Chapter 6

Vitamin C Transporter (SVCT2) Distribution in Developing and Adult Brains

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Abstract

Vitamin C is the major antioxidant molecule in the central nervous system (CNS), reaching concentrations of 10 mM in neurons and 400 μM in the cerebrospinal fluid (CSF). Uptake of vitamin C by brain cells is performed through the co-transporter of ascorbic acid and sodium isoform 2, SVCT2, which is expressed in cells from the choroid plexus, neurons, oligodendrocytes, and ependymal cells. SVCT2 expression has also been described in cells at the neurogenic niche, specifically in proliferative type C cells. In this chapter, we will describe recently published studies of SVCT2 expression during brain development and define its polarization in cells from the radial glia (neuronal precursors within the CNS) and vitamin C-mediated effects in regulating genes associated with the maintenance of CNS stem cell pluripotency. We will discuss the differential biological effect that vitamin C generates in neurons versus astrocytes and how the oxidized form of vitamin C, dehydroascorbic acid (DHA), produced by neurons in conditions of oxidative stress must leave this cell to be incorporated by astrocytes. In this context, we will discuss recent literature, which shows that DHA regulates glycolytic metabolism in neurons. In parallel, we will analyze vitamin C recycling by astrocytes, which reduce DHA into ascorbic acid (AA), increasing the antioxidant potential of the brain. Data discussed in this chapter will provide an updated view of SVCT2 distribution in the brain and will also describe how vitamin C recycling participates in normal or pathological brain function.

Keywords: ascorbic acid, dehydroascorbic acid, SVCT2, bystander effect, recycling, brain

1. Introduction

Vitamin C is a small, water-soluble molecule that possesses two dissociable protons with pK values of 4.2 and 11.8. At physiological pH, the reduced form of vitamin C, ascorbic acid

(AA), predominates and is specifically incorporated by neurons through isoform 2 of the cotransporter of sodium and ascorbate (SVCT2) [1]. Once AA loses its protons, it is oxidized into dehydroascorbic acid (DHA), which diffuses among nervous cells through the facilitative glucose transporters, GLUT1 and GLUT3 [2–4]. Most mammals are able to synthesize vitamin C from glucose in the liver; however, primates, including humans, lack the enzyme that catalyzes the last step in vitamin C biosynthesis. Thus, they must obtain vitamin C from the diet [5, 6]. The most relevant function of vitamin C is as an antioxidant agent. Given that the brain has one of the highest metabolic rates of all organs and is under constant oxidative stress, vitamin C is, therefore, critical for the maintenance of brain function and protection of central nervous system (CNS) structures [5].

2. Distribution and function of SVCT2 in the adult brain

Many studies indicate that SVCT2 is a transporter that is preferentially expressed in neurons from different areas in the adult brain, such as the cortex, hippocampus, hypothalamus, and cerebellar precursors [1, 7–11]. SVCT2 mediates uptake of AA, an important molecule for antioxidant defense and general metabolic needs of neurons. Although SVCT2 was originally thought to be a neuronal transporter, there is increasing evidence demonstrating its expression in glial cells in the CNS [4, 7, 8, 10, 12–14]. Indeed, SVCT2 has been reported in cortical microglia, where its function remains unknown [10]. In hypothalamic tanycytes, it may participate in maintaining the high parenchymal concentrations of vitamin C that characterize this region [8]. Furthermore, in choroid plexus cells, it facilitates entry of AA into the brain through the blood-cerebrospinal fluid (CSF) barrier [4, 7, 10, 12]. In Schwann cells, SVCT2 promotes axonal myelination in the peripheral nervous system [14]. Under physiological conditions, SVCT2 protein has not been detected in astrocytes from the gray matter, but its mRNA has been reported in marginal astrocytes in the subpial surface at the entorhinal cortex [13]. In contrast, SVCT2 mRNA is induced in the brains of animals with middle cerebral artery occlusion as an experimental model of ischemia/reperfusion in the brain, suggesting that expression of this transporter is induced under pathological conditions [15]. Moreover, our group has recently defined that SVCT2 protein is induced in reactive astrocytes in various *in vivo* pathological models that generate severe reactive astrogliosis. Specifically, such events involve neuroinflammation generated by intracerebroventricular injection of adenovirus-GFP or the bacterial enzyme, neuraminidase, mechanical damage of the brain cortex evaluated at 5 and 10 days post-injury, and astrogliosis observed in the brain cortex and hippocampus from "Kindled" rats, a widely used model of epilepsy at the mesial temporal lobe [16, 17]. *In vitro* studies support these findings by showing that SVCT2 is induced in astrocytes that have been cultured for long periods and express markers of astrocytic activation [3]. However, we did not observe a positive correlation between SVCT2 expression and the moderate and focal reactive astrogliosis surrounding amyloid plaques in postmortem brain samples from patients with Alzheimer's disease. Altogether, data indicate that SVCT2 is induced in astrocytes during pathophysiological events that produce severe reactive astrogliosis, which may be important to enhance antioxidant defense in order to protect glial cells.

3. Expression and polarization of SVCT2 during brain development

Studies published more than a decade ago have unequivocally demonstrated the expression and functionality of SVCT2 in cultured cortical neurons from mouse embryos [18]. Similarly, studies addressing the effect of physiological brain concentrations of AA have shown that this nutrient promotes differentiation of embryonic cortical precursors into neurons and astrocytes [19]. Expression and polarization of SVCT2 have been studied during rat and human embryonic development in the cortex at 9 weeks of gestation [20, 21]. In lissencephaly, SVCT2 is localized in radial glial cells in the ventricular zone, where it is polarized toward the ventricular cavity, and in subapical and intermediate apical progenitors in the subventricular zone [21]. In gyrencephalic brains, SVCT2 localization is also associated with progenitors of the inner and outer areas of the subventricular zone [21]. *In vitro* studies with J1ES cells, a cell model used to evaluate radial glial cell differentiation, indicate that both AA and SVCT2 are key regulators in maintaining the radial phenotype. They also may regulate the pluripotency of neural stem cells, as AA induces the expression of the pluripotency gene, *Nanog*, through activation of the JAK/STAT signaling pathway and inhibition of retinoic acid-dependent neuronal differentiation [21, 22].

During postnatal brain development, levels of SVCT2 mRNA and protein follow an inverse relation with AA concentrations [23]. Thus, high concentrations of AA exist during late embryonic and early postnatal development periods at both the cortex and cerebellum, diminishing later in adulthood; however, levels of SVCT2 mRNA and protein are low during embryonic development but increase with age [23]. By means of confocal and immune-structural studies, our research group has identified SVCT2 in the Golgi apparatus of pyramidal neurons from the deep layers of the brain cortex (layers IV to VI) in early postnatal mice (days 1 and 5 after birth). These findings indicate that SVCT2 is induced during cell arborization and synaptic maturation of deep neurons in the brain cortex. We also identified the expression of a short isoform of SVCT2 (SVCT2sh), which is unable to transport AA and regulate the functional capacity of the active transporter [1]. This finding and its biological consequence in the postnatal brain will be discussed in the following section.

SVCT2 knock-out mice (*SLC23a2* null) have been critical to understanding the physiological importance of SVCT2 in the postnatal brain [24]. Although the intrauterine development of these animals looks normal, homozygote mice that lack SVCT2 present with brain cortex hemorrhage and are unable to expand their lungs, resulting in death a few minutes following birth. Also, SVCT2-null animals have undetectable levels of AA in their brain tissue, supporting the essential role of this transporter in AA entry into cells of the CNS. The defects characterized in SVCT2 knock-out animals suggest that their death was due to CNS problems, possibly generated by a deficit in individual neuronal function, formation of aberrant connections in the cortex and/or other brain areas, or lack of neuronal differentiation in the absence of AA [9]. Further studies have demonstrated that SVCT2 expression is pivotal for neuronal differentiation and maturation, since cultures of hippocampal neurons from knock-out mice have reduced neurite growth, glutamate receptor aggregation, and spontaneous activity [9]. Lentiviral-mediated overexpression of SVCT2 in cells of neuronal linage (N2a) allowed us to demonstrate a three- to

four-fold increase in the percentage of cells with increased filopodia numbers and processes that were positive for the dendritic marker, microtubule-associated protein 2 (MAP2) [25]. Furthermore, overexpression of SVCT2 combined with AA treatment promotes phosphorylation of the mitogen-activated protein kinase (MAPK), ERK1/2, a key signaling pathway intermediate that participates in the differentiation and maturation of cortical neurons [25].

The hypothesis that SVCT2 and AA can potentiate neurogenesis during postnatal development has also been validated by Pastor et al. [26], who reported the postnatal expression of SVCT2 in the neurogenic niche from rat and human brains after 1 month of extra-uterine life [26]. This is a restricted area that borders the wall of lateral ventricles in which a stream of progenitor cells that migrate toward the olfactory bulb originate (called the rostral migratory stream [RMS]). SVCT2 is abundant in cells with high proliferative capacity in the neurogenic niche (also known as type C cells) and migratory progenitor cells present at the RMS. In contrast, among the different cell types of the neurogenic niche, SVCT2 is only present at low levels in astrocytes or type B cells and neuroblasts [26]. These findings suggest a role for AA in the maintenance of proliferative cells, which, in turn, is supported by studies indicating that treatment with AA improves the genetic reprogramming of human fibroblasts into induced pluripotent stem cells (iPSC), enhancing the efficiency of the process and allowing the generated iPSC to present similar epigenetic patterns to those present in embryonic stem cells [27]. Moreover, AA supplementation of neurosphere cultures obtained from rat embryonic brain tissue and a teratocarcinoma cell line from P19 mice shows the functionality of SVCT2-mediated uptake of AA as well as neuronal differentiation revealed by the increased expression of the marker, βIII tubulin [26].

In conclusion, SVCT2 is expressed at low levels during embryonic development, and its localization is restricted to the ventricular zone from cerebral ventricles, where the bodies of the radial glia are present. During this period, SVCT2 and AA are key players in regulating pluripotency and the proliferative capacity of neural stem cells. During postnatal development, AA and SVCT2 are important for postmigratory neurons that are beginning the process of arborization and synaptic maturation.

4. Regulation of SVCT2-mediated vitamin C uptake in neurons by shSVCT2

As mentioned in the previous chapter, SVCT2 expression and function are important for the arborization and synaptic maturation of post-mitotic neurons present in the postnatal brain cortex. However, during this period, SVCT2 function is tightly regulated by the co-expression of a shorter isoform of SVCT2, SVCT2sh, which is unable to transport AA. This isoform was initially identified in human fetal brain cDNA [28] and is generated by deletion of an internal sequence of 345 bp in the mRNA, resulting in the translation of a smaller protein completely lacking intracellular domains 5 and 6 and part of domain 4. SVCT2sh was initially thought to be a possible dominant negative of SVCT2 through protein-protein interaction [28]. However, Forster resonance energy transfer (FRET) studies, a decade later using SVCT2-CFP

and SVCT2sh-YFP in the neural linage cell line, N2a [1], showed that the proteins interact at both the intracellular space and the cell membrane. The functional consequence of the interaction reduces the affinity of SVCT2 for its substrate, AA, in neuronal cells, suggesting that SVCT2 activity and intracellular concentrations of AA are tightly modulated during postnatal development in order to generate a precise physiological intracellular dose of AA to trigger the neuronal differentiation and synaptic maturation in a particular period of development.

5. Vitamin C recycling

5.1. General aspects

In order to understand the concept of vitamin C recycling, it is necessary to remember that vitamin C is found in two forms: (1) a reduced form, AA, that enters the cells specifically through SVCT transporters and (2) an oxidized form, DHA, that enters the cells through the facilitative glucose transporters, GLUT1, GLUT2, GLUT3, GLUT4, and GLUT8 [29–31]. These two forms of vitamin C represent two different molecules with independent functions and characteristics, although they are interconvertible by means of oxidation-reduction reactions [32]. Because every cell in the body expresses glucose transporters, all cells can uptake vitamin C in the form of DHA [2]. However, for intracellular accumulation of AA and antioxidant protection, the cell needs to reduce DHA into AA, a process that depends on glutathione and NADPH [33–35]. Therefore, the ability of a cell to accumulate AA via reduction of DHA is limited by its antioxidant enzymatic capacity. Interestingly, two different cell populations have been identified: cells with high and those with low antioxidant enzymatic capacity. Cells with low antioxidant enzymatic capacity preferentially use AA for protection against oxidative damage. Once AA accomplishes its antioxidant function, it is oxidized into DHA. Because these cells cannot reduce DHA back into AA, the presence of neighboring cells with high reducing power is crucial. Hence, DHA leaves the cell through glucose transporters and enters neighboring cells that possess an elevated concentration of antioxidant enzymes to reduce DHA into AA. Finally, AA can be accumulated or released into the extracellular space to maintain constant AA concentrations. Therefore, ideal conditions exist *in vivo* to allow coupling between cells in order to efficiently recycle vitamin C.

5.2. Vitamin C recycling between astrocytes and neurons

The idea of vitamin C recycling in the brain originated from the observation that during nutritional deficiency of vitamin C, high AA concentrations in the brain are maintained for a longer period compared to all the other tissues [36, 37]. Thus, efficient mechanisms must exist to maintain AA concentrations in the brain. As mentioned before, in order for vitamin C recycling to occur, a cell with low reducing power that utilizes AA as an antioxidant is required in addition to a cell with high reducing power that is constantly incorporating DHA and releasing AA to the extracellular space. The ideal conditions for vitamin C recycling converge in the CNS, where we find two cell populations that couple in order to recycle vitamin C. On one hand, we find the neuron that is able to intracellularly accumulate up to 10 mM AA but rapidly

oxidizes it into DHA, which leaves the cell [4]. Furthermore, the neuron possesses a very limited capacity to reduce DHA back into AA due to low concentration of antioxidant enzymes and its elevated metabolic rate [38]. Accordingly, isolation of neurons from the neighbor cells that recycle DHA compromises their metabolism, resulting in neuronal death [32, 39].

On the other hand, another cell type is found in the CNS, the astrocytes. For a long time, astrocytes were only thought to be scaffold cells in the brain, without any physiological function. However, today we know that astrocytes perform critical physiological activities, such as (1) the uptake of extracellular glutamate for recycling into glutamine to avoid neuronal excitotoxicity; (2) the release of lactate to be used by neurons for energy production; (3) the release of interleukin-6 (IL-6) and tumor growth factor-beta (TGF-beta) neuroprotective cytokines; (4) the synthesis of apolipoprotein E for axonal growth; and (5) the release of trophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF), among others [40]. Moreover, astrocytes are required for vitamin C recycling in the CNS due to their ability to uptake DHA from the extracellular space and, in virtue of its elevated reducing power, to efficiently reduce it back into AA [41, 42]. After that, astrocytes release AA to the extracellular space to maintain stable AA concentrations [43]. Besides, astrocytes participate in the neuronal antioxidant defense by releasing AA during pathological conditions that involve changes in the osmotic pressure or ischemic/reperfusion damage [44]. Experiments using neuronal-astrocyte co-cultures have demonstrated that the presence of astrocytes ameliorates neuronal death induced by glutamate, H_2O_2, or DHA *in vitro* [39, 45], suggesting that astrocytes and vitamin C recycling are of vital importance for antioxidant defense in the brain.

6. Relevance of DHA recycling

6.1. Effects of DHA in neurons

As previously described, the neuron utilizes AA as the main antioxidant agent, but it is unable to maintain vitamin C in its reduced form. The effects triggered by the accumulation of DHA in neurons have begun to be elucidated recently. The first studies were focused in determining the effect of the extracellular oxidation of AA over the redox state of the neuron, showing that incubation of brain slices with exogenous AA produces an increase in thiobarbituric acid reactive substances (TBARS) [46–48], an indicator of oxidative damage. However, this effect was prevented by treatment with glutathione, indicating that AA oxidation might induce an increase in reactive oxygen species (ROS). It is widely accepted that AA oxidation generates ROS through the Fenton reaction, which occurs in the presence of metals, such as Fe in acidic pH, producing H_2O_2. AA creates the conditions for the Fenton reaction to occur, so AA might induce an increase in ROS and trigger cell death due to extracellular accumulation of H_2O_2 [49, 50]. Nevertheless, no increase in TBARS occurs when DHA uptake is prevented by inhibition of GLUT1 in brain slices, indicating that the pro-oxidant effect requires DHA uptake by nervous cells and does not depend directly on AA oxidation and accumulation of H_2O_2 [47]. Additionally, as previously described, the pro-oxidant effect

of AA disappears when it is co-incubated with glutathione, even when glutathione reduces DHA into AA [33]. Thus, we can conclude that extracellular DHA accumulation due to an oxidative environment stimulates its uptake by neurons, which, in turn, induce oxidative stress because neurons possess low reducing power. Although these results constitute strong evidence suggesting that DHA might be triggering oxidative stress and neuronal death, the ultimate intracellular effect as well as the DHA target has not been identified. Further studies of our research group have shed light on the actual effect of DHA on neurons. Radioactive vitamin C uptake analyses have shown that neurons are able to incorporate both AA and DHA; however, their ability to reduce DHA is limited up to 40% after which they start to oxidize AA back into DHA. Moreover, neurons cannot keep AA in its reduced form for long periods, since it consumes 80% of intracellular AA after 1 h of uptake. However, in the presence of DHA, neurons consume 90% of glutathione in 1 h. Therefore, our group has defined that the neuronal target of DHA is glucose metabolism since incubation with DHA induces a 75% arrest in glycolysis accompanied by increased activity of the pentose phosphate pathway, possibly to regenerate glutathione and avoid oxidative damage [32]. In conclusion, as shown in **Figure 1**, neurons need neighboring cells to recycle vitamin C in order to maintain stable concentrations of AA.

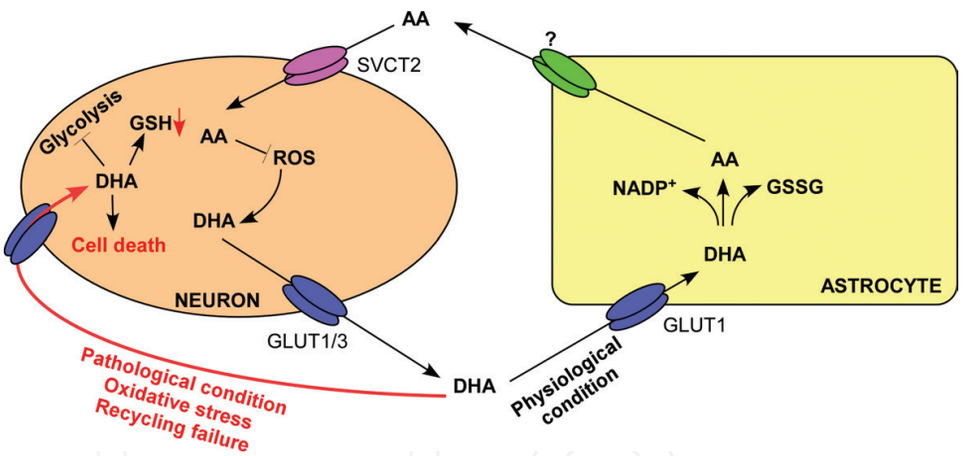

Figure 1. Recycling of vitamin C in physiological and pathophysiological condition. Under physiological conditions, neurons uptake AA through the SVCT2 transporter. AA fulfills its antioxidant function intracellularly, oxidizing to DHA. Following DHA efflux from the neuron through GLUTs 1/3, astrocytes incorporate DHA that was released from the neuron, to reduce it intracellularly to AA at the expense of GSH and NADPH. Finally, the astrocyte releases AA into the extracellular medium to maintain a homeostatically stable concentration of AA. In pathophysiological conditions, vitamin C recycling is deficient; therefore, DHA accumulates in the extracellular space, recycling in an autocrine fashion toward the neuron. Accumulation of DHA in the neuron arrests glycolysis, consumes glutathione, and finally triggers cell death.

6.2. Relevance of astrocytes for DHA recycling

In order to confirm the hypothesis that the presence of vitamin C-recycling cells is necessary, our research group performed studies of cell viability in neurons subjected to oxidative damage induced by H_2O_2 and incubation with DHA. The aim of this experiment was

to elucidate the dichotomy existing between the protective and toxic effects that DHA may produce. Given that the literature proposes antioxidant effects for DHA treatment in pathological conditions [51], we incubated neurons with H_2O_2, the major ROS accumulated in the extracellular space during pathological conditions of the brain (e.g., ischemia/reperfusion), and expected a depletion of the antioxidant enzymatic defense of the neuron [52]. In this experimental setting, we incubated neurons with DHA to assess for any antioxidant effect. Interestingly, treatment with H_2O_2 and DHA induced 50 and 70% cell death after 3 and 6 h, respectively, compared with cells that were incubated with H_2O_2 alone [39]. The findings that neurons are inefficient in reducing DHA and that DHA accumulation induces cell death led us to the following question: how do neurons avoid DHA accumulation in physiological and/or pathological conditions? The answer is the presence of another population of brain cells: the astrocytes. To analyze if astrocytes prevent the toxic effects of DHA, neuron-astrocyte cocultures using the sandwich technique were used to assess any protective effect of astrocytes on neuronal survival in the presence of H_2O_2 and DHA. Neurons were incubated with H_2O_2, and astrocytes were added to the culture just before DHA was added. Notably, neurons cocultured with astrocytes showed 100% survival after 3 and 6 h of treatment, compared with neurons cultured alone [39], demonstrating that astrocytes are highly efficient in the recycling of DHA from the extracellular medium and that accumulation of DHA due to deficient astrocyte recycling or severe oxidative conditions is toxic for neurons.

7. Conclusions

The relationship between vitamin C and brain pathologies remains a topic of discussion in the current literature, especially given that the oxidized form of vitamin C, DHA, has been largely ignored. DHA is actually a molecule that has great relevance for the treatment of various pathologies, including cancer, although conclusions observed when using DHA should be taken with caution. Indeed, DHA was originally proposed as a molecule that would be beneficial in pathological conditions, such as ischemia and reperfusion [51]. Unfortunately, the research group that proposed DHA as a treatment for ischemia and reperfusion subsequently determined that its administration in primate models did not replicate the results observed in rodents [53]. In these experiments, the researchers did not consider that the observations could be attributed to a failure in vitamin C recycling. Although vitamin C deficiency in the brain has been associated with increased oxidative stress, which could be stimulating ideal conditions to trigger certain pathologies, including Alzheimer's disease [54], DHA has recently been proposed as a molecule that would trigger neuronal death [39]. As shown in **Figure 1**, we propose that CNS pathologies could be associated with reduced astrocytic vitamin C recycling, resulting in the accumulation of DHA in neurons, which would trigger cell death.

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References

[1] Salazar K, Cerda G, Martinez F, Sarmiento JM, Gonzalez C, Rodriguez F, et al. SVCT2 transporter expression is post-natally induced in cortical neurons and its function is regulated by its short isoform. Journal of Neurochemistry. 2014;**130**:693-706. DOI: 10.1111/jnc.12793

[2] Nualart FJ, Rivas CI, Montecinos VP, Godoy AS, Guaiquil VH, Golde DW, et al. Recycling of vitamin C by a bystander effect. The Journal of Biological Chemistry. 2003;**278**:10128-10133. DOI: 10.1074/jbc.M210686200

[3] Astuya A, Caprile T, Castro M, Salazar K, Garcia Mde L, Reinicke K, et al. Vitamin C uptake and recycling among normal and tumor cells from the central nervous system. Journal of Neuroscience Research. 2005;**79**:146-156. DOI: 10.1002/jnr.20326

[4] Nualart F, Mack L, Garcia A, Cisternas P, Bongarzone ER, Heitzer M, et al. Vitamin C transporters, recycling and the bystander effect in the nervous system: SVCT2 versus gluts. Journal of Stem Cell Research and Therapy. 2014;**4**:209. DOI: 10.4172/2157-7633.1000209

[5] Rice ME. Ascorbate regulation and its neuroprotective role in the brain. Trends in Neurosciences. 2000;**23**:209-216

[6] May JM. Vitamin C transport and its role in the central nervous system. Subcellular Biochemistry. 2012;**56**:85-103. DOI: 10.1007/978-94-007-2199-9_6

[7] Tsukaguchi H, Tokui T, Mackenzie B, Berger UV, Chen XZ, Wang Y, et al. A family of mammalian Na^+-dependent L-ascorbic acid transporters. Nature. 1999;**399**:70-75. DOI: 10.1038/19986

[8] García L, Salazar K, Millan C, Rodriguez F, Montecinos H, Caprile T, et al. Sodium vitamin C cotransporter SVCT2 is expressed in hypothalamic glial cells. Glia. 2005;**50**:32-47. DOI: 10.1002/glia.20133

[9] Qiu S, Li L, Weeber EJ, May JM. Ascorbate transport by primary cultured neurons and its role in neuronal function and protection against excitotoxicity. Journal of Neuroscience Research. 2007;**85**:1046-1056. DOI: 10.1002/jnr.21204

[10] Mun GH, Kim MJ, Lee JH, Kim HJ, Chung YH, Chung YB, et al. Immunohistochemical study of the distribution of sodium-dependent vitamin C transporters in adult rat brain. Journal of Neuroscience Research. 2006;**83**:919-928. DOI: 10.1002/jnr.20751

[11] Oyarce K, Silva-Alvarez C, Ferrada L, Martinez F, Salazar K, Nualart F. SVCT2 is expressed by cerebellar precursor cells, which differentiate into neurons in response to ascorbic acid. Molecular Neurobiology. 2017. DOI: 10.1007/s12035-016-0366-5

[12] Angelow S, Haselbach M, Galla HJ. Functional characterisation of the active ascorbic acid transport into cerebrospinal fluid using primary cultured choroid plexus cells. Brain Research. 2003;**988**:105-113

[13] Nualart F, Salazar K, Oyarce K, Cisternas P, Jara N, Silva-Alvarez C, et al. Typical and atypical stem cells in the brain, vitamin C effect and neuropathology. Biological Research. 2012;**45**:243-256. DOI: 10.4067/S0716-97602012000300006

[14] Gess B, Lohmann C, Halfter H, Young P. Sodium-dependent vitamin C transporter 2 (SVCT2) is necessary for the uptake of L-ascorbic acid into Schwann cells. Glia. 2010;**58**:287-299. DOI: 10.1002/glia.20923

[15] Berger UV, Lu XC, Liu W, Tang Z, Slusher BS, Hediger MA. Effect of middle cerebral artery occlusion on mRNA expression for the sodium-coupled vitamin C transporter SVCT2 in rat brain. Journal of Neurochemistry. 2003;**86**:896-906

[16] Morales JC, Alvarez-Ferradas C, Roncagliolo M, Fuenzalida M, Wellmann M, Nualart FJ, et al. A new rapid kindling variant for induction of cortical epileptogenesis in freely moving rats. Frontiers in cellular neuroscience. 2014;**8**:200. DOI:10.3389/fncel.2014.00200

[17] Cole-Edwards KK, Musto AE, Bazan NG. c-Jun N-terminal kinase activation responses induced by hippocampal kindling are mediated by reactive astrocytes. The Journal of Neuroscience. 2006;**26**:8295-8304. DOI: 10.1523/JNEUROSCI.1986-05.2006

[18] Castro M, Caprile T, Astuya A, Millan C, Reinicke K, Vera JC, et al. High-affinity sodium-vitamin C co-transporters (SVCT) expression in embryonic mouse neurons. Journal of Neurochemistry. 2001;**78**:815-823

[19] Lee JY, Chang MY, Park CH, Kim HY, Kim JH, Son H, et al. Ascorbate-induced differentiation of embryonic cortical precursors into neurons and astrocytes. Journal of Neuroscience Research. 2003;**73**:156-165. DOI: 10.1002/jnr.10647

[20] Caprile T, Salazar K, Astuya A, Cisternas P, Silva-Alvarez C, Montecinos H, et al. The Na^+-dependent L-ascorbic acid transporter SVCT2 expressed in brainstem cells, neurons, and neuroblastoma cells is inhibited by flavonoids. Journal of Neurochemistry. 2009;**108**:563-577. DOI: 10.1111/j.1471-4159.2008.05788.x

[21] Silva-Alvarez C, Salazar K, Cisternas P, Martinez F, Liour S, Jara N, et al. Apical polarization of SVCT2 in apical radial glial cells and progenitors during brain development. Molecular Neurobiology. 2016. DOI: 10.1007/s12035-016-0081-2

[22] Wu H, Wu Y, Ai Z, Yang L, Gao Y, Du J, et al. Vitamin C enhances Nanog expression via activation of the JAK/STAT signaling pathway. Stem Cells. 2014;**32**:166-176. DOI: 10.1002/stem.1523

[23] Meredith ME, Harrison FE, May JM. Differential regulation of the ascorbic acid transporter SVCT2 during development and in response to ascorbic acid depletion. Biochemical and Biophysical Research Communications. 2011;**414**:737-742. DOI: 10.1016/j.bbrc.2011.09.146

[24] Sotiriou S, Gispert S, Cheng J, Wang Y, Chen A, Hoogstraten-Miller S, et al. Ascorbic-acid transporter Slc23a1 is essential for vitamin C transport into the brain and for perinatal survival. Nature Medicine. 2002;**8**:514-517. DOI: 10.1038/nm0502-514

[25] Salazar K, Martinez M, Ulloa V, Bertinat R, Martinez F, Jara N, et al. SVCT2 overexpression in neuroblastoma cells induces cellular branching that is associated with ERK signaling. Molecular Neurobiology. 2016;**53**:6668-6679. DOI: 10.1007/s12035-015-9553-z

[26] Pastor P, Cisternas P, Salazar K, Silva-Alvarez C, Oyarce K, Jara N, et al. SVCT2 vitamin C transporter expression in progenitor cells of the postnatal neurogenic niche. Frontiers in Cellular Neuroscience. 2013;**7**:119. DOI: 10.3389/fncel.2013.00119

[27] Esteban MA, Wang T, Qin B, Yang J, Qin D, Cai J, et al. Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. Cell Stem Cell. 2010;**6**:71-79. DOI: 10.1016/j.stem.2009.12.001

[28] Lutsenko EA, Carcamo JM, Golde DW. A human sodium-dependent vitamin C transporter 2 isoform acts as a dominant-negative inhibitor of ascorbic acid transport. Molecular and Cellular Biology. 2004;**24**:3150-3156

[29] Rumsey SC, Daruwala R, Al-Hasani H, Zarnowski MJ, Simpson IA, Levine M. Dehydroascorbic acid transport by GLUT4 in Xenopus oocytes and isolated rat adipocytes. Journal of Biological Chemistry. 2000;**275**:28246-28253. DOI: 10.1074/jbc.M000988200

[30] Rumsey SC, Kwon O, Xu GW, Burant CF, Simpson I, Levine M. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. Journal of Biological Chemistry. 1997;**272**:18982-18989

[31] Corpe CP, Eck P, Wang J, Al-Hasani H, Levine M. Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. Journal of Biological Chemistry. 2013;**288**:9092-9101. DOI: 10.1074/jbc.M112.436790

[32] Cisternas P, Silva-Alvarez C, Martinez F, Fernandez E, Ferrada L, Oyarce K, et al. The oxidized form of vitamin C, dehydroascorbic acid, regulates neuronal energy metabolism. Journal of Neurochemistry. 2014;**129**:663-671. DOI: 10.1111/jnc.12663

[33] Li X, Qu ZC, May JM. GSH is required to recycle ascorbic acid in cultured liver cell lines. Antioxidants & Redox Signaling. 2001;**3**:1089-1097. DOI: 10.1089/152308601317203594

[34] May JM, Qu Z, Li X. Requirement for GSH in recycling of ascorbic acid in endothelial cells. Biochemical Pharmacology. 2001;**62**:873-881

[35] Corti A, Casini AF, Pompella A. Cellular pathways for transport and efflux of ascorbate and dehydroascorbate. Archives of Biochemistry and Biophysics. 2010;**500**:107-115. DOI: 10.1016/j.abb.2010.05.014

[36] Hughes RE, Hurley RJ, Jones PR. The retention of ascorbic acid by guinea-pig tissues. The British Journal of Nutrition. 1971;**26**:433-438

[37] Englard S, Seifter S. The biochemical functions of ascorbic acid. Annual Review of Nutrition. 1986;**6**:365-406. DOI: 10.1146/annurev.nu.06.070186.002053

[38] Hediger MA. New view at C. Nature Medicine. 2002;**8**:445-446. DOI: 10.1038/nm0502-445

[39] Garcia-Krauss A, Ferrada L, Astuya A, Salazar K, Cisternas P, Martinez F, et al. Dehydroascorbic acid promotes cell death in neurons under oxidative stress: A protective role for astrocytes. Molecular Neurobiology. 2016;**53**:5847-5863. DOI: 10.1007/s12035-015-9497-3

[40] Allaman I, Belanger M, Magistretti PJ. Astrocyte-neuron metabolic relationships: For better and for worse. Trends in Neurosciences. 2011;**34**:76-87. DOI: 10.1016/j.tins.2010.12.001

[41] Korcok J, Wu F, Tyml K, Hammond RR, Wilson JX. Sepsis inhibits reduction of dehydroascorbic acid and accumulation of ascorbate in astroglial cultures: Intracellular ascorbate depletion increases nitric oxide synthase induction and glutamate uptake inhibition. Journal of Neurochemistry. 2002;**81**:185-193

[42] Daskalopoulos R, Korcok J, Tao L, Wilson JX. Accumulation of intracellular ascorbate from dehydroascorbic acid by astrocytes is decreased after oxidative stress and restored by propofol. Glia. 2002;**39**:124-132. DOI: 10.1002/glia.10099

[43] Wilson JX, Dragan M. Sepsis inhibits recycling and glutamate-stimulated export of ascorbate by astrocytes. Free Radical Biology & Medicine. 2005;**39**:990-998. DOI: 10.1016/j.freeradbiomed.2005.05.020

[44] Wilson JX. Antioxidant defense of the brain: A role for astrocytes. Canadian Journal of Physiology and Pharmacology. 1997;**75**:1149-1163

[45] Kim EJ, Won R, Sohn JH, Chung MA, Nam TS, Lee HJ, et al. Anti-oxidant effect of ascorbic and dehydroascorbic acids in hippocampal slice culture. Biochemical and Biophysical Research Communications. 2008;**366**:8-14. DOI: 10.1016/j.bbrc.2007.11.050

[46] Song JH, Shin SH, Ross GM. Prooxidant effects of ascorbate in rat brain slices. Journal of Neuroscience Research. 1999;**58**:328-336

[47] Song JH, Shin SH, Ross GM. Oxidative stress induced by ascorbate causes neuronal damage in an *in vitro* system. Brain Research. 2001;**895**:66-72

[48] Song JH, Shin SH, Chung IM. Effects of glutamate on dehydroascorbate uptake and its enhanced vulnerability to the peroxidation in cerebral cortical slices. Experimental & Molecular Medicine. 2002;**34**:419-425. DOI: 10.1038/emm.2002.59

[49] Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR, et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:13604-13609. DOI: 10.1073/pnas.0506390102

[50] Chen Q, Espey MG, Sun AY, Lee JH, Krishna MC, Shacter E, et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid *in vivo*. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:8749-8754. DOI: 10.1073/pnas.0702854104

[51] Huang J, Agus DB, Winfree CJ, Kiss S, Mack WJ, McTaggart RA, et al. Dehydroascorbic acid, a blood-brain barrier transportable form of vitamin C, mediates potent cerebroprotection in experimental stroke. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:11720-11724. DOI: 10.1073/pnas.171325998

[52] Hyslop PA, Zhang Z, Pearson DV, Phebus LA. Measurement of striatal H_2O_2 by microdialysis following global forebrain ischemia and reperfusion in the rat: Correlation with the cytotoxic potential of H_2O_2 *in vitro*. Brain Research. 1995;**671**:181-186

[53] Ducruet AF, Mack WJ, Mocco J, Hoh DJ, Coon AL, D'Ambrosio AL, et al. Preclinical evaluation of postischemic dehydroascorbic acid administration in a large-animal stroke model. Translational Stroke Research. 2011;**2**:399-403. DOI: 10.1007/s12975-011-0084-2

[54] Dixit S, Bernardo A, Walker JM, Kennard JA, Kim GY, Kessler ES, et al. Vitamin C deficiency in the brain impairs cognition, increases amyloid accumulation and deposition, and oxidative stress in APP/PSEN1 and normally aging mice. ACS Chemical Neuroscience. 2015;**6**:570-581. DOI: 10.1021/cn500308h

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