Betaine - the Dark Knight of the brain

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Abstract

The role of betaine in the liver and kidney has been well documented, even from the cellular and molecular point of view. Despite literature reporting positive effects of betaine supplementation in Alzheimer’s, Parkinson’s, and Schizophrenia, the role and function of betaine in the brain are little studied and reviewed. Beneficial effects of betaine in neurodegeneration, excitatory and Inhibitory imbalance, and oxidative stress in the central nervous system have been collected and analyzed with the aim of understanding the main role of betaine in the brain. There are many “dark” aspects needed to complete the picture. The understanding of how this osmolyte is transported across neuron and glial cells is also controversial, as the expression levels and functioning of the known protein capable to transport betaine expressed in the brain, betaine-GABA transporter 1 BGT-1, is itself not well clarified. The reported actions of betaine beyond BGT-1 related to neuronal degeneration and memory impairment are the focus of this work. With this review, we underline the scarcity of detailed molecular and cellular information about betaine action. Consequently, the requirement of detailed focus on and study of the interaction of this molecule with CNS components to sustain the therapeutic use of betaine.

Focused Review

Betaine - the Dark Knight of the brain

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**Keywords**
Betaine, GABA, neurodegeneration, oxidative stress, Alzheimer’s, Parkinson’s, dementia, epilepsy, schizophrenia, PTSD

**Introduction**

Betaine, also known as glycine betaine and N,N,N trimethyl glycine, is an osmolyte found across animals, plants, and microorganisms. It is a zwitterionic amino acid derivative that can be endogenously produced by the oxidation of choline[1], and exogenously absorbed as a dietary nutrient. The name betaine comes from its discovery from *beta vulgaris* (beets) in the 1860s, but later it was found at high concentration in other dietary sources like wheat bran, spinach, and seafood[2-4]. In mammals, from the physiological point of view, betaine serves primarily two roles: as one of the major osmolytes accumulated in the tissues for cell volume regulation, mainly in kidney, and as a methyl donor for the toxic metabolite, homocysteine (Hcy), to convert it into methionine [1,4]. The daily betaine uptake in human diet ranges from 1-2.5g/day, based on individual consumption. The study on red blood cell physiology at high betaine doses showed mild perturbance and suggested safe daily betaine intake was 9-15g/day [2]. The active absorption of betaine across the enterocytes is thought to be via sodium/chloride dependent amino acid transport system (SLC36A1, SLC36A2), and also passive sodium independent [1,2,5,6]. A rapid adsorption and distribution up to 1-3mM within 1-2h of intake was recorded in human studies on betaine supplementation [2,7]. However, the tissue concentration for an osmolyte, as expected, is higher than plasma concentration [8]. Apart from diet and supplementation, betaine can be synthesized via a two-step irreversible process using choline in mitochondria (see figure 1). Firstly, the enzyme choline dehydrogenase oxidates choline into betaine aldehyde. And then, betaine aldehyde is converted to betaine by the same enzyme in the presence of nicotinamide adenine dinucleotide (NAD$^+$). This betaine is catabolized via transmethylation reactions involved in vital biological processes [2]. This transmethylation is catalysed by betaine-homocysteine methyltransferase (BHMT), which detoxifies Hcy by converting it into methionine and producing S-adenosylmethionine (SAM).

*Figure 1 near here*

**The dual role and distribution**

Betaine helps maintain the intracellular osmotic pressure, as it binds little to nothing with protein surfaces and enables cellular control of water surface tension. Thus, it stabilizes protein structure and function, while protecting cells, proteins, and enzymes from osmotic stress. This role is relevant, especially in the kidney, where betaine can be present in extraordinary concentrations (>100mM) [1]. Other than kidney, betaine is also found in human liver and brain. However, the role of osmolyte and methyl donor has been studied in liver and kidney, and much less in the nervous system. Moreover, recently it was shown that BHMT, one of the enzymes involved in the detoxification of homocysteine, is present not only in liver and kidneys, but it is also expressed in the intestine and in white adipose tissue suggesting a role of betaine also in these tissues [4,9,10].

Betaine is a potential therapeutic against alcohol-induced and metabolic associated diseases, and heavy metal toxicity in liver [4,11]. Betaine supplementation was also shown to have a role in muscle strength and power [12]. It helps to improve body composition in both males and females, but improvement of muscular performance only in males has been reported [13]. Betaine also helps against heat tolerance and increases

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resilience against thermal stressors[14]. At the same time, the increasing number of studies showing beneficial effects of betaine in cognition, early-stage neuronal development, and in reducing neurodegeneration and memory impairment, suggesting an important role of betaine in human (neuro)physiology[7].

Betaine: a therapeutic nutrient

Traditional eastern medicines have effectively used herbs and food ingredients as therapeutics for several different diseases. The primary advantage of such substances over modern medicine would be the absence of any severe side effects. One approach by modern pharmacologists has been to integrate these herbs and nutrients with currently effective drug administration and develop new therapies. Betaine is one such stable, natural, and non-toxic substance that has shown beneficial effects in several diseases.

Homocystinuria, a sulphur metabolism pathway disorder characterized by increased accumulation of homocysteine in cells and plasma, can cause osteoporosis, arteriosclerosis, dislocated eye lenses, intellectual disability, and neurodegenerative pathologies such as Alzheimer’s, Parkinson’s, and dementia[15]. As a treatment, betaine therapy (6-9 g/day of oral administration) is used to decrease Hcy levels by converting it to methionine, and thus increasing the flux through re-methylation pathway. Since 2020, betaine is an FDA approved drug marketed as Cystadane®. This treatment has not been reported to cause any severe side effects except mild body odour and a rare possibility of cerebral edema due to hypermethioninemia[15,16]. Apart from homocystinuria, betaine supplementation also shows beneficial effects on diseases such as alcohol-induced liver diseases, hepatic steatosis, heart disease, dehydration, heat tolerance etc[1,2,4,12,14,17]. The positive role of betaine is not limited to the diseases involved with liver and kidney; increasing number of papers and studies demonstrate the beneficial role of betaine in the brain as well. However, the positive effects in the CNS are very little understood and reviewed. In this work, we present an overview of papers highlighting the potential therapeutic role of betaine in neuronal disease and disorders and when known the cellular or molecular mechanism involved.

The presence of betaine in the brain

The betaine/GABA transporter 1 (BGT-1), a member of the solute carrier family 6, can transport γ-aminobutyric acid (the primary inhibitory neurotransmitter in the CNS)[18] and also betaine across the blood-brain barrier[7]. Compared to liver and kidneys, the reported amount of betaine in the brain is relatively low[8,19].

Since the relationship between blood plasma concentration and tissue accumulation of betaine is not very related, there are some anomalies in the reported betaine blood plasma concentration (1-3nM)[8,20-22]. Knight et al. have shown time, dose, and osmolarity-dependent betaine accumulation in the hippocampal tissues of mice[7]. They showed that the active betaine accumulation also affects the accumulation of other osmolytes in nervous tissues. Under isotonic conditions, betaine significantly reduces the accumulation of creatine, taurine, myo-inositol, but not glutamate. On contrary, under hyperosmotic conditions, betaine increases the accumulation of glycine and glutamate. Also, to be noted that the betaine intracellular accumulation reaches at peak (8h post first exposure) around 12mM, which is four times higher than the given extracellular concentration (3nM). This work suggests that apart from being an osmolyte and serving as a methyl donor, betaine could also influence GABA production/recycling and GABAergic pathways, which resonates with the findings of Kunisawa et al that showed mediation of GABAergic pathways by betaine[23].

Effects of betaine against neurological diseases and disorders

To have healthy physiological functioning and stable control of neuronal circuits, the excitatory/inhibitory (E/I) balance of the brain must be maintained. Since E/I ratio is essential to maintain and regulate signalling transmission, the inhibitory system represents a key point to re-stabilize the neural network function when a
predominance of excitation over inhibition rises, as in brain disorders[24]. GABA is the primary inhibitory neurotransmitter in the adult CNS and the imbalance in its levels can be related to many neurodevelopmental and neurodegenerative diseases such as autism spectrum disorder (ADS), Schizophrenia, epilepsy, depression but also Parkinson’s and Alzheimer’s disease etc[25].

Figure 2 near here

Role in epilepsy

The GABA transporters regulate GABA synaptic concentration by the cellular uptake and regulate GABA activity, consequently are often predominant targets for the antiepileptic and anticonvulsant drugs[26]. Since the majority of GABA uptake in the CNS is done by GABA transporter 1 (GAT1), it has been a pharmaceutical target to treat disorders related to GABAergic imbalance. The FDA-approved antiepileptic drug Tiagabine is a selective inhibitor of GAT1[27]. The synergic anticonvulsant effect of tiagabine with the selective inhibitor EF1502 on GAT1 and BGT1 both raised a functional role of BGT-1 in regulating diffused GABA from synaptic regions[28,29]. However, the seizure threshold experiments on BGT-1 knock-out mice showed no alteration and ruled out a role of BGT-1 in seizure susceptibility[30]. Given the limitations of this study, it is possible that the BGT-1 still might be playing a role in epileptic seizures and GABAergic imbalance in CNS. Also, the great uncertainty in the localization of BGT-1 in the brain and in cell cultures also questions the role of betaine in the brain[19].

Role in stress-related disorders

The stress-induced psychiatric disorders like depression, anxiety, and post-traumatic stress disorder (PTSD) are associated with abnormalities in GABAergic neurotransmission functioning[25,31,32]. The study of water-immersion restraint strain (WIRS) induced stress (in mice) resulting in memory impairments showed amelioration by betaine[23]. This improvement could be inhibited by antagonists of BGT-1, GABA_A, and GABA_B receptors. Also, the betaine treatment post-WIRS, significantly decreased the expression of GABA transaminase (GABA-T), the enzyme responsible for breaking down GABA when not needed. GAT1, GAT3, and BGT-1 expressed in astrocytes regulate GABA levels[33], and inhibition of GABA-T also increases GABA levels in synaptic cleft[34]. As betaine is transported by BGT-1 and decreases GABA-T expression, betaine could be asserting its positive effects by changing GABA levels in the CNS. Thus, betaine does not work only as a substrate of BGT-1, but it could also interact largely with the entire GABAergic system.

In psychology, social defeat is seen as a form of stress that could cause depression, anxiety, PTSD etc[35]. The resilience against such stress can be mediated by adaptive changes in neural circuits of neurotransmitters (like GABA) and molecular pathways[36]. Anhedonia is one of the main symptoms of stress-related disorders. While studying the role of brain-gut-microbiota axis in such disorders, it was found that the mice subjected to chronic social defeat stress (CSDS), when given betaine supplementation showed resilience to anhedonia. This indicates that betaine supplementation could be used as a prophylactic nutrient to prevent or minimize the relapse by stress in patients of such psychiatric disorders[37].

Neuroprotective role against Alzheimer’s and dementia

Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease that is characterized by progressive impairment of cognition, memory, and intellectual functions. So far, there have not been many approved agents that ameliorate cognition and the overall function of AD patients, and different therapeutical approaches have been developed. It is known that the amyloid-ß (Aß) aggregation play an essential role in neuronal degeneration in AD; hence, it is an important biomarker in AD-like pathologies[38]. Aß is generated by amyloid precursor protein (APP) via sequential cleavage from β- and γ-secretases. The generation and deposition of Aß has been associated with altered oxidative stress, inflammation, tau phosphorylation and synaptic dysfunction, contributing to the progression of the disease and eventually leading to death. Also, the increased levels of Hcy have been associated with the onset of AD, along with hyper-homocysteinemia[39].
As betaine is an effective methyl donor to convert Hcy to methionine, a therapeutic approach is being developed to use betaine supplementation to target increased Hcy and cascade the AD progression. Chai et al have shown that betaine supplementation ameliorates AD-like Hcy-induced memory deficits, enhances long-term potentiation, and increases dendritic branch numbers and density of dendritic spines[40]. It also attenuated the tau phosphorylation and Aβ accumulation by altering APP processing[40,41]. In a study, Sun et al demonstrated that subjects treated with betaine supplementation showed amelioration in cognitive deficit resulting in better recall of words, improved visual-spatial capacity etc. [42]. Their results showed betaine supplementation (200 μg/kg for a month) reverses Aβ accumulation and stimulates the regulation of memory-related protein (NR1, NR2A, and NR2B). Overall, betaine has been suggested to be an effective therapeutic tool to treat AD and could be combined with other drugs to provide a successful therapy.

Vascular dementia (VaD) is the other most common type of dementia in aged people after AD and lacks effective therapy. Chronic cerebral hypoperfusion (CCH) is thought to be the primary reason behind cognitive impairment in VaD patients. A study of betaine administration in rats showed that memory deficits induced by CCH were ameliorated[43]. The CCH-induced synaptic protein loss was restored and oxidative stress was suppressed by betaine. This experimental evidence yet suggests a therapeutical application of betaine in neurodegenerative disease[43].

**Protective role against Parkinson’s**

Parkinson’s disease (PD) would be the second most prevalent neurodegenerative disease (only after AD) characterized by uncontrolled muscular activity, an increase in the metabolic concentrations of sulphate and nitrate compounds, a decline in dopamine levels due to neuronal degeneration, and sleep disturbance. In PD patients, oxidative stress in the brain is evident and this stress causes oxidative damage[44]. The dopaminergic drug laevo-3-4-dihydroxyphenylalanine (Levodopa, LD) reduces the effects of PD effectively and is prescribed prominently. However, LD administration also increases plasma levels of Hcy[45]. To cross the blood-brain barrier and to avoid peripheral toxicity, LD is administered with a dopa decarboxylase inhibitor such as benserazide, which by catechol-o-methyltransferase elevates Hcy levels further. The clinical and experimental trials in PD patients show that a high accumulation of Hcy could contribute to accelerated neurodegeneration and the onset of atherosclerotic and neuropsychiatric symptoms[46,47]. Hence, such treatment overall poses a risk of hyper-homocysteinemia in PD patients leading them towards other neurodegenerative diseases such as AD and dementia[48].

Alirezaei et al studied the effects of betaine administration on oxidative stress and increased Hcy levels induced by LD/benserazide treatment of PD patients[49]. They demonstrated the neuroprotective qualities of betaine against LD-induced oxidative stress in the brain tissues of rats. Betaine could elevate antioxidant levels and decrease lipid peroxidation and Hcy levels. Also, the inhibitory effects of betaine on the neurotoxic nitric oxide in microglial cells show the effectiveness of betaine as a therapeutic against neurodegenerative diseases and suggests that betaine would be useful for reducing NO-dependent inflammation in the brain[50].

Rotenone is an inhibitor of mitochondrial complex I, breaks ATP production and by enhancing the production of mitochondrial ROS causes apoptosis, inducing neurotoxicity. It is widely used as a model of the pathogenesis of PD. Neuronal cell death is one of the major factors behind cognitive decline in AD and PD. It was demonstrated that betaine performs neuroprotective effects against rotenone-induced neurotoxicity in PC12 cells[51]. The increasing oxidative stress and inflammation in the brain can cause brain ischemia and ischemic stroke. The betaine treatment of PC12 cells (with oxidative stress induced by H2O2) resulted in decreased pro-inflammatory cytokine production and reduced oxidative stress[52]. Betaine also increased the expression of antioxidative enzymes and nonenzymatic genes. These results showcase how betaine can and should be considered as a protector against oxidative stress and neurodegeneration.
A therapeutic agent for Schizophrenia

Schizophrenia has always had sleep dysfunction as one of its primary descriptions\cite{53}. The sleep pressure, the driving force of the homeostatic process, builds up during wakefulness and dissipates when asleep. Sleep pressure and sleep disturbance are associated with onsets for patients with psychosis. It is shown that with increasing high sleep pressure, specific metabolomic alterations occur like decreased levels of betaine in the whole brain\cite{54}. Hence, betaine is proposed as one of the biomarkers for the diseases and treatments associated with sleep deprivation.

The neurons in schizophrenic brains tend to undergo gross morphological changes. The neuronal morphogenesis-related traits are significantly alleviated by a high-betaine diet, suggesting that betaine through a neuroprotective mechanism could be effective for refractory Schizophrenia patients\cite{55}. Also, in CHDH (a gene for betaine synthesis) deficient mice the remnants of schizophrenia-related molecular perturbations in the brain were recorded. It was shown that betaine supplementation induced improvements in cognitive performance dependent on genetic background\cite{56}. With such results, betaine has been recommended as a psychotherapeutic for patients with schizophrenia.

Discussion

Given its dual role, betaine has always been considered an important nutrient for human physiology. But the evident growing literature highlights a possible third role: the positive effects of betaine in CNS disorders. The betaine in the liver and kidney has been well studied, reviewed, and understood. Despite the reported benefits of betaine supplementation in improving brain conditions, the mechanism of action yet is not clear.

In this review, we highlight the positive role and therapeutic potential of betaine in brain-associated diseases like AD, PD, dementia, Schizophrenia, depression, PTSD, epilepsy, and anhedonia reported in recent literature. Although BGT-1 can actively uptake betaine in the brain, the little expression and lack of understanding around its role in CNS raise questions over the mechanism behind accumulation and its impact on the brain. Kunisawa et al showed that the interaction of betaine with GABAergic pathway could not be limited to just BGT-1\cite{23}. Their results indicate a possible betaine interaction with GABA\textsubscript{A} and GABA\textsubscript{B} receptors. The work from Ibi\cite{57} et al supports this hypothesis by showing that the prevention of cognitive impairment by betaine is mediated by BGT-1, but not its antioxidant effects. Despite the lower and questionable expression of BGT-1 in the brain\cite{19}, the beneficial effects of betaine appear to be asserted via active transport by BGT-1. Although these effects are not solely driven by BGT-1 and involvement of some other mechanisms is highly possible.

Under hyperosmotic conditions, betaine can significantly influence the uptake of glycine and glutamine\cite{7}. Since glycine is a precursor to GABA and glutamine to glutamate, a possible role for betaine in maintaining the balance between inhibitory and excitatory neurotransmission cannot be denied. Also, the effects of betaine against stress-induced diseases and memory loss indicate a connection with the GABAergic pathway in CNS.

Betaine certainly has neuroprotective properties that prevent the progression of neurodegenerative diseases like AD, PD, dementia, and depression. Along with choline, folic acid, vitamin B6, and B12, betaine in the maternal diet is correlated with early neuronal development and attenuation of cognitive function at the later stage of life\cite{58}. One way betaine helps is by reducing the Hey levels in the neurons and promoting the expression of memory-related proteins\cite{40,41}. Also, it can convert homocysteine to methionine and increase SAM, which protects the brain against a variety of toxic agents causing oxidative stress\cite{59}.

As a therapeutic, betaine is already in use as an FDA-approved drug to treat homocystinuria. The reported side effects (for 6-9g/day) are relatively mild such as gastrointestinal illness, mild body odour, increased urination, feeling dry mouth, preference for salty food etc\cite{60}. The excess of betaine is also associated with cardiovascular disease and pulmonary hypertension\cite{61,62}. While there are very few side effects
recorded for betaine[63, 64], there is still not enough information on the long-term effects of regular betaine supplementation.

Conclusion

Although betaine is not considered an essential osmolyte, its beneficial and therapeutic roles in human physiology make it important in the diet. The role of betaine in diseases related to liver and kidney is better understood. The reported effects of betaine in the past two decades confirm its neuroprotective and antioxidative qualities with a positive correlation with the GABAergic pathway, but very little is known about the mechanisms using which betaine asserts these effects. Hence, with a protective role and very little information about its working methods, betaine is indeed the dark knight of the human brain.

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Conflict of interest

The authors declare that there is no conflict of interest.

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**Figure Caption**

**Figure 1: Cellular metabolism of betaine.** Betaine is found in beets, wheat bran, seafood, and spinach. Once it enters the cell through betaine/GABA transporter 1 (BGT-1- SLC6A12) and proton/amino acid transporters (PAT1 and PAT2, SLC36A1 and A2 respectively), it is metabolized according to the methionine cycle to synthesize methionine from homocysteine. Also, in the mitochondria, betaine can be directly synthesized from choline in small quantities. All the pathway enzymes refer human metabolism: Choline dehydrogenase (CDH); Betaine-aldehyde dehydrogenase (BADH); Betaine-homocysteine methyltransferase (BHMT); Methionine synthase (MS); Methionine adenosyltransferase (MAT).

**Figure 2: Schematic for the role of betaine in the brain.** Betaine plays an active role in neuroprotection and against oxidative stress in cells. In neurodegenerative disease as Alzheimer’s, Parkinson’s, and in Schizophrenia, betaine can be a possible therapeutic. Although betaine is actively regulated by BGT-1 in GABAergic pathways, the complete mechanism behind to be determined.