REVIEW

System x$_c^-$ cystine/glutamate antiporter: an update on molecular pharmacology and roles within the CNS

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System x$_c^-$ is an amino acid antiporter that typically mediates the exchange of extracellular L-cystine and intracellular L-glutamate across the cellular plasma membrane. Studied in a variety of cell types, the import of L-cystine through this transporter is critical to glutathione production and oxidative protection. The exchange-mediated export of L-glutamate takes on added significance within the CNS, as it represents a non-vesicular route of release through which this excitatory neurotransmitter can participate in either neuronal signalling or excitotoxic pathology. When both the import of L-cystine and the export of L-glutamate are taken into consideration, system x$_c^-$ has now been linked to a wide range of CNS functions, including oxidative protection, the operation of the blood–brain barrier, neurotransmitter release, synaptic organization, viral pathology, drug addiction, chemosensitivity and chemoresistance, and brain tumour growth. The ability to selectively manipulate system x$_c^-$, delineate its function, probe its structure and evaluate it as a therapeutic target is closely linked to understanding its pharmacology and the subsequent development of selective inhibitors and substrates. Towards that goal, this review will examine the current status of our understanding of system x$_c^-$ pharmacology and the structure–activity relationships that have guided the development of an initial pharmacophore model, including the presence of lipophilic domains adjacent to the substrate binding site. A special emphasis is placed on the roles of system x$_c^-$ within the CNS, as it is these actions that are among the most exciting as potential long-range therapeutic targets.

Abbreviations

4-S-CPG (S)-4-carboxyphenylglycine; 4-S-SPG, sulphonic acid phenylglycine; ACPA, amino-3-carboxy-5-methyl isoxazole propionic acid; *trans*-ACPD,1-aminocyclopentane-*trans*-1,3-dicarboxylic acid; ApcT, amino acid polyamine organo-cation transporter; ARE, antioxidant response element; ASC, alanine–serine–cysteine uptake systems; β-L-ODAP, L-β-N-oxalyl-L-a,β-diaminopropionate; BSO, buthionine sulphoximine; CPPG (R,S)-4-[4′-carboxyphenyl]-phenylglycine; dbcAMP, dibutyryl cyclic AMP; EAA, excitatory amino acid; EAAT, excitatory amino acid transporter; GSH, glutathione; HAT, heteromeric amino acid transporter family; iGluR, ionotropic glutamate receptor; KA, kainate; L-CysH, L-cysteine; L-Cys$_2$, L-cystine; L-*trans*-2,4-PDC, L-*trans*-2,4-pyrrolidine dicarboxylate; mGluR, metabotropic glutamate receptor; NAC, N-acetyl-cysteine; NACPA, S-2-naphthyl-ethyl-ACPA; NEIH, 5-naphthylethyl isoxazole-4-(2,4-dinitrophenol)hydrazone-dinitrophenol; Nrf2, nuclear factor erythroid-2-related factor; QA, quisqualate; ROS, reactive oxygen species; SAR, structure–activity-relationship; SLC, solute carrier gene families, SSZ, sulphasalazine, *aka* 6-oxo-3-(2-[4-(N-pyridin-2-ylsulphamoyl)phenyl]diazenyl)cyclohexa-1,4-dienecarboxylic acid; Sx$_c^-$, System x$_c^-$; TMDs, transmembrane domains; TSA, R,S-4-sulphothienylglycine; xCT, transporters subunit of Sx$_c^-$ in the SLC7A11 superfamily

System x$_c^-$ (Sx$_C^-$) is an amino acid antiporter that typically mediates the exchange of extracellular L-cystine (L-Cys$_2$) and intracellular L-glutamate (L-Glu) across the cellular plasma membrane. Initial characterizations of this transporter most often focused on the influx of L-Cys$_2$, as in many cells this uptake serves as a rate-limiting step in providing the intracellular L-cysteine (L-CysH) required for the synthesis of glutathione (GSH). Within the context of the central nervous system (CNS), recent attention has shifted to the other half of the exchange reaction, as the efflux of L-Glu through the exchanger carries with it the potential to contribute to excitatory signalling and/or excitotoxic pathology. When both sides of the Sx$_c^-$-mediated exchange are taken into consideration, the range of CNS processes to which this transport system has now been linked is rather surprising, and includes oxidative protection (Shih et al., 2006a), the operation of the blood brain barrier (Hosoya et al., 2002), neurotransmitter release (Baker et al., 2002), synaptic organization (Augustin et al., 2007), viral pathology (Espey et al., 1998), drug addiction (Baker et al., 2003; Knackstedt et al., 2008; Kalivas, 2009), chemosensitivity and chemoresistance (Huang et al., 2005), and brain tumour growth (Chung et al., 2005; Chen et al., 2009; de Groot and Sontheimer, 2010). As with many transporters, the ability to selectively manipulate Sx$_C^-$ activity, delineate its function, probe its structure and evaluate it as a therapeutic target is closely linked to understanding its pharmacology and the subsequent development of selective inhibitors and substrates. Towards that goal, this review will examine the current status of our understanding of Sx$_C^-$ pharmacology and the structure–activity relationships (SARs) that will likely guide the development of ligands with greater specificity and potency. A special emphasis is placed on the roles of Sx$_C^-$ within the CNS, as it is these actions that are amongst the most exciting as potential long-range therapeutic targets.

Structure

Sx$_C^-$ is a member of the heteromeric amino acid transporter (HAT) family (a.k.a. glycoprotein-associated amino acid exchangers) (Broer and Wagner, 2002; Verrey et al., 2003; Palacin et al., 2005). These transporters are heterodimers composed of one of two types II N-glycosylated 'heavy chains' (4F2hc or rBAT, Solute Carrier SLC3 family) covalently linked via a disulphide bond to one of several hydrophobic, non-glycosylated 'light chains' (SLC7 family). In the instance of Sx$_C^-$, the heterodimer is composed of 4F2hc (≈80 kDA, a.k.a. CD98 or FRP1) and xCT (SLC7A11, ≈40 kDa). The xCT subunit is credited with the transport activity of the dimer, while 4F2hc acts in the trafficking of the light chain and is required for cell surface expression. Reconstitution studies with the HAT dimer rBAT/b°,+AT (SLC7A9), however, demonstrate that light chains may be fully functional in the absence of its corresponding heavy subunit (Reig et al., 2002). Structural studies, including those based upon cysteine-scanning and thiol-modifying reagent accessibility, indicate that xCT has 12 transmembranes domains (TMDs), intracellular N and C termini, and a re-entrant loop between TMD 2 and 3 that appears to participate in substrate binding and/or permeation (Gasol et al., 2004; Jimenez-Vidal et al., 2004). More recently,

the crystallization of a representative APC (amino acid, polyamine, organo-cation) transporter from the thermophile *Methanocaldoccus jannaschii* has provided much greater insight into HAT structure (Shaffer et al., 2009). ApcT acts as a sodium-independent transporter with a broad-specificity (e.g. substrates include L-Glu, L-alanine, L-serine, L-glutamine and L-phenylalanine). The transporter, which was crystallized in an apo (no substrate bound), inwards-facing form, exhibits an inverted repeat of five TMD helicies reminiscent of the LeuT transporter (Yamashita et al., 2005). Figure 1 shows a homologous structure for xCT that was constructed by threading the human xCT sequence through the ApcT structure (Figure 1).
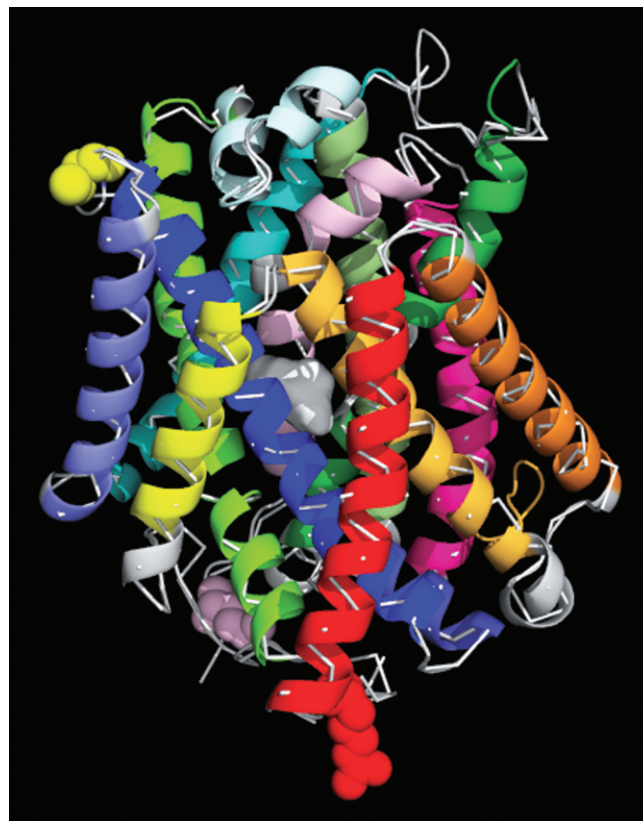

Figure 1

An xCT homology model was constructed by threading the hxCT sequence over the crystal structure of ApcT (RCSB: pdb3GIA) from *M. jannaschii* (GI:1591319) as reported by Goaux and co-workers (Shaffer et al., 2009) using FASTA (Pearson and Lipman, 1988) followed by multiple sequence alignments with ClustalW (Larkin et al., 2007). The structure of ApcT is shown as a white thread overlayed with the helical ribbons of the 12 TMDs of xCT: TMD 1A/B in light pink; TMD2 in dark pink; IL1 in dark blue; TMD3 in bright blue; TMD4 in light purple; TMD5 in teal, TMD6A/B in dark green; TMD7 in olive green; EL4A/B in silver; TMD8 in light green; TMD9 in yellow; TMD10 in gold; TMD11 in orange and TMD12 in red. Truncated intracellular N (light pink) and C (red) termini are shown with spheres. The cysteine-158 that participates in the disulphide bond with 4F2hc is highlighted with yellow spheres. The protein is shown in its inwardly facing Apo-form. A hypothetical substrate is depicted by the centrally located white surface as predicted by Schaffer et al., based upon the binding site of LeuT.

Localization

Sx_c^- has been characterized in a wide variety of cells, both outside (e.g. fibroblasts, macrophages, hepatocytes and endothelial cells) (Bannai, 1986; Sato et al., 1999; Hosoya et al., 2002; Sasaki et al., 2002) and inside the CNS [e.g. astrocytes (Cho and Bannai, 1990; Allen et al., 2001; Gochenauer and Robinson, 2001), microglia (Piani and Fontana, 1994), retinal Muller cells (Kato et al., 1993), immature cortical neurons (Murphy et al., 1990), and glioma cell lines (Cho and Bannai, 1990; Ye et al., 1999)]. The characterization of Sx_c^- distribution by using antibodies to the xCT and 4F2hc subunits show the transporter to be localized to neurons and glia in the CNS. Furthermore, Sx_c^- was highly expressed at the borders of the CNS and periphery including the vascular endothelial cells, ependymal cells, choroid plexus and the leptomeninges (Burdo et al., 2006). Similarly, expression of xCT and 4F2hc was also detected immunohistochemically in the brush border membranes of the kidney and duodenum. Work focusing on the distribution of mRNA for xCT in the mouse brain complements the antibody labelling studies in that xCT mRNA was highly detected in the meninges and specific regions facing the cerebral ventricles (Sato et al., 2002). It must be noted, however, that the detection of either xCT mRNA or xCT immunoreactivity does not necessarily confirm the expression of functional Sx_c^- at the level of the plasma membrane (Lim et al., 2007).

Pharmacology

Initial studies characterizing Sx_c^- focused primarily on non-CNS cells and concentrated on the uptake of L-Cys$_2$; differentiating the specificity, kinetics and ionic dependence of Sx_c^- from other transport systems. Thus, Sx_c^- was shown to (i) utilize either L-Glu or L-Cys$_2$ as substrates, with each acting as a competitive inhibitor of the other; (ii) function as an antiporter, mediating the 1:1 exchange of an intracellular and extracellular amino acid; (iii) act in a sodium-independent, chloride-dependent, electroneutral manner; and (iv) transport L-Glu or L-Cys$_2$ in an anionic form (Bannai and Kitamura, 1980, 1981; Waniewski and Martin, 1984; Cho and Bannai, 1990). In most of the cell types examined, the K_m values for the uptake of either L-Glu or L-Cys$_2$ were typically in the 50–100 µM range, although more recent studies with differentiated astrocytes yielded lower K_m values close to 20 µM (Seib et al., 2011).

Substrate activity

Delineating the specificity of Sx_c^- was central to these early (as well as more recent) studies and is reviewed in greater detail below. These findings not only provide insight into the SARs that govern Sx_c^- selectivity and the development of pharmacophore models, the resulting identification of inhibitors (and substrates) can yield needed small molecule probes with which to assess physiological and pathological roles of the transporter. A majority of these investigations have been based upon competitive assays that essentially screen structurally similar small molecules for the ability to block the transport of radiolabelled L-Glu or L-Cys$_2$ under conditions

selective for Sx_c^--mediated uptake. In this manner, the demonstration that none of the class-defining excitatory amino acid (EAA) receptor agonists [e.g. NMDA, kainate (KA), aminomethyl-isoxazole propionic acid (AMPA) and 1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD)] nor the well-known excitatory amino acid transporter (EAAT) inhibitors [e.g. L- or D-aspartate, L-trans-2,4-pyrrolidine dicarboxylate (L-trans-2,4-PDC) and dihydrokainate] could block Sx_c^- served to readily demonstrate that the transporter has a distinct pharmacological profile within the EAA system (Patel et al., 2004; Balazs et al., 2006; Beart and O'Shea, 2007; Bridges and Patel, 2009). While these competition assays shed light on whether or not a compound binds to the transporter, it is important to note that the resulting inhibitory data do not differentiate alternative substrates from non-substrate inhibitors. Indeed, studies on the EAAT's suggest that the chemical requirements for binding and translocation are related, but not necessarily identical to one another (Bridges and Esslinger, 2005). Following substrate activity often requires the synthesis of radiolabelled derivatives or the quantification of a secondary measure, such as the substrate-coupled conduction currents employed to study the electrogenic EAATs (Esslinger et al., 2005) (Sx_c^- is electroneutral). In the instance of Sx_c^-, a protocol originally developed to measure the release of L-Glu from synaptosomes (Nicholls et al., 1987) has been modified to quantify Sx_c^- activity by coupling the efflux of L-Glu through the transporter to its extracellular metabolism by glutamate dehydrogenase and the production of NADPH (Patel et al., 2004; Warren et al., 2004). As will be described in greater detail below, this approach has allowed us to demonstrate that the different classes of compounds that inhibit Sx_c^- also exhibit a wide range of substrate activities.

Acyclic amino acids (Figure 2)

The compounds within this series, which also includes L-Glu and L-Cys$_2$, (i) represent some of the most straight forward structural mimics of the endogenous substrates; (ii) were amongst the earliest Sx_c^- inhibitors identified and (iii) were critical to establishing tolerated limits of stereo-specificity, backbone length and charged functional group substitutions (Bannai and Kitamura, 1980; Waniewski and Martin, 1984; Bannai, 1986; Patel et al., 2004). The transporter exhibits a demonstrable stereo-selective preference for L-amino acids (e.g. D-Glu and D-Cys$_2$ exhibit little or no inhibitory activity), although it is not absolute, as D-serine-O-sulphate is a moderately effective inhibitor (Patel et al., 2004). The inactivity of D- and L-aspartate demonstrates that decreasing the chain length of carboxylic acids results in a loss of inhibitory activity. Given the backbone length of L-Cys$_2$, it is not surprising that dicarboxylic amino acids L-α-aminoadipate and L-α-aminopimelate exhibit inhibitory activities comparable to L-Glu (Bannai, 1986; Patel et al., 2004). These longer dicarboxylate acids appear, however, to be less suitable than L-Cys$_2$ or L-Glu as substrates of Sx_c^-. The inactivity of L-homocystine and L-djenkolate as inhibitors also demonstrates that there is a limit to the ability of the binding site to accommodate increasing chain length (Patel et al., 2004). Substitutions of the distal carboxylate group on L-Glu analogues further demonstrate that SO_3^- (e.g. L-homocysteate, S-sulpho-L-cysteine, L-serine-O-sulphate) and SO_2^- groups (e.g. inhibition by
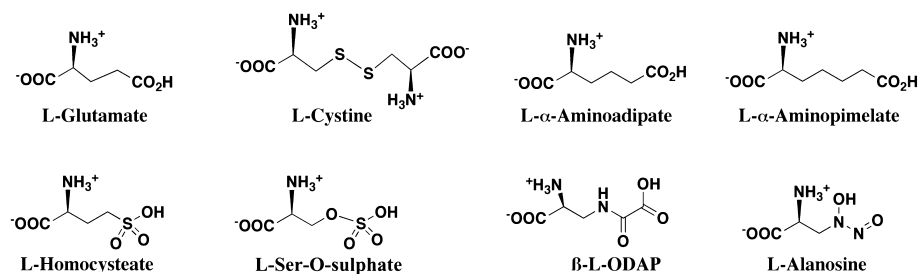
Figure 2
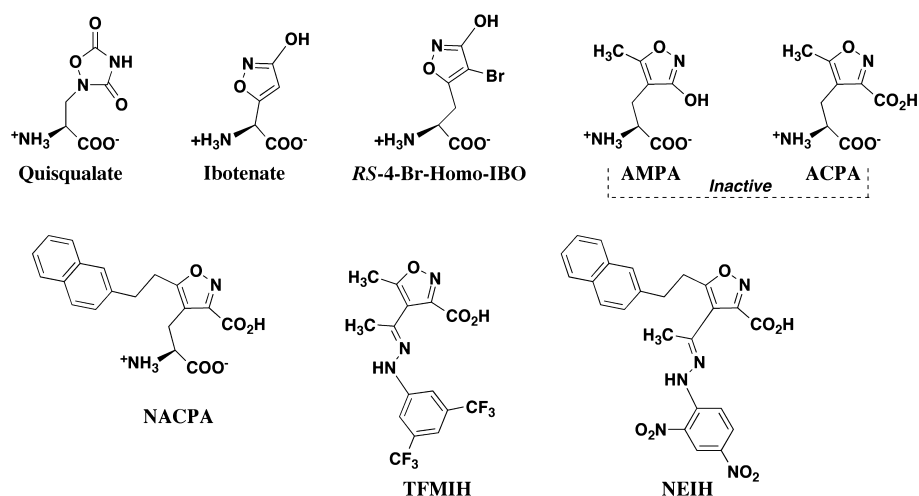Acyclic amino acid substrates and inhibitors of the system x$_c^-$ transporter.

Figure 3
Isoxazole and related heterocyclic substrate and inhibitors of the system x$_c^-$ transporter.

L-homocysteine sulphinate) are tolerated within the binding site, while PO$_3^{2-}$ groups are not (e.g. lack of inhibition by L-serine-O-phosphate). The extent to which the distal portion of L-Glu analogues can be modified and still retain potent inhibitory activity are illustrated by two novel natural products: the excitotoxin L-β-N-oxalyl-L-α,β-diaminopropionate (β-L-ODAP, K_i ≈ L-Cys$_2$ ≈ 40–100 μM) derived from *Lathyrus sativus* and the antimetabolite, antitumour agent L-alanosine from *Streptomyces alanosinicus* (Warren *et al.*, 2004; Huang *et al.*, 2005). Interestingly, both of these compounds are also substrates of the transporter, suggesting that portions of their *in vivo* actions may reflect or be dependent upon intracellular accumulation via Sx$_c^-$.

Isoxazoles and related heterocyclics (Figure 3)

The recognition that quisqualate (QA) is a potent inhibitor of Sx$_c^-$ [K_i ≈ 5 μM (Patel *et al.*, 2004)] was significant for several reasons. It provided direct evidence that Sx$_c^-$ was not only present in the CNS but also highlighted the importance of L-Cys$_2$ uptake, GSH production and oxidative protection. Thus, the toxicity of QA in select neuronal cell lines and immature cortical neurons was shown to be attributable to Sx$_c^-$ inhibition and reduced oxidative protection, rather than EAA receptor-mediated excitotoxicity (Murphy *et al.*, 1989;

1990). The action of QA also provided a pharmacological link between previously indentified Cl-dependent, L-Glu binding sites on astrocytes and the L-Cys$_2$/L-Glu exchanger (Pin *et al.*, 1984; Bridges *et al.*, 1986; 1987; Kessler *et al.*, 1987). Lastly, the conformationally constrained structure of QA provided early SAR data for pharmacophore modelling, as well as prompted the characterization of isoxazoles as potential inhibitors. In contrast to QA, AMPA [which replaced QA as the defining agonist for AMPA receptors (Monaghan *et al.*, 1989)] is inactive as an Sx$_c^-$ inhibitor. Other isoxazoles such as ibotenate (K_i ≈ 30 μM), and (RS)-4-Br-homoibotenate (K_i ≈ 20 μM), proved, however, to be very potent inhibitors of the transporter (Patel *et al.*, 2004). Interestingly, while all the compounds exhibited inhibitory activity, each displayed a different ability to act as substrates. For example, ibotenate is transported by Sx$_c^-$ as readily as is L-Cys$_2$ (RS)-4-Br-homoibotenate only about half as well, and QA only about a third as well. These findings clearly demonstrate that conformational restriction alone does not necessarily limit the ability of a compound to be transported and set a foundation to begin delineating the ligand characteristics that differentiate binding from translocation. The demonstration that QA was a substrate (albeit only a moderate one) also provided a greater mechanistic understanding of the process of 'QA sen-
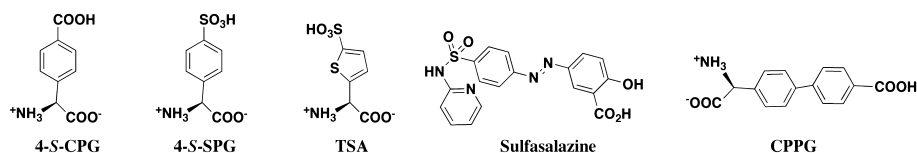
Figure 4

Benzoic acid and aromatic sulphonic acid inhibitors of the system x_c^- transporter.

sitization', where QA applied to brain slices can be sequestered in cells only to be subsequently released through Sx_c^- and activate EAA receptors (Chase *et al.*, 2001). Collectively, the actions of these isoxazoles (as well a majority of the other compounds described in this review) also serve to highlight a major challenge in the development of selective pharmacological probes of Sx_c^-, as each is equally well known for its action at other sites within the EAA system. Thus, QA acts as an agonist at AMPA and mGluR receptors, ibotenate as an NMDA receptor and non-selective mGluR agonist (*RS*)-4-Br-homoibotenate as an AMPA receptor agonist, and (*RS*)-5-Br-willardiine as a kainate receptor agonist (Monaghan *et al.*, 1989; Balazs *et al.*, 2006).

Prompted by the dichotomy that some isoxazoles are potent Sx_c^- inhibitors, while others are not, more recent work continued to examine the isoxazole ring as a scaffold for the further development of inhibitors. Building on the extensive work of Krogsgaard-Larsen and colleagues (Brauner-Osborne *et al.*, 2000), a series of derivatives based upon AMPA, amino-3-carboxy-5-methyl isoxazole propionic acid (ACPA) and non-amino acid biosteres of ACPA acid-hydrazones were synthesized in which additional substitutions were made to the isoxazole core at the 4 and 5 positions of the ring (Patel *et al.*, 2010). While neither AMPA nor ACPA are active as inhibitors, a subset of derivatives containing increasingly larger lipophilic groups, such as napthyl ($K_i \approx$ 50 μM), and trifluoromethyl-phenyl ($K_i \approx$ 60 μM), moieties, were found to block Sx_c^- with affinities comparable with L-Cys2. The resulting SAR data revealed not only a divergence point that distinguishes the binding sites of Sx_c^- and GluR2, it also demonstrated the presence of lipophilic binding domains adjacent to the substrate site on the Sx_c^- transporter. Significantly, exploiting the presence of such lipophilic pockets has been key to developing very potent and selective EAAT inhibitors (Shimamoto *et al.*, 2004; Dunlop *et al.*, 2005). Indeed, the structural alignment of these inhibitors [e.g. *S*-2-naphthyl-ethyl-ACPA (NACPA) and bis-trifluoromethyl-phenyl-isoxazole-4-hydrazone (TFMIH)] suggested the presence of two distinct lipophilic domains (see pharmacophore section below). That this appears to be the case was confirmed by the inhibitory activity of a 'hybrid' isoxazole that included lipophilic substituents at each position, 5-naphthylethyl isoxazole - 4 - (2, 4 - dinitrophenol) hydrazone - dinitrophenol (NEIH, $K_i \approx$ 100 μM). Exchange reactions also confirmed that none of these inhibitors could act as Sx_c^- substrates, suggesting that while the binding site could accommodate the added steric bulk, an effective interaction between the added groups and the lipophilic domains on the transporter precluded translocation (Patel *et al.*, 2010). While the derivatized isolazoles have not yet yielded ligand affinities comparable with

what has been achieved for the EAAT system, these blockers have been key to localizing lipophilic domains that can be targeted in the next generation of inhibitors.

Benzoic and related aromatic acids (Figure 4)

Two of the most potent Sx_c^- inhibitors characterized to date are (*S*)-4-carboxyphenylglycine (4-*S*-CPG, $K_i \approx$ 1–5 μM) (Ye *et al.*, 1999; Patel *et al.*, 2004) and sulphasalazine (SSZ; (6-oxo-3-(2-[4-(*N*-pyridin-2-ylsulphamoyl)phenyl]diazenyl)-cyclohexa-1,4-dienecarboxylic acid), $IC_{50} < 1$ μM) (Gout *et al.*, 2001). 4-*S*-CPG was initially developed and characterized as a group I metabotropic receptor antagonist (Bedingfield *et al.*, 1995), again highlighting the issue of cross-reactivity among Sx_c^- blockers (Patel *et al.*, 2010). The observation that 4-*S*-CPG effectively blocked the release of L-Cys2 from glial tumour cells (see pathology section below) provided the critical pharmacological link to Sx_c^- (Ye *et al.*, 1999). In this manner, *S*-4-carboxy,3-hydroxyphenylglycine and *S*-3-carboxy,4-hydroxyphenylglycine were also identified as inhibitors, although less potent that 4-*S*-CPG. Similar analogues not possessing a second carboxylate group (e.g. 3-*S*-hydroxyphenylglycine, *RS*-35-dihydroxyphenylglycine) were essentially inactive. In contrast to ibotenate or QA, 4-*S*-CPG was essentially inactive as a Sx_c^- substrate when examined in the fluorometric exchange assay (Patel *et al.*, 2004). More recently, a 4-*S*-CPG derivative in which the distal carboxylate was replaced with a sulphonic acid (4-*S*-SPG) was also found to inhibit Sx_c^- (Etoga *et al.*, 2010). Unlike the analogous anionic substitution between L-Glu and L-homocysteate, 4-*S*-SPG was markedly less potent than 4-*S*-CPG, inhibiting uptake even less effectively than L-Cys2. Both *R*- and *S*-4-sulphophenylalanine were also shown to lack inhibitory activity at Sx_c^-. Interestingly, when the phenyl ring of 4-*S*-SPG was replaced with a thiophene (*R,S*-4-sulphothienylglycine), inhibitory activity increased to a level comparable with L-Cys2 ($K_i \approx$ 75 μM; Etoga *et al.*, 2010). Similar to 4-*S*-CPG, SSZ was identified as a Sx_c^- inhibitor within the context of assessing its action on tumour cells (Gout *et al.*, 2001). Originally developed as a pro-drug containing sulphapyridine and 5-amino salicylic acid, SSZ has been used as an anti-inflammatory agent to treat Chron's disease and inflammatory bowel disorders. SSZ is also a very potent (≈4-*S*-CPG) inhibitor of Sx_c^-. Although SSZ possess a benzoic acid moiety common to many of the inhibitors, it stands out amongst the other compounds described in this review in that it lacks the prototypical L-α-amino acid head group. Comparisons with 4-*S*-CPG also led to synthesis of [(*R,S*)-4-[4′-carboxyphenyl]-phenylglycine; CPPG] (Etoga *et al.*, 2010), which in some ways might be considered a hybrid between 4-*S*-CPG and SSZ. While CPPG proved to be as active as L-Cys2 as an inhibitor,
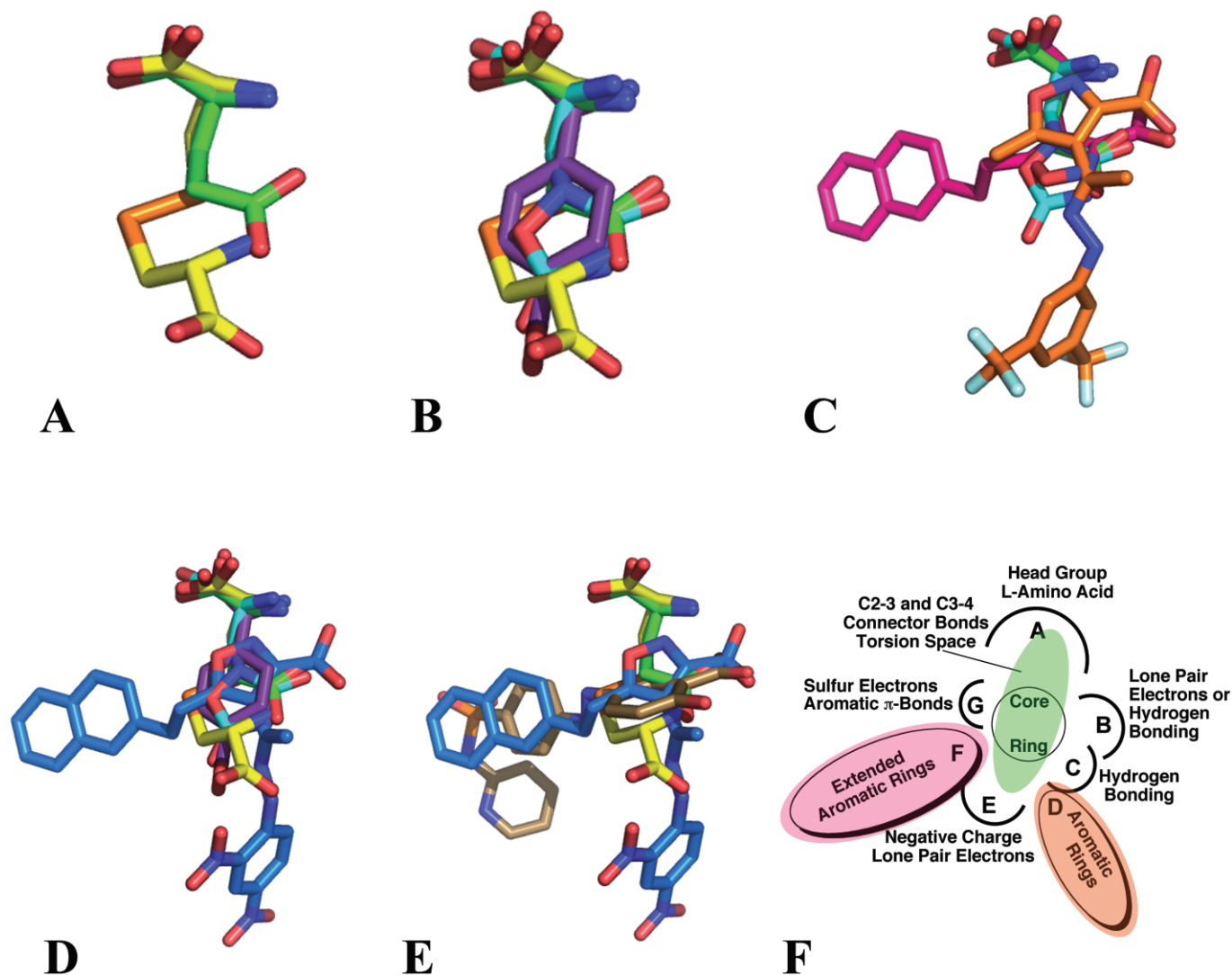
Figure 5
A ligand-based, superposition, 3D pharmacophore model for the substrate binding on Sx$_c^-$. A–E. L-Glu in green; L-Cys$_2$ in yellow; QA in teal; 4-S-CPG in purple; NACPA in red; TFMIH in orange; NEIH in blue and SSZ in bronze. F. Pharmacophore binding template.

it failed to match the potency of either 4-S-CPG or SSZ. As will be discussed below, this may suggest that portions of SSZ interacts effectively enough with the other domains within the substrate binding site of Sx$_c^-$, that the interactions with an L-α-amino acid head group are no longer required.

Modelling the binding site

Pharmacophore models provide a strategy to collectively analyse and visualize the SARs that govern ligand-binding site interactions. This approach, which relies on the 3D superposition of low energy conformation of substrates and inhibitors, has been successfully employed in the characterization of the EAATs (Esslinger *et al.*, 2005; Mavencamp *et al.*, 2008). In the instance of Sx$_c^-$, a pharmacophore model has been developed (Figure 5) using L-Glu, L-Cys$_2$, QA and 4-S-CPG (panel B) as a training set and aligning these ligands in a manner that maximizes overlap of the α-C, α-COOH, α-NH$_2$

and distal COOH groups or related isosteric moieties (Patel *et al.*, 2010). One of the first postulates to emerge from this comparison of minimized structures was that L-Cys$_2$ may be binding to Sx$_c^-$ in a pseudo chair-like conformation that maximizes hydrogen bonding between the 4-position sulphur atom and a proton of the C7 terminal amine group (Figure 5A). The resulting ring-like structure is consistent with NMR spectroscopic findings (Edmonds and Summers, 1969; Hunston *et al.*, 1982) and aligns with the central core rings of QA and 4-S-CPG (Figure 5B). Overlaying this conformation of L-Cys$_2$ and L-Glu then leads to the likelihood that the distal carboxylate groups of the two substrates may be interacting with different sub-domains within a larger substrate binding site. The available COOH–COOH distances are also consistent with the inactivity of L-aspartate (too short) and the ability of the substrate site to accommodate L-α-aminoadipate. Furthermore, such an arrangement provides an explanation for the activities of 4-S-CPG and QA in which

the positions of the distal carboxylate-mimics are relatively fixed as a consequence of the rigid structure of the inhibitors.

The pharmacophore model also provides a framework on which to superimpose the inhibitors containing appended steric and lipophilic bulk as a way to identify the relative positions of the corresponding domains within the binding site that interact or accommodate these groups (Patel *et al.*, 2010). As depicted in Figure 5, modelling of NACPA, TFMIH and NEIH led to the identification of previously unrecognized 'lipophilic pockets' on the transporter that are adjacent to the substrate binding domains. While the hybrid molecule NEIH clearly establishes the presence of two such pockets, the presence of multiple anionic groups on the substrates and inhibitors makes it more difficult to definitively assign the relative position of these lipophilic substituents with respect to each of the inhibitor COOH groups with the specific α- and distal COOH groups of L-Glu and L-Cys$_2$. In the overlay shown in Figure 5C, the α-C, α-COOH and α-NH$_2$ of NACPA is aligned with the L-α-amino acid head group of L-Glu and L-Cys$_2$. In this configuration, the naphthyl ring of NACPA delineates one of the two lipophilic pockets. In turn, overlaying the naphthyl groups of NACPA and NEIH positions the 3-COOH group extending from the isoxazole ring of NEIH such that it aligns with the distal COOH group of L-Glu and delineates a second lipophilic pocket occupied by the 2,4-dinitrophenyl moiety (Figure 5D and E). Similarly, overlaying the 3,5-bis-trifluoromethyl-phenyl group of TFMIH with the 2,4-dinitrophenyl group of NEIH also aligns the 3-COOH group extending from the isoxazole ring of NEIH to the distal COOH moiety of L-Glu (Figure 5D). The issue of the relative positioning of lipophilic groups also arises in the case of SSZ, as the pyridylsulphamoylphenyl portion of SSZ can be correlated to either of the lipophilic regions depending upon whether the COOH group of the salicyclic moiety of SSZ is aligned with either the α- or distal COOH group of L-Glu. Although the pictured lipophilic group alignments are consistent with one another, it is readily acknowledged that the positions could be reversed, resulting in a switch in the alignments between the COOH groups and related isosteric moieties. Regardless of the exact positioning, however, the activities of NACPA, NEIH and TFMIH highlight that interaction with these lipophilic domains appears significant enough to overcome less than optimal binding of the COOH and NH$_2$ biosteres (evidenced by the inactivity of AMPA and ACPA) or, in the instance of SSZ, the lack of a prototypical L-α-amino acid head group. A pharmacophore template summarizing the modelling results (Figure 5F) depicts the overall binding site as a collection of sub-domains that can interact with an L-amino acid head group, a distal COOH or similar biostere, a hydrogen bonding group equivalent to the distal L-amino acid moiety of L-Cys$_2$ or a second heteroatom of an isoxazole ring, a lone pair electron or π-electron density associated with the sulphur atoms of L-Cys$_2$ or the aromatic ring of 4-*S*-CPG, and two distinct lipophilic pockets that can accommodate aromatic rings. Importantly, the presently identified group of inhibitors indicates that a ligand needs to interact with a subset, but not necessarily all, of these domains to effectively bind and inhibit Sx$_c^-$. The continued advancement of delineating the pharmacology of Sx$_c^-$ will utilize these models in two ways: (i) developing the next generation of inhibitors to optimize interactions with the lipohlilic domains as a strategy to increase potency and (ii) structurally aligning and docking the ligand-based models to the homology-based protein models of Sx$_c^-$.

Physiological and pathological roles

Regulation

Given the functional importance of Sx$_c^-$ in cell types both within and outside the CNS, it is not surprising that multiple mechanisms exist through which to regulate its expression. Indeed, amino acid deprivation, xenobiotic exposure and oxidative stress have all been shown to trigger the up-regulation of Sx$_c^-$ (Bannai, 1984; Bannai *et al.*, 1989; Sato *et al.*, 1995; Ishii *et al.*, 2000). The best-characterized of these mechanisms are those linked to the Nrf2-ARE signalling pathway (Figure 6) in which the transcription of a specific gene is governed by the presence of a *cis*-acting regulatory DNA element (i.e. the antioxidant response element, ARE) in its promoter. Numerous enzymes associated with the synthesis of GSH, the inactivation of reactive oxygen species (ROS) and phase II detoxification contain AREs (Ishii *et al.*, 2000) (Prochaska *et al.*, 1985; Talalay, 1989; Spencer *et al.*, 1991; Limon-Pacheco *et al.*, 2007), and at least four ARE motifs have been identified in the promoter region sequence of mouse xCT (Sasaki *et al.*, 2002). The expression of these ARE-containing genes is up-regulated by the binding of nuclear factor erythroid-2-related factor (Nrf2), a CNC-bZIP transcription factor (Jaiswal, 2004; van Muiswinkel and Kuiperij, 2005; Vargas and Johnson, 2009; Giudice *et al.*, 2010) following its translocation into the nucleus. Electrophilic or oxidative stress can also lead to direct phosphorylation of Nrf2 by kinases in several cascades [e.g. protein kinase C, phosphatidyl-inositol 3-kinase, PKR-like endoplasmic reticulum kinase, JNK, ERK and p38 MAPK (MK14)] that also facilitates translocation into the nucleus (Kobayashi and Yamamoto, 2006; Kandil *et al.*, 2010). The regulatory control provided by these pathways appears to vary among cell types (Sasaki *et al.*, 2002; Qiang *et al.*, 2004), and several studies point to existence of alternate mechanisms of Sx$_c^-$ regulation (Sasaki *et al.*, 2002; Sato *et al.*, 2004; Lewerenz *et al.*, 2009).

In CNS tissue, treatment of primary astrocytes with dibutyryl cyclic AMP (dbcAMP) has been shown to differentiate the glial cells into a more *in vivo*-like, activated phenotype that includes the increased expression of several proteins associated with the glutamate system, including: glutamine synthetase, EAATs and Sx$_c^-$ (Hertz *et al.*, 1998; Gochenauer and Robinson, 2001; Daginakatte *et al.*, 2008). More recently, a closer examination of these dbcAMP-treated astrocytes reveals that Sx$_c^-$ expression may also directly be regulated by GSH levels in a phenotype dependent manner (Seib *et al.*, 2011). Sx$_c^-$ activity is markedly up-regulated in dbcAMP-treated astrocytes in which GSH levels have been depleted with synthesis blocker buthionine sulphoximine (BSO), a response not observed in untreated protoplasmic astrocytes. Interestingly, an Nrf2-dependent increase in Sx$_c^-$ activity is observed when the electrophile tBHQ is added to the protoplasmic astrocytes, but not in differentiated cultures, suggesting a distinct regulatory mechanism. Changes in Sx$_c^-$ activity induced by BSO also correlate to increases in both xCT mRNA
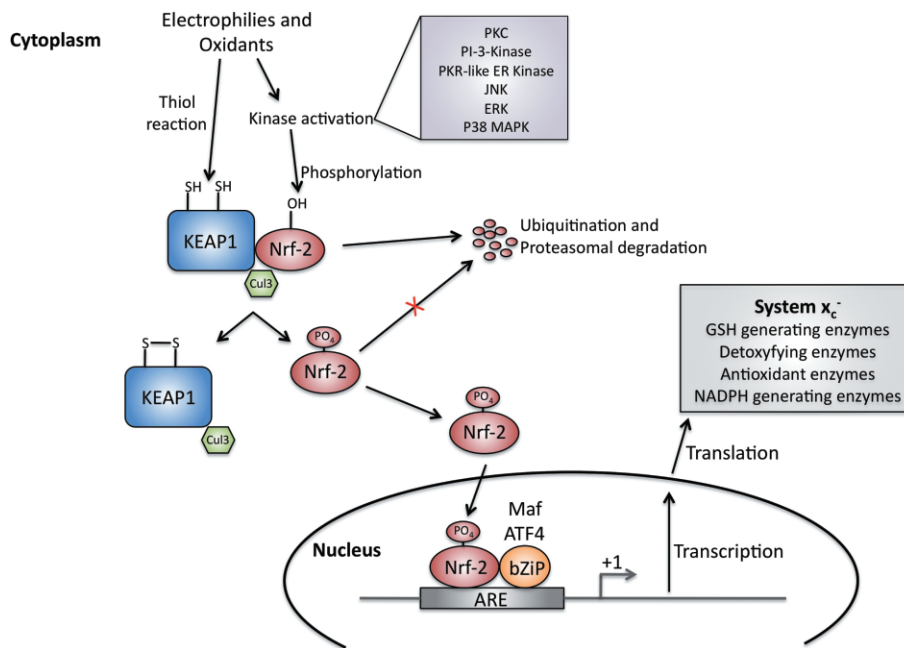
Figure 6

Nrf-2/ARE activation pathway. Under basal conditions Nrf-2 is dimerized with KEAP1 and continuously targeted for degradation due to ubiquitination by Cul3. Electrophilies and oxidants can disrupt the dimerization of Nrf2 and KEAP directly by modifiying cysteine residues in KEAP1 or through phosphorylation of Nrf2 at Ser40 by protein kinases. Nrf2 is then free to translocate into the nucleus, bind with an adaptor protein (e.g. Mafs and ATF4) and increase ARE-driven transcription.

and protein and are temporally linked to GSH levels. Furthermore, the effect of BSO cannot be mimicked by H_2O_2 treatment or reversed by the addition of general antioxidants, suggesting that it is a change in GSH level that triggers the induction. Consistent with such a mechanism, co-administration of permeable GSH pro-drug GSH-ethylester and BSO prevents the up-regulation of Sx$_c^-$ activity.

Studies seeking drug candidates to induce the up-regulation of EAAT expression as a potential point of intervention in amyotrophic lateral sclerosis (ALS) demonstrated that some β-lactam antibiotics could increase expression of EAAT2 through an action at the promoter level (Rothstein *et al.*, 2005). One of the more effective of these compounds, Ceftriaxone, was recently reported to also produce an increase in Nrf-2 and xCT expression in HT22 hippocampal cells and fibroblasts, as well as protect these cells from L-Glu-induced exicitotoxicty (Lewerenz *et al.*, 2009). Such an induction has also been postulated to provide a novel therapeutic approach in inflammatory neurodegenerative disease (for review, see Chen and Kunsch, 2004; Vargas and Johnson, 2009). In those instances where an increase in Sx$_c^-$ activity may be of therapeutic benefit (see physiology and pathology section below), pharmacological strategies targeting enhanced expression provide an alternative to screening for potential allosteric activators.

CNS GSH production and oxidative protection via Sx$_c^-$

The generation and maintenance of GSH levels in all mammalian cells is critical to the detoxification of xenobiotics and

the prevention of oxidative stress and damage. This latter effect is especially relevant to the CNS given its high levels of oxygen consumption, the abundance of enzymes and metabolites that can generate ROS and its limited antioxidant capacity (Halliwell, 2006). As a transporter, Sx$_c^-$ acts as a key component in GSH production by providing L-Cys$_2$ as a synthetic precursor (Figure 7). The combination of micromolar concentrations of L-Cys$_2$ in the CSF and millimolar concentrations of L-Glu in the astrocyte serve to drive the exchange, after which the intracellular L-Cys$_2$ is rapidly reduced to L-CysH and enzymatically incorporated into GSH (Sagara *et al.*, 1993). While both astrocytes and neurons have been shown to express the xCT subunit of Sx$_c^-$, mature neurons exhibit little or no Sx$_c^-$ activity and appear even less capable of directly mediating the uptake L-Cys$_2$. GSH production in neurons is thus dependent upon the uptake of L-CysH that is provided by astrocytes via the 'cystine/cysteine cycle' (Dringen *et al.*, 1999; Wang and Cynader, 2000). Essentially, the L-Cys$_2$ that is transported into the astrocytes by Sx$_c^-$ is reduced and subsequently released as either L-CysH itself or GSH, which can also serve as an L-CysH source following its extracellular metabolism by γ-glutamyl-transpeptidase (GGT) and aminopeptidase N. As previously mentioned, the observation that QA toxicity in immature cortical neurons could be attributed to blocking Sx$_c^-$ and the resultant oxidative stress (and not EAA receptor mediated excitotoxicity) highlighted the significance of the transporter in these pathways (Murphy *et al.*, 1989; Cho and Bannai, 1990; Shih *et al.*, 2003; 2006a). Under conditions of primary or secondary oxidative stress, astrocytes also are capable of contributing to neuroprotection by rapidly providing neurons with increased levels
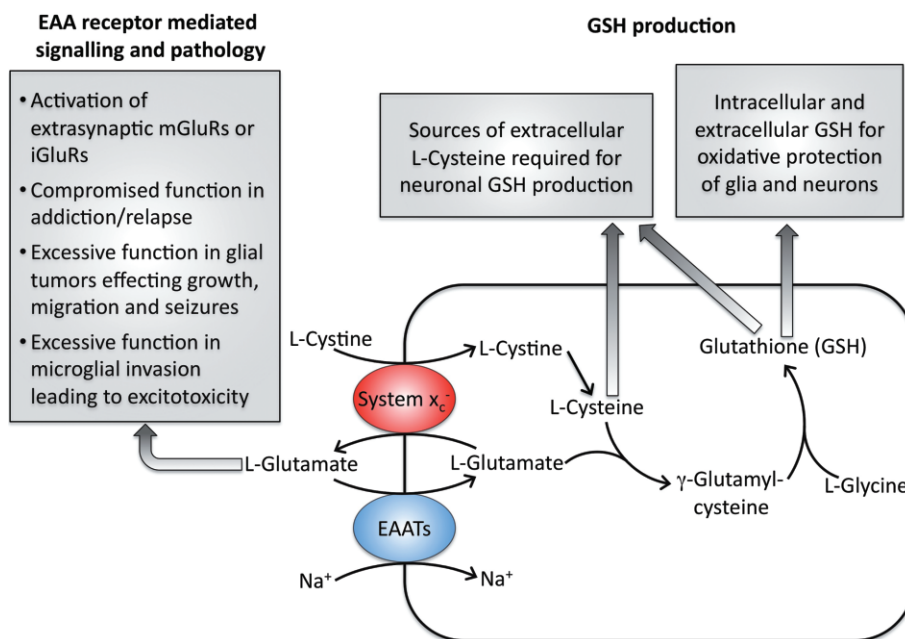
Figure 7
Functional roles of the system x_c^- transporter in the CNS.

of GSH precursors via the Nrf2-dependent induction of the GSH synthesis (Sagara *et al.*, 1993; Dringen *et al.*, 1999; Wang and Cynader, 2000; Guebel and Torres, 2004) (Shih *et al.*, 2006a).

Given that the maintenance of intracellular GSH levels is critical to most cells, those that do not express Sx_c^-, as is the case with mature neurons, presumably accumulate either L-CysH or L-Cys₂ through alternative systems. Other members of SLC7 family are also capable of transporting these precursors. Thus, CysH is a substrate of the 4F2hc-linked SLC7A10 transporter Asc-1. The SLC7A9 transporter b°,+AT (SLC7A9) can mediate the uptake of Cys₂, although it is dimerized with rBAT instead of 4F2hc. (Verrey *et al.*, 2003). As its name implies, functionally defined ASC (alanine–serine–cysteine) system members ASCT1 and ASCT2 (SLC1A4 and A5, respectively) transport L-CysH in a sodium-dependent manner (Zerangue and Kavanaugh, 1996a; Kanai and Hediger, 2004). L-CysH is also a substrate of sodium-dependent neutral amino acid transporters SNAT1, SNAT 2 and SNAT4, which are members of the SLC38 family and are typically associated with glutamine and asparagine uptake (Mackenzie and Erickson, 2004). Amongst the alternative routes of entry for L-CysH or L-Cys₂ within CNS cells, the most intriguing is associated with the EAATs (Zerangue and Kavanaugh, 1996b; McBean, 2002; Chen and Swanson, 2003). In particular, L-CysH was shown to be transported by the neuronal transporter EAAT3 (EAAC-1) with a maximal flux rate comparable with that of L-Glu (Zerangue and Kavanaugh, 1996b). Consistent with such a role, GSH levels are negatively regulated by the interaction of EAAC-1 with the glutamate transport-associated protein 3–18 (GTRAP3-18) (Watabe *et al.*, 2007) and mice homozygous deficient for EAAT3, exhibit not only increased neurodegeneration with age, but also decreased

neuronal levels of GSH concentrations and an increased vulnerability to oxidative stress (Aoyama *et al.*, 2006).

Source for extrasynaptic excitatory signalling

The L-Cys₂-coupled export of L-Glu through Sx_c^- takes on additional significance within the CNS as it represents a non-vesicular route by which this excitatory transmitter can access EAA receptors, particularly those present in extrasynaptic locales. Given the presence of Sx_c^- on astrocytes, L-Glu released in this manner also represents a novel mechanism for glial-neuronal communication. Work by Kalivas, Baker and co-workers demonstrate that the extracellular L-Glu released through Sx_c^- has a physiological role in the tonal regulation of extrasynaptic type 2/3 mGluR receptors and the control of neurotransmitter release throughout the CNS (Baker *et al.*, 2002; Xi *et al.*, 2003; Mohan *et al.*, 2011). In both the striatum and nucleus accumbens, extrasynaptic activation of type 2/3 mGluR receptors inhibits the release of L-Glu and/or dopamine. Aberrant changes in Sx_c^- activity and L-Glu homeostasis at these synapses have been implicated in the pathophysiology of drug addiction (Kalivas *et al.*, 2009; Madayag *et al.*, 2010). In rat models of chronic, self-administered cocaine addiction, a series of neuroadaptations have been identified that underlie the disease including: a decrease in extra-synaptic L-Glu concentrations in the nucleus accumbens, a decrease in L-Cys₂ and L-Glu exchange, and an increase in glutamatergic transmission. Restoring Sx_c^- activity by applying L-CysH or *N*-acetyl-CysH (NAC) can return extra-synaptic L-Glu to control levels (Baker *et al.*, 2003); restoring normal glutamatergic transmission and in doing so prevents cocaine primed drug seeking and relapse (Baker *et al.*, 2003; Amen *et al.*, 2011). A similar mechanism has been implicated for nicotine addiction and suggests tar-

geting L-Glu homeostasis is a potential method for treating addiction (Knackstedt and Kalivas, 2009; Knackstedt et al., 2009).

The ability of Sx$_c^-$ to contribute to neuronal signalling through the release of L-Glu is, however, not uniformly accepted. Thus, while a L-Cys$_2$-mediated efflux of L-Glu was shown to be sufficient to activate non-NMDA receptors in cerebellar slices and NMDA receptors in hippocampal slices (Warr et al., 1999; Cavelier and Attwell, 2005), it was concluded that the combination of CSF L-Cys$_2$ levels (typically in the 0.1–0.5 µM range) and the K_m for L-Cys$_2$ at Sx$_c^-$ (typically reported in 50–100 µM range), would preclude Sx$_c^-$ from contributing to ambient L-Glu levels. Such a conclusion, however, must be tempered somewhat by assay results quantifying the uptake of ^{35}S-L-Cys$_2$ into slices of nucleus accumbens, which yielded K_m values in the 2–4 µM range (Baker et al., 2003). More recent studies on primary cultures of differentiated astrocytes also yielded K_m values for L-Glu transport that were two- to fivefold lower than observed in many glial cell lines (Seib et al., 2011). L-Cys$_2$ levels in the 0.1–0.3 µM range were able to produce an Sx$_c^-$-mediated efflux of L-Glu that decreased the synaptic release of L-Glu as a consequence presynaptic mGluR2 activation (Moran et al., 2005). These different findings point to the likelihood that the ability of L-Glu released from Sx$_c^-$ to activate EAA receptors probably varies among circuits.

Beyond its role in GSH production and oxidative protection within the different cell types of the mammalian eye (Lim et al., 2005; 2007; Li et al., 2007) additional insight into the role of the Sx$_c^-$ in extrasynaptic signalling comes from a closer examination of retinal circuits (Hu et al., 2008). Electron microscopy studies reveal the ultra-structural localization of Sx$_c^-$ (based upon xCT expression) to the photoreceptor (rod and cone) ribbon synapse with ON bipolar cells of the outer plexiform layer. Using the cation channel probe agmatine (AGB) as a fluorescent reporter, it was shown that L-Glu released through Sx$_c^-$ could activate mGluR 6 receptors that, in turn, would close non-selective cation channels on the post synaptic ON bipolar cells. Consistent with the participation of Sx$_c^-$, the ability of L-Cys$_2$ to reduce this AGB fluorescence was blocked by co-incubation with 4-S-CPG. The findings suggest that signalling within the photoreceptor synapse incorporates both vesicular release and Sx$_c^-$-mediated exchange as a source of L-Glu.

Evidence validating Sx$_c^-$ as a source of ambient L-Glu and its contribution to signalling and plasticity has also come from work with *Drosophila melanogaster*. Studies on an xCT homologue *genderblind* in *D. melanogaster* suggest that the transporter influences courtship behaviour through its regulation of ambient extracellular L-Glu *in vivo* (Grosjean et al., 2008). The loss of this gene reduces extracellular L-Glu levels by about half. Furthermore, changes in ambient L-Glu levels arising from altered Sx$_c^-$ activity were shown to influence both the function (i.e. desensitization) and localization (i.e. clustering) of ionotropic glutamate receptors (iGluRs) in the *Drosophila* CNS (Augustin et al., 2007; Featherstone, 2010).

Source for excitotoxicity

When the efflux of L-Glu through Sx$_c^-$ becomes excessive, its character within the CNS switches from that of a potential excitatory transmitter to that of a well-characterized and pathologically relevant excitotoxin (Waxman and Lynch, 2005; Lau and Tymianski, 2010). Early recognition that Sx$_c^-$ could be a source of excitotoxic L-Glu came from an examination of microglia cells that, owing to a marked need for oxidative protection, express high levels of the transporter (Piani and Fontana, 1994). Thus, under conditions of CNS infection or trauma, activation of microglia from a resting state by pro-inflammatory stimuli, as well as migration into localized areas, not only have beneficial neuroprotective and neurothrophic effects, but the ability to release substantial levels of L-Glu (Espey et al., 1998; Qin et al., 2006; Shih et al., 2006b; Barger et al., 2007). Given the ubiquitous involvement of microglia in CNS disease and pathology, it is not surprising that this ability to exacerbate neurodegeneration through the conversion of oxidative stress to excitotoxic stress via Sx$_c^-$ has been linked to a variety of disorders, including: Alzheimer's disease (Barger and Basile, 2001), Parkinson's disease, AIDS (Zeng et al., 2010), bacterial infection/LPS (Taguchi et al., 2007) and multiple sclerosis (Domercq et al., 2007). Ironically, the observations that microglia express high levels of Sx$_c^-$ and that transporter-mediated efflux of L-Glu provides a non-synaptic pathway of mGluR signalling also prompted the examination of this mechanism within the non-neural cells of the immune system. These studies revealed that L-Glu released by dendritic cells acts through mGluRs on T cells to modulate T-cell activation and that this release occurs through Sx$_c^-$ (Pacheco et al., 2006; 2007).

The specific up-regulation of Sx$_c^-$ in astrocytes by interleukin-1β (IL-1β) and subsequent activation of mGluR1 receptors has also been demonstrated to enhance hypoxic neuronal injury (Fogal et al., 2005; 2007). Neurons co-cultured with astrocytes were found to be more susceptible to hypoxic neuronal cell death after treatment of cultures with IL-1β, an affect that was mediated through increased expression and efflux of L-Glu through Sx$_c^-$. Confirming the role of Sx$_c^-$, the IL-1β-induced increase in L-Cys$_2$ uptake (and L-Glu efflux) could be blocked with 4-S-CPG and was absent in astrocytes prepared from mice carrying a mutation in the SLC7A11 gene (Jackman et al., 2010).

Probably the strongest links between the Sx$_c^-$-mediated efflux of L-Glu and CNS pathology have been made during the course of investigation on brain tumours, where Sx$_c^-$ is a major contributor to the growth, survival and expansion of malignant gliomas (de Groot and Sontheimer, 2010). The high levels of xCT expression in astrocytomas and gliomas, compared with normal astrocytes, likely reflects the increased growth and metabolic rates associated with tumour cells (Ye et al., 1999). More specifically, however, the import of L-Cys$_2$ through Sx$_c^-$ is needed to meet the increasing demand for cellular GSH as the tumour outgrows its blood supply, becomes hypoxic and is challenged by the accompanying build up of ROS and NO (Ogunrinu and Sontheimer, 2010). From a therapeutic perspective, the increased GSH levels can also represent an enhanced ability of glioma cells to protect themselves from both radiation and chemotherapeutic agents (Dai et al., 2007; Liu et al., 2007). Thus, while not yet induced specifically by the inhibition of Sx$_c^-$, depletion of GSH using the synthesis inhibitor L- BSO rendered gliomas more sensitive to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-based chemotherapy and D-54 human glioma xenografts to radiotherapy (Lippitz et al., 1990; Allalunis-

Turner *et al.*, 1991). Even greater attention has focused on the Sx$_c^-$-mediated efflux of L-Glu from the glial tumours and its impact on brain tumour aetiology. At one level, the released L-Glu can act as an excitotoxin through the over activation of ionotropic EAA receptors, induce neurodegeneration and clear the area for tumour expansion (Ye *et al.*, 1999). Thus, both glioma cultures *in vitro* and implanted glioma cells *in vivo* have been shown to release L-Glu (Ye and Sontheimer, 1999; Behrens *et al.*, 2000; Takano *et al.*, 2001). Co-cultures of glioma and neuronal cells demonstrated that the released L-Glu was sufficient to induce excitotoxic damage and that the neuronal loss could be reduced by either by preventing its efflux through Sx$_c^-$ (4-*S*-CPG or SSZ) or inhibiting its action at NMDA receptors (MK-801).

Even if the L-Glu does not reach concentrations that are excitotoxic, the excessive activation of these receptors in peritumoural regions provides an explanation for the seizures commonly observed in the early stages of the disease (Sontheimer, 2008). Lastly, the released L-Glu also is capable of mediating an autocrine/paracrine signal that enhances invasive and migratory properties through the activation of Ca^{2+} permeable AMPA receptors on the glioma cells themselves (Lyons *et al.*, 2007; Lastro *et al.*, 2008). All of these relationships have highlighted the potential of Sx$_c^-$ as a therapeutic target in tumour cells, particularly gliomas. Blockers could be envisioned as acting by any number of mechanisms, some of which might be synergistic, including: metabolic inhibition, increasing oxidative stress, radio-sensitization, decreasing chemoresistance, reducing invasiveness or preventing peritumoural seizures. Indeed, SSZ (Chart 3), which was originally developed for anti-inflammatory applications before its Sx$_c^-$ inhibitory actions were discovered (see Pharmacology section), has been shown to reduce GSH and tumour proliferation in both gliomas (Lyons *et al.*, 2007) and small-cell lung cancer (Guan *et al.*, 2009). Inhibition of Sx$_c^-$ activity also inhibited cell metastasis in esophageal squamous carcinoma cells through caveolin-1/β-catenin pathway (Chen *et al.*, 2009). Treatment of astrocytoma cells with SSZ depleted GSH levels and induced caspase-mediated apoptosis (Chung *et al.*, 2005) Importantly, the antitumour effects of SSZ have been shown to be exclusively via inhibition of Sx$_c^-$-mediated L-Cys$_2$ uptake and GSH production as opposed to the reported anti-inflammatory effects of SSZ acting via NfkB (Chung and Sontheimer, 2009). Unfortunately SSZ, as well as most of the other Sx$_c^-$ inhibitors described earlier in this review, suffer from problems related to specificity, potency and pharmacokinetic properties. Progress on mapping out how SSZ is interacting with the domains on the Sx$_c^-$ binding site should, however, provide insight into optimizing new derivatives.

Conclusion

In summary, considerable progress has been made in the last few years detailing the physiological and pathological significance of both the import of L-Cys$_2$ and the export of L-Glu through the Sx$_c^-$ antiporter, particularly as associated with the CNS. Not surprisingly, this greater appreciation for the functional importance of Sx$_c^-$ has also created a demand for pharmacological reagents with which to selectively manipulate its

activity. What is also appealing about this challenge is that there is a need for both specific inhibitors (e.g. to reduce Sx$_c^-$ function in glioma) and substrates (e.g. to increase Sx$_c^-$ in addiction). A survey of presently available Sx$_c^-$ ligands, unfortunately, reveals that the most potent blockers tend to lack specificity, while the more recently developed inhibitors that hold a promise for increased selectivity have yet to exhibit the desired affinity. Fortunately, all of these analogues have been of value in the development of an Sx$_c^-$ pharmacophore model that is beginning to delineate the requisite domains on the transporter, including lipophilic pockets, that can be exploited in the synthesis future ligands to the benefit both specificity and potency.

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Conflict of interest

The authors Bridges, Natale and Patel have no conflicts of interests.

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