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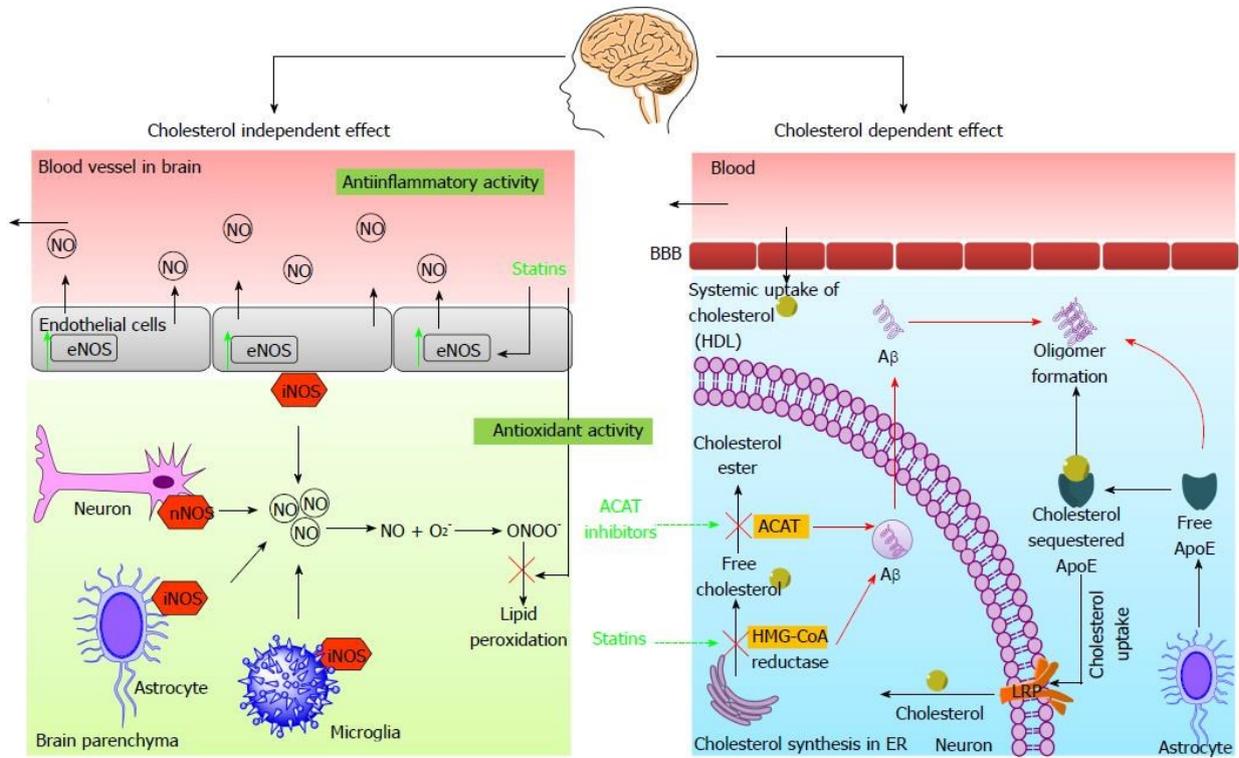
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ABSTRACT

Vitamin C, or ascorbic acid, is important as an antioxidant and participates in numerous cellular functions. Although it circulates in plasma in micromolar concentrations, it reaches millimolar concentrations in most tissues. These high ascorbate cellular concentrations are thought to be generated and maintained by the SVCT2 (Slc23a2), a specific transporter for ascorbate. The vitamin is also readily recycled from its oxidized forms inside cells. Neurons in the central nervous system (CNS) contain some of the highest ascorbic acid concentrations of mammalian tissues. In addition to its well known role as an antioxidant, the vitamin serves as a co-factor in several important enzyme reactions, including those involved in the synthesis of catecholamine, carnitine, cholesterol, amino acids, and certain peptide hormones. Intracellular ascorbate serves several functions in the CNS, including antioxidant protection, peptide amidation, myelin formation, synaptic potentiation, and protection against glutamate toxicity. The importance of the SVCT2 for CNS function is supported by the finding that its targeted deletion causes widespread cerebral hemorrhage. Once in cells, it is rapidly reduced to ascorbate. The highest concentrations of ascorbate in the body are found in the brain and neuroendocrine tissues such as adrenal, although the brain is the most difficult organ to deplete of ascorbate. Combined with regional asymmetry in ascorbate distribution within different brain areas, these facts suggest an important role for ascorbate in the brain. Neuronal ascorbate content is maintained by this protein also has relevance for human disease, since ascorbate supplements decrease infarct size in ischemia-reperfusion injury models of stroke, and since ascorbate may protect neurons from the oxidant damage associated with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. The aim of this review is to assess the role of the SVCT2 in regulating neuronal ascorbate homeostasis and the extent to which ascorbate affects brain function and antioxidant defenses in the CNS. In all of its known functions, ascorbate serves as a one-electron donor, generating the ascorbate free radical (AFR) (Fig 1). The AFR is reduced back to ascorbate within cells by NADH- and NADPH-dependent reductases that have a high affinity for the low concentrations of the radical generated. If the AFR accumulates significantly in areas not accessible to these enzymes, or if its concentration exceeds their capacity, two molecules of the AFR react or dismutate to form one molecule each of ascorbate and dehydroascorbic acid (DHA).

Keywords: Brain SVCT2 Ascorbate transport Dehydroascorbate Neurons Glutamate



INTRODUCTION

The trans-plasma membrane gradient of ascorbate is generated by specific ascorbate transporters that were first cloned in 1999. Two isoforms of these transporters are known, which although similar in amino acid sequence and structure, have different tissue distributions. Urinary loss of ascorbate decreases ascorbate concentrations in plasma and in most tissues, but not the brain. The SVCT2 is especially important in brain, as discussed later. The nomenclature for both isoforms has evolved since cloning of the protein in 1999. Once DHA has entered cells, it is rapidly reduced to ascorbate. First, DHA concentrations in blood plasma are 2 μM or less, whereas ascorbate concentrations are 40-60 μM. Second, glucose will compete in most cells for uptake of this low concentration of DHA on the GLUT. Third, and perhaps most compelling, ascorbate concentrations are low in cells lacking the SVCT. Two examples highlight this point. First, ascorbate levels are the same in erythrocytes and the plasma from which they were taken. This is despite the observations that human erythrocytes express high numbers of GLUT1. and that glucose does not compete well for DHA uptake in this cell type. Key here is that failure to concentrate ascorbate in human erythrocytes corresponds to lack SVCT proteins. The second example is that knockout of the SVCT2 results in almost undetectable ascorbate concentrations. Nonetheless, DHA uptake and reduction could well preserve intracellular ascorbate over short time periods in areas of high oxidant stress, where significant amounts of extracellular ascorbate are oxidized. Vitamin C may be more than simply a “micronutrient” in the central nervous system (CNS), since it is present in millimolar concentrations in neuron-rich areas. There are two novel aspects of how ascorbate enters the CNS that distinguish its uptake from that seen in other organ systems (Fig.2). First, although ascorbate transport across the blood-brain barrier occurs, it is very slow, and second, the ability to maintain a steep ascorbate concentration gradient from blood to neuronal cells is generated by a two step mechanism: first into the cerebrospinal fluid (CSF), and then into the brain cells.

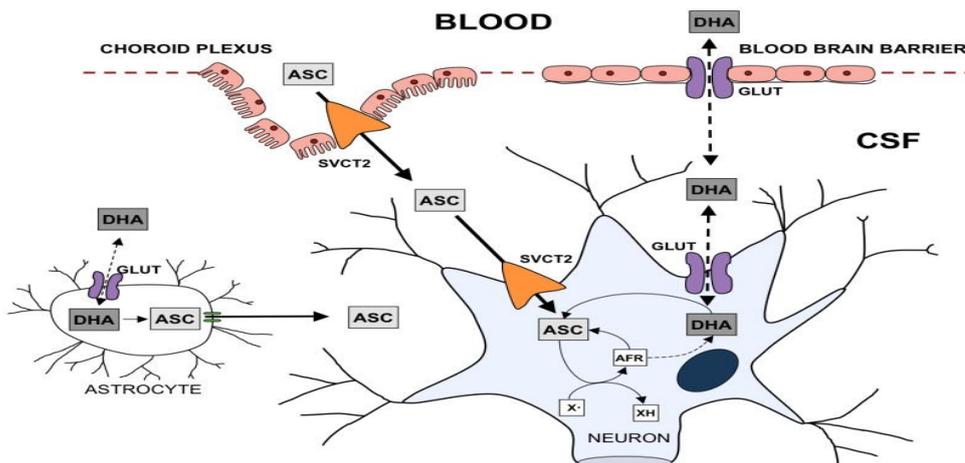


Figure-2

Ascorbate uptake and metabolism in the CNS

SVCT2 mRNA has been demonstrated by in situ hybridization in the choroid plexus epithelium, where it mediates high affinity and sodium-dependent ascorbate transport across the basolateral membrane into the cells. Intracellular ascorbate then exits the cells into the CSF across the apical membrane of the choroid plexus ependymal cells although it is not known how this occurs. In humans CSF ascorbate concentrations tend to be slightly lower at 160 μM but still show a pronounced gradient from plasma values of 40-60 μM . These ependymal modified glial cells showed high affinity, sodium-, and energy-dependent ascorbate uptake in culture. However, they were not thought to move ascorbate from the CSF into the brain interstitium.

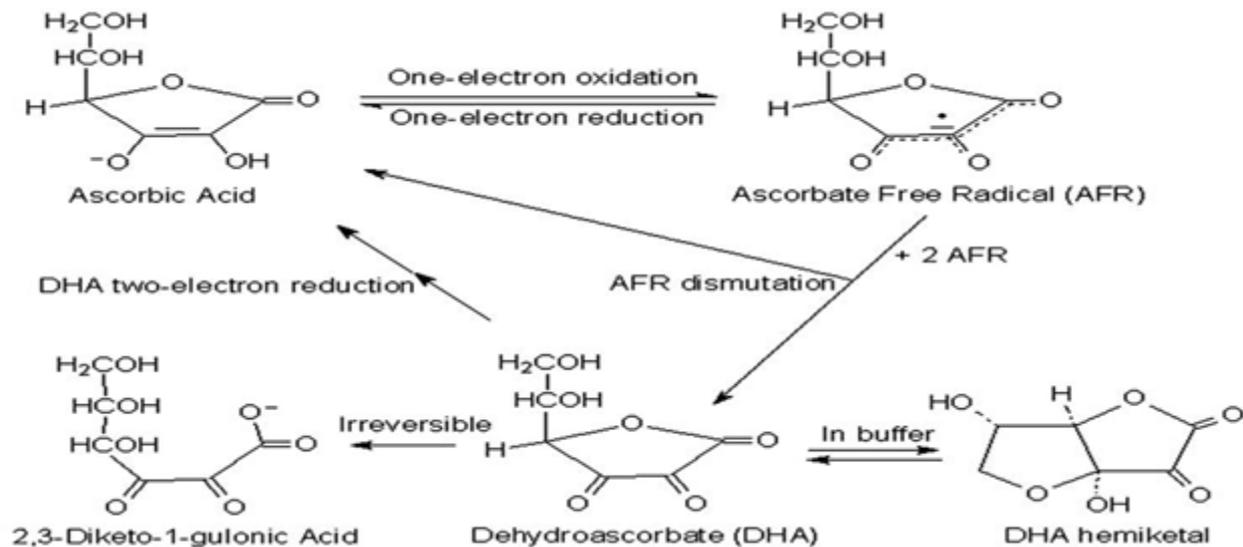


Figure 3

The junctions between endothelial cells that form the blood-brain barrier are very tight relative to other capillary beds. Also in contrast to most large vessel endothelial cells that have been studied, brain capillary endothelial cells do not express the SVCT2 protein in vivo. Concordance of lack of the SVCT2 in brain capillary endothelium and lack of ascorbate transport across the blood-brain barrier endothelium provides support for the notion that the SVCT2 mediates ascorbate movement out of the blood in most other tissues. Such a mechanism would involve uptake of ascorbate from blood on the SVCT2, transit of ascorbate across the endothelial cell, and exit from the basolateral side of the cell by some as yet unknown mechanism. It remains to be seen whether ascorbate might also transit across less tightly apposed endothelia by paracellular movement between the cells. In contrast to ascorbate, if DHA is present in blood in significant quantities, it can enter the CNS more rapidly than ascorbate. The proposed route of entry involves uptake of DHA on GLUT1 in the blood-brain barrier endothelium, exit from the cells on GLUT1 on the basolateral side of the cells. DHA can then diffuse to neurons or glial cells where it can again be taken up, reduced to ascorbate, and retained by the cells. Ascorbate generated by DHA reduction within endothelial cells might also somehow exit the cells on their basolateral margins and thus enter the CNS. In human erythrocytes this mechanism appeared to require interaction of GLUT1 with the integral membrane protein stomatin. There remains the problem of how that ascorbate would exit the cells into the brain interstitium. Nevertheless, under conditions of oxidant stress, enough DHA might be generated from ascorbate in the blood or even in the endothelial cells to allow its passage into the brain.

REVIEW

The functions of ascorbate in the CNS and brain are numerous. Although all actions of ascorbate involve donation of a single electron, they can be divided into those considered antioxidant and non-antioxidant in nature. Many of the latter functions involve monovalent reduction of Fe^{3+} or Cu^{2+} at the active sites of dioxygenase enzymes in hydroxylation reactions. Regarding the antioxidant functions, ascorbate directly acts to scavenge oxygen- or nitrogen-based radical species generated during normal cellular metabolism. At the millimolar concentrations present in neurons in vivo, ascorbate will effectively scavenge superoxide, a major diffusible byproduct of rapid neuronal mitochondrial metabolism. Ascorbate in aqueous compartments can also recycle α -tocopherol in membranes by reducing the α -tocopheroxyl radical back to α -tocopherol. Ascorbate has been shown to spare/recycle α -tocopherol in lipid bilayers and in erythrocytes. Given the lipid-rich environment of the brain, the sparing or recycling α -tocopherol may be a very important role for ascorbate, as will be discussed below in regard to animal studies of combined vitamin C and E deficiencies¹⁴. Ascorbate at the same concentration also induced synaptic maturation of the neurons, based on finding increased numbers of miniature excitatory post-synaptic currents in the cultured neurons. The ascorbate effects were not mimicked by other antioxidants, such as GSH and vitamin E, leading to the conclusion that they occurred by some other mechanism. Although the effects of ascorbate were associated with an increase in intracellular ascorbate, this study did not distinguish between effects on intra- and extracellular

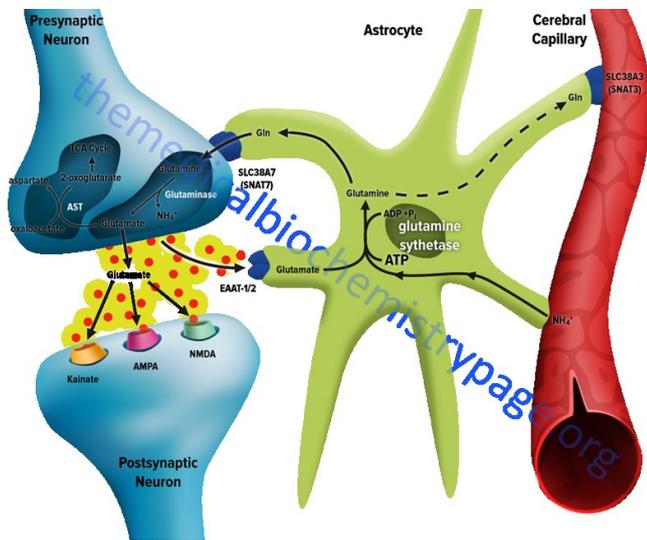
extracellular ascorbate, nor did it consider whether neuronal differentiation and maturation could be induced by lower amounts of ascorbate. The non-antioxidant functions of ascorbate in brain and neural-derived tissues center on neurotransmitters. For example, it is well established that ascorbate is essential for catecholamine biosynthesis in neural tissues, serving as a co-factor for dopamine β -hydroxylase in the conversion of dopamine to norepinephrine. Ascorbate inside the granule is oxidized to the AFR, either in hydroxylation reactions or in simply scavenging radicals that might otherwise react with redox-sensitive catechols in the granule¹⁵. This AFR is then reduced back to ascorbate by interaction with the reduced form of a cytochrome b561 in the granule membrane, which is maintained in the reduced form by accepting an electron from cytoplasmic ascorbate. Ascorbate in chromaffin granules is then secreted concomitantly with catecholamines from cultured chromaffin cells and in vivo by human adrenal glands, the latter in response to adrenocorticotrophin stimulation¹³. Ascorbate has been proposed to function as a neuromodulatory of both dopamine- and glutamate-mediated neurotransmission (reviewed). More recent studies relating ascorbate modulation of glutamate dynamics with changes in rat behavior show that such modulation is complex, since it depends on the site in the brain studied, level of behavioral activity, and level of extracellular ascorbate. For example, ascorbate has been shown to protect neurons from excitotoxicity induced by activation of the NMDA receptor and it prevented glutamate-induced cell damage and death in cultured cerebellar granule cells. This could be due to redox modulation of the receptor itself by ascorbate, or to direct scavenging of ROS generated by receptor activation¹⁶. A more severe stress of ischemia releases large amounts of ascorbate from brain cells, which is associated with glutamate uptake by neurons and glia. Removal of extracellular glutamate by such a process, would also decrease excitotoxicity caused by activation of cell surface and synaptic glutamate receptors. It was hoped that knowledge of the regional distribution of ascorbate throughout the brain would provide vital clues as to its neuromodulatory function. Areas such as the forebrain that typically show the highest levels of ascorbate are rich in catecholamine innervations⁵. However, there does not appear to be any clear relationship between extracellular ascorbate levels and any neurotransmitters, and the question as to how such modulation is achieved has yet to be clarified.¹⁷ Pharmacological manipulations highlight the strength of the relationship between ascorbate and dopaminergic function. Treatment with amphetamine, which affects dopaminergic, cholinergic and glutamatergic activity, increased levels of both dopamine and ascorbate in the caudate nucleus. Apomorphine (a dopamine receptor agonist) increased striatal dopamine and ascorbate, whereas the inverse is true of haloperidol (a dopamine receptor antagonist) which decreased both. These results reflect a well-regulated interaction between dopamine and ascorbate function, especially in the striatum⁸. The increases in extracellular ascorbate levels that are seen following activity or amphetamine administration are not uniform across brain areas and may implicate the heteroexchange system between ascorbate and glutamate

in the control of these changes. There are rich glutamatergic projections from the cortex to the neostriatum. When the cortex is damaged, severing these connections, basal ascorbate levels decrease in the neostriatum and activity-induced increases in extracellular release are also diminished²³. There is also evidence that ascorbate is involved in the regulation of both acetylcholine and catecholamine release from synaptic vesicles. Less is known about the role of ascorbate in collagen synthesis in brain than in other organs, but minimal amounts seem essential for blood vessel and neural sheath formation¹⁸.

OBSERVATION

Milder variants of this defect in humans have been associated with small vessel disease and hemorrhagic stroke²⁴. Ascorbate-dependent collagen synthesis has also been linked to formation of the myelin sheath that surrounds many neuronal processes¹². Scurvy causes severe lassitude and asthenia in humans. Although the disease has been associated with paraparesis in humans, death appears to be due more to complications of systemic collagen dysfunction rather than to a distinct neurologic syndrome. This likely relates to the fact that ascorbate is avidly retained by the CNS during ascorbate deficiency. Indeed, as described by²⁹ James Lind in his Treatise on Scurvy in 1772, even in sailors whose organs were ravaged by hemorrhage and edema in scurvy? Whereas this suggests that decreases in CNS ascorbate do not play a major role in the signs and symptoms of generalized scurvy²⁷, it also suggests that the strong retention of ascorbate in the CNS reflects its importance to neuronal function. Thus, even a modest decrease in CNS ascorbate accelerated signs of vitamin E deficiency in this model and led to significant neuronal loss. Perhaps the most dramatic acute oxidant stress in the CNS is the ischemia-reperfusion injury that occurs with ischemic stroke. Ischemia initially depletes intracellular GSH and ascorbate in brain. If reperfusion with oxygen rich blood occurs, the ROS generated due to abnormal mitochondrial metabolism will extend tissue damage to areas with decreased oxidant defenses. This dramatic decrease in infarct size with acute dosing was surprising, given that ascorbate normally enters the CNS slowly through the choroid plexus, as discussed above¹¹. It is important to note that improvement occurred despite the fact that such high DHA doses may be toxic to some cell types, such as insulin-secreting cells of islets. In contrast to results with DHA, no effects were observed for comparable injections of ascorbate. Protection by DHA infusion was observed in both ischemia alone and ischemia-reperfusion models²⁶. So loss of the ability to synthesize ascorbate may have favored development of alternate routes or of more rapid transit of ascorbate into the CNS in primates¹. The studies in which brain ascorbate was measured clearly support the notion that increases in brain ascorbate concentrations can be beneficial in ischemia or ischemia-reperfusion models of stroke. They also allay to some extent the concern raised by a human study of acute parenteral ascorbate prophylaxis before ischemia-reperfusion related to abdominal vascular surgery¹⁹. In that study intravenous ascorbate infusion (2 g given 2 h before ischemia was induced) increased tissue release of ROS, possibly due to a Fenton-type reaction of

ascorbate with free iron in ischemic areas²⁵. This was thought in turn to account for the increased generation of lipid peroxides and inflammatory cytokines observed. That opposite results were seen following middle cerebral artery occlusion suggests that ascorbate did not encounter significant amounts of free iron in the brain. Such iron would likely be outside living cells, where it would encounter only non-reactive DHA in the DHA infusion studies²². This DHA would be taken up and to ascorbate intracellularly, where it would not have exposure to free iron.



DISCUSSION :

The results discussed thus far suggest that ascorbate plays a role in both sustaining normal function of the CNS and in ameliorating damage induced by pathological conditions that increase generation of ROS (e.g., severe antioxidant vitamin depletion, lack of the SVCT, and ischemia-reperfusion injury).²⁰ As alluded to above, a consistent factor in these pathologies is the need to maintain intracellular, as opposed to extracellular, ascorbate.² The concept that intracellular ascorbate is crucial for protection against oxidant stress is also supported by results of several *in vivo* studies. Finally, the neuroprotective role of intracellular ascorbate highlights a crucial role for the SVCT2 in preserving intracellular ascorbate. After 7-14 days of growth in defined media lacking ascorbate, intracellular ascorbate was very low in SVCT2-expressing neurons (100 μ M or less), and undetectable in SVCT2-deficient neurons. Moreover, following middle cerebral artery occlusion in rats, SVCT2 mRNA²⁴ increased over several hours in the peri-infarct penumbra, both in neurons and in glia. Together, the results of these studies strongly support a protective role for ascorbate and for the SVCT2 in brain during acute ROS generation. There are again two mechanisms by which ascorbate can enter neurons and glia from the interstitial space (Fig.2): 1) transport of ascorbate on the SVCT2 and 2) oxidation of ascorbate to DHA and uptake of DHA on the GLUTs and subsequent intracellular reduction. The SVCT2 is found in greater abundance in neuron-dense areas of the brain such as cortex, hippocampus and cerebellum. In most non-neuronal cell types, the SVCT2 has a high affinity for ascorbate ($K_m = 20-40 \mu$ M).

This suggests that ascorbate uptake and content in neurons will be limited by the number of SVCT2 proteins expressed, and affinity for ascorbate becomes relevant only when ascorbate deficiency is present²³. Oxidant stress due to ischemia-reperfusion injury has been shown to increase SVCT2 mRNA expression in both neurons and glia²². As reviewed most recently by Rice, ascorbate release from astrocytes and neurons has been linked in numerous studies to uptake of glutamate on one or more of its specific transporters¹⁴. First, both glutamate uptake and ascorbate efflux were attenuated when cell swelling was prevented by culture in hypertonic media. Second, ascorbate readily moved into glutamate-stimulated astrocytes down a concentration gradient, which would occur across a channel, but not on an exchange transporter.¹⁴ Third, inhibitors of volume-sensitive organic anion channels blocked ascorbate efflux, but had no effect on glutamate uptake.²² Finally, high intracellular ascorbate concentrations did not enhance glutamate uptake, as would be expected in a heteroexchange mechanism. Glutamate-induced ascorbate release has also been demonstrated in neuronal cells. Ascorbate released into the brain interstitium and CSF in response to glutamate appears to have antioxidant and neuromodulatory effects, as discussed in the next section.²³ Oxidative stress in the brain with focus on neurodegenerative diseases has been extensively reviewed, so only aspects relevant to ascorbate will be considered here. Neurons appear to be especially sensitive to ascorbate deficiency, perhaps because they have 10-fold higher rates of oxidative metabolism than supporting glia.¹ This neuronal sensitivity is most apparent when ascorbate supply is low in conditions in which there is excess oxidant stress¹³. The involvement of reactive oxygen species in neurodegenerative disorders explains the enthusiasm for ascorbate as an antioxidant therapeutic approach, although its complicated interactions with neurotransmitter systems as described above make it difficult to discern the specific mechanisms involved¹¹. Alzheimer's disease is caused by a combination of genetic and lifestyle factors and it is established that oxidative stress plays a key role in the pathogenesis of the disease contributing to the degeneration of the basal forebrain cholinergic system and general cell death.⁵ Alzheimer's disease patients have been found to have lower plasma and CSF ascorbate levels despite adequate nutritional intake. Positive relationships have been shown between ascorbate supplement use and reduced disease incidence and also with disease-related markers of oxidative stress although these beneficial results are not universal¹⁶. Interpretation of the data is hindered by the nature of the studies as population or epidemiological studies have high levels of variability¹⁰ inherent in their design. Despite the large amount of information that can be collected from participants, and complicated statistical techniques that can be used to control for variability in the data, individual differences can still have a significant influence on results. The most important contributors to variability are education and career, diet, exercise, alcohol consumption, cigarette smoking and general health and illness across the lifetime⁵.

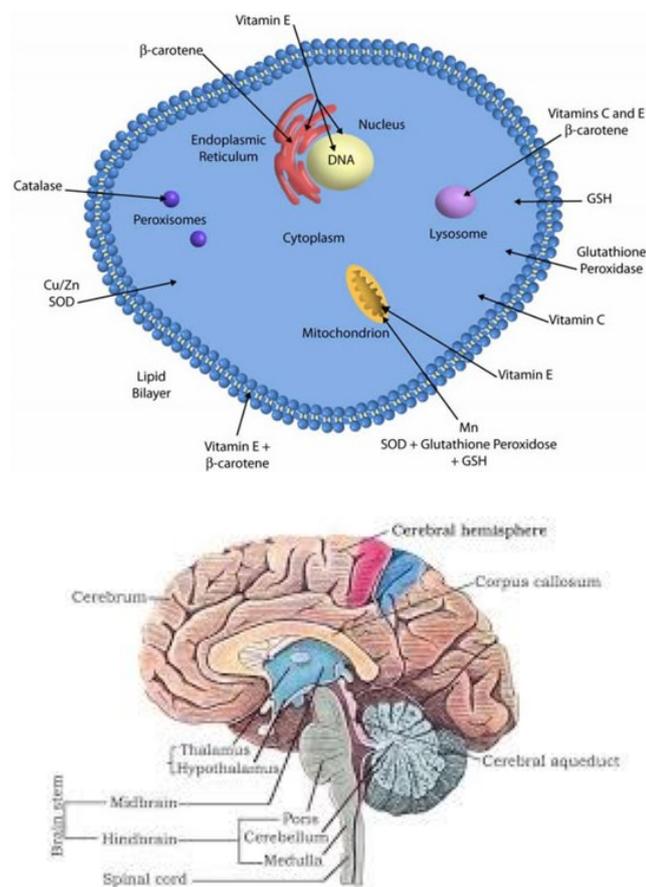
Any combination of the above factors may contribute to the lack of consistency in findings among these studies. Nevertheless, there is further evidence to support the potential for vitamin C as a therapeutic avenue for Alzheimer's disease. It also protected SH-SY5Y neuroblastoma cells from β -amyloid induced apoptosis. Scopolamine (which impairs memory in rodents) is often used as a pharmacological model for Alzheimer's disease. As noted earlier, ascorbate blocked or attenuated the effects of scopolamine in several different types of studies. Furthermore, ascorbate has been shown to be an effective acetyl cholinesterase inhibitor, the most common form of treatment used for Alzheimer's disease⁶. These results may seem contradictory, since scopolamine can also inhibit acetyl cholinesterase; however, the net effect of vitamin C appears to be a boost to cholinergic system functioning, although this relationship needs further investigation. Parkinson's disease involves severe decreases in dopaminergic signalling in the central nervous system, specifically the motor cortex. Oxidative injury is also thought to play a key role in the pathogenesis of the disease⁷. Given the range of data presented above concerning the relationship between ascorbate and dopaminergic function it is understandable that ascorbate is being investigated for its therapeutic potential in this disease. Although population studies concerning ascorbate intake show no protection against development of Parkinson's disease, there are a number of lines of evidence that suggest that ascorbate as a pharmacological agent may be of more benefit⁴. Ascorbate has been shown to improve bioavailability of levodopa (which can then be converted into dopamine) in members of an elderly Parkinson's disease population with low baseline bioavailability for levodopa⁹.

CONCLUSIONS

That ascorbate is important for neuronal maturation and function, as well as for protection of the brain against oxidant stress is well supported by the evidence presented in this review. The vitamin is maintained at high concentrations in brain and in neurons in particular relative to other organs. In addition, strong homeostatic mechanisms maintain brain and neuronal ascorbate concentrations within very tight limits³. Thus, not only is it difficult to deplete brain ascorbate, but it is also difficult if not impossible to increase levels for more than a short period above those set by uptake and recycling mechanisms. Whereas oral supplements generally increase brain ascorbate by only 20% at most, larger relative increases may occur if significant oxidant stress has caused localized ascorbate deficiency in brain areas affected by neurodegeneration or inflammation. Study of the role of ascorbate in human brain function has been limited, but with the availability of suitable mouse models, ascorbate deficiency or excess can be studied in more detail, particularly with regard to effects of the vitamin on brain development, neurotransmitter function and responses to inflammatory or oxidant stresses, such as might exist in cerebral atherosclerosis or in several neurodegenerative diseases⁸.

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