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Minireview

Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia

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Abstract **Mild to moderate hyperhomocysteinemia is a risk factor for neurodegenerative diseases. Human studies suggest that homocysteine (Hcy) plays a role in brain damage, cognitive and memory decline. Numerous studies in recent years investigated the role of Hcy as a cause of brain damage. Hcy itself or folate and vitamin B12 deficiency can cause disturbed methylation and/or redox potentials, thus promoting calcium influx, amyloid and tau protein accumulation, apoptosis, and neuronal death. The Hcy effect may also be mediated by activating the *N*-methyl-D-aspartate receptor subtype. Numerous neurotoxic effects of Hcy can be blocked by folate, glutamate receptor antagonists, or various antioxidants. This review describes the most important mechanisms of Hcy neurotoxicity and pharmacological agents known to reverse Hcy effects.**

Keywords: Homocysteine; Brain; Folate; Vitamin B12; Dementia; Oxidative stress

1. Homocysteine metabolism in the central nervous system

The role of micronutrients in neuronal development and degeneration has been established [1–4]. Homocysteine (Hcy) is a non-essential sulfur-containing amino acid that is derived from methionine metabolism. Hcy catabolism depends on folate, vitamin B12, and vitamin B6. Therefore, plasma concentration of Hcy may indicate the nutritional status of the B-vitamins [5]. The role of folate in neurolation during early embryonic life has been well documented. Hcy delays the closure of the neural tube in chick embryo by inhibiting the transmethylation pathway [6]. This data has been supported by the effect of folate supplementation in preventing neural tube defects. Deficiency of one of the cofactors in Hcy metabolism can cause impaired myelination and severe neurological damage. A link between Hcy and disorders in the nervous system was first documented in patients with severe cystathionine beta synthase (CBS) deficiency [7,8]. Mental retardation, cerebral atrophy, and seizures have been reported in such patients [7–10].

The remethylation of Hcy into methionine is mediated by methionine synthase and its cofactor vitamin B12. 5-Methyltetrahydrofolate donates a methyl group in this reaction. Hcy remethylation is an important source of methyl groups in the brain [11]. Numerous methylation reactions take place in the brain including synthesis and degradation of neurotransmitters, membrane phospholipids and controlled DNA-methylation. The alternative remethylation pathway of Hcy via betaine-homocysteine methyl transferase seems to be absent in the brain (Fig. 1) [12,13].

The homocysteine transsulfuration pathway is important for Hcy catabolism and is considered a major source of glutathione in the liver. The situation in the brain however, is less clear. Cystathionine beta synthase and cystathionase catalyze the transsulfuration of Hcy into cysteine, the precursor of glutathione. The enzyme CBS has been detected in the human brain [14]. Data about cystathionase in the brain has not been consistent [15–17]. Moreover, there have been few studies that showed large regional variations in cystathionase activity in the brain [16,17]. Moreover, cysteine and, to a lesser degree cystathionine, were utilized by astroglial culture to produce glutathione [18]. In contrast, Hcy and methionine were not able to pass the transsulfuration pathway to be converted into glutathione in astroglial culture [18].

In general, available data does not support a major role for Hcy transsulfuration in the production of cysteine necessary for glutathione synthesis in the brain [11,18,19]. Cysteine is the rate-limiting substrate for the synthesis of glutathione [19,20], which has been shown to be transported via a Na-dependent glutamate transporter in astrocytes [19]. It is likely that cysteine precursors (cystathionine, homocysteine, and methionine) may have a major role for maintaining brain glutathione by maintaining blood cysteine. However, studies are still needed to clarify this view.

Homocysteine transport in the brain has not been fully explored. Early animal studies indicated that Hcy can be transported via a specific saturable receptor in addition to simple diffusion [21–23]. Human neuronal cells are capable of producing Hcy under normal conditions [24]. An increased production of Hcy has been documented in neuronal cells incubated in folate deficient media [24]. These results demonstrated that Hcy can be produced within the brain itself. Regional variations in Hcy metabolism within the brain have not been inves-

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Abbreviations: Hcy, homocysteine; HHCY, hyperhomocysteinemia; AD, Alzheimer disease; Aβ, amyloid beta; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine

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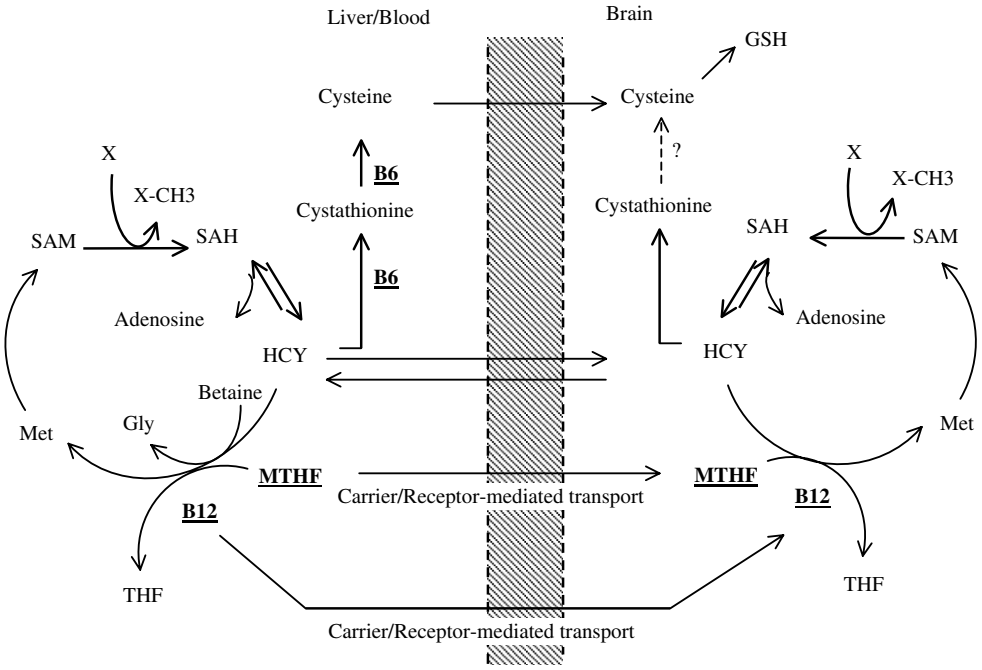

Fig. 1. Homocysteine metabolism. SAM, *S*-adenosyl methionine; SAH, *S*-adenosylhomocysteine; HCY, homocysteine; Met, methionine; Gly, glycine; THF, tetrahydrofolate; MTHF, 5-methlytetrahydrofolate; GSH, glutathione.

tigated. Moreover, Hcy has been shown to compromise the integrity of the blood–brain barrier in a mice model of hyperhomocysteinemia (HHCY) [25]. However, the research done on the ability of Hcy to enter the brain via the blood–brain barrier and the effect of secondary HHCY (renal patients) on brain Hcy has not been sufficient. Current evidence suggests that Hcy can be imported from the plasma into the brain and vice versa probably via specific, bi-directional cellular transporters [21].

Concentrations of Hcy in the brain and cerebrospinal fluid (CSF) are elevated in several neurological diseases [26–30]. Elevation of Hcy in the CSF parallels that in serum; however serum concentrations are 20–100-fold higher than concentrations in the CSF. Antifolate treatment (methotrexate) lowered concentrations of folate and *S*-adenosyl methionine (SAM) and increased Hcy in serial CSF samples [31]. This indicates that a methotrexate neurotoxic effect might be explained by folate depletion and/or increased Hcy in the brain. Furthermore, severe HHCY (>100 μM) in children with CBS deficiency was associated with a 10-fold increase in concentrations of Hcy in CSF [32]. Treating homocystinuric patients with betaine lowered concentrations of Hcy in their plasma and CSF [32]. This data suggests that Hcy may cross the blood–brain barrier probably in both directions. The finding that Hcy compromises the integrity of the blood–brain barrier provided further support to this view [25].

2. Homocysteine is linked to neurodegenerative diseases and aging

Neurodegenerative diseases include a wide group of disorders of various ethologies and clinical features. These diseases share one important feature; that is a disorder in protein structure which can cause deterioration of certain nerve cells or neurons. Dementia, Alzheimer's disease (AD), Parkinson's disease and stroke are examples of neurodegenerative diseases that are common in elderly people. Mild cognitive impairment is a major health problem in elderly people that will progress into dementia in about 50% of the cases [33]. AD is a multifactorial disease that is related to both genetic and acquired factors. The pathological hallmark of AD is the accumulation of neurofibrillary tangles, neuritic plaques, and the insoluble amyloid beta (Aβ) [34,35]. This process occurs 10–20 years before the cognitive decline [36]. Pathological mechanisms involved in neurodegenerative diseases include apoptosis, neuronal death, oxidative stress, overactivation of glutamate receptors, mitochondrial dysfunctions and activation of caspases [37–39]. Brains of patients with AD showed abnormal redox balance and oxidative damage to proteins, and nucleic acids [40,41].

Hcy promotes neuronal degeneration and thus it contributes to psychiatric and age-related neurodegenerative diseases. In the general population, the interest regarding Hcy metabolism in relation to neurodegenerative diseases arises from the fact that plasma concentration of Hcy increases with age [5,42]. Hcy has been implicated as a risk factor for vascular disease as well as brain atrophy [43]. Concentrations of Hcy above 11.9 μmol/L were associated with approximately 3-fold higher risk for white matter damage when compared to concentrations below 8.6 μmol/L [44]. Epidemiological and longitudinal studies suggested a causal link between Hcy and cognitive impairment [45]. This may be due to cerebrovascular as well as to direct neurotoxic mechanisms [46]. Changes of Hcy overtime predicted the decline in memory scores in elderly subjects who were followed for a mean of 6 years [45]. Several follow up studies demonstrated a positive association between Hcy at baseline and a worsening in some measures of cognitive function after several years [47–49].

Elevated concentrations of Hcy (>12.0 μmol/L) and methylmalonic acid (MMA >271 nmol/L) (a functional metabolic marker for vitamin B12 deficiency) are common in the neuropsychiatric population in the absence of haematological manifestations [50]. Numerous studies have confirmed that low concentrations of folate, vitamin B12, and/or B6 are common in demented elderly persons. Serum concentrations of B-vitamins were negatively related to deficits in neurocognitive tests [4]. Moreover, concentrations of Hcy were a stronger predictor of cognitive decline than low vitamin concentrations [51]. Nevertheless, it remains unclear whether Hcy causes dementia, or if it is a surrogate marker for folate or B12 deficiencies, and disturbed transmethylation reactions in the brain.

Despite the fact that in vitro and in vivo studies strongly suggested a causal role for Hcy in the neurological diseases, clinical studies were not conclusive. The association between HHCY (Hcy >12.0 or 15.0 μmol/L) and cognitive decline, dementia or AD was positive in the majority of retrospective and prospective studies [47–49]. However, a causal role can only be confirmed by vitamin supplementation studies. Two issues to be considered in these studies are; first HHCY is one of several other factors that may enhance the total risk. Second, in chronic conditions such as AD, or following acute lesions such as stroke, neurons will degenerate and lost neurons or axons will not be replaced. This may explain that vitamin supplementation did not improve cognitive function in elderly patients with vascular lesions [52]. The role of Hcy as an independent risk factor for stroke has been confirmed by many investigators and recent data demonstrated an improvement in stroke mortality in Canada and the United States after folate fortifications [53]. Large scale-controlled studies are needed before a definite conclusion can be drawn. In general, it seems that vitamin supplementation or higher intake should be initiated at an earlier age, before the clinical onset of dementia.

3. Mechanisms of homocysteine toxicity in the central nervous system

Hcy is toxic to neuronal cells [54–56]. Neurological damage has been reported in mice deficient in CBS enzyme (Cbs −/+ or Cbs −/−), where Hcy increased by approximately 2–50-fold in comparison to wild type mice, depending on the genotype and the type of diet [25,57,58]. These animals show alterations in neuronal plasticity, suffer from severe retardation and die early [59]. Animals exposed to Hcy accumulate this compound in the brain [60], suffer from restricted growth, neural or cognitive dysfunction [60,61], and impaired brain energy metabolism [62]. Moreover, HHCY has been implicated in neural plasticity and neurodegenerative disorders in human studies [63]. This review summarizes the role of Hcy as a neuronal toxin in relation to other biological mechanisms in dementia (Table 1).

3.1. Homocysteine and glutamate receptors

Hcy is an endogenous glutamate receptor agonist [54,64–66] that is prone to act on *N*-methyl-D-aspartate (NMDA) receptor subtype [54,67]. Homocysteic acid, an oxidative product of Hcy, is produced by brain cells and released in response to excitatory stimulation [68]. Homocysteic acid functions as an excitatory neurotransmitter by activating NMDA receptor [69]. The neurotoxicity of homocysteic acid in the brain can be blocked by using a selective NMDA antagonist [66,70].

By binding to NMDA receptor [23], Hcy indirectly enhances calcium influx [54,56,70,71]. Interestingly, in the presence of low (i.e., normal; 10 μmol/L) concentrations of glycine, Hcy acts as a partial antagonist of the glycine site of the NMDA receptor, and inhibits receptor-mediated activity (Hcy role as antagonist or neuroprotective) [54]. The toxicity of Hcy in the presence of normal glycine may be observed when Hcy is at high concentrations (i.e., Hcy = 100 μmol/L). In contrast, when glycine levels increase in the nervous system (after stroke, head trauma, or migraine) [72], a relatively low concentration of Hcy (i.e., Hcy = 10 μmol/L) can be excitotoxic (agonist) by binding and activating NMDA [54,73,74]. These results have suggested that Hcy may contribute to cerebral damage in patients with migraine, after stroke or after ischemia [54]. Therefore, depending on glycine concentrations, Hcy may either block the glycine site of the NMDA receptor or may act as an antagonist at the glutamate site of this receptor [54].

An another line of evidence suggested that Hcy can act via a non-NMDA receptor mechanism or by activating the group I metabotrophic glutamate receptor [73]. Hcy has been shown to induce an extra-cellular signal regulated kinase in the hippocampus. This effect was blocked by three types of glutamate receptor antagonists (NMDA, non-NMDA, and metabotrophic glutamate receptor) [71]. By activating ionotrophic and metabotrophic receptors, Hcy may indirectly increase intracellular calcium levels and activate several kinases [71].

3.2. Homocysteine and oxidative stress

Hcy metabolism is regulated by the redox potential in the cell [75,76]. The activities of several enzymes that mediate the clearance of Hcy are regulated by this oxidative status (i.e., methionine synthase, CBS) [75,77,78]. For example, the activity of CBS is increases under conditions of oxidative stress, thus converting more Hcy into cysteine and glutathione. Disruption of the transsulfuration pathway (Cbs +/−) disturbs redox homeostasis and reduces cysteine levels [57], thus contributing to neuronal damage. In contrast, the activity of methionine synthase is lower in the case of increased reactive oxygen species (ROS). Rats fed a methionine rich diet showed elevated concentrations of Hcy in blood (20 vs. 7 μM in the control rats) and a lowered activity of glutathione peroxidase [79]. Hcy-induced oxidative stress may be worsened in case of a reduced glutathione production.

Hcy itself can undergo autooxidation, thus causing the disruption of redox homeostasis and affecting the redox signaling pathways in vascular and neuronal cells [75,80,81]. Hcy has been found to induce neurological dysfunction via oxidative stress [82,83]. This effect can be explained by enhancing the production of ROS, and oxidative deactivation of nitric oxide. Moreover, Hcy causes brain lipid peroxidation by blocking NMDA receptor [84]. Antioxidant treatment restores several toxic effects of Hcy [84].

The role of oxidative stress in neurodegeneration has been intensively studied. Oxidative stress was one important mechanism for Hcy toxicity in neuronal cells [85]. Hcy directly increased the neurotoxicity of Aβ by inducing oxidative stress [83]. The cytotoxicity of Hcy was mitigated by antioxidants like *N*-acetyl cysteine, vitamin E or C [83,86,87]. Antioxidants (vitamin E or C) also prevented memory dysfunction [86] and ATPase activity caused by Hcy in rats [87]. Other studies showed an effect of folate deficiency on the CNS [85,88]. Folate deprivation induced a marked increase in Hcy and ROS and

Table 1
Summary of some studies testing the neurotoxic effects of HHCY or vitamin deficiency

Authors	Model	↑ Hcy or ↓ B-vitamins	Mechanisms	Neuroprotective agent
Ho et al. [85]	SH-SY-5Y neuroblastoma Human primary cortical neurons	Folate free medium	↑ Hcy in the medium ↑ Cytosolic Ca^{2+} ↑ Aβ ↑ Oxidative stress ↑ P-tau ↑ Apoptosis	DZA (SAH-hydrolase inhibitor), NMDA antagonist
Ho et al. [85]		Hcy 10–250 μM	↑ Calcium influx	NMDA antagonist
Parsone et al. [55]	Neuroblastoma SK.N.SH Medulloblastoma TE 671 Glioblastoma U-87 MG	Hcy 0–200 μM	↑ Cell death	
Robert et al. [71]	Cbs −/− and Cbs +/+ mice	Blood Hcy ≈ 50 fold higher in Cbs −/− mice Brain Hcy 1.7 vs. 0.2 μM/mg cell proteins	↑ Calcium influx ↑ ERK 1/2 activation	NMDA antagonist, Na^{+}-channel blocker, NMDA antagonist
Robert et al. [71]	Mouse hippocampal slices	Hcy 100 and 200 μM for 10–30 min	Hcy as a glutamate agonist (NMDA and non-NMDA receptors)	
Hasegawa et al. [121]	Primary rat cortical neurons	HA 10 nM to 100 μM Hcy 10–100 μM	↓ Extracellular Aβ42 ↑ Intracellular Aβ42	γ-Secretase inhibitor
Vitvitsky et al. [57]	Cbs +/− and Cbs +/+ mice: control diet/↑ Met ↓ folate diet Cbs −/− mice	Mean Hcy 10.6 vs. 5.7 μM Mean Hcy 64.5 vs. 21.4 μM	↓ Brain CBS activity ↓ Brain cystein ↓ Brain glutathione	–
Lipton et al. [54]	Primary rat cortical cultures, mixed neurons/ Glia	Hcy = 5 mM Hcy = 100–150 μM and Glycine = 10 μM Hcy = 100–150 μM and Glycine = 50 μM	↑ Calcium influx	NMDA antagonists, Catalase, SOD, Memantin
Kruman et al. [56]	Primary rat hippocampal cells	Hcy 0.5–250 μM	↑ Apoptosis and necrosis *at Hcy* 250 μM; ↑ DNA-damage ↑ Poly-ADP-ribose polymerase ↑ Caspase-3 activity ↑ P53 immunoactivity ↑ Oxidative stress ↑ Mitochondrial ROS	Poly-ADP-ribose Polymerase inhibitors
Lee et al. [141]	Rats control diet; 3 g/kg Hcy for 3 week; then folate 8 mg/kg for 8 week	Plasma Hcy increased by 400% at 10 week in comparison to animals on the control diet	↑ Damaged vessels ↓ Expression of glucose transporter-1 in the brain	Folate
Baydas et al. [79]	Rats fed a control diet or a diet rich of Met	Plasma Hcy was 20 vs. 7 μM in the Met group versus the control diet	↑ Lipid peroxidation ↓ Glutathione peroxidase ↓ Neuronal cell adhesion molecule Impaired learning and memory performance	Melatonin; lowered Hcy Lowered lipid peroxidation increased neuronal cell adhesion molecule
Baydas et al. [145]	Rat hippocampus	Diet (Met) induced HHCY	Oxidative stress Caspase 3 activation Cytochrome *C* translocation	Melatonin

(*continued on next page*)

Table 1 (*continued*)

Authors	Model	↑ Hcy or ↓ B-vitamins	Mechanisms	Neuroprotective agent
Tjiattas et al. [146]	Chicks primary neuronal cells	Folate deficient media for 2 h or Hcy 250 μM for 2 h	↑ cytosolic Ca^{2+} ↑ Oxidative stress ↓ Mitochondrial membrane potential	DZA (SAH-hydrolase inhibitor), NMDA antagonists, Inhibitors of Ca^{2+} efflux
Jara-Prado et al. [84]	Rat brain synaptosomes	Hcy 5–1000 μM	↑ Brain lipid peroxidation ↑ NMDA receptor stimulation ↑ NOS activation	NMDA receptor antagonists, *N*-acetyl cysteine, Non-specific NOS inhibitors
Pacheco-Quinto et al. [147]	Cbs −/+; APP; PS1 mice	Hcy = 7.9 vs. 12 μM in Cbs +/+ vs. Cbs −/+	↑ Brain Aβ-40 and Aβ-42	–
Shea et al. [107]	ApoE deficient and normal mice Iron induced oxidative stress	Folate supplemented or Folate deficient diet	Oxidative stress in the brain	Folate and vitamin E
Fuso et al. [112]	Human neuroblastoma	Folate and B12 deficient media	Hypomethylation of nt 451–454 in the promoter region of PS1	SAM
Algaidi et al. [60]	Rats	Chronic Hcy injection	Spatial reference memory learning	–
Christie et al. [148]	Rats	Chronic Hcy injection for 4 weeks	hippocampal plasticity and a slow-onset disruption in synaptic transmission	–
Lee et al. [149]	Rat brain	Diet containing 0.3% Hcy	↓ Cerebrovascular eNOS activity ↓ Glucose Transporter-1 ↑ VCAM-1	Dietary folate
Kamath et al. [25]	CBS +/− mice	Vitamin deficient methionin rich diet for 8 weeks	Damage to the blood–brain barrier	–

Hcy, homocysteine; Met, methionine; Cbs, cystathionine beta synthase; APP, amyloid precursor protein; PS1, Presenilin 1; Aβ, amyloid beta; DZA, 3-diazaadenosine; SOD, superoxidedismutase; NOS, nitric oxide synthase.

increased Aβ-induced apoptosis, while folate supplementation prevented the generation of ROS by Aβ [85]. Treatment with the *S*-adenosyl hydrolase inhibitor, 3-deaza adenosine, provides neuroprotection in normal and apolipoprotein E-deficient mice and in cultured neuronal cells deprived of folate and vitamin E and subjected to oxidative challenge [89]. It is however, not known whether the effect of folate deficiency on the brain is independent on or mediated by Hcy elevation.

The transsulfuration pathway represents the metabolic link between antioxidant and methylation metabolism [90]. There is evidence suggesting an antioxidant role for SAM. SAM caused increased glutathione production, lowered lipid peroxidation by almost 65% [91] and prevented neuronal death in an experimental model of ischemia (oxidative stress) [92]. In vivo studies were able to demonstrate an improvement in the blood–brain barrier after transient cerebral ischemia in the presence of SAM [93]. Moreover, chronic SAM treatment (22 months) increased concentrations of glutathione and lowered lipid peroxidation in rat brain [94]. More evidence has been provided by clinical studies where B-vitamins mitigate oxidative damage when administered immediately after acute ischemic stroke [95]. At least some of the neuroprotective effects of SAM can be related to its important role in enhancing the transsulfuration pathway and increasing the production of glutathione.

A new and interesting link has been recently reported between folate deficiency and lowered melatonin production [96]. Melatonin is an indole hormone that is produced in the pineal gland from serotonin via a methyltransferase enzyme (hydroxyl-indole-*O*-methyltransferase) that utilizes SAM as a methyl donor. Melatonin regulates circadian rhythm and has a significant neuroprotective role that is mostly related to its antioxidant effects [97]. Melatonin can directly scavenge free radicals [98,99] and can increase the expression and activities of several antioxidant enzymes [100]. Melatonin prevents oxidative stress and death of neurons exposed to Aβ [101,102]. Recent studies showed that melatonin protects against the neurotoxicity of Hcy by mechanisms related to its antioxidant effects and its ability to modulate apoptosis [79,103]. Oxidative stress and the accumulation of oxidatively damaged proteins increase with age and age-related pathologies. On the other hand, aging is also associated with increased plasma concentrations of Hcy [5,104] and its oxidized form, homocysteic acid. Careful review of the literature suggests that melatonin is probably not effective in managing the cognitive manifestations of dementia [105]. However, melatonin lowered Aβ in

young but not in old animals [106]. This data indicates that Aβ accumulation is probably not reversible later in life.

Another mechanism by which Hcy-associated-oxidative stress can cause neuronal damage, is increased hyperphosphorylated tau (P-tau) protein (discussed below). B-vitamins have been found to modulate the impact of genetic factors on neurodegeneration. In accordance with this, ApoE knockout mice exhibited increased brain oxidative damage and cognitive deficits when they were folate deficient [107,108]. This may imply that patients with certain risk factors (i.e., ApoE4, APP) may be more susceptible to oxidative stress caused by folate deficiency.

3.3. Homocysteine and hypomethylation

HHCY, folate or vitamin B12 deficiency can cause lowered SAM and elevated *S*-adenosyl homocysteine (SAH), the potent competitive inhibitor for methyltransferases. Experimental hyperhomocystemia led to increased concentrations of Hcy and SAH in the brain [109]. The importance of methylation for the CNS has been reviewed elsewhere [63]. Synthesis and catabolism of several neurotransmitters as well as maintaining DNA-methylation are all important biological reactions where the methyl groups are required.

Folate or vitamin B12 deficiency can cause decreased SAM. On the other hand, increased concentration of Hcy is associated with increased production of SAH via the reversible reaction mediated by SAH-hydrolase. A lower ratio of SAM/SAH causes DNA damage and thereby apoptosis, which is one important explanation for Hcy neurotoxicity [56]. In accordance with this, SAM reduced apoptosis (by 50%) that is caused by Hcy in cortical neuronal cells [110]. Supplementing SAM after ischemia improves the blood–brain barrier and neuronal survival [93] and protects against disturbances in brain phospholipids [111].

Hcy can increase neuronal death and DNA damage [56]. Hypomethylation of DNA and altered gene expression are two important mechanisms leading to neuronal damage caused by elevated Hcy. Deficiency of folate or vitamin B12 caused low SAM and DNA-hypomethylation in cultured neuroblastoma cells, an effect mitigated by SAM [112]. Presenilin 1 (PS1) gene expression is one important example that has been tested in relation to methylation [112–114]. PS1 is a γ-secretase that mediates the formation of Aβ from amyloid precursor protein (APP) [112]. DNA hypomethylation causes accelerated APP processing and Aβ production through the upregulation of the PS1 gene. Moreover, exogenous SAM can silence the PS1 gene thus reducing Aβ production. Silencing the PS1 gene could be a target therapy for AD patients [113,114]. These results suggest that treating folate and vitamin B12 deficiency can protect against Aβ accumulation in dementia by supplying methyl groups.

Another important biological reaction where hypomethylation can increase brain damage in AD is the dephosphorylation of P-tau protein. The enzyme protein phosphatase 1 (PPM1) is involved in the regulation of protein phosphatase 2A, the enzyme that dephosphorylate tau protein [115]. The methylation by PPM1 is SAM-dependent; hence reduced methylation capacity can increase P-tau (discussed below).

3.4. Homocysteine, tau protein and amyloid beta

Extensive Aβ deposition in the brain increases as a part of the normal aging process. A prominent feature of the AD brain is the widespread cerebral deposition of Aβ within senile plaques and in cerebral and meningeal blood vessel [116,117]. Concentrations of Aβ are low in CSF samples from AD patients and vascular dementia, and this decrease is related to cognitive decline.

A positive association between plasma concentrations of Hcy and that of Aβ1–40 has been recently reported in aging and neurodegenerative disease [118]. A homocysteine-responsive endoplasmic reticulum protein (Herp) has been recently identified and found to enhance the γ-secretase activity and thereby Aβ1–40 accumulation in the brain [119]. On the other hand, Hcy can be toxic to neurons and can increase their vulnerability to being damaged by Aβ [83,120]. Homocysteic acid results in the accumulation of intracellular and extracellular Aβ 42 in neuronal cells [121]. In vascular smooth muscle cells, Hcy increased Aβ toxicity and caspase-3 activation in a dose dependent manner [122]. To sum up, current data suggests that Hcy accelerates dementia by stimulating Aβ deposition in the brain.

Tau protein is another important protein in the human brain that has been implicated in memory decline and dementia. Tau is an intracellular microtubule-associated protein that participates in forming the neurofibrillary tangles (NFTs). NFTs correlate with cognitive deficits, neurodegenerative disorders and dementia. The physiological function of tau is to facilitate tubulin assembly and to stabilize microtubules. Tau phosphorylation seems to influence its distribution and can modulate its association with plasma membrane. Increased hyperphosphorylated tau (P-tau) may be related to a lower phosphatase activity or to an increased kinases activity (Fig. 2). Increased Aβ may accelerate tau accumulation by activating the kinases that phosphorylate tau (GSK3beta, phosphatidylinositol 3-kinase (PI3K), MAP kinase) [123]. The enzyme protein phosphatase 2A (PP2A) can dephosphorylate P-tau in paired helical filaments, making tau detached and accessible to proteolysis [124]. Factors that influence the activity of kinases (decrease) or phosphatases (increase) may therefore hold a therapeutic potential for AD.

Several studies have shown that the expression or the activity of PP2A was reduced in brain tissues from AD patients in comparison to controls [125,126]. The expression of the PP2A protein was also low in fibroblasts from AD patients [127] although mRNA PP2A was increased. These results suggest a failure in post-translational modifications or in the stability of the enzyme.

A possible link between AD and reduced PP2A protein methylation in hyperhomocysteinemia has been hypothesized [128]. The attachment of the catalytic subunit B to the methylated subunit C of the enzyme is essential for the activity of the enzyme [129]. Low concentrations of SAM or a low SAM/SAH ratio results in a lower activity of PP2A and increased P-tau [128,130]. Accordingly, the protein level of phosphatase methyltransferase 1, and that of the methylated C subunit of PP2A were approximately 40% lower in frontal and temporal extracts from AD patients in comparison to that of the controls [126]. In addition, PP2A protein activity and gene expression were markedly reduced in the brains of AD patients [125]. As a whole, this data strongly suggests that alterations in SAM-metabolism or SAM/SAH ratio may contribute to the ethiology of AD by inhibiting dephosphorylation of tau.

Experimental folate deficiency in neuronal cells has provided additional evidence. Folate deprivation in neuroblastoma cells, induced a marked elevation in Hcy level and ROS, in addition to an increase in the immunoreactivity of P-tau by 66% [85]. The increment of P-tau was reversible after adding folate to the cultures [85]. These results suggested that P-tau accumulates in folate deficiency, but this phenotype is probably revers-

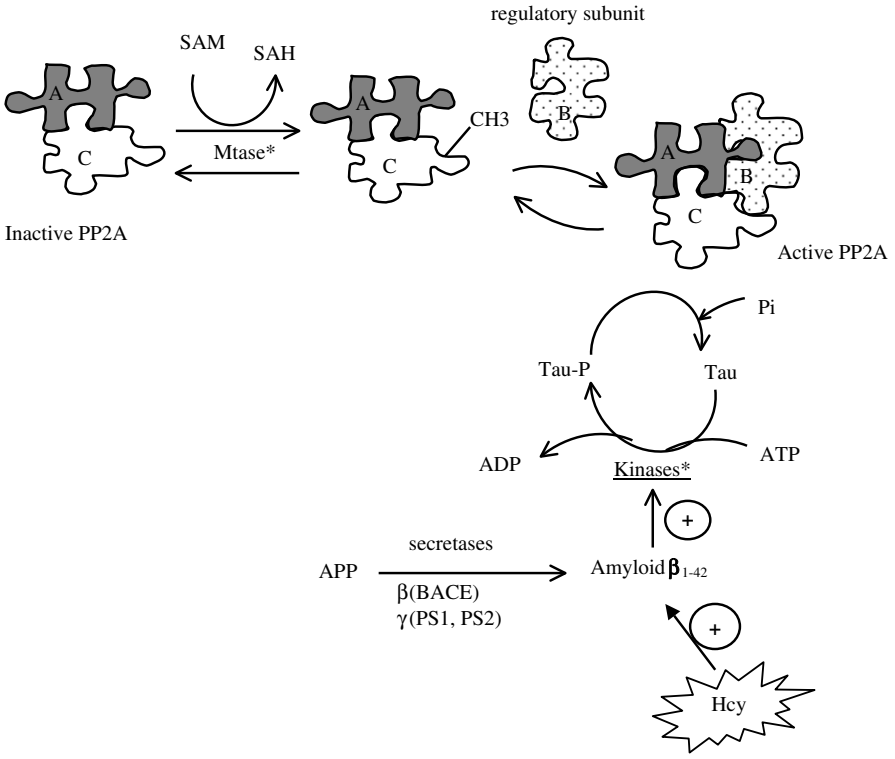

Fig. 2. The role of SAM, and Hcy in phospho-tau and amyloid beta metabolism. PP2A, protein phosphatase 2A; Tau-p, phosphorylated tau; PS, presenilin; Mtase, methyltransferase; APP, amyloid precursor protein. * kinases are; GSK3-beta, phosphatidylinositol 3-kinase (PI3K), MAP kinase.

ible, at least in short term deficiency. Because reducing P-tau might lead to improved memory function [131], Hcy-lowering treatment may improve memory function via reducing P-tau.

3.5. Miscellaneous

Oxidative modifications of thiol residues in functional proteins predispose these molecules to homocysteinylation [132–134] thereby causing structural and functional disruptions. Homocysteine thiolactone is a highly active thioester that is formed by an enzymatic conversion of Hcy in error editing aminoacyl-tRNA [135]. Homocysteine thiolactone can react with some cellular molecules and modify their structure and functions [136,137]. Possible structural modifications of some functional proteins by Hcy in the central nervous system have not been tested.

Hyperhomocysteinmeia is associated with increased plasma concentrations of asymmetric dimethylarginine (ADMA), a potent endogenous inhibitor of nitric oxide (NO)-synthase. A recent study suggested that Aβ enhances the production of NO and mitochondrial dysfunction as well as apoptosis [138]. NO plays a significant role in mediating neuronal death. Therefore, ADMA may play a role in brain aging and Alzheimer's disease by regulating NO synthesis [139]. However, this data was not confirmed by all studies [140].

Homocysteine has been linked to vascular damage. This effect could be related to lowering the bio-availability of endothelial NO which is a potent vasodilator. This is in agreement with the finding that folate supplementation in animals can reduce the endothelial damage in brain microvasculator [141].

Mild to moderate HHCY can be a secondary manifestation in patients with Parkinson disease treated with L-dopa [142]. Increased concentrations of Hcy in those patients can be explained by *O*-methylation of L-dopa by the enzyme catechol-*O*-methyltransferase, a SAM-dependent enzyme, that is up-regulated in L-dopa treated patients [143]. Although HHCY can be a secondary manifestation in patients with Parkinson disease, it is thought to be a risk factor for vascular diseases and cognitive impairment or dementia in those patients. Vitamin treatment lowered plasma concentrations of Hcy by about 40% in L-dopa treated patients and this treatment could be preventive in those patients [142].

Other mechanisms that have been reported are; impairment of brain energy metabolism and the inhibition of Na^+, K^+-ATPase activity caused by the accumulation of the metabolites in patients with homocysteinuria [62,144].

After reviewing the current literature, a few critical points should be considered. First, most studies that have been conducted on cultured neuronal cells have utilized supraphysiological concentrations of Hcy (Table 1) which may not resemble the situation even in HHCY subjects. Second, because Hcy is an intracellular product, and cells incubated with high concentrations of Hcy may incorporate only a limited amount of that added to the culture, results from various studies can not be generalized to the case of HHCY patients. Third, most animal based studies have used dietary manipulations or genetically modified animals. Metabolic and genetic differences between human and rodents may limit the extrapolation of the data between these two species. Other critical points in using mice models of HHCY have been reviewed elsewhere [58].

4. Summary and conclusions

Mild to moderate hyperhomocysteinemia (Hcy 15–50 μmol/L) is an established risk factor for neurodegenerative diseases.

HHCY (Hcy 15–50 μmol/L) can be acquired in the case of cofactor deficiency (vitamin B12, B6, and folate), increased requirements of the vitamins, certain medications, or diseases (i.e., renal failure). Inherited genetic disorders related to Hcy metabolism, folate and vitamin B12 transport or metabolism cause more severe HHCY (Hcy = 70 –100 μmol/L) (methionine synthase, CBS, or transcobalamin deficiency). Various Hcy lowering treatments have been tested and clinical improvements have been documented, especially in cases where the intervention started at an early stage of the disease.

A causal link between HHCY and disorders in the central nervous system has been suggested. However, available results from treatment studies are very limited. Large controlled studies are required. Nevertheless, by rationalizing the available results, prevention seems more feasible than treatment and early intervention seems crucial in order to achieve a significant effect. Therefore, increasing vitamin intake in non-demented elderly people is recommended.

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