up to 20% of total energy consumption. The Cr/PCr/CK system also plays essential roles in CNS development. Different studies showed that CK isoforms are found highly concentrated in cerebellum (especially glomeruli structures of granular layer), choroid plexus and hippocampal granular and pyramidal cells (Hemmer *et al.* 1994). It must be noted that hippocampus is important for learning and memory function and can be severely affected in Alzheimer disease (AD). B-CK is much higher than uMtCK in cerebellar Bergmann glial cells and hypothalamus, where it plays essential functions in regenerating ATP for glutamate clearance during excitatory synaptic transmission (Oliet *et al.* 2001). Knock-out for one CK isoform (B-CK or uMt-CK) showed behavioral abnormalities and defects in formation and maintenance of hippocampal mossy fiber connections. Double knock-out mice displayed decreased body weight and severely impaired spatial learning, lower nest building activity and

reduction of hippocampal size (Jost *et al.* 2002; Streijger *et al.* 2005). All these studies demonstrate the key function of CK in brain energy metabolism (Hemmer and Wallimann 1993).

Apart of its functions in energy, Cr may play other roles, as recently suggested in particular in CNS. Cr was suggested as essential CNS osmolyte. Astrocytes placed in hyperosmotic shock significantly increase their Cr uptake, suggesting that Cr can work as compensatory osmolyte (Alfieri *et al.* 2006). Conversely, astrocytes exposed to hypo-osmotic swelling conditions stimulate the release of their osmotically active Cr (Bothwell *et al.* 2001). In contrast, ammonium-exposed MCEC *in vitro* stimulate their Cr uptake (Bélanger *et al.* 2007), suggesting that cells making BBB (MCEC and astrocytes lining them) behave differentially during swelling. Cr was also proposed as appetite and weight regulator, by acting on specific hypothalamic nuclei (Galbraith *et al.* 2006).

Creatine: a co-transmitter in CNS?

Cr and GAA can affect GABA-ergic neurotransmission as partial agonists or antagonists on post-synaptic $GABA_A$ receptors, depending on local GABA concentration (De Deyn *et al.* 1991; Neu *et al.* 2002; Cupello *et al.* 2008). These data stimulated research showing that in organotypic cultures of rat cortex, caudate putamen and hippocampus slices, Cr is released from neurons in a similar manner as classical neurotransmitters. This electrically-evoked exocytotic Cr release mechanism is action potential-dependent, being dependent from Ca^{++}, inhibited by the Na^+-channel blocker tetrodotoxin and enhanced by the K^+-channel blocker 4-amino-pyridine (Almeida *et al.* 2006b). According to these *in vitro* studies, Cr may thus also be considered as a neuromodulator or co-transmitter in CNS, which may modulate the activity

of post-synaptic receptors such as $GABA_A$ (Almeida *et al.* 2006a). Interestingly, rat brain synaptosomes were identified recently as expressing SLC6A8, which allows their active accumulation of Cr (Peral *et al.* 2010). This suggests the presence of a Cr recapture mechanism in axon terminal membrane, which would fit with a neurotransmitter/co-transmitter function of Cr in CNS (Almeida *et al.* 2006a).

AGAT, GAMT and SLC6A8 in adult brain

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 (Wyss and Kaddurah-Daouk 2000; Brosnan and Brosnan 2007; da Silva *et al.* 2009). However, Cr is synthesized in the mammalian brain (Van Pilsum *et al.* 1972) as well as in primary brain cell cultures and nerve cell lines (Daly 1985; Dringen *et al.* 1998; Braissant *et al.* 2002). AGAT and GAMT are expressed in CNS, for which we provided the first detailed analysis demonstrating their expression in all the main structures of the adult rat brain, in every main cell types (neurons, astrocytes and oligodendrocytes; Braissant *et al.* 2001) (**Figure 2**). Particularly high levels were found in telencephalon and cerebellum. AGAT was further shown in rat retina (Nakashima *et al.* 2005), while our data on GAMT were confirmed in mouse and human (Schmidt *et al.* 2004; Tachikawa *et al.* 2004).

Organotypic rat cortical cultures, primary brain cell cultures (neuronal, glial or mixed) and neuroblastoma cell lines have a Cr transporter activity (Daly 1985; Möller and Hamprecht 1989; Almeida *et al.* 2006b; 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, particularly in

those associated with learning, memory and general limbic functions (Guimbal and Kilimann 1993; Schloss *et al.* 1994; Happe and Murrin 1995; Saltarelli *et al.* 1996). We provided the first detailed analysis demonstrating that SLC6A8 is found in neurons and oligodendrocytes but, in contrast to AGAT and GAMT, cannot be detected in astrocytes (Braissant *et al.* 2001). We also showed that in contrast to its absence in astrocytes lining microcapillaries, SLC6A8 is present in MCEC (BBB; **Figure 2**). These data were confirmed later in rat and mouse (Ohtsuki *et al.* 2002; Tachikawa *et al.* 2004; Nakashima *et al.* 2004; Acosta *et al.* 2005; Tachikawa *et al.* 2008; Mak *et al.* 2009).

AGAT, GAMT and SLC6A8 in developing brain

The Cr/PCr/CK system plays essential roles in energy homeostasis during vertebrate embryonic development (Wallimann *et al.* 1992). Many structures of vertebrate embryo express CKs at early stages (Lyons *et al.* 1991; Dickmeis *et al.* 2001), and Cr concentrations between 5 and 8 mmol/kg wet weight were measured in CNS of rat and human fetus (Miller *et al.* 2000; Kreis *et al.* 2002). Parts of CNS developmental needs for Cr are provided by active transport of Cr from mother to embryo, Cr accumulating in chorioallantoic placenta and yolk sac at concentrations higher than found in maternal and fetal blood, then diffusing down its concentration gradient into fetal circulation (Davis *et al.* 1978).

AGAT, GAMT and SLC6A8 are well expressed during vertebrate embryogenesis (Schloss *et al.* 1994; Sandell *et al.* 2003; Schmidt *et al.* 2004; Braissant *et al.* 2005; Wang *et al.* 2007; Ireland *et al.* 2009), and probably play essential roles in developing CNS as their deficiencies

lead to neurological symptoms in early infancy and severe neurodevelopmental delay (see below).

Working on rat, we have provided the first detailed analysis of AGAT, GAMT and SLC6A8 expression in developing embryonic CNS (Braissant *et al.* 2005). AGAT and GAMT are expressed in the whole developing CNS parenchyma. However, their low level (GAMT in particular) at early developmental stages suggests that embryonic CNS depends on external Cr supply, be it from embryonic periphery or from maternal origin. This is coherent with SLC6A8 expression in 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 brain before differentiation of BBB (Braissant *et al.* 2005; Braissant *et al.* 2007).

Functions of AGAT, GAMT and SLC6A8 in CNS: Synthesis or uptake of creatine by the brain ?

Total Cr levels and CK activity are well correlated in mammalian CNS (Wyss and Kaddurah-Daouk 2000), their highest levels being reached in brain cells described with high and fluctuating energy demands, where AGAT, GAMT and SLC6A8 are expressed (Hemmer *et al.* 1994; Wang and Li 1998; Braissant *et al.* 2007).

SLC6A8 absence in astrocytes, particularly in their feet sheathing MCEC, made us suggest that in mature brain, BBB has a limited permeability for Cr, despite SLC6A8 expression by MCEC and their capacity to import Cr (Braissant *et al.* 2001; Braissant *et al.* 2007). *In vivo* data confirmed this hypothesis: the blood to brain transport of Cr is effective in rodents, 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 CNS Cr (Stöckler *et al.* 2007; Schulze and Battini 2007). Consequently, the brain may depend more on its own Cr synthesis through AGAT and GAMT expression than on Cr supply from blood (Braissant *et al.* 2007; Braissant and Henry 2008). The effective but limited passage of Cr from blood to CNS through BBB may occur through the limited surface of CNS microcapillary endothelium that is free of astrocytic feet (Virgintino *et al.* 1997; Ohtsuki 2004) (**Figure 2**).

One strong argument in favor of the "brain endogenous Cr synthesis" hypothesis comes from Cr measures in CSF of Cr-deficient patients (see Braissant and Henry 2008, and references therein). SLC6A8 deficient patients present normal Cr levels in CSF, but cannot import it from periphery (Cecil *et al.* 2001; DeGrauw *et al.* 2002). In contrast, GAMT-deficient patients show strongly decreased Cr levels in CSF, but can import it from blood (Schulze *et al.* 1997). This also suggests that CNS Cr synthesis 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 endogenous Cr synthesis" hypothesis might seem contradictory with *in vivo* characteristics of SLC6A8 deficiency, which, despite AGAT and GAMT expression in CNS, shows absence (or very low level) of brain Cr by magnetic resonance spectroscopy (MRS) (Salomons *et al.* 2001). This apparent contradiction is probably explained by AGAT, GAMT

and SLC6A8 expression patterns in CNS. AGAT and GAMT are found in every CNS cell type (Braissant *et al.* 2001), 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 (Braissant and Henry 2008) (**Figure 2**). This GAA transfer most probably occurs through SLC6A8, as recently shown by Cr and GAA competition studies, and the use of stable isotope-labeled GAA demonstrating its conversion to Cr by GAMT activity (Braissant *et al.* 2010). These observations may explain Cr absence in CNS of SLC6A8-deficient patient, despite normal expression of AGAT and GAMT in their brain (Braissant and Henry 2008). Recent studies also demonstrate the potential role of SLC6A8 (and taurine transporter) for GAA transport across BBB and at blood-cerebrospinal fluid barrier, as well as in brain parenchymal cells (Tachikawa *et al.* 2008; Tachikawa *et al.* 2009).

While we have shown that AGAT and GAMT can be found in all brain cell types (Braissant *et al.* 2010), various studies demonstrated high levels of GAMT within 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 by glial. However, this probably depends on the CNS region considered, as in cortex, only 20% of astrocytes express GAMT, in comparison with 48% of neurons (Braissant *et al.* 2010).

Creatine deficiency syndromes

Inborn errors of Cr biosynthesis and transport, called Cr deficiency syndromes and due to deficiencies in AGAT, GAMT and SLC6A8 (**Figures 3 to 5**), are characterized by an absence or a severe decrease of Cr in CNS, as measured by MRS (Stöckler *et al.* 1994; Item *et al.*

2001; Salomons *et al.* 2001; Stromberger *et al.* 2003). AGAT and GAMT deficiencies are autosomal recessive diseases, while SLC6A8 deficiency is a 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 appear rarer. The prevalence of all combined Cr deficiencies was estimated at 2.7% of all mental retardation (Lion-François *et al.* 2006). CNS is the main organ affected in Cr deficiency syndromes, whose patients show severe neurodevelopmental delay and develop, in early infancy, mental retardation, disturbance of active and comprehensible speech, autism, automutilating behavior and hypotonia (Stöckler *et al.* 1996b; Schulze *et al.* 1997; de Grauw *et al.* 2002; Battini *et al.* 2002). Patients with GAMT deficiency exhibit a more complex phenotype, including intractable epilepsy, extrapyramidal movement syndromes and abnormalities in basal ganglia (Stromberger *et al.* 2003; Schulze 2003; Mercimek-Mahmutoglu *et al.* 2006). GAMT-deficient patients accumulates GAA due to the block in GAMT enzymatic activity, including in the brain where GAA accumulation is probably due to the combined CNS endogenous AGAT activity (Braissant and Henry, 2008), as well as to a facilitated crossing of BBB by GAA due to increased GAA versus decreased Cr in their blood (Tachikawa *et al.* 2009) (**Figure 4**). GAA toxicity in CNS, and particularly its epileptogenic action (Schulze *et al.* 2001), may occur through disturbances of GABAergic neurotransmission (see above; 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), that could be caused by impairment of GAA transport, through deficient SLC6A8, from AGAT- to GAMT-expressing cells (Braissant *et al.* 2010) (**Figure 5**).

The diverse phenotypic neurological spectrum observed in Cr deficiency syndromes show the importance of Cr for psychomotor development and cognitive functions and might be explained by the wide pattern of AGAT, GAMT and SLC6A8 genes in mammalian brain (**Figures 2 to 5**), which has been documented in every main regions of rat (AGAT, GAMT and SLC6A8), mouse (GAMT and SLC6A8) and human (GAMT) CNS (see above). The potential role of Cr as co-transmitter on the widely distributed GABA postsynaptic receptors (Almeida *et al.* 2006b) might also contribute to this phenotypic diversity.

AGAT- and GAMT-deficient patients can be treated with Cr, which strongly improves their neurological status and CNS development (Stöckler *et al.* 1996a; Schulze *et al.* 1998; Item *et al.* 2001; Battini *et al.* 2002) (**Figures 3 and 4**). For GAMT-deficient patients, combined arginine restriction and ornithine substitution coupled to Cr treatment decrease GAA and improve clinical outcome (Schulze *et al.* 1998; Schulze *et al.* 2001; Schulze 2003). 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 CNS Cr in SLC6A8-deficient patients (**Figure 5**), who remain with mental retardation, severe speech impairment, and progressive brain atrophy (Cecil *et al.* 2001; Bizzi *et al.* 2002; de Grauw *et al.* 2002). Attempts to treat SLC6A8-deficient patients with arginine and glycine as precursors of Cr gave encouraging results in two SLC6A8-deficient patients (Chilosi *et al.* 2008; Wilcken *et al.* 2008), while it failed to improve the neurological status of four others (Fons *et al.* 2008). 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).

Pre-symptomatic treatment of AGAT- and GAMT-deficient patients

Two recent studies have shown that the pre-symptomatic treatment of AGAT and GAMT deficiencies appears to prevent the phenotypic expression of these diseases (Schulze and Battini, 2007). An AGAT-deficient boy, brother of two already affected AGAT-deficient sisters, was diagnosed at birth with the same homozygous mutation as his sisters, and treated orally since the age of 4 months with Cr monohydrate (Battini *et al.* 2006). Similarly, a GAMT-deficient girl, sister of an already affected GAMT-deficient brother, was diagnosed at birth with the same heterozygous mutations as her brother, and treated orally since the age of 22 days with Cr monohydrate (Schulze *et al.* 2006). Both patients, over a follow-up of more than 2 years, did not develop the characteristic CNS phenotypic expression of AGAT and GAMT deficiencies (Schulze and Battini 2007). These two cases suggest that Cr plays essential roles in the development of CNS higher cognitive functions, like speech acquisition, during the first months and years of life, and that treatment with Cr before irreversible damage occurs may prevent clinical symptoms of AGAT and GAMT deficiencies permanently. As described above, the pre-symptomatic treatment with Cr in post-natal stages and during the first years of life may also facilitate the entry of Cr into the brain, at stages where BBB is not as tightly regulated as in more mature stages (Virgintino *et al.* 1997; Engelhardt, 2003), and where SLC6A8 expression on BBB and choroid plexus may still facilitate entry of peripheral Cr into the brain (Braissant *et al.* 2005; Ireland *et al.* 2009), in contrast to adulthood (Braissant *et al.* 2001).

The GAMT $^{-/-}$ mouse

So far, only one *in vivo* model of Cr deficiencies has been described: the GAMT knock-out mouse ($GAMT^{-/-}$) in which the first exon of the murine GAMT gene has been disrupted (Schmidt *et al.* 2004). As GAMT-deficient patients, $GAMT^{-/-}$ mice have markedly decreased Cr and increased GAA levels in CNS and in body fluids (urine, serum, CSF) (Renema *et al.* 2003), and slowly replenish their brain Cr when fed with Cr. $GAMT^{-/-}$ mice show increased neonatal mortality, muscular hypotonia and decreased male fertility. The most obvious symptom observed is a reduction of body weight throughout life, more pronounced in females than males. While biochemical alterations of $GAMT^{-/-}$ mice are comparable to those found in GAMT-deficient patients, their neurological and behavioral analysis reveals only mild cognitive impairment and no severe neurological symptoms despite the important accumulation of GAA in CNS (Schmidt *et al.* 2004; Torremans *et al.* 2005). In particular, no severe symptoms like epileptic seizures or ataxia are observed. One explanation for this contrast to GAMT patients may be the use of GAA as CK substrate, leading to the formation of phosphorylated GAA which may play the same role as PCr in providing high-energy phosphates (Ellington 2001; Renema *et al.* 2003). If this were true, the same compensation mechanism by GAA may also occur in GAMT-deficient patients, who can accumulate phosphorylated GAA at least in their muscles (Schulze *et al.* 2003; Ensenauer *et al.* 2004). No *in vivo* models of AGAT and SLC6A8 deficiencies have been published so far.

Secondary creatine deficiencies in CNS

Apart of the primary Cr deficiency syndromes, several other CNS pathologies cause a secondary Cr deficiency in brain cells.

Hyperammonemia

Excess of ammonium (NH_4^+) is toxic for CNS. In adults, liver failure can result in hyperammonemia and lead to a potentially severe neuropsychiatric disorder named hepatic encephalopathy, which progressively leads to altered mental status and coma (Beal and Martin 1998). In pediatric patients, hyperammonemia can be caused by various inherited or acquired disorders, the most frequent being urea cycle diseases, which can cause irreversible damages to the developing brain with presentation symptoms such as cognitive impairment, seizures and cerebral palsy (Leonard and Morris 2002; Gropman and Batshaw 2004). In CNS, NH_4^+ exposure alters several amino acid pathways and neurotransmitter systems, cerebral energy, nitric oxide synthesis, axonal and dendritic growth and signal transduction pathways (Cagnon and Braissant 2007; Cagnon and Braissant 2008; Cagnon and Braissant 2009) eventually leading to energy deficit, oxidative stress and cell death (Braissant 2010a). In particular, NH_4^+ exposure generates a secondary Cr deficiency in brain cells, both *in vivo* and *in vitro* (Ratnakumari *et al.* 1996; Choi and Yoo 2001; Braissant *et al.* 2002). NH_4^+ appears to inhibit AGAT enzymatic activity and to differentially alter 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 2010b).

Stroke

Stroke is the rapidly developing loss of brain functions due to disturbances in CNS blood flow, and resulting in insufficient oxygen and glucose delivery to support brain cell homeostasis (Donnan *et al.* 2008). Distinction is made between ischemic stroke, due to thrombotic or embolic events interrupting blood supply to the brain, and hemorrhagic stroke, due to the rupture of a blood vessel or an abnormal vascular structure. Ischemic stroke is the

most frequent, representing about 87% of all cases of stroke (Donnan *et al.* 2008). The Cr/PCr system is known to allow the regeneration of ATP even in absence of oxygen and glucose, but for a very limited amount of time. In the brain in particular, PCr levels are limited, and rapidly become depleted after anoxia or ischemia, the PCr decrease preceding the fall in ATP (Lipton and Whittingham 1982; Obrenovitch *et al.* 1988). Moreover, studies on different *in vivo* models for brain ischemia have demonstrated a rapid diminution in CNS total Cr (Peres *et al.* 1992; Gideon *et al.* 1992; Lei *et al.* 2009). In ischemic patients, total Cr levels are also significantly lower than in normal volunteers (Mathews *et al.* 1995). This lower Cr level causes a decrease in high energy phosphates production, and leads to a failure in most energy-dependent processes necessary for cell survival, such as ion pumping, neuronal depolarization or presynaptic re-uptake of excitatory amino acids (Nicholls and Attwell 1990). This in turn favors the accumulation of excitotoxic glutamate in CNS extracellular space, eventually leading to neuronal death by necrosis or apoptosis (Dirnagl *et al.* 1999; Zhu *et al.* 2004).

Gyrate atrophy of the choroid and retina

Gyrate atrophy of the choroid and retina (GA), an IEM causing chorioretinal dystrophy starting in childhood and that can lead to blindness in the fourth to seventh decade of life, is caused by mutations in ornithine δ-aminotransferase (OAT) (Valle *et al.* 1981). GA generates a secondary Cr deficiency in skeletal muscle as well as in brain cells, as OAT deficiency leads to an important accumulation of ornithine which inhibits AGAT reaction, therefore depleting GAA for Cr synthesis (Sipilä 1980), as shown in brain and skeletal muscle of GA animal models and patients (Wang *et al.* 1996; Valayannopoulos *et al.* 2009). While GA patients may develop with normal intelligence, electroencephalography and magnetic resonance imaging analysis have however demonstrated unspecific abnormalities and premature degenerative

changes in CNS (Näntö-Salonen *et al.* 1999; Valtonen *et al.* 1999). GA neurological symptoms may thus possibly be related to a secondary Cr deficiency in CNS (Näntö -Salonen *et al.* 1999; Valayannopoulos *et al.* 2009).

Therapeutic potential of creatine 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 (Beal 2000; Chaturvedi and Beal 2008). The cellular energy state plays key roles in regulating and initiating necrosis and apoptosis in brain cells, since mitochondria are known as essential in controlling specific apoptotic pathways (Green and Reed 1998) (**Figure 6**).

The dominant role of mitochondria is to supply and regulate energy, in the form of ATP, for the cell. In addition, mitochondria are involved in a range of other processes, such as cellular growth and differentiation, and cell cycle control (McBride *et al.* 2006). Their dysregulation can lead to alterations in Ca^{++} homeostasis, production of reactive oxygen species (ROS) and cell death (apoptosis) (Steeghs *et al.* 1997; Green and Reed 1998; McBride *et al.* 2006). Mitochondria can release several pro-apoptotic proteins into cytosol which in turn can induce cell death (Primeau *et al.* 2002). This release is under control of ROS, allowing formation of mitochondrial permeability transition pores (mPTP), a continuum between inner and outer mitochondrial membranes (Adhihetty and Beal 2008) (**Figure 6**). mPTP are associated with different death mechanisms leading to apoptosis and necrosis (Bernardi *et al.* 1998). mPTP formation and opening is facilitated by several factors, like accumulation of Ca^{++}, reduction in

membrane potential, increase in inorganic phosphate, decrease in ATP and ADP, and elevation in oxidative stress (Di Lisa and Bernardi 2005). In particular, mPTP are localized on the mitochondrial membrane beside MtCK, with which they interact. MtCK suppresses pore opening and potentially decreases apoptotic susceptibility, which is itself stabilized by the presence of Cr (O'Gorman *et al.* 1997; Adhihetty and Beal 2008). Thus, Cr can play essential roles in stabilizing mitochondrial function and in decreasing neuronal cell death (**Figure 6**).

The mechanisms of neuroprotection by Cr differ depending on the brain pathology, but several studies have shown that Cr supplementation can improve the bioenergetic deficit associated with these disorders (Gualano *et al.* 2010).

Huntington disease

Huntington disease (HD) is caused by a CAG triplet expansion in exon 1 of huntingtin gene, resulting in an elongated polyglutamine expansion in huntingtin protein. The precise roles of huntingtin are unknown so far, but hypotheses have been made for functions in intracellular transport, autophagy, transcription, signal transduction and mitochondrial function (Beal and Ferrante 2004; Gauthier *et al.* 2004; Ross 2004). Huntingtin is a cytosolic protein expressed ubiquitously in vertebrates, including in CNS (Bender *et al.* 2005). HD symptoms are progressive motor dysfunction, emotional disturbance, dementia and weight loss (Klein and Ferrante 2007).

Mutated huntingtin has a toxic effect in neural tissue, with transcriptional dysregulation, proapoptotic signaling, oxidative injury, inflammatory reactions and mitochondrial dysfunctions (Ryu and Ferrante 2005). $HD^{-/-}$ mice showed an important interaction between

energy metabolism dysfunction, mitochondrial abnormalities and excitotoxicity in HD pathogenesis (Brouillet and Beal 1993; Beal 1995; Beal 2000), and that Cr plays important roles in stabilizing intracellular Ca^{++}, buffering intracellular energy reserves, inhibiting mPTP and decreasing extracellular glutamate (Ferrante *et al.* 2000; Andreassen *et al.* 2001; Dedeoglu *et al.* 2003; Ryu and Ferrante 2005) (**Figure 6**). Cr supplementation of $HD^{-/-}$ mice increased their life span, decreased their brain atrophy and delayed the formation of mutant huntingtin aggregates (Ferrante *et al.* 2000). Recently, a phase II clinical trial on safety and tolerability of Cr in HD patients showed that Cr supplementation made an indicator of oxidative-induced damage to DNA (8-hydroxy-2'-deoxyguanosine) undetectable in the serum (Hersch *et al.* 2006). A phase III clinical trial has now been approved and is currently ongoing in various centers.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is caused by a loss of motor neurons in CNS, particularly in brainstem and motor cortex, which leads to skeletal muscle atrophy, paralysis and death. ALS is caused by a variety of genetic mutations, the most common being located in superoxide dismutase 1 gene (Hervias *et al.* 2006). Different studies with G93A transgenic mice, an animal model for ALS, have shown decreased ATP levels and impairment in respiratory chain activity, inducing a significant decrease in mitochondrial Ca^{++} loading capacity, oxygen consumption, and ATP synthesis in CNS mitochondria (Mattiazzi *et al.* 2002; Damiano *et al.* 2006) (**Figure 6**). Cr supplementation in G93A mice improved their motor performance, extended their survival, protected against neuronal loss in substantia nigra and motor cortex, and finally decreased oxidative damage in mitochondria (Klivenyi *et al.* 1999).

Despite these promising results, human clinical trials testing the efficacy of Cr in ALS patients showed no evidence for Cr therapeutic potential on survival and/or disease progression in patients (Groeneveld *et al.* 2003; Shefner *et al.* 2004).). While mitochondrial dysfunction is essential in the motor neuron death cascade in G93A mice, it is not known whether it plays similar roles in inducing motor neuron degeneration in ALS patients (Wong *et al.* 1995; Kong and Xu 1998; Swerdlow *et al.* 1998; Borthwick *et al.* 1999). Other reasons for the discrepancy in Cr responsiveness between G93A mice and ALS patients may be the time of starting Cr treatment (40 days before onset of disease in mice, as compared to an average of 500 days after onset of symptoms in patients), as well as the Cr dose given to ALS patients that may have been inefficient as compared to the dose given to G93A mice (Groeneveld *et al.* 2003; Shefner *et al.* 2004; Rosenfeld *et al.* 2008).

Parkinson disease

Mitochondrial dysfunction and oxidative damage play important roles in the pathogenesis of Parkinson disease (PD), which manifests by a loss and/or dysfunction of dopaminergic neurons in substantia nigra (Beal 1995; Beal 2003) and intraneural protein inclusions called Lewy bodies (Lin and Beal 2006). Principal symptoms are progressive bradykinesia, rigidity, tremor and gait abnormalities (Adhihetty and Beal 2008). Mitochondrial dysfunction in PD decreases ATP synthesis and increases ROS production. ROS inactivate MtCK and decrease cytosolic CK activities, thus shutting down energy metabolism (Bindoff *et al.* 1989; Parker *et al.* 1989) (**Figure 6**). Mutations in several genes also appear to affect mitochondrial metabolism in PD (Thomas and Beal 2007). Cr supplementation in PD animal models resulted in significant protection against both CNS dopamine depletion and neuronal loss of

neurons in substantia nigra (Matthews *et al.* 1999). Finally, several double-blinded, phase II clinical trials of Cr in early PD patients indicated that Cr supplementation is not futile and should be considered for phase III clinical trials (Bender *et al.* 2006).

Alzheimer disease

Alzheimer disease (AD), the most common form of dementia, is characterized by a loss of neurons in cerebral cortex and specific subcortical regions (Wenk 2003). This neuronal loss is associated with deposits of extracellular plaques (amyloid-β peptide and cellular material) outside and around neurons, and deposits of intracellular neurofibrillary tangles (aggregation of the microtubule-associated protein tau in a hyperphosphorylated form) (Bouras *et al.* 1994; Tiraboschi *et al.* 2004).

At the molecular level, AD lesions show inactive CKs in association with depositions enriched in Cr (Bürklen *et al.* 2006). This loss of bioenergetic function appears due to an excessive production of ROS by mitochondria and the absence of translocation of MtCK from cytosol to mitochondria due to lack of a protein chaperone-like activity (Li *et al.* 2006) (**Figure 6**). Cr supplementation does not improve cellular bioenergetics at late stages of AD, and the question remains open whether improvement can occur earlier. Other Cr functions have been considered, like protection against oxidative-induced CK inactivation by a delay in ROS action (Aksenov *et al.* 2000). Cr supplementation appears neuroprotective against glutamate and β-amyloid toxicity in rat hippocampal neurons (Brewer and Wallimann 2000).

Ischemic stroke

As described above, stroke generates a significant decrease in CNS total Cr pools. Several studies have investigated the potential neuroprotective effects of Cr supplementation to protect CNS against stroke deleterious mechanisms. Cr supplementation of organotypic cultures of hippocampal slices placed in anoxic conditions appears to replenish their PCr content, protect synaptic transmission and enhance survival of hippocampal neurons (Whittingham and Lipton 1981; Balestrino *et al.* 1999; Balestrino *et al.* 2002) (**Figure 6**). Total Cr is also increased *in vivo* in the ischemic CNS of rat supplemented with Cr (Wick *et al.* 1999). Moreover, Cr supplementation exerts neuroprotective effects against cerebral ischemia in mice, by inhibiting mitochondrial cytochrome c release and downstream caspase-3 activation (Zhu *et al.* 2004). To counteract the poor penetration of Cr from periphery to CNS, the direct administration of Cr into cerebral ventricles, aimed at bypassing BBB, protected CNS from damage of global ischemia in rat (Lensman *et al.* 2006). Similarly, Cr-derived compounds that can cross biological membranes in a Cr transporter-independent manner also showed neuroprotective effects against brain tissue anoxia (Lunardi *et al.* 2006; Perasso *et al.* 2008).

Hyperammonemia

As described above, NH_4^+ exposure generates a secondary Cr deficiency in brain cells, both *in vitro* and *in vivo*. As Cr is essential, during CNS development, to buffer the energetic levels necessary, in growth cones, for axonal and dendritic elongation, and as NH_4^+ exposure impairs axonal growth (Braissant *et al.* 2002), we investigated whether a Cr co-treatment under NH_4^+ exposure could be neuroprotective. We could show that Cr supplementation can protect axonal growth under NH_4^+ exposure (Braissant *et al.* 2002). This protection by Cr depends on the presence of glial cells. As NH_4^+ exposure inhibits axonal growth and decreases Cr, while

Cr co-treatment under NH_4^+ protects axonal growth, methods to efficiently sustain Cr concentration in the developing hyperammonemic CNS should be assessed. As described above, Cr can cross from blood to brain through BBB under physiological conditions, but with a low permeability, partly because astrocytes lining BBB do not express SLC6A8. MCEC, at BBB, express SLC6A8 (Braissant *et al.* 2001; Ohtsuki *et al.* 2002). NH_4^+ exposure increases both SLC6A8 and Cr uptake in MCEC (Bélanger *et al.* 2007). As we demonstrated that SLC6A8 is induced in NH_4^+-exposed astrocytes (Braissant *et al.* 2008), BBB of the hyperammonemic CNS might thus be more permeable to Cr than under physiological conditions, and supplying oral Cr to hyperammonemic neonates or infants might likely contribute to protect their brain (Braissant 2010a; Braissant 2010b).

Conclusion

The main function of Cr, in energy metabolism, is to allow ATP regeneration through CK enzymatic activity. In recent years, new roles of Cr have been suggested in CNS, like a function of central neuromodulator or even true neurotransmitter and roles in appetite and weight regulation by acting on specific hypothalamic nuclei.

Several studies investigated the brain biosynthetic pathway and transport of Cr, and suggested that due to a poor permeability of BBB for Cr, CNS must secure parts of its needs in Cr by endogenous synthesis. We have recently shown that in many brain structures, AGAT and GAMT are dissociated between different cells, suggesting that to allow brain synthesis of Cr, GAA must be transported from AGAT- to GAMT-expressing cells, most probably through SLC6A8 (Braissant and Henry 2008; Braissant *et al.* 2010).

Given the essential functions of Cr played in CNS, several studies have investigated its neuroprotective potential in numerous brain pathologies, both on neurodegenerative animal models and in patients, with ongoing clinical trials in phase II and III. Cr supplementation appears to exert neuroprotective effects in HD and PD, but not in AD nor in ALS. Cr may also be used as neuroprotective agent under stroke, ischemia or hyperammonemic states, for which further studies are needed.

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Figure legends

Figure 1: Synthesis and function of creatine (Cr). Cr synthesis requires the presence of two enzymes, L-arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT); cells take up Cr by a specific transporter, SLC6A8. Mitochondrial or cytosolic creatine kinases (CK) convert Cr to its high-energy counterpart phosphocreatine (PCr). PCr dephosphorylation yields energy, as ADP is converted to ATP. Besides its function in cellular energy, Cr may also be involved in neurotransmission.

Figure 2: Model of Cr synthesis and transport in CNS, illustrating the diversity of AGAT, GAMT and SLC6A8 expression by 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. **3)** Cell expressing only SLC6A8 (“users” of Cr). **4)** Cells silent for AGAT, GAMT and SLC6A8. While microcapillaries express SLC6A8, astrocytic feet lining

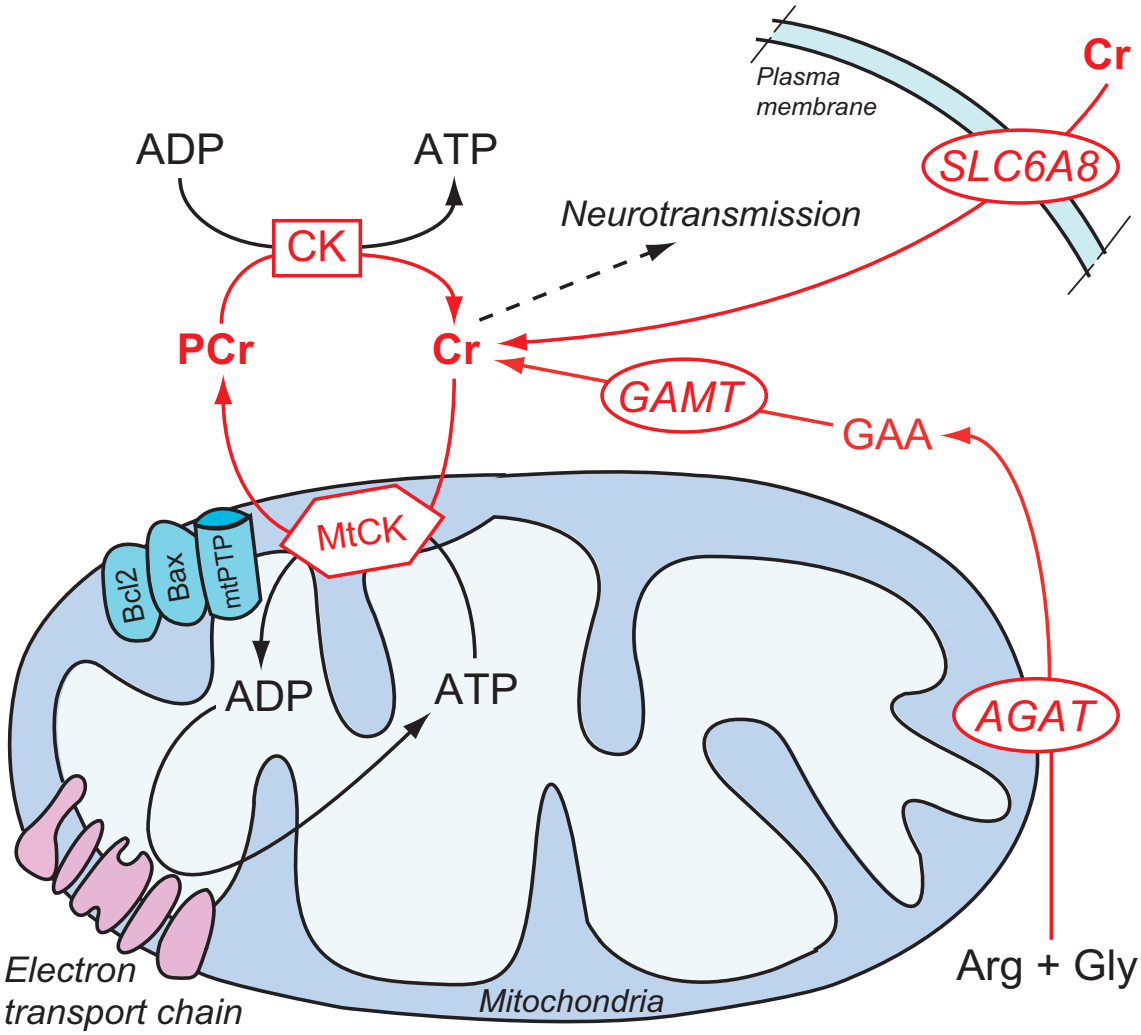
them do not. This implies that only low amounts of peripheral Cr can enter the brain through the limited endothelial surface that is free of astrocytic feet, and that CNS must also ensure its own endogenous synthesis of Cr. So far, the way Cr (and GAA) can leave cells is poorly known. Cr: creatine; AGAT: L-arginine:glycine amidinotransferase; GAMT: guanidinoacetate methyltransferase; GAA: guanidinoacetate; SLC6A8: Cr transporter.

Figure 3: Model of AGAT deficiency in CNS. See Figure 2 for abbreviations.

Figure 4: Model of GAMT deficiency in CNS. See Figure 2 for abbreviations.

Figure 5: Model of SLC6A8 deficiency in CNS. See Figure 2 for abbreviations.

Figure 6: Involvement of mitochondria and the Cr/PCr system in brain cell death. In Hungtington (HD), Parkinson (PD) and Alzheimer (AD) diseases, as well as in amyotrophic lateral sclerosis (ALS), stroke and hyperammonemia (NH_4), the impairment of mitochondria produces reactive oxygen species (ROS) by the electron transport chain. ROS inactivate mitochondrial creatine kinase (MtCK) by changing its octameric conformation to a dimeric inactivated form, leading to phosphocreatine (PCr) depletion. Dimeric MtCK and ROS modify the structure and open mitochondrial permeability transition pores (mPTP), leading to the release of pro-apoptotic factors in cytosol and to cell death. Creatine (Cr) supplementation allows the regeneration of the cell Cr pool. Moreover, in HD, PD, stroke and hyperammonemia, Cr supplementation may stabilize octameric MtCK and prevent the opening of mPTP, thus avoiding cell death.


Plasma
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GAMT
GAA
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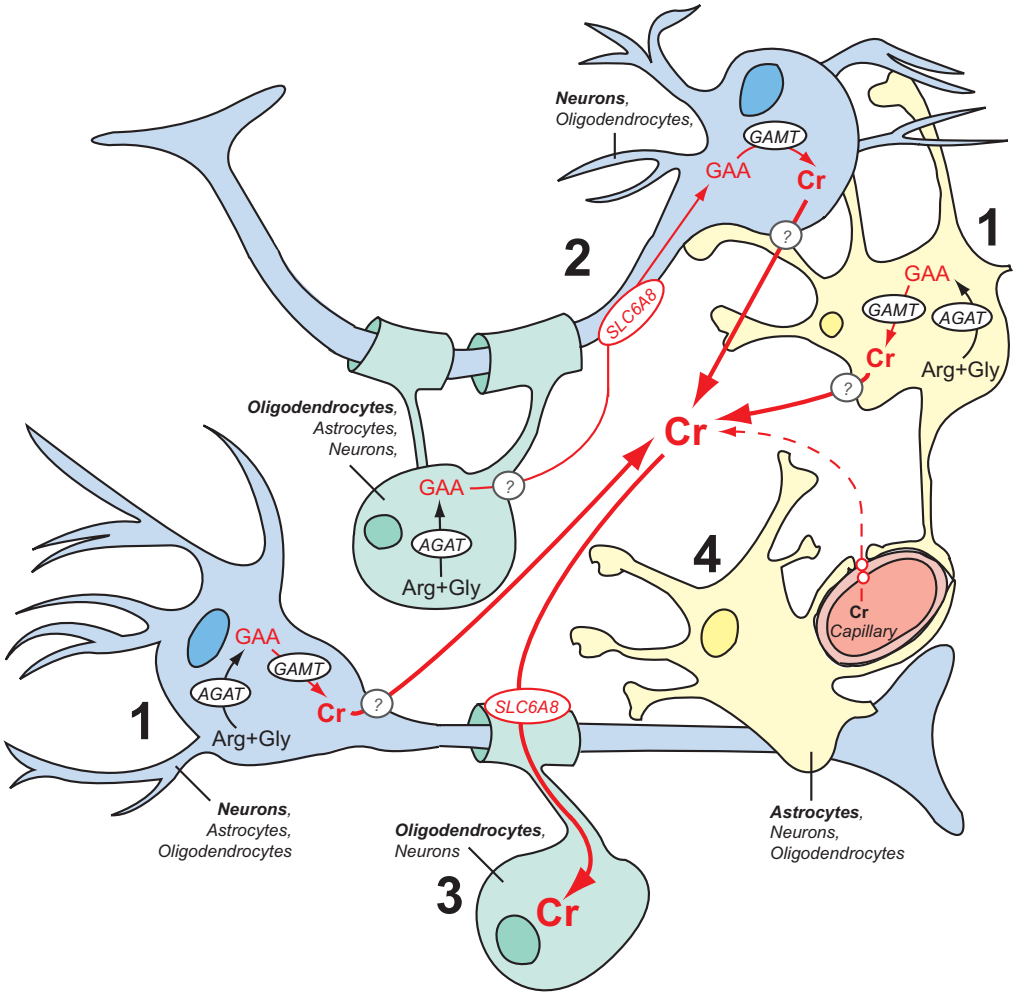
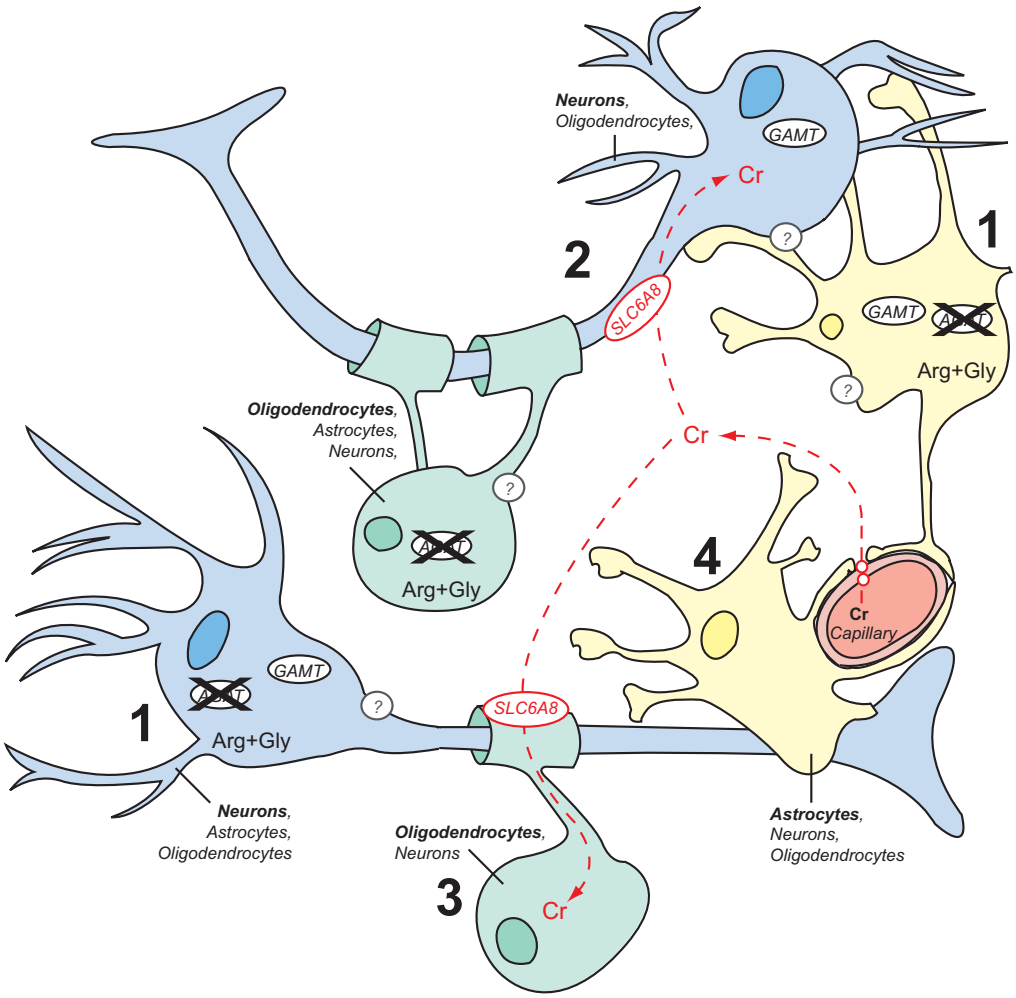
AGAT
Electron
transport chain
Mitochondria
Arg + Gly


Neurons,
Oligodendrocytes,
GAMT
GAA
Cr
2
1
?
GAA
GAMT
AGAT
Cr
Arg+Gly
SLC6A8
Oligodendrocytes,
Astrocytes,
Neurons,
GAA
?
AGAT
Arg+Gly
Cr
4
Cr
Capillary
GAA
GAMT
AGAT
1
Arg+Gly
Cr
?
SLC6A8
Neurons,
Astrocytes,
Oligodendrocytes
Oligodendrocytes,
Neurons
3
Cr
Astrocytes,
Neurons,
Oligodendrocytes


Neurons,
Oligodendrocytes,
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GAMT
AGAT
Arg+Gly
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Astrocytes,
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Cr
4
AGAT
Arg+Gly
Cr
Capillary
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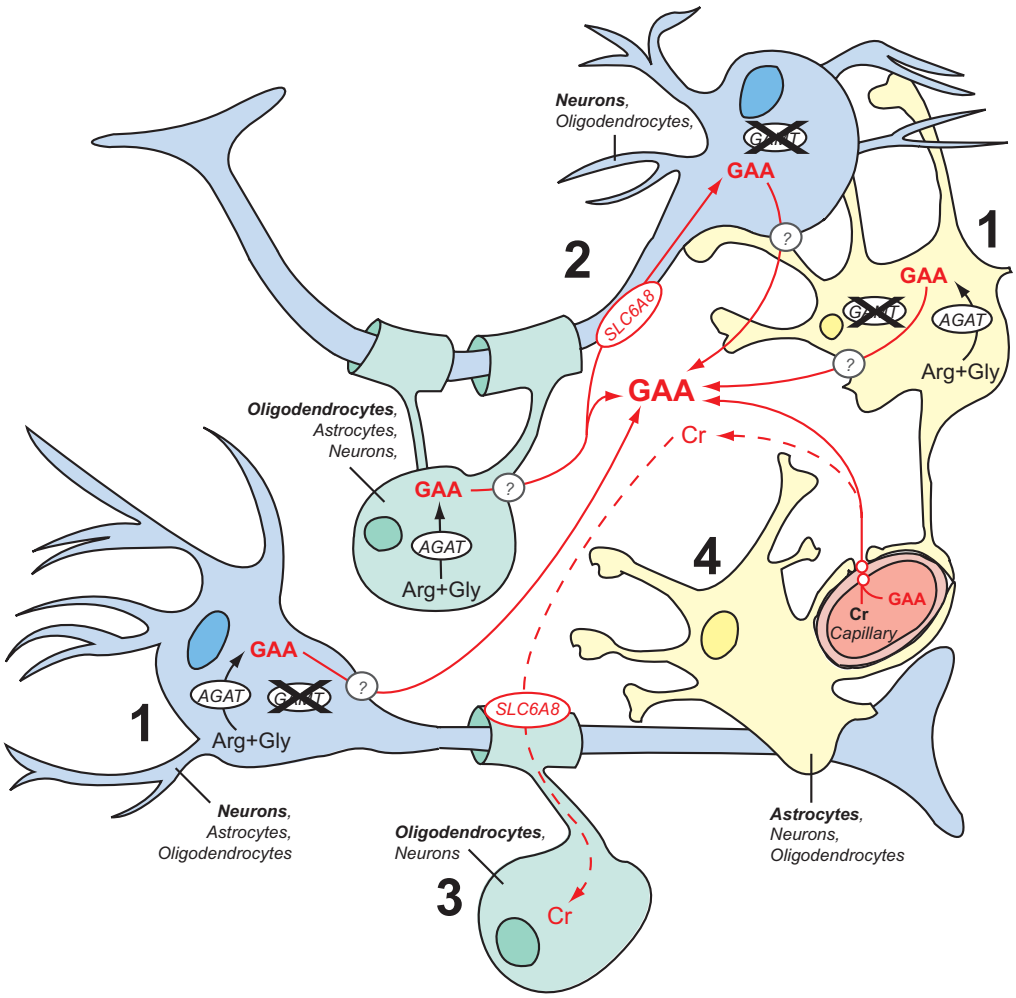
Neurons,
Astrocytes,
Oligodendrocytes
Oligodendrocytes,
Neurons
3
Cr
Astrocytes,
Neurons,
Oligodendrocytes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Arg+Gly
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Cr
Oligodendrocytes,
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Neurons,
Astrocytes,
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Oligodendrocytes,
Neurons
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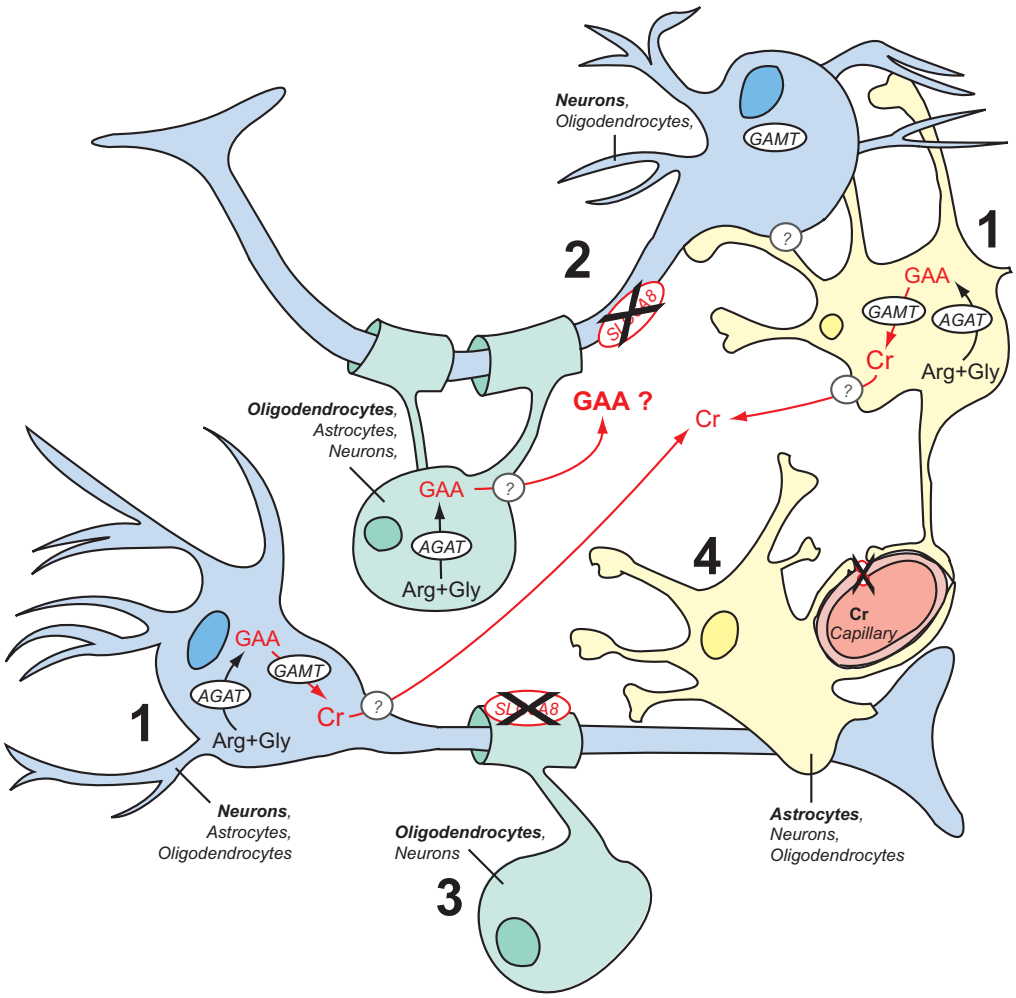
Cr
Astrocytes,
Neurons,
Oligodendrocytes


Neurons,
Oligodendrocytes,
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GAMT
AGAT
Cr
Arg+Gly
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SLC6A8
GAA ?
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Oligodendrocytes,
Astrocytes,
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AGAT
Arg+Gly
?
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GAMT
AGAT
Cr
Arg+Gly
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SLC6A8
Neurons,
Astrocytes,
Oligodendrocytes
Oligodendrocytes,
Neurons
3
Astrocytes,
Neurons,
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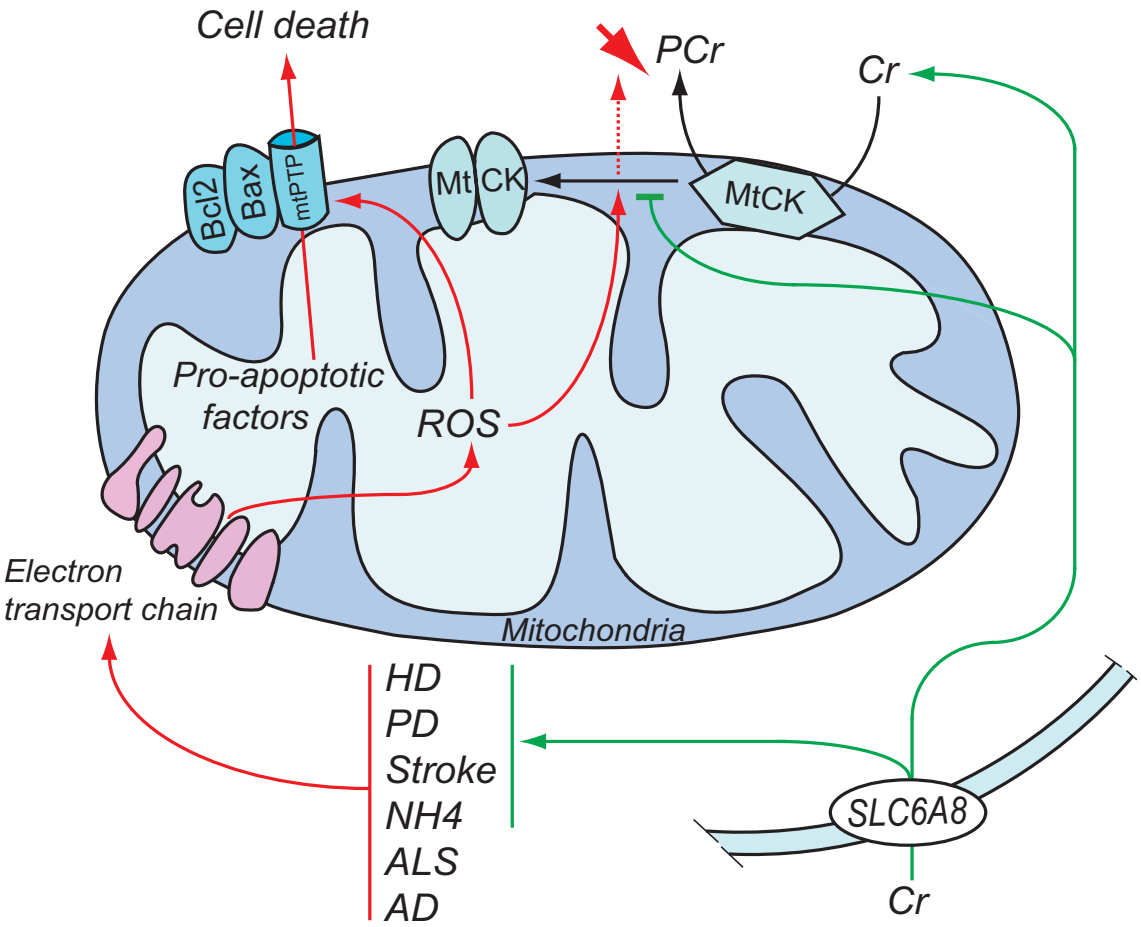
Cell death
PCr
Cr
Bcl2
Bax
mtPTP
Mt CK
MtCK
Pro-apoptotic
factors
ROS
Electron
transport chain
Mitochondria
HD
PD
Stroke
NH4
ALS
AD
SLC6A8
Cr