Amino Acids (2008) 34: 347–355
DOI 10.1007/s00726-007-0555-7
Printed in The Netherlands

Amino Acids

SVCT1 and SVCT2: key proteins for vitamin C uptake

Review Article

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Received March 14, 2007
Accepted April 18, 2007
Published online June 1, 2007;

Summary. Vitamin C is accumulated in mammalian cells by two types of proteins: sodium-ascorbate co-transporters (SVCTs) and hexose transporters (GLUTs); in particular, SVCTs actively import ascorbate, the reduced form of this vitamin.

SVCTs are surface glycoproteins encoded by two different genes, very similar in structure. They show distinct tissue distribution and functional characteristics, which indicate different physiological roles. SVCT1 is involved in whole-body homeostasis of vitamin C, while SVCT2 protects metabolically active cells against oxidative stress. Regulation at mRNA or protein level may serve for preferential accumulation of ascorbic acid at sites where it is needed.

This review will summarize the present knowledge on structure, function and regulation of the SVCT transporters. Understanding the physiological role of SVCT1 and SVCT2 may lead to develop new therapeutic strategies to control intracellular vitamin C content or to promote tissue-specific delivery of vitamin C-drug conjugates.

Keywords: Ascorbic acid transport – Self-regulation – Vitamin C deficiency – Pharmacological applications

Abbreviations: AP-1, Activator protein-1; AP-2, activator protein-2; EGR-1, early growth response 1; FAST-1, fork-head activin signal transducer 1; GLUTs, hexose transporters; HIF, hypoxia-inducible factor; NFY, nuclear factor Y; SMAD-3, mothers against decapentaplegic homolog 3; SVCTs, sodium-vitamin C transporters; TM, trans-membrane; USF, upstream stimulatory factor; XBP, X-box-binding-protein

Discovery

Independently from their biosynthetic ability, all animal cells are strictly dependent on the presence of functional vitamin C transporters, which determine the distribution of this molecule between extra- and intra-cellular fluids. Both the reduced (ascorbic acid) and the oxidised (dehydroascorbic acid) forms of vitamin C can be transported across the plasma membrane, although by distinct carriers. Dehydroascorbic acid can be internalized by facilitative hexose transporters (GLUT1, GLUT3 and GLUT4) (Rumsey et al., 1997, 2000) and then reduced to ascorbic acid by different enzymatic systems. Conversely, the reduced form is imported by an active mechanism, requiring two sodium-dependent vitamin C transporters (SVCT1 and SVCT2), cloned for the first time in 1999 (Tsukaguchi et al., 1999).

SVCT1 and SVCT2 belong to a family of nucleobase transporters, which includes *Aspergillus nidulans* uric acid-xanthine permease A (UapA) and general purine permease (UapC), bacterial xanthine transporter (PbuX), uracil transporter (UraA) and membrane-bound uracil permease (PyrP) (Faaland et al., 1998; Meintanis et al., 2000). These two proteins have no structural homology with any other mammalian membrane transporter. In humans, the family of ascorbate transporters includes SVCT1 (previously identified as yolk-sac permease-like molecule 3, YSPL3) and SVCT2 (yolk-sac permease-like molecule 2, YSPL2, or KIAA0238) and two orphan members, named SVCT3 (yolk-sac permease-like molecule 1, YSPL1) and SVCT4 (Takanaga et al., 2004). Characterization of SVCT1 and SVCT2 indicates that they have a high sequence homology; nonetheless they exert distinct functions, depending on their cellular distribution.

Gene structure

The two human isoforms have been cloned (Daruwala et al., 1999; Rajan et al., 1999), using an amplification

strategy based upon the previously cloned orthologs in rats (Tsukaguchi et al., 1999). SVCT1 and SVCT2 are encoded by the *SLC23A1* and *SLC23A2* genes, as the HUGO nomenclature reassigned in 2003. The *SLC23A1* gene is 16,096 bp long, contains 15 exons and maps to human chromosome 5q31.2–31.3 (Wang et al., 2000; Eck et al., 2004). The *SLC23A2* gene maps to chromosome 20p12.2–12.3 and is ten times larger, being 158,398 bp long and containing 17 exons (Stratakis et al., 2000; Eck et al., 2004).

Data on SVCT promoters are scarce and incomplete. Analysis of the SVCT1 genomic structure reveals the presence of classical CAAT and TATA 1 boxes, together with two AP-1 and one GATA1 binding sites (Erichsen et al., 2001). Concerning the *SLC23A2* regulatory regions, two putative promoters (P1 and P2) are located immediately upstream of two first exons (named exon1a and exon1b) in human vascular smooth muscle cells; P1 and P2 originate two 5' untranslated region (5′ UTR) variants, as the putative translation start site is located in exon 3 (Rubin et al., 2005). When cloned into a reporter construct, P1 and P2 show promoter activity, with P2 being more efficient than P1. Analysis of transcripts abundance further confirmed this result, as the exon1b variant is preferred in several cultured cells (Rubin et al., 2005). By computer analysis, P1 appears to have single sites for XBP/USF, NFY, HIF, FAST-1 and SMAD3 transcription factors, while multiple cis-acting elements are found in P2, including 4 zinc finger transcription factor sites, 3 SP1 sites, 2 EGR1 sites, an AP2 site, a metal transcription factor site and a Myc-associated zinc finger protein site (Rubin et al., 2005).

The open reading frames (ORFs) of the two genes are of comparable size (1,791 and 1,953 for *SLC23A1* and *SLC23A2*, respectively); moreover, the exon-intron borders are in similar positions in the two genes (Eck et al., 2004) and the main difference is the position of introns in the 5′ and 3′ UTR (Eck et al., 2004). Homology among transcripts from different species (mouse, rat, pig and human) is about 86–95 and 89–95% for SVCT1 and SVCT2 mRNAs, respectively (Daruwala et al., 1999; Clark et al., 2002; Obrenovich et al., 2006). These data have suggested a duplication of a common ancestral gene, which has been calculated to occur prior to the divergence of bony fish and tetrapods, an event dating 450 million years ago (Kumar and Hedges, 1998; Eck et al., 2004). The hypothesis of gene duplication is sustained by the observation that neighbouring genes of both *SLC23A1* and *SLC23A2* are conserved in human and mouse (Eck et al., 2004).

Both genes have been screened for genetic variants and, interestingly, only *SLC23A1* has been found to contain non-synonymous single nucleotide polymorphisms. This suggests that the coding region of the larger gene could be under a more conservative selective pressure (Eck et al., 2004).

Protein structure

Biochemical and structural studies are lacking, since no purified proteins have been as yet obtained. Thus, all the available information mainly comes from predictions based on the deduced primary amino acid sequence and Western blot analysis.

The exact amino acid length of the proteins is different in various species: rat SVCT1 and SVCT2 have 604 and 592 amino acids (Tsukaguchi et al., 1999), while mouse proteins are 605 (Faaland et al., 1998) and 647 amino acids long (Gispert et al., 2000), respectively. Human SVCT1 lacks a seven amino acid fragment in the N-terminus, thus resulting in a protein of 598 amino acids, whereas human SVCT2 contains 650 residues (Faaland et al., 1998; Daruwala et al., 1999; Rajan et al., 1999; Wang et al., 1999; Liang et al., 2001). The overall amino acid identity of SVCT1 and SVCT2 is 65% in human and rat (Tsukaguchi et al., 1999) and 60% in mouse (Faaland et al., 1998).

Mouse and rat isoforms appear to have a mass between 65 and 75 kDa (Wu et al., 2003a, b; Jin et al., 2005; May et al., 2005; May and Qu, 2005; Mun et al., 2006); for SVCT2, two bands, possibly related to the glycosilation state, can be identified by the use of specific antibodies (Garcia Mde et al., 2005). Concerning human SVCTs, information about their molecular mass is controversial. Some papers report SVCT1 and SVCT2 molecular weights and glycosilation state close to those found in mouse and rat (Liang et al., 2002; Lutsenko et al., 2004; Kang et al., 2007), but these data mainly derive from over-expression of transfected human SVCTs. For at least SVCT2, the in vivo situation appears to be different: indeed, experiments carried out in different models (neurons, platelets and melanoma) show a single band at about 50 kDa (Li et al., 2003; Godoy et al., 2007; Savini et al., 2007).

A putative structure for SVCT1 and SVCT2 has been predicted by hydropathy analysis. Kyte-Doolittle plots for the two transporters are super imposable (Faaland et al., 1998; Liang et al., 2001) and indicate that both are trans-membrane (TM) proteins. The predicted structure contains 12 membrane-spanning domains, with both the N- and the C-termini (102 and 81 amino acids) located on the cytoplasmic side of the membrane. The extracellular loop between the 7 and 8 TM domains contains a

series of conserved proline residues, which are needed for structure stability and transport efficiency (Liang et al., 2001). Other conserved proline residues, putatively located within the TM segments, are thought to be important for determination of protein structure (Liang et al., 2001). A small C-terminus fragment (PICPVFKGFS, amino acids 563–572), encoding a β-turn motif and homologous to other sodium-dependent transporters (Cheng et al., 2002; Sun et al., 2003), is required for targeting SVCT1 to the apical membrane of intestinal cells (Maulen et al., 2003; Subramanian et al., 2004; Boyer et al., 2005).

Non-functional splice variants have been identified both for human SVCT1 and SVCT2. The SVCT1 variant, isolated from the human colon adenocarcinoma cell line CaCo-2, contains an extra four amino acids insert (VGLH) in the second extracellular loop between the 3 and 4 TM domains (Wang et al., 1999). The SVCT2 variant is a truncated protein resulting from a 345 bp deletion, which excludes the 5 and 6 TM domains and part of the 4 TM domain. Unlike the full length isoform, the truncated protein is unable to promote ascorbate transport, but it probably regulates ascorbate uptake in tissues where it is the predominant carrier (Lutsenko et al., 2004).

Functional properties

The functional aspects of SVCT1 and SVCT2 have been studied in several experimental models. Nonetheless, kinetic properties of cloned SVCT1 and SVCT2 appear different depending on cell, tissue, or species from which the transporters were cloned, as well as on the expression system employed. Moreover, as shown in Table 1, the same model can give different K_m values in different laboratories, a phenomenon that may be attributable to specific experimental procedures. Finally, other information come from in vivo measurements, performed in cells where one type of transporter was lacking or where a differential analysis of the two components was done (Maulen et al., 2003). For the human isoforms, K_m values appear to be 65–237 μM for SVCT1 and 8–62 μM for SVCT2. Recently, it was reported that K_m value for SVCT2 expressed in 10 different primary cells and cell lines is close to 20 μM (Godoy et al., 2007). The two isoforms have also different capacity for ascorbic acid as, in all heterologous expression studies, human SVCT1 exhibits a higher V_{max} value than SVCT2. Thus, SVCT2 is a high affinity/low capacity transporter, whereas SVCT1 is a high capacity/low affinity carrier.

For both isoforms, the ascorbate transport occurs with a pH optimum of about 7.5 (Liang et al., 2001). At pH 5.5, about 50–60% inhibition occurs with a mechanism that is unrelated to the ionic state of ascorbic acid, since it is present as ascorbate anion in this range. It has been suggested that the inhibition during acidification of the aqueous compartments is mostly due to a reduced binding affinity of the transporters, through protonation of four conserved histidine residues (Liang et al., 2001).

Both transporters show a stringent specificity for L-ascorbic acid, since D-ascorbic acid, dehydroascorbic acid, ascorbic acid 2-phosphate, ascorbic acid 2-sulphate and L-gulono-c-lactone are not substrates for SVCT1 or SVCT2 (Rumsey et al., 1999; Tsukaguchi et al., 1999).

SVCT functions are obligatorily dependent on sodium ions. Replacement of Na^+ with K^+, Li^+ or coline almost completely abolishes ascorbate uptake (Rajan et al., 1999). The vitamin crosses cell membranes against an

Table 1. Apparent K_m values of SVCT1 and SVCT2 in different experimental models

Model	SVCT2 Apparent K_m (μM)	SVCT1 Apparent K_m (μM)	Reference
Human exogenous expression			
X. laevis oocytes	22	237	Daruwala et al. (1999)
X. laevis oocytes	21	107	Corpe et al. (2005)
X. laevis oocytes	58	90	Wang et al. (2000)
X. laevis oocytes	–	149	Song et al. (2002)
HRPE cells	62	–	Rajan et al. (1999)
CHO cells	14.6	103	Boyer et al. (2005)
COS-1 cells	29	65	Liang et al. (2002)
HRPE cells	–	74	Wang et al. (1999)
HEK-293 cells	13	–	Godoy et al. (2007)
Human endogenous expression			
Melanoma cells	17	–	Godoy et al. (2007)
Skin fibroblasts	27	–	Corpe et al. (2005)
Platelets	29	–	Savini et al. (2007)
Melanocytes	20	–	Astuya et al. (2005)
Endothelial cells	13,2	–	Seno et al. (2004)
Chondrocytes	62	–	McNulty et al. (2005)
Neuroblastoma cells	113		May et al. (2006)
Colonic cells	8	125	Maulen et al. (2003)
Other species			
Pig endothelial cells	27	–	Best et al. (2005)
Mouse myotubes	24	–	Savini et al. (2007)
Mouse neurons	103	–	Castro et al. (2001)
Mouse cortical neurons	115	–	Qiu et al. (2007)
Mouse tanycytes	20	–	Garcia et al. (2005)
Mouse osteoblasts	35	–	Wu et al. (2003a)
Mouse macrophages	41	–	May et al. (2005)
Rat myotubes	17	–	Savini et al. (2007)
Rat astrocytes	32	–	Korcok et al. (2000)
Rat SVCTs (in *X. laevis* oocytes)	10–100	29	Tsukaguchi et al. (1999)

electrochemical barrier. The energy is provided by the favourable inward gradient for Na^+, which in turn is sustained by the continuous extrusion of Na^+ by the Na^+/K^+-ATPase. Indeed, ouabain, a specific inhibitor of the Na^+/K^+-ATPase, almost completely abrogates ascorbate transport (Castro et al., 2001; Garcia Mde et al., 2005). The relationship between transport rate and sodium concentration is sigmoidal, indicating the involvement of more than one Na^+ ion per molecule of ascorbate transported. Indeed, the Na^+: ascorbic acid stoichiometry is 2:1. The transport is electrogenic (there is a net positive charge movement inside cells) for SVCT1 (Tsukaguchi et al., 1999), but not for SVCT2 (Godoy et al., 2007).

Only very recently, the detailed mechanism of action of SVCT2 has been elucidated (Godoy et al., 2007). SVCT2 is modulated by Ca^{2+} and Mg^{2+} ions, that switch the transporter from an inactive into an active form. The protein functionality is also influenced by a reciprocal interaction between Na^+ and ascorbic acid, determining the binding order and transport stoichiometry. In the absence of Ca^{2+} and Mg^{2+}, SVCT2 exists in an inactive state, at Na^+ concentrations below 20 mM the transporter shows a low affinity conformation and finally, at elevated Na^+ concentrations, high affinity conformations, modulated by the two substrates, are present. In addition, SVCT2 may be localized intracellularly or on the plasma membrane. In the first case, it should be in the low affinity conformation, as it is bound to Mg^{2+}, exposed to Na^+ concentrations below 20 mM and to fluctuations in cytoplasmic Ca^{2+} concentrations. On the contrary, when it is exposed on the cell surface, the transporter is bound to Mg^{2+} and Ca^{2+}, exposed to 135 mM extracellular Na^+ concentrations and, thus, it would be in the high affinity conformation. Godoy et al. (2007) explain the absence of electrogenicity for SVCT2 with three hypotheses: (i) currents are undetectable because they are too low, (ii) a cation efflux may occur simultaneously with the Na^+ and ascorbate influx or (iii) a cation efflux occurs at the end of the process, when the transporter returns to the initial conformation.

Regulation

Ascorbate transport is mainly controlled by carriers availability, and thus it depends on the number of SVCT proteins present in the plasma membrane (which in turn is related to enhanced synthesis, slowed degradation, activation of non-functional carriers or cellular redistribution), as well as their substrate affinity. So, transcriptional, translational and post-translational modifications of SVCTs allow a fine-tuned regulation of vitamin C uptake (Liang et al., 2002).

In vitro and in vivo studies have revealed a transcriptional control mechanism for SVCTs activity by hormones and intracellular signalling molecules. For example, SVCT2 mRNA levels are increased by foetal bovine serum and epidermal growth factor in a human trophoblast cell line (Biondi et al., 2007) and by glucocorticoids (Fujita et al., 2001), zinc (Wu et al., 2003b) and calcium/phosphate ions (Wu et al., 2004) in osteoblastic cells. In vivo, SVCT2 mRNA is over-expressed following ischemic brain injury (Berger et al., 2003). Moreover, SVCT1 expression decreases in rat hepatocytes during ageing (Michels et al., 2003) and SVCT2 expression is down-modulated during differentiation of rat and mouse muscle cells (Savini et al., 2005).

Recently, we demonstrated a translational control for SVCT2 in human platelets. In response to activation by thrombin or phorbol esters, platelets increase endogenous SVCT2 protein levels (Savini et al., 2007), thus suggesting a role for protein synthesis in controlling ascorbate uptake. Indeed, platelets are anucleated cells so that the involvement of transcription factors in the regulation of expression is ruled out. Nonetheless, different transcripts are localized in polysomes of resting platelets, so that translational mechanisms may contribute to replenishment of molecules lost during platelet activation.

Post-translational modifications represent an additional level of regulation. Different putative regulatory sites are present throughout the amino acid sequence of SVCT1 and SVCT2 (Fig. 1). The N-terminus contains more than 20% of acidic residues, that can act as a regulatory domain deputed to interaction with other proteins (Liang et al., 2001). Regulation based on protein–protein interaction has been demonstrated for the alternatively spliced form of SCVT2, that can act as a dominant negative isoform (Lutsenko et al., 2004). Both carriers also possess putative glycosilation and phosphorylation sites. Conserved N-linked glycosilation sites are located in the second and third extracellular loop (Asn 138, 144 and 230 in human SVCT1; Asn 188 and 196 in human SVCT2) (Faaland et al., 1998; Tsukaguchi et al., 1999; Liang et al., 2001). One conserved protein kinase A (PKA)-dependent (Thr 557 of human SVCT1) and several protein kinase C (PKC)-dependent (Thr 15, Ser 396, Ser 454, Ser 540 and Ser 574 in human SVCT1; Thr 9, Ser 299, Ser 455, Ser 513, Thr 629 in human SVCT2) phosphorylation sites are present in different portions of the protein. In humans, the PKC enzymatic activity appears to be isoform-specific: phosphorylation alters SVCT2 transport activity by chang-

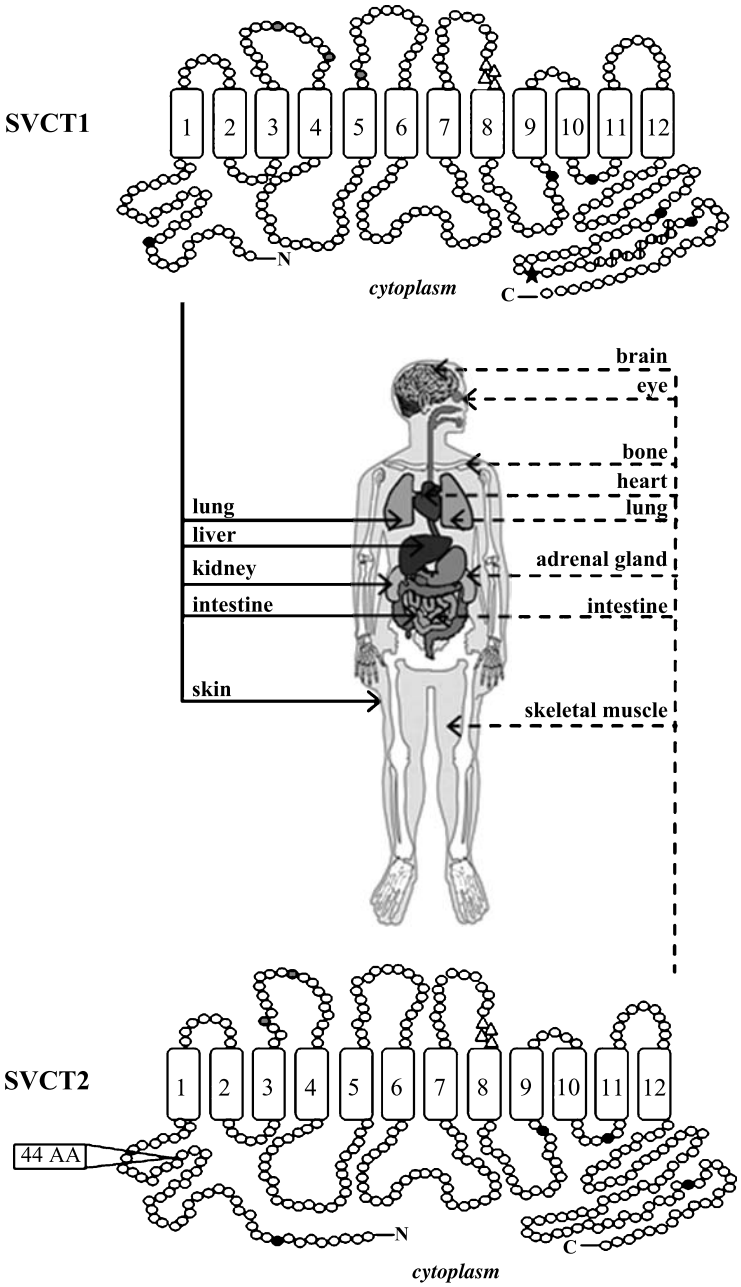

Fig. 1. Model and tissue distribution of human SVCT1 and SVCT2. Potential membrane spanning domains are numbered from 1 to 12. Grey circles represent N-linked glycosylation sites; black circles represent protein kinase C phosphorylation sites, while residues for protein kinase A phosphorylation are marked with a star. The C-terminus fragment required for apical membrane targeting (datched circles) and significant proline residues (triangles) are also shown. Solid lines indicate SVCT1 distribution; dotted lines indicate SVCT2 distribution

ing protein conformation, while, in the case of SVCT1, it inhibits the cytoplasm-to-membrane translocation. Redistribution from cytosol to membrane appears to be a common mechanism of regulation for SVCT1, since UVB irradiation uses a similar way to activate SVCT1 in keratinocytes (Kang et al., 2007).

An interesting finding is that the two Na^+-dependent transporters can be subjected to a fine-tuned regulation by their own substrate. Indeed, elevated levels of ascorbate in the intestinal lumen leads to down-regulation of SVCT1 mRNA in enterocytes (MacDonald et al., 2002), although the exact mechanisms involved are still unknown; down regulation may be due to transcriptional repression or, alternatively, to decrease in mRNA stability. Ascorbate regulates its bioavailability, controlling not only intestinal SVCT1 expression, but also SVCT2-mediated cellular uptake in other body compartments. Indeed, ascorbate supplementation or deprivation influence its own transport in osteoblasts (Dixon and Wilson, 1992) and astrocytes (Wilson et al., 1990); in addition, we recently demonstrated a substrate-mediated translational control for SVCT2 in platelets (Savini et al., 2007). Finally, a feedback mechan-

ism has been demonstrated also in human lung epithelial cells, where a loss of intracellular ascorbate is compensated by active uptake thanks to a marked increase of SCVT2 expression (Karaczyn et al., 2006).

Physiological roles

Distribution and kinetic parameters suggest that the primary role of SVCT1 is maintenance of the whole-body homeostasis, through dietary absorption and renal reabsorption, while SVCT2 is crucial for ascorbate uptake in metabolically active and specialized tissues, thus protecting them from oxidative stress.

Information about the physiological functions of SVCT1 and SVCT2 has been gained, in the last years, from animals (Sotiriou et al., 2002) and cultured cells (McNulty et al., 2005) knocked out for the transporter genes. In a milestone paper, Sotiriou et al. (2002) demonstrated that mice knocked out for Slc23a2 (and, thus, completely deficient in SVCT2) have a normal development in uterus, but they die within minutes of birth. Although the cause of death has not been ascertained in the animals, Slc23a2$-/-$ mice show diffuse cerebral haemorrhage and fail to breath, suggesting that death is due to impairment of functions in the central nervous system. Unlike knockout animals, heterozygous SVCT2 (Slc23a2$+/-$) mice survive to adulthood, but they have low ascorbate levels in several tissues prior to birth, as well as in the brain at 9–11 months of age. This study evidenced an indispensable need for SVCT2 during prenatal life, further corroborated by recent findings on the role of SVCT2 on placental transport (Biondi et al., 2007). A human trophoblast cell line and human first-trimester chorionic villi express SVCT2, which is crucial for vitamin C transplacental transfer from the maternal to the fetal circulation. Thus, the placental transport mediated by SVCT2 is important in rodents, where vitamin synthesis begins late in gestation, and may be more crucial in primates, where the fetal demand is always met by the mother. It is noteworthy that some genetic variants of human vitamin C transporters have been related to preterm delivery (<37 weeks' gestation), a leading cause of neonatal mortality and birth-related morbidity (Erichsen et al., 2006).

Respiratory failure and intracerebral haemorrhage seen in knock-out newborns immediately after birth underline the critical function of the SVCT2 transporter in specific body districts. Below, we will report some examples, based on studies performed in organs where vitamin C is naturally abundant or where it plays a crucial role.

a) Central nervous system

In brain, SVCT2 is present in neuroepithelial cells of the choroid plexus, allowing the ascorbate transport in cerebrospinal fluid, as well as in neurons (Tsukaguchi et al., 1999). It acts by a two-step mechanism, transporting ascorbate from plasma into the cerebrospinal fluid and then into neurons (Qiu et al., 2007). A more specific role of SVCT2 in ascorbate retention has been recently demonstrated: as expected, cultured hippocampal and cortical neurons derived from knock-out mice are completely deficient on ascorbic acid, whereas wild-type neurons show small amounts of intracellular ascorbate, even if the vitamin was lacking in the culture medium, thus suggesting a role for the transporter in the re-uptake of ascorbate lost from the cells. The biological significance of SVCT2 in brain appears to be strictly related to neuronal development, functional maturation and antioxidant responses. Indeed, SVCT2-deficient neuronal cells showed reduced neurite outgrowth and neuronal activity, as well as increased susceptibility to oxidative damage (Qiu et al., 2007).

b) Respiratory system

Both SVCTs are expressed in the respiratory system, although it is yet unclear whether the two proteins have different roles. An immunohistochemical study reveals that SVCT1 and SVCT2 are located in the apical portion of respiratory epithelial cells, from trachea to terminal bronchiole and some alveolar cells, suggesting that the SVCT proteins transport ascorbate from the airway surface liquid into the respiratory epithelial cells (Jin et al., 2005). Fischer et al. (2004) proposed that SVCT transporters may be essential for the ascorbate-dependent activation of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel which regulates epithelial surface fluid secretion.

c) Intestine

Likely in the respiratory system, SVCT transporters are both present in the intestine (Maulen et al., 2003), although they are differently distributed and play distinct roles. SVCT1, being expressed on the apical membrane, transports vitamin C across the intestinal barrier (MacDonald et al., 2002), while SVCT2 is located at the basolateral surface, where it should be involved in vitamin C transport from blood into enterocytes (Boyer et al., 2005).

d) Kidney

Kidney plays a crucial role in maintaining vitamin C homeostasis through excretion and reabsorption. Immunohistochemistry analysis shows that SVCT1 is expressed in medullary rays and the brush border of proximal tubule (Lee et al., 2006), whereas SVCT2 is absent. A polar distribution of SVCT1 has also been demonstrated in a kidney cell line (Subramanian et al., 2004).

e) Adrenal gland

SVCT2 is the main isoform in adrenal glands and plays a key role in adrenal chromaffin cell functions. In SVCT2-null mice, adrenal cells are less capable of producing and storing catecholamines (although these hormones are not significantly decreased in brain and heart); cells become hypofunctional and eventually apoptotic, with an altered mitochondrial morphology. Moreover, SVCT2-null mice show impaired plasma corticosterone levels (Bornstein et al., 2003; Patak et al., 2004).

f) Bone and cartilage

As mentioned above, SVCT2 expression is enhanced by a variety of factors involved in bone development, such as Ca^{2+} (Wu et al., 2003a), Zn^{2+} (Wu et al., 2003b) and dexamethasone (Fujita et al., 2001). Thus, SVCT2 overexpression leads to osteoblast differentiation, mineralization and calcium deposition (Wu et al., 2004). SVCT2 also mediates ascorbate uptake in chondrocytes, allowing cartilage to store vitamin C needed for the synthesis of type II collagen in the extracellular matrix (McNulty et al., 2005).

g) Eye

SVCT2 is the major determinant of vitamin C concentration in lens. Mouse lens epithelial cells show vitamin C levels lower than humans, a phenomenon ascribed to SVCT2 levels. Indeed, the ascorbate uptake is significantly improved by SVCT2 transient transfection and not by vitamin C supplementation (Obrenovich et al., 2006).

Future directions

Cloning of SVCTs has led to the structural characterization of vitamin C transport, as well as the distribution and physiological role of the two proteins. Nonetheless, further research is needed to better understand how these transporters are regulated and if their therapeutic alteration may be useful in prevention of vitamin C deficiency. Increasing knowledge on regulatory mechanisms will also allow to discover potential interference with apparently unlinked drugs. Just an example, some cyclo-oxygenase inhibitors (diclofenac and indomethacin) down-regulate SVCT2 expression, suggesting caution in their therapeutic use during pregnancy (Biondi et al., 2007). Pharmacological applications of vitamin C transport are under investigation, since kinetic features of SVCTs can be exploited for specific targeting of some drugs. Indeed, Dalpiaz et al. (2005) conjugated an anticonvulsant drug (nipecotic acid) with ascorbate, thus improving its brain delivery through SVCT2; the interesting finding is that the drug alone does not interact with SVCT2, but it becomes able to interact with SVTC2 and to perform in vivo anticonvulsant activity only when conjugated to ascorbate.

Acknowledgement

This work was supported by grant from the Italian MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca).

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