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REVIEW

The solute carrier 6 family of transporters

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The solute carrier 6 (SLC6) family of the human genome comprises transporters for neurotransmitters, amino acids, osmolytes and energy metabolites. Members of this family play critical roles in neurotransmission, cellular and whole body homeostasis. Malfunction or altered expression of these transporters is associated with a variety of diseases. Pharmacological inhibition of the neurotransmitter transporters in this family is an important strategy in the management of neurological and psychiatric disorders. This review provides an overview of the biochemical and pharmacological properties of the SLC6 family transporters.

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Abbreviations
ADHD, attention deficit hyperactivity disorder; MDMA, 3,4-methylenedioxymetamphetamine; NMDA, N-methyl-D-aspartate; NSS, neurotransmitter sodium symporters; OCD, obsessive-compulsive disorder; PDZ, PSD-95/Discs-large/ZO-1; SSRI, selective 5-HT reuptake inhibitors; SNRI, 5-HT/noradrenaline reuptake inhibitors; TCA, tricyclic antidepressants

Introduction and overview

The solute carrier family 6 (SLC6) has 20 members in the human genome (Chen *et al.*, 2004; Broer, 2006). It comprises transporters for neurotransmitters, proteinogenic amino acids, betaine, taurine and creatine. The neurotransmitter transporters were the first identified members, and hence, it is also known as the family of neurotransmitter sodium symporters (NSS) or the Na^+/Cl^--dependent transporter family (Nelson, 1998; Beuming *et al.*, 2006). Sequence similarity allows subdividing the SLC6 family into four branches, namely the GABA transporter branch, the monoamine transporter branch and the amino acid transporter branches (I) and (II) (Figure 1).

The GABA transporter branch contains transporters for GABA, betaine, taurine and creatine. GABA is the major inhibitory neurotransmitter in the brain. Inhibition of GABA transporters will result in reduced clearance after synaptic release and therefore enhances the action of inhibitory synapses. Consequently, GABA transporter drugs are used to treat not only seizures but also pain and anxiety (Clausen *et al.*, 2006). Taurine and betaine are both osmolytes (Lang, 2007), and creatine is a storage compound for high-energy phosphate bonds to replenish ATP, particularly in muscle and brain (Wallimann *et al.*, 2011).

The monoamine transporter branch contains the neurotransmitter transporters for dopamine, 5-HT and noradrenaline. These neurotransmitters play a modulatory role in the CNS, affecting the activity of many pathways. They are particularly involved in the modulation of mood, aggression, anxiety, depression, addiction, appetite, attention etc. (Hahn and Blakely, 2007; Ramamoorthy *et al.*, 2011). In general, inhibition of monoamine transporters will result in reduced clearance of monoamine transmitters after synaptic release, resulting in a more intense and prolonged signal. Certain drugs, in addition, elicit non-synaptic release of monoamine neurotransmitters through the transporter.

The amino acid transporter branch (I) (Figure 1) comprises transporters for glycine, proline and the general amino acid transporter $ATB^{0,+}$ which is broadly specific for neutral (0) and cationic (+) amino acids. Glycine is not only the major inhibitory neurotransmitter in the spinal cord but also modulates glutamatergic neurotransmission in the cortex by binding to the NMDA receptor. The glycine transporter GlyT1 is widely expressed in the brain, and it is thought to modulate glycine concentrations in the cortex, whereas GlyT2 is

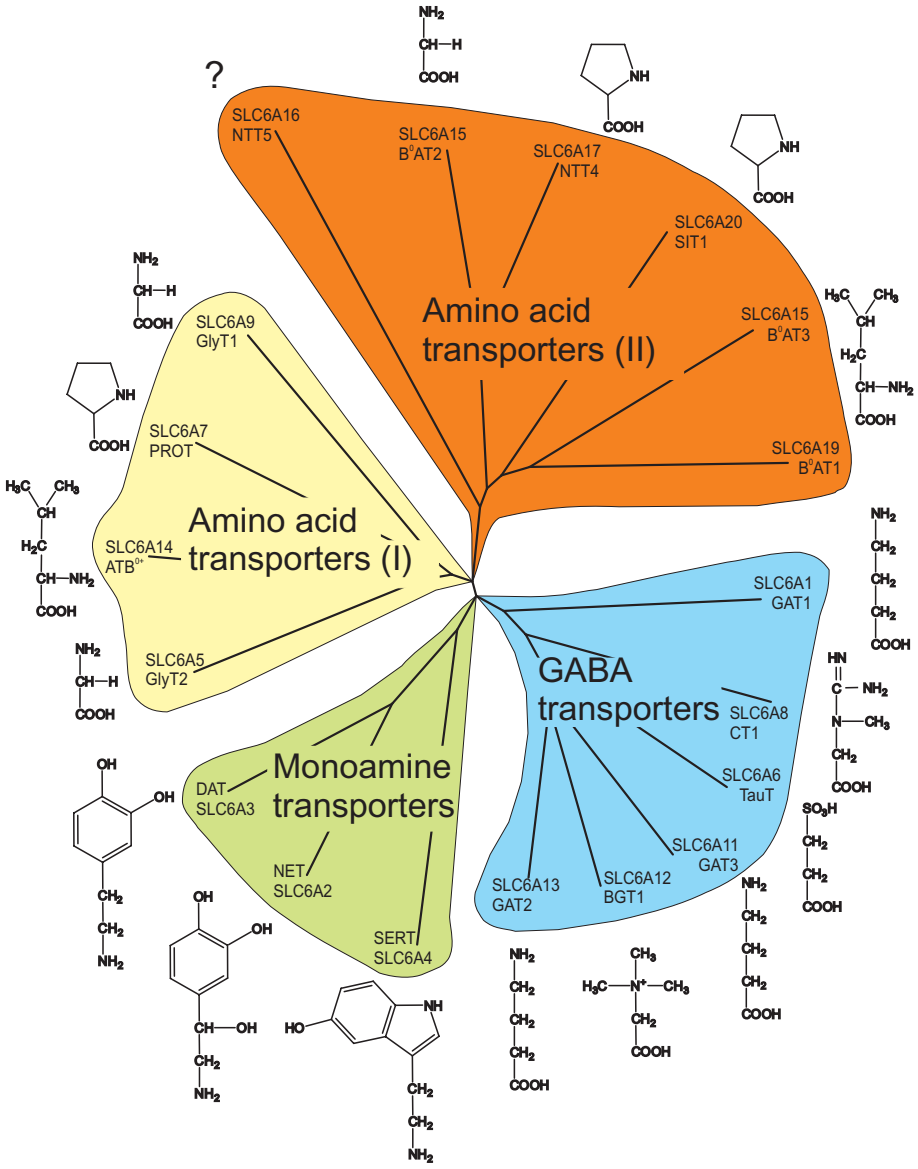

Figure 1

Sequence similarity of SLC6 transporters. Peptide sequences of all human SLC6 members were aligned using T-coffee (Notredame *et al.*, 2000), and similarities were visualized using Treeview (Page, 1996). The main substrate for each transporter is shown next to the name. Subfamilies are indicated.

mainly found in the spinal cord. Glycine transporters are targeted for treatment of neuropathic pain and schizophrenia (Aragon and Lopez-Corcuera, 2005; Javitt, 2009). The proline transporter PROT1 is almost exclusively expressed in the brain, where its physiological role remains unclear. The general amino acid transporter $ATB^{0,+}$ is found in lung and other epithelia and is thought to be involved in the clearance of amino acids from secreted fluids (Mager and Sloan, 2003).

The amino acid transporter branch (II) contains amino acid transporters involved in epithelial and brain amino acid transport (Broer, 2008). Most members of this branch accept a variety of neutral amino acids and therefore are involved in amino acid homeostasis. The epithelial transporters mediate the absorption of amino acids in the intestine and the re-absorption of amino acids from the primary urine in the kidney. The physiological role of the neural members of this branch is ill-understood. Most likely, they provide metabolic precursors for tricarboxylic acid cycle intermediates to allow production of neurotransmitters.

Nomenclature

In addition to the SLC nomenclature, members of the SLC6 family are referred to by transporter names indicating substrate preference. Some transporters have been named independently by different groups or were renamed after their function was discovered. Table 1 provides a list, ordered by

Table 1

Overview and nomenclature of the SLC6 family

SLC number	Common name	Alias	Protein variation	Comments	Reference
SLC6A1	GAT1	GATA			Guastella *et al.* (1990)
SLC6A2	NET	NAT1, NET1	C-t var1		Pacholczyk *et al.* (1991)
			C-t var2		
SLC6A3	DAT				Giros *et al.* (1991); Kilty *et al.* (1991)
SLC6A4	SERT	5-HTT			Blakely *et al.* (1991); Hoffman *et al.* (1991)
SLC6A5	GlyT2		GlyT2a		Smith *et al.* (1992a)
			GlyT2b		
SLC6A6	TauT				Liu *et al.* (1992a); Smith *et al.* (1992b); Uchida *et al.* (1992)
SLC6A6P				Pseudogene	
SLC6A7	PROT				Fremeau *et al.* (1992)
SLC6A8	CT1	CRTR			Mayser *et al.* (1992); Guimbal and Kilimann (1993)
SLC6A9	GlyT1		GlyT1a		Guastella *et al.* (1992); Liu *et al.* (1992b)
			GlyT1b		
			GlyT1c		
			GlyT1d		
			GlyT1e		
SLC6A10	CT2			Pseudogene	
SLC6A11	GAT3	GATB			Borden *et al.* (1992); Clark *et al.* (1992)
		GAT4 (mouse)			
SLC6A12	BGT1	GAT2 (mouse)			Lopez-Corcuera *et al.* (1992); Yamauchi *et al.* (1992)
SLC6A13	GAT2	GAT3 (mouse)			Borden *et al.* (1992)
SLC6A14	$ATB^{0,+}$	β-alanine carrier			Sloan and Mager (1999)
SLC6A15	B^0AT2	v7-3, NTT73, SBAT1			Uhl *et al.* (1992)
SLC6A16	NTT5				Farmer *et al.* (2000)
SLC6A17	NTT4	RXT1			Uhl *et al.* (1992); Liu *et al.* (1993b)
SLC6A18	B^0AT3	XT2, XTRP2	6 splice variants in mouse		Wasserman *et al.* (1994); Nash *et al.* (1998)
SLC6A19	B^0AT1	XT2s1			Broer *et al.* (2004)
SLC6A20	SIT1	IMINO, XT3, XTRP3	SLC6A20a		Smith *et al.* (1995); Nash *et al.* (1998)
			SLC6A20b		
			In rodents		
SLC6A21P				Pseudogene	

SLC number, which gives the most commonly used name and alternative names. Some transporters have splice variants resulting in a different peptide sequence, and these are listed as well. In this review, transporter nomenclature follows Alexander *et al.*, (2011).

Substrates and mechanism

The four branches of the SLC6 family have different substrate preferences (Figure 1 and Table 2). The monoamine transporter branch comprises transporters for the biogenic amines 5-HT (SERT), noradrenaline (NET) and dopamine (DAT). However, the substrate specificity is overlapping. DAT, for instance, can also transport noradrenaline and NET has a high affinity for dopamine (Gether *et al.*, 2006). Furthermore, a recent study has provided evidence that SERT is capable of transporting dopamine, however, with lower substrate affinity, higher maximum velocity and the requirement for higher Na^+ and Cl^- to sustain transport (Larsen *et al.*, 2011). Similarly, there is substantial substrate promiscuity in the GABA transporter branch. For example, β-alanine is not only a substrate for the taurine transporter but also for GABA transporters GAT-2 and GAT-3 and the amino acid transporter $ATB^{0,+}$.

Table 2

Endogenous substrates and transport mechanism

Transporter	Endogenous substrate	K_M-values	Mechanism	Reference
SLC6A1/GAT1	GABA	11 μM	1S:2Na(S):1Cl(S)	Loo *et al.* (2000)
SLC6A2/NET	noradrenaline	0.4 μM	1S:1Na(S):1Cl(S)	Pacholczyk *et al.* (1991); Gu *et al.* (1996)
	dopamine	0.7 μM		
SLC6A3/DAT	dopamine	2.5 μM	1S:2Na(S):1Cl(S)	Gu *et al.* (1994); Sonders *et al.* (1997)
	noradrenaline	20 μM		
SLC6A4/SERT	5-HT	0.45 μM	1S:1Na(S):1Cl(S):1 K(A)	Ramamoorthy *et al.* (1993); Rudnick (1998)
SLC6A5/GlyT2	glycine	27 μM	1S:3Na(S):1Cl(S)	Roux and Supplisson (2000); Rees *et al.* (2006)
SLC6A6/TauT	taurine	5 μM	1S:2Na(S):1Cl(S)	Ramamoorthy *et al.* (1994)
	β-alanine	56 μM		
SLC6A7/PROT	proline	6–10 μM (rat)	Not tested	Fremeau *et al.* (1992)
SLC6A8/CT1	creatine	77 μM	Not tested	Nash *et al.* (1994)
SLC6A9/GlyT1	glycine	72 μM	1S:2Na(S):1Cl(S)	Kim *et al.* (1994); Roux and Supplisson (2000)
SLC6A10	pseudogene			
SLC6A11/GAT3	GABA	7 μM	1S:2Na(S):1Cl(S)	Borden *et al.* (1994); Karakossian *et al.* (2005)
SLC6A12/BGT1	GABA	36 μM	1S:3Na(S):1Cl(S)	Borden *et al.* (1995); Rasola *et al.* (1995); Matskevitch *et al.* (1999)
	betaine	934 μM		
SLC6A13/GAT2	GABA	3.7 μM	1S:2Na(S):1Cl(S)	Sacher *et al.* (2002); Christiansen *et al.* (2007)
SLC6A14/$ATB^{0,+}$	Neutral and cationic amino acids	Non-polar 6–100 μM Polar 100–600 μM Cationic 76–100 μM	1S:2Na(S):1Cl(S)	Sloan and Mager (1999)
SLC6A15/B^0AT2	BCAA, Met, Pro	40–200 μM	1S:1Na(S)	Takanaga *et al.* (2005a); Broer *et al.* (2006)
SLC6A16/NTT5	Unknown			
SLC6A17/NTT4	BCAA, Met, Pro, Ala, Gln	360–5000 μM (rat)	1S:1Na(S)	Parra *et al.* (2008); Zaia and Reimer (2009)
SLC6A18/B^0AT3	Gly, Ala	900–2300 μM (mouse)	1S:2Na(S):1Cl(S)	Singer *et al.* (2009); Vanslambrouck *et al.* (2010)
SLC6A19/B^0AT1	Neutral amino acids	1–12 mM (mouse)	1S:1Na(S)	Broer *et al.* (2004)
SLC6A20/IMINO	Pro, OH-Pro, betaine	130 μM–200 μM (rat/mouse)	1S:2Na(S):1Cl(S)	Kowalczuk *et al.* (2005); Takanaga *et al.* (2005b)

BCAA, branched chain amino acid; Mechanism: (S) symport (A) antiport; K_M values are given for human isoforms unless indicated otherwise.

Glycine is a substrate of the specific glycine transporters GlyT1 and GlyT2 and of the general amino acid transporters B^0AT1, B^0AT3 and $ATB^{0,+}$. Betaine is transported by BGT-1 and also by SIT1. The overlapping substrate specificities within the SLC6 family can be rationalized: All substrates of the GABA transporter branch have a carboxyl group and an amino group in the β- or γ- position (Figure 1). In the case of creatine, the amino group is part of the guanidino group, and in the case of betaine, the amino-group is methylated. The exception is taurine, which has a sulphonate group in the β-position. Monoamine transporters accept decarboxylated derivatives of aromatic amino acids, while all other members transport amino acids. In some cases, site-directed mutagenesis of the substrate binding site has been used to alter the substrate specificity of SLC6 transporters (Dodd and Christie, 2007; Vandenberg *et al.*, 2007).

About half of the transporters in the SLC6 family co-transport their substrate(s) together with two Na^+ ions and one Cl^- ion (Table 2). The number of co-transported Na^+ ions can, however, vary from 1 (NET, SERT, B^0AT1, B^0AT2, NTT4) to 3 (GlyT2, BGT1). Furthermore, chloride is not universally used as a co-transported ion (B^0AT1, B^0AT2, NTT4). SERT is unique in its use of K^+ as the anti-ported ion, resulting in an overall electroneutral transport mechanism (Rudnick, 1998). Two examples illustrate the fine-tuning of transport mechanisms to physiological demands: GlyT1 uses the co-transport

of two Na^+ ions, while GlyT2 uses three Na^+-ions (both together with Cl^-) to accumulate glycine (Supplisson and Roux, 2002). As a result, the accumulative power of GlyT1 is less than that of GlyT2. GlyT1 is expressed in astrocytes in the cortex and is thought to allow a significant extracellular glycine concentration, enough to co-activate neighbouring NMDA receptors through the glycine site. Small concentration changes of the co-transported ions or of the membrane potential could thus result in glycine release or removal, thereby modulating glutamatergic neurotransmission. GlyT2, by contrast, is expressed in glycinergic neurons, allowing optimal removal of the neurotransmitter from the synaptic cleft, leaving only traces of extracellular glycine. The second example is the occurrence of channel-like properties in monoamine transporters. Due to the high affinity of these transporters for their substrates, turnover is generally slow. Channel-like properties may allow an increased transport rate when neurotransmitter concentrations are elevated (Galli *et al.*, 1996; DeFelice and Goswami, 2007). The removal of neurotransmitter is ensured by metabolic inactivation or sequestration into synaptic vesicles. Inadvertently, the channel-like mechanism is triggered by amphetamine binding in the case of the DAT (Sitte *et al.*, 1998; Kahlig *et al.*, 2005), which then results in release of the neurotransmitter, explaining the pharmacological effects of amphetamine (Robertson *et al.*, 2009; Leviel, 2011). A similar mechanism underlies 5-HT release by 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy') (Rudnick and Wall, 1992). Ion channel-like features triggered by substrates and drugs are particularly obvious in SERT, as its transport mechanism is electroneutral and any currents observed are caused by uncoupled movements of ions (Mager *et al.*, 1994).

Pharmacology of SLC6 transporters

The pharmacology of the SLC6 family has been developed to different extents (Table 3) (Gether *et al.*, 2006; Iversen, 2006; Kristensen *et al.*, 2011). Due to their role as drug targets, a wide variety of inhibitors and synthetic substrates are available for monoamine transporters, whereas GABA and glycine transporter pharmacology is less well developed. All other transporters in the family remain largely unexplored (Table 4).

The first generation of monoamine transporter drugs were identified in the 1950s and included the tricyclic antidepressants (TCAs), such as imipramine and desimipramine, that exert their action on SERT and/or NET (Moltzen and Bang-Andersen, 2006). Because of side effects caused by additional affinity for several different receptors and for cardiac sodium channels (Gillman, 2007), these antidepressants have been largely superseded by compounds devoid of ectopic binding. These include compounds targeting solely SERT ('selective 5-HT reuptake inhibitors', SSRIs) such as escitalopram, fluoxetine and paroxetine (Wong and Bymaster, 1995), compounds targeting solely NET ('selective noradrenaline reuptake inhibitors', NRIs), such as reboxetine (Andersen *et al.*, 2009) and compounds targeting both NET and SERT ('dual uptake inhibitors' or '5-HT/ noradrenaline reuptake inhibitors', SNRIs) including venlafaxine, desvenlafaxine and duloxetine (Wong and Bymaster, 2002). Notably, these newer classes of uptake inhibitors have not only shown efficacy in treatment of major depression but also demonstrated their usefulness in anxiety, obsessive-compulsive disorder (OCD) and eating disorders. Interestingly, sibutramine, which has been used as an appetite suppressant, might act as 'a triple uptake inhibitor' because it is rapidly metabolized to its desmethyl and didesmethyl congeners that display high affinity for all three monoamine transporters (Glick *et al.*, 2000). More recently, specific triple uptake inhibitors are being developed (Bettati *et al.*, 2010).

The monoamine transporters are also target for illicit and widely abused drugs such as cocaine and amphetamines. Cocaine is a rapidly acting non-selective high-affinity inhibitor of all three monoamine transporters (Eshleman *et al.*, 1999). Nonetheless, studies on DAT knock-out mice and knock-in mice expressing a cocaine-insensitive DAT strongly suggest that the stimulatory properties of cocaine are the result of its interaction with DAT (Giros *et al.*, 1996; Chen *et al.*, 2006). Several analogues of cocaine have been developed over the years with both higher affinity and improved selectivity for the different monoamine transporters (Table 3). These compounds have served and still serve as important tool compounds; however, some are also considered candidates for treating cocaine addiction (Newman and Kulkarni, 2002; Dutta *et al.*, 2003; Loland *et al.*, 2008). For example, the benztropine analogue JHW007 does not possess the same strong stimulatory properties as cocaine and was found to antagonize the effect of cocaine on behaviour in rats (Desai *et al.*, 2005). Possibly, this is the result of a much slower onset and longer duration of action than that of cocaine, an effect that may be caused by JHW 007's ability to stabilize a different more closed conformation of the transporter, compared with cocaine (Loland *et al.*, 2008). The compound modafinil, which is used to treat narcolepsy, is also a DAT inhibitor with weaker action than cocaine and little potential for abuse (Zolkowska *et al.*, 2009). Accordingly, modafinil might also be a promising therapeutic agent for cocaine addiction (Dackis *et al.*, 2005; Hart *et al.*, 2008; Minzenberg and Carter, 2008).

Some drugs targeting the monoamine transporters are not simple inhibitors but are transporter substrates (Sitte *et al.*, 1998; Gether *et al.*, 2006). They include amphetamine, metamphetamine and MDMA, compounds that are capable of promoting reverse transport of the endogenous substrate and thus monoamine release via the monoamine transporters to the extracellular environment (Fuller *et al.*, 1988; Sulzer *et al.*, 2005). Amphetamine and metamphetamine act primarily on DAT and NET, whereas SERT and NET are targets for MDMA (Green *et al.*, 2003; Sulzer *et al.*, 2005). Amphetamine-induced reverse transport is a complex process that is not only the simple result of facilitated exchange (Pifl *et al.*, 1999; Scholze *et al.*, 2002; Leviel, 2011) but is also likely to involve a channel-like mode of the transporter (Kahlig *et al.*, 2005). The underlying mechanism appears to require binding of Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) to the distal C-terminus of DAT (Fog *et al.*, 2006). This binding facilitates phosphorylation of N-terminal serine residues that in turn changes the transporter from an 'unwilling' to a 'willing' state for reverse transport (Khoshbouei *et al.*, 2004; Fog *et al.*, 2006). Binding of syntaxin1a to DAT might also be critical for the process (Binda *et al.*, 2008). Methylphenidate is a derivative of amphetamine that is widely used in the

Table 3

Inhibitors of SLC6 transporters

Transporter	Inhibitor	K_i	Application	Selectivity	Reference
SLC6A1/GAT1	Tiagabine	110 nM (h)	Seizures, neuropathic pain	GAT1>>>GAT2, GAT3, BGT1	Kvist *et al.* (2009)
	NNC-711	1.4 μM (h)	Research tool	GAT1>>>GAT2, GAT3, BGT1	Kvist *et al.* (2009)
	SKF89976A	130 nM (h)	Research tool	GAT1>>>GAT2, GAT3, BGT1	Dhar *et al.* (1994)
	CI-966	260 nM (h)	Research tool	GAT1>>>GAT2, GAT3, BGT1	Dhar *et al.* (1994)
	Nipecotic acid	19 μM (h)	Research tool	GAT1>GAT3>GAT2>BGT1	Kvist *et al.* (2009)
	Guvacine	15 μM (h)	Research tool	GAT1>GAT3>GAT2,BGT1	Kvist *et al.* (2009)
	(R)-EF1502	8.9 μM (h)	Research tool	GAT1>GAT2,BGT1>GAT3	Kvist *et al.* (2009)
	THPO	1300 μM (h)	Research tool	GAT1=GAT2=GAT3=BGT1	Kvist *et al.* (2009)
SLC6A2/NET	Amphetamine	70 nM (h)	Drug	NET>DAT>>SERT	Han and Gu (2006)
	Nisoxetine	5.3 nM (h)	Ligand for quantification	NET>>SERT=DAT	Eshleman *et al.* (1999)
	Talopram	8.9 nM (h)	Research tool	NET>>SERT>>DAT	Andersen *et al.* (2011)
	Talsupram	2.5 nM (h)	Research tool	NET>>SERT>DAT	McConathy *et al.* (2004)
	Reboxetine	3 nM (h)	Therapeutic drug	NET>>SERT>>DAT	Andersen *et al.* (2009)
	Atomoxetine	5 nM (h)	Therapeutic drug	NET>>SERT>DAT	Andersen *et al.* (2009)
	χ-MrIA	1260 nM (h)	Research tool	NET>>>SERT=DAT	Sharpe *et al.* (2001)
	Nomifesine	16 nM (h)	Therapeutic drug	NET>DAT>>SERT	Tatsumi *et al.* (1997)
	Mazindole	3.3 nM (h)	Therapeutic drug	NET>DAT>SERT	Eshleman *et al.* (1999)
	MDMA	1190 nM (h)	Drug of abuse	NET=SERT>DAT	Han and Gu (2006)
	Desipramine	0.8 nM (h)	Therapeutic drug	NET>SERT>>DAT	Tatsumi *et al.* (1997)
	Methylphenidate (Ritalin)	34 nM (h)	Therapeutic drug	NET>DAT>>SERT	Markowitz *et al.* (2006)
SLC6A3/DAT	Benztropine	42 nM (h)	Research tool	DAT>NET>>>SERT	Eshleman *et al.* (1999)
	JHW 007	24.6 nM (r)	Research tool	DAT>>SERT=NET	Agoston *et al.* (1997)
	GBR12935	22.4 nM (h)	Ligand for quantification	DAT>NET>SERT	Eshleman *et al.* (1999)
	RTI-55 (β-CIT)	1.3 nM (rt)	Ligand for quantification	DAT=SERT>NET	Carroll *et al.* (1995)
	Cocaine	278 nM (h)	Drug of abuse	DAT=SERT=NET	Eshleman *et al.* (1999)
	CFT	27.2 nM (h)	Research tool	DAT=NET>SERT	Eshleman *et al.* (1999)
	Bupropion	520 nM (h)	Therapeutic drug	DAT>>SERT>NET	Tatsumi *et al.* (1997)
	Modafinil	4800 nM (h)	Therapeutic drug	DAT>NET>SERT	Zolkowska *et al.* (2009)

Table 3

Continued

Transporter	Inhibitor	K_i	Application	Selectivity	Reference
SLC6A4/SERT	Fluvoxamine	2 nM (h)	Therapeutic drug	SERT>>DAT>NET	Tatsumi *et al.* (1997)
	Escitalopram	2.5 nM (h)	Therapeutic drug	SERT>>>NET>>DAT	Owens *et al.* (2001)
	DASB	1.1 nM (h)	PET ligand	SERT>>>NET=DAT	Wilson *et al.* (2000)
	Sertraline	0.13 nM (h)	Therapeutic drug	SERT>>NET>DAT	Tatsumi *et al.* (1997)
	Paroxetine	0.13 nM (h)	Therapeutic drug	SERT>>>NET>DAT	Tatsumi *et al.* (1997)
	Fluoxetine	0.8 nM (h)	Therapeutic drug	SERT>>NET>DAT	Tatsumi *et al.* (1997)
	Desvenlafaxine	40 nM (h)	Therapeutic drug	SERT>NET>>DAT	Deecher *et al.* (2006)
	Imipramine	1.4 nM (h)	Therapeutic drug	SERT>>NET>>DAT	Tatsumi *et al.* (1997)
	Duloxetine	0.8 nM (h)	Therapeutic drug	SERT>NET>>DAT	Bymaster *et al.* (2001)
SLC6A5/GlyT2	ORG 25543	16 nM (h)	Research tool	GlyT2>GlyT1	Caulfield *et al.* (2001)
	5,5-Diaryl-2-amino-4-pentenoate	300 nM (h)	Research tool	GlyT2>GlyT1	Isaac *et al.* (2001)
	N-Arachidonylglycine	3 μM (h)	Research tool	GlyT2>GlyT1	Wiles *et al.* (2006)
SLC6A6/TauT	Diaminopropionic acid	100 μM (m)	Research tool		Liu *et al.* (1992a)
	Hypotaurine	10 μM (m)	Research tool		Liu *et al.* (1992a)
	β-guanidino-ethanesulfonic acid	50 μM (m)	Research tool		Liu *et al.* (1992a)
	β-alanine	100 μM (r)	Research tool	TauT=GAT3	Smith *et al.* (1992b)
SLC6A7/PROT	Leu-Enkephalin	2.1 μM (r)	Research tool		Fremeau *et al.* (1996)
	GGFL	0.3 μM (r)	Research tool		Fremeau *et al.* (1996)
	Benztropin	0.75 μM (h)	Research tool		Yu *et al.* (2009)
	LP-403182	0.11 μM (h)	Research tool		Yu *et al.* (2009)
SLC6A8/CT1	Guanidinopropionate	n.d.	Research tool		Guimbal and Kilimann (1994)
	Guanidinobutyrate	n.d.	Research tool		Guimbal and Kilimann (1994)
SLC6A9/GlyT1	Sarcosine	55 μM (r)	Research tool	GlyT1>>GlyT2	Mallorga *et al.* (2003)
	NFPS (ALX 5407)	3 nM (r)	Research tool	GlyT1>>>GlyT2	Mallorga *et al.* (2003)
	ORG 24598	32 nM (r)	Research tool	GlyT1>>>GlyT2	Mallorga *et al.* (2003)
	LY2365109	16 nM (h)	Research tool	GlyT1>>>GlyT2	Perry *et al.* (2008)
	CP-802,079	16 nM (h)	Research tool	GlyT1>>>GlyT2	Martina *et al.* (2004)
	Lu AA20465	150 nM (h)	Research tool	GlyT1>>>GlyT2	Smith *et al.* (2004)
	SSR130800	1.9 nM (h)	Research tool	GlyT1>>>GlyT2	Boulay *et al.* (2008)

SLC6A10	n.a.				
SLC6A11/GAT3	β-alanine	36 μM (h)	Research tool	GAT3=GAT2>BGT1>GAT1	Kvist *et al.* (2009)
	(S)-SNAP-5114	50 μM (h)	Research tool	GAT3=GAT2>BGT1>GAT1	Kvist *et al.* (2009)
	THPO	2200 μM (h)	Research tool	GAT1=GAT2= GAT3=BGT1	Kvist *et al.* (2009)
SLC6A12/BGT1	Betaine	590 μM (h)	Research tool	BGT1>GAT2>GAT1,GAT3	Kvist *et al.* (2009)
	NNC 05–2090	1.4 μM (m)	Research tool	BGT1>GAT1,GAT3,GAT2	Thomsen *et al.* (1997)
	THPO	2100 μM (h)	Research tool	GAT1=GAT2=GAT3=BGT1	Kvist *et al.* (2009)
SLC6A13/GAT2	β-alanine	42 μM (h)	Research tool	GAT2=GAT3>BGT1>GAT1	Kvist *et al.* (2009)
	(S)-SNAP-5114	130 μM (h)	Research tool	GAT2=GAT3>BGT1>GAT1	Kvist *et al.* (2009)
	THPO	1500 μM (h)	Research tool	GAT1=GAT2=GAT3=BGT1	Kvist *et al.* (2009)
SLC6A14/ATB$^{0,+}$	1-Methyltryptophan	250 μM (m)	Research tool		Hatanaka *et al.* (2001)
	N^G-monomethyl-L-arginine (L-NMMA)	0.77 mM (m)	Research tool		Hatanaka *et al.* (2001)
	N^G-nitro-L-arginine (L-NNA)	0.56 mM (m)	Research tool		Hatanaka *et al.* (2001)
SLC6A15/B^{0}AT2	Pipecolic acid	0.9 mM (m)	Research tool		Broer *et al.* (2006)
SLC6A16/NTT5	unknown				
SLC6A17/NTT4	unknown				
SLC6A18/B^{0}AT3	unknown				
SLC6A19/B^{0}AT1	unknown				
SLC6A20/IMINO	Sarcosine	3.2 mM (m)	Research tool	Inhibits GlyT1	Kowalczuk *et al.* (2005)
	Pipecolic acid	0.09 mM (m)	Research tool	IMINO>B^{0}AT2	Kowalczuk *et al.* (2005)
	MeAIB	0.78 mM (m)	Research tool		Kowalczuk *et al.* (2005)
	Betaine	0.2 mM (m)	Research tool	Inhibits BGT1	Kowalczuk *et al.* (2005)

Selectivity: >less than 10-fold, >>10- to 100-fold, >>>100- to 1000-fold.
Reported are K_i values for heterologously expressed transporters unless indicated otherwise. The species is indicated (h, human; r, rat; m, mouse).
Rt, rat tissue, n.a., not applicable, n.d., not determined.

Table 4

SLC6 transporters as drug targets

Transporter	Drug/Drug class	Indication	Comment
GAT1	Tiagabine	Epilepsy, neuropathic pain	
NET	NRI	ADHD, depression	
	NDRI	ADHD, depression, obesity	
	SNRI	Depression, neuropathic pain	
	Tricyclic antidepressants	Depression, neuropathic pain	
DAT	NDRI	ADHD, depression, obesity	
SERT	SSRI	Depression, anxiety, OCD	
	SNRI	Depression, neuropathic pain	
TauT	Taurine	No specific condition	Dietary Supplement
CT1	Creatine	Athletic sport	Dietary Supplement
		Creatine deficiency syndromes	

NRI, noradrenaline reuptake inhibitor; NDRI noradrenaline/dopamine reuptake inhibitor; SNRI, serotonin/noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.

treatment of attention-deficit hyperactivity disorder (ADHD). This compound differs from amphetamine in not having dopamine-releasing properties (Sulzer *et al.*, 2005). It acts, compared with cocaine, as a relatively weak DAT and NET inhibitor, and as a consequence, this compound can also have a potential in treating cocaine abuse (Goldstein *et al.*, 2010). Buproprion is another amphetamine derivative acting primarily as an inhibitor at NET and DAT (Dwoskin *et al.*, 2006). It is registered as an atypical antidepressant and as smoking cessation agent. Moreover, buproprion has a modest weight-reducing effect that appears to be enhanced when administered in combination with an opioid receptor antagonist (Dwoskin *et al.*, 2006).

The GAT-1-specific inhibitor tiagabine is used to treat seizures and neuropathic pain and is the only GABA transporter inhibitor currently registered as a therapeutic agent (Clausen *et al.*, 2006). Tiagabine is also one of the only GABA inhibitors showing selectivity among the GABA transporters, although several inhibitors have been developed. In general, GABA transporter inhibitors display rather low affinity and selectivity for the transporters; and, thus, there is a strong need for improved inhibitors of this subgroup of SLC6 transporters (Table 3). Such new compounds might not only serve as investigatory tool but also as new therapeutic agents; for example, there is evidence that BGT-1 is an alternative target to GAT-1 for treatment of epilepsy (Madsen *et al.*, 2009).

In recent years, an increasing number of inhibitors of the two glycine transporters GlyT1 and GlyT2 have been identified (Table 3). Development of these inhibitors has been motivated by data indicating that blocking synaptic glycine uptake is beneficial in psychotic disease and neuropathic pain (Lechner, 2006; Dohi *et al.*, 2009). The inhibitor *N*-methyl glycine (sarcosine) was the basis for several potent inhibitors, for instance (*R*)-NPTS and ORG 24598 (Mallorga *et al.*, 2003). Non-sarcosine GlyT1 inhibitors include LY2365109 (Perry *et al.*, 2008), CP-802 079 (Martina *et al.*, 2004), Lu AA20465 (Smith *et al.*, 2004) and SSR130800 (Boulay *et al.*, 2008). Overall, fewer inhibitors are known for GlyT2. The competitive and selective inhibitor ORG 25543 was discovered first (Caulfield *et al.*, 2001) and followed by other classes of compounds (Isaac *et al.*, 2001).

The efficacy of inhibitors is also affected by functional redundancy among neurotransmitter transporters. As a result, use of a specific inhibitor of one transporter may have a result different from that using a broadly specific inhibitor. GAT1 is the most abundant GABA transporter in the cortex. GAT-1-deficient mice show typical signs of increased inhibitory neuronal activity, such as reduced locomotor and general activity, abnormal gait and constant tremor, but have a normal life span and reproduce normally (Chiu *et al.*, 2005). This suggests that other GABA transporters can replace GAT-1 activity or that other components of GABA signalling adapt to reduced neurotransmitter clearance (Bragina *et al.*, 2008). Similarly, there is overlap between the function of noradrenaline and dopamine transporters. For example, there is evidence that NET is responsible for clearance of extracellular dopamine in the prefrontal cortex (see Carboni and Silvagni, 2004). By contrast, there appears to be little overlap between the function of glycine transporters GlyT1 and GlyT2, due to differential localization (Eulenburg *et al.*, 2005). The amino acid transporters NTT4 and B°AT2 seem to have a very similar distribution in the brain, providing redundancy for the uptake of essential amino acids into neurons (Masson *et al.*, 1996).

Pathology and clinical significance

Members of the SLC6 family are believed to play a role in a variety of disease states. In a number of cases, mutations in a transporter are associated with an inherited Mendelian disorder. In some cases, mutations contribute to more complex multifactorial diseases and in others disorders of unknown aetiology can be treated by inhibitors of SLC6 transporters. Table 5 provides an overview of disease states associated with the SLC6 family.

Table 5

Disease states associated with SLC6 family transporters

Transporter	Disease state	Inheritance	Variation	Reference
NET	ADHD	Complex	Promoter variants	Kim *et al.* (2006)
	Depression	Complex		Hahn and Blakely (2007)
	Orthostatic intolerance	Complex	A457P	Shannon *et al.* (2000)
	Blood pressure	Complex		Halushka *et al.* (1999)
SERT	Autism/OCD	Complex	VNTR, SNPs	Cook *et al.* (1997); Sutcliffe *et al.* (2005)
	OCD	Complex	I425V	
	Anxiety/Depression	Complex	VNTR	Ozaki *et al.* (2003)
			VNTR	Lesch *et al.* (1996)
			L255M	
DAT	ADHD	Complex	3′ VNTR	Hahn and Blakely (2007)
	Bipolar	Complex	Several SNPs	Grunhage *et al.* (2000)
	Tourette syndrome	Complex	3′ VNTR	Tarnok *et al.* (2007)
GlyT2	Hyperekplexia	Mendelian	Various mutants	Rees *et al.* (2006)
Creatine	X-linked mental retardation	Mendelian	Various mutants	Salomons *et al.* (2001)
B^0AT1	Hartnup disorder	Mendelian	Various mutations	Kleta *et al.* (2004); Seow *et al.* (2004)
B^0AT2	Major depression	Complex	Downstream of the gene	Kohli *et al.* (2011)
IMINO	Iminoglycinuria	Mendelian	T199M	Broer *et al.* (2008)

VNTR, Variable number of tandem repeats; a short nucleotide sequence organized into clusters of tandem repeats.

Creatine deficiency syndrome is a rare disorder caused by mutations in the creatine transporter CT1 (Salomons *et al.*, 2001). It causes creatine deficiency in the brain resulting in mental retardation. Creatine is an important storage compound for high-energy phosphate bonds, which replenish ATP during times of high energy consumption (Wallimann *et al.*, 2011). Mutations in the glycine transporter GlyT1 cause hyperekplexia, an exaggerated startle syndrome, resulting from increased inhibition in motor circuits (Harvey *et al.*, 2008). Mutations in B^0AT1 are associated with Hartnup disorder, an amino acid malabsorption syndrome, which can cause skin rash and ataxia in young individuals (Seow *et al.*, 2004). Mutations in IMINO are associated with iminoglycinuria, a benign disorder affecting reabsorption of glycine and proline in the kidney (Broer *et al.*, 2008).

The noradrenergic system plays an important role in depression, attention, vigilance, learning, memory and has been proposed to contribute to ADHD (Hahn and Blakely, 2007). It is also involved in blood pressure regulation through its role in the peripheral nervous system. DNA and protein variants of NET are associated with disorders of the noradrenergic system. An inactivating mutation in NET is associated with orthostatic intolerance (Shannon *et al.*, 2000; Hahn *et al.*, 2003), and another variant was identified by analysing candidate genes for blood-pressure homeostasis (Halushka *et al.*, 1999). Interestingly, this variant has also been associated with major depression (Haenisch *et al.*, 2009). However, NET is only one component in the noradrenergic systems, and other variations in receptors, synthesizing and degrading enzymes, and also in environmental factors, all can contribute to the phenotype of these disorders.

The dopaminergic system is an important mediator of motor function, cognition, mood, reward and addiction. The dopaminergic system is associated with a variety of disorders, such as ADHD, bipolar disorder, autism, schizophrenia, drug abuse, Parkinson's disease and Tourette's syndrome (Hahn and Blakely, 2007). Of interest, DAT knock-out mice display a behavioural phenotype that in part resembles symptoms seen in ADHD patients (Gainetdinov and Caron, 2000) and in agreement a single point mutation ($Ala^{559}Val$) has been identified in patients with ADHD (Mazei-Robison *et al.*, 2005). Furthermore, two inactivating mutations in DAT have been linked to the rare autosomal-recessive disease, infantile parkinsonism-dystonia (Kurian *et al.*, 2009).

5-HT plays a role in mood, aggression, response to alcohol, appetite, sleep, cognition and sexual and motor activity. It is likely to contribute to a range of mental illnesses such as depression, suicide, anxiety, autism, OCD, eating disorders, schizophrenia and alcohol abuse (Hahn and Blakely, 2007). Indeed, many polymorphisms have been identified in SERT, including, for example, both rare protein variants in patients with various neuropsychiatric disorders and an intensively studied promoter variant (Hahn and Blakely, 2007). The general significance of the promoter variant (5HTTLPR) has been debated (Munafo *et al.*, 2009; Risch *et al.*, 2009). Nonetheless, several studies have linked the short 's' allele to anxiety-related personality traits, increased risk for neuropsychiatric disorders, impulsive behaviour and impaired response to antidepressant treatment (Hahn and Blakely, 2007; Serretti *et al.*, 2007; Homberg and Lesch, 2011). It has been proposed that these phenotypic traits in people carrying the 's' allele are caused by hypervigi-

lance and thereby supersensitivity to environmental cues. This supersensitivity might cause enhanced emotional responses that could contribute to development of pathological conditions (Homberg and Lesch, 2011).

Recently, a genome-wide association study has suggested B°AT2 as a candidate gene involved in major depression (Kohli *et al.*, 2011). It should be noted that SNPs associated with major depression are significantly downstream of the gene, and a more causal relation needs to be established.

The role of taurine transport in human physiology is still ill understood. Taurine transporter deficient mice show a variety of pathological features, such as subtle derangements of renal osmoregulation, changes in neuroreceptor expression and loss of long-term potentiation in the striatum (Warskulat *et al.*, 2007). They develop clinically relevant age-dependent disorders, for example, visual, auditory and olfactory dysfunctions, non-specific hepatitis and liver fibrosis (Warskulat *et al.*, 2004). It is thought that taurine is an important osmolyte for cells, but the relative vulnerability of cells to lack of taurine has yet to be assessed. No human disorder has been identified involving the taurine transporter.

Distribution

Members of the SLC6 family are found in a wide variety of tissues (Table 6). The neurotransmitter transporters are mainly found in the CNS. However, many neurotransmitters are also used as signalling molecules by peripheral neurons and chromaffin cells. The amino acid transporters are not only found in epithelial cells, mostly in the intestine and kidney, but also in brain, lung and testis. Taurine and creatine transporters are expressed widely. Apart from NTT4 (Parra *et al.*, 2008) and PROT (Velaz-Faircloth *et al.*, 1995), two amino acid transporters found in vesicular compartments in the brain, all other SLC6 family transporters are located primarily in the plasma membrane. Neurotransmitter transporters in the brain are either localized in the presynaptic membrane to recapture released neurotransmitters or are localized in astrocytes, which remove neurotransmitters and modulate neurotransmission by a variety of mechanisms. Monoamine transporters, for example, are found in the plasma membrane of the presynaptic neurons using the corresponding neurotransmitter. By contrast, the GlyT1 is expressed in astrocytes and its distribution correlates with that of the NMDA receptor (Smith *et al.*, 1992a). As a result it is thought to modulate glutamatergic neurotransmission. During development, it is in addition important for removal of the inhibitory neurotransmitter glycine (Eulenburg *et al.*, 2010). The glycine transporter GlyT2 is found mainly in neurons of the spinal cord, where it recaptures glycine released from inhibitory neurons (Liu *et al.*, 1993a). The GABA transporter GAT-1 is found in inhibitory neurons throughout the cortex. GAT-2 is found in the leptomeninges

Table 6

Tissue and cellular distribution of SLC6 family transporters

Transporter	Endogenous substrate	Main organ	Other tissues	Subcellular
SLC6A1/GAT1	GABA	brain	Bladder, liver, parathyroid	PM, presynaptic
SLC6A2/NET	noradrenaline	brain adrenal gland	Intestine, kidney, placenta, testis	PM, presynaptic
SLC6A3/DAT	dopamine	brain	thymus	PM, presynaptic
SLC6A4/SERT	5-HT	brain	Bone, intestine, thymus	PM
SLC6A5/GlyT2	glycine	spinal cord	Brain, eye	PM, presynaptic
SLC6A6/TauT	taurine	ubiquitous		PM
SLC6A7/PROT	proline	brain		Vesicular
SLC6A8/CT1	creatine	ubiquitous		PM
SLC6A9/GlyT1	glycine	widely		PM
SLC6A10	pseudogene	n.a.		
SLC6A11/GAT3	GABA	brain	Eye, spinal cord	PM
SLC6A12/BGT1	betaine, GABA	kidney	Brain, liver	PM
SLC6A13/GAT2	GABA	kidney, liver	Brain, eye	PM
SLC6A14/ATB$^{0,+}$	Neutral and cationic amino acids	lung	Pituitary, colon, mammary gland	PM
SLC6A15/B^{0}AT2	BCAA, Met, Pro	brain	Eye, muscle, placenta	PM
SLC6A16/NTT5	Unknown	testis	Blood, bone	unknown
SLC6A17/NTT4	BCAA, Met, Pro, Ala, Gln	brain	Eye, pituitary, pancreas	Vesicular
SLC6A18/B^{0}AT3	Gly, Ala	kidney		PM
SLC6A19/B^{0}AT1	Neutral amino acids	kidney, intestine	skin	PM
SLC6A20/SIT1	Pro, OH-Pro, betaine	intestine	Brain, eye	PM

BCAA, branched chain amino acid; PM, plasma membrane.

surrounding the brain, while GAT-3 has been reported in astrocytes and neurons. The GABA-betaine transporter BGT-1 is also found in neurons but not in the presynaptic membrane. Amino acid transporters of the SLC6 family, such as B^0AT1, B^0AT3 and IMINO, are found in the apical membrane of epithelial cells in the kidney and intestine, where they remove amino acids from the lumen (Romeo *et al.*, 2006; Vanslambrouck *et al.*, 2010). In addition, there are two neutral amino acid transporters (NTT4 and B^0AT2), which are found in neurons throughout the brain (Masson *et al.*, 1996).

Regulation

Many members of the SLC6 family are highly regulated, involving multiple protein–protein interactions. These can be direct interactions between the transporter and another protein or involve a post-translational modification such as phosphorylation (see, e.g., Ramamoorthy *et al.*, 2011) or ubiquitination (Miranda and Sorkin, 2007) of a transporter, mostly at the N- or C-terminus, followed by recognition of the modified sequence by other proteins (Figure 2). In most cases, this results in changes of transporter localization, with changes of catalytic activity being less common (Apparsundaram *et al.*, 2001; Zhu *et al.*, 2005). Changes of transporter localization

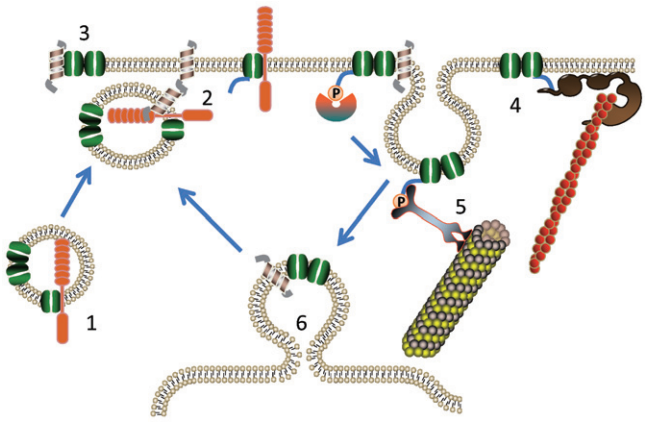

Figure 2

Regulation of SLC6 transporters. Major mechanisms regulating transporter activity and localization are illustrated. In (1), in order to be trafficked to the cell surface, transporters (shown in green) often need to dimerize or associate with a trafficking subunit (orange). At (2), fusion of vesicles with the plasma membranes occurs through interaction of t-SNARE and v-SNARE proteins, shown as helices. Once in the cell membrane (3), a variety of neurotransmitter transporters interact with the t-SNARE protein syntaxin1A (helix) or with the trafficking subunit (orange). To stabilise localisation in the cell membrane (4), transporters can interact with scaffolding proteins, such as PDZ domain-binding proteins (shown as brown globular structures). These can anchor transporters to the cytoskeleton, shown as orange filaments. Internalization of transporters frequently starts with phosphorylation of the N-terminus or C-terminus of transporters and this phosphorylated form is recognized by adapter proteins (5), causing internalization and subsequent removal of the transporter to endosomal compartments (6). Internalization often occurs in specialized lipid domains, such as lipid rafts.

can occur in different ways: first, attachment of a protein can result in movement of the transporter from intracellular membranes to the cell surface; second, attachment of a protein can result in withdrawal of the transporter from the cell surface; third, attachment of a protein can result in stabilization and retention of the protein at the cell surface. Monoamine transporters in addition are regulated by substrate binding, which can affect phosphorylation and trafficking. A variety of SLC6 transporters undergo continuous constitutive internalization into endosomal compartments conceivably followed by recycling back to the plasma membrane (Torres *et al.*, 2003; Melikian, 2004; Eriksen *et al.*, 2010; Ramamoorthy *et al.*, 2011). This process can be affected by activation of kinases, binding of substrate and interaction with other proteins (Figure 2). The number of studies demonstrating protein–protein interactions and protein phosphorylation are too numerous to be listed exhaustively. Instead, the reader is referred to recent reviews in this area (Melikian, 2004; Sitte *et al.*, 2004; Torres, 2006; Eriksen *et al.*, 2010; Ramamoorthy *et al.*, 2011). A few examples serve to illustrate these different mechanisms; (i) Movement to the cell surface by protein–protein interactions. Two different mechanisms are observed, either transporters dimerize before exit from the endoplasmic reticulum can occur (Schmid *et al.*, 2001; Farhan *et al.*, 2006; Horschitz *et al.*, 2008), or they need to associate with an accessory protein, such as collectrin or ACE2 (B^0AT1 and B^0AT3) (Danilczyk *et al.*, 2006; Kowalczuk *et al.*, 2008; Vanslambrouck *et al.*, 2010). These mechanisms are not exclusive, because SERT, for instance, requires both dimerization and binding to the cargo protein SEC24C to reach the surface (Sucic *et al.*, 2011). (ii) Withdrawal from the cell surface. Many neurotransmitter transporters are withdrawn from the surface into endosomal compartments after treatment of cells with agents that activate PKC (Ramamoorthy *et al.*, 2011). In most cases, this is accompanied by increases in transporter phosphorylation on the amino-terminus or the carboxyl-terminus. However, mutagenesis of canonical phosphorylation sites in DAT did not affect down-regulation (Foster *et al.*, 2002). It rather appears that PKC-mediated down-regulation requires sequestration of the transporters in specific membrane domains, such as lipid rafts (Jayanthi *et al.*, 2004; Cremona *et al.*, 2011). For DAT, PKC-mediated down-regulation might also depend on N-terminal ubiquitination (Miranda and Sorkin, 2007) and/or binding of the Ras-like GTPase, Rin, to an endocytic motif in the C-terminus (Navaroli *et al.*, 2011). Whether similar mechanisms account for other SLC6 transporters and whether other kinds of adaptor proteins are required remains unclear. (iii) Stabilization of transporters in the membrane/synapse: Many SLC6 transporters contain a C-terminal PDZ (PSD-95/Discs-large/ZO-1 homology) binding sequence, enabling binding to 'scaffolding' proteins containing PDZ domains. As an example, the PDZ domain protein syntenin-1 binds the C-terminus of GlyT2, and mutation of the PDZ binding motif reduces synaptic localization of this transporter, suggesting that syntenin-1/ or another PDZ domain protein is involved in stabilizing the transporter at synaptic sites (Armsen *et al.*, 2007). Of interest, DAT and NET both bind to PICK1 (protein interacting with C-kinase-1), and this interaction was suggested to play a role in synaptic targeting of DAT (Torres *et al.*, 2001). However, later results have questioned this function, and the functional significance of

the DAT/PICK1 interaction remains unsettled (Bjerggaard *et al.*, 2004).

A notable but still poorly understood protein–protein interaction is the interaction of syntaxin1A with members of the SLC6 family. Syntaxin1A has been shown to interact with GAT1 (Beckman *et al.*, 1998), DAT (Lee *et al.*, 2004; Binda *et al.*, 2008), NET (Sung *et al.*, 2003), SERT (Quick, 2003), GlyT1 and GlyT2 (Quick, 2006). Syntaxin 1A is a t-SNARE protein, which resides in the plasma membrane and catalyses the fusion of synaptic and other vesicles with the plasma membrane. Syntaxin1A appears to interact directly with the N-terminus of several neurotransmitter transporters exerting a variety of effects. First, it seems to be important for movement of transporters from vesicular compartments to the plasma membrane (Geerlings *et al.*, 2001) as shown by decreased trafficking of NET to the surface after partial proteolysis of syntaxin1A with botulinum toxin C1 (Sung *et al.*, 2003). Second, as suggested for SERT, interaction of the transporter with syntaxin1A can reduce transport activity by interfering with an interaction between the N-terminus and intracellular loop 4 (Quick, 2003). Syntaxin1A also abolishes the transport-associated ion conductance in SERT (Quick, 2003). Finally, syntaxin1A binding can promote reverse transport of substrate such as, for example, amphetamine-induced efflux of dopamine in DAT (Binda *et al.*, 2008). Taken together, while there is no question that syntaxin 1A affects neurotransmitter transporter activity in a variety of ways, the physiological rationalization of these processes is far from complete. Interestingly, collectrin, which traffics B^0AT1 and B^0AT3 to the membrane, is thought to interact with Snapin, another t-SNARE protein (Fukui *et al.*, 2005).

Biochemistry

The structure of SLC6 neurotransmitter transporters has been probed intensively, using site-directed mutagenesis, alanine scanning, cysteine accessibility and other approaches (Kanner, 2006; Kristensen *et al.*, 2011). The advent of the high-resolution structure of the bacterial homologue, the leucine transporter LeuT, however, has provided a quantum leap to our understanding of the SLC6 structure (Yamashita *et al.*, 2005). Overall SLC6 transporters have 12 transmembrane helices, 10 of which constitute the core of the transporter. The additional two helices might be involved in transporter dimerization (Just *et al.*, 2004). The first 10 helices are arranged in a pseudo-twofold symmetric pattern, named the 5 + 5 inverted repeat fold (Figure 3). The first five helices can be converted into the second five helices by a simple symmetry operation. Interestingly, the '5 + 5 inverted repeat' of LeuT has been identified also in structures of other prokaryotic transporters, which belong to families that lack significant sequence identity to LeuT or other SLC6 transporters and, accordingly, were not expected to be structurally related (see Abramson and Wright, 2009; Forrest and Rudnick, 2009). Hence, the 5 + 5 inverted repeat fold observed in LeuT appears to characterize several families of secondary active transporters that are likely to operate via a conserved molecular mechanism (see Abramson and Wright, 2009; Forrest and Rudnick, 2009; Krishnamurthy *et al.*, 2009; Forrest *et al.*, 2011). Of particular interest are helices 1 and 6,

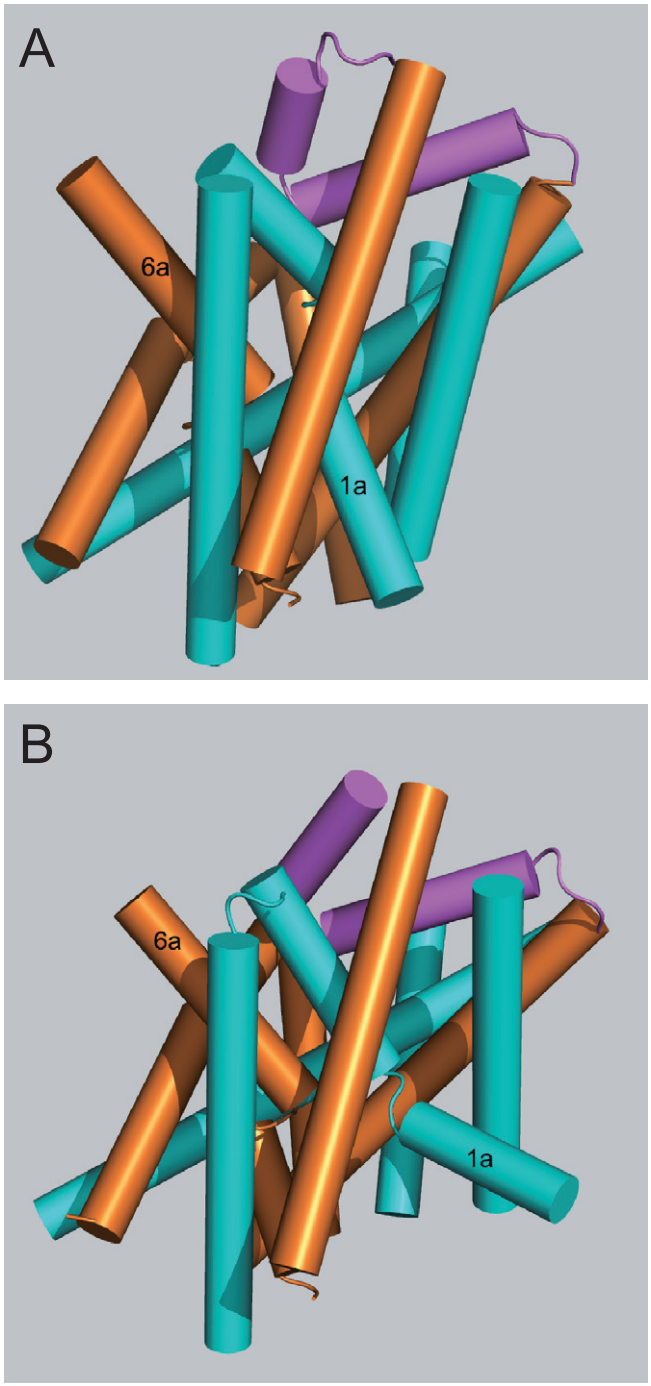

Figure 3

Structure of SLC6 family transporters. The structure of the prokaryotic leucine transporter LeuT is used as a template to generate homology structures of SLC6 transporters. In (A) the structure of LeuT is shown in the open-outside conformation (Protein database 3TT1) and in (B) as the open-inside conformation (Protein database 3TT3). Its structure is characterized by an inverted repeat of a group of five helices. Helices 1–5 are shown in blue and helices 6–10 in orange. Extracellular loop 4 is shown in magenta. Helices 11 and 12 are omitted for clarity. Note the significant movement of helix 1b to allow substrate access on the inside. Extracellular loop 4 blocks access from the outside in the open-inside conformation.

which are unwound in the center allowing backbone contacts with Na^+ ions and substrate (Table 7). Helix 1 and 6 are therefore subdivided into helix 1a/1b and helix 2a/2b. Using site-directed mutagenesis, Krishnamurthy and Gouaux (2012) were able to crystallise LeuT in a substrate-free outward-open and inward-open conformation, providing insight into the transport mechanism. It appears that helices 1,2,5,6 and 7 make significant moves during the transport cycle; while the remaining helices form a scaffold. In particular, helix 1a bends around the center opening up the cytosolic access. In the inward-open conformation, access from the outside is blocked by helices 1b and 6a and extracellular loop 4 (Figure 3).

All SLC6 family members have a high-affinity substrate binding site in the centre of the membrane, called the S1 site. In the initial LeuT structure, which was captured in an occluded outside-facing conformation (Yamashita *et al.*, 2005), access to the S1 site from the extracellular side is prevented by a network of interactions extracellular to S1 that is generated by side chains from TM1, TM3, TM6 and TM10. This includes a highly conserved Arg in TM1 (Arg^{30} in LeuT) that interacts via a pair of water molecules with an Asp in TM10 (Asp^{404} in LeuT) (Yamashita *et al.*, 2005). Access to S1 from the intracellular medium is obstructed by a large layer of protein intracellular to S1 that contains a network of interactions formed primarily by residues at the cytoplasmic ends of TM1, TM6 and TM8 (Yamashita *et al.*, 2005). A highly conserved key residue is Tyr^{268} (LeuT numbering) that by forming a cation–π interaction with Arg^5 in the N-terminus just below TM1 stabilizes a salt bridge between the arginine and Asp^{369} at the bottom of TM8 (Yamashita *et al.*, 2005). Together, these external and internal networks are believed to form dynamic gates that sequentially allow access to the substrate binding site from the extracellular and intracellular environments, respectively, during the transport cycle.

It is assumed that substrate binding together with Na^+ leads to conformational changes that close the external gate and occlude the substrate. In the case of LeuT, two Na^+ ions bind together with the substrate in the active site. Recent site-directed spin labelling and electron paramagnetic resonance (EPR) analysis on purified LeuT demonstrated the Na^+-dependent formation of a dynamic outward-facing intermediate that exposes the primary substrate binding site and the conformational changes that occlude this binding site upon subsequent binding of leucine (Claxton *et al.*, 2010). The free energy of the new closed conformation is such that a larger conformational change can now occur fairly easily (by thermal movement), leading to the open-inside structure with access to the cytosol. In the last step, the gate on the inside opens allowing the substrate to be released. Notable is the bending of helix 1a, which provides access from the cytosol in the open-inside structure. This latter event might be triggered by binding of a second substrate to the outer vestibule in LeuT; hence, steered molecular dynamics have suggested the existence of a second substrate binding site (S2) in the vestibule extracellular to the primary binding site (S1) (Shi *et al.*, 2008). The existence of this S2 site has been further supported by single molecule FRET studies of conformational changes in the LeuT (Zhao *et al.*, 2011). However, the existence and role of this binding site is controversial, and other sets of data have supported the presence of only one substrate binding site in the LeuT structure (Piscitelli *et al.*, 2010). The structural basis of transport-associated conductances also remains unclear but appears to be in equilibrium with the inward facing conformation in the case of the SERT (Schicker *et al.*, 2012). It is tempting to speculate that movements of extracellular loop 4 could allow formation of a continuous, water-accessible, pathway through the transporter.

In the context of the transport mechanism of the SLC6 family, chloride co-transport is another important feature. A plausible chloride binding site has been identified in chloride-dependent members of the SLC6 family (Forrest *et al.*, 2007; Zomot *et al.*, 2007). In Cl^--independent members of the family, such as LeuT and Tyt1 (another prokaryotic transporter), the site is occupied by a negatively charged glutamate residue. In Tyt1, substrate transport measured after reconstitution into liposomes was stimulated by an inversely oriented pH gradient, and correspondingly, mutation of Ser^{331} in the Cl^- binding site of GAT1 to glutamate conveyed pH dependency to this transporter. The data suggest that in Cl^- independent members of the family, a proton may bind and unbind during the transport cycle and thus be counter-transported by a Na^+/substrate–coupled H^+ anti-port mechanism, possibly to facilitate the return step of the 'empty' transporter. This ensures a charge balance among the SLC6 transporters with similar mechanistic features but different molecular solutions (Zhao *et al.*, 2010).

The availability of the LeuT structures also provides relevant clues for the pharmacology of the SLC6 transporters. In LeuT, the substrate binding pocket is lined by residues from helices 1, 3, 6 and 8 (Table 7). The pocket has two regions: first, unwound segments of helices 1 and 6 form backbone contacts with the α-amino and α-carboxyl group of the substrate; second a hydrophobic pocket is formed by aliphatic side chains from TM1, TM3 and TM6 (Tyr^{108}, Phe^{253}, Ser^{356}, Phe^{259}, Ser^{355} and Ile^{359}) (Yamashita *et al.*, 2005). Functional studies and homology modelling suggests that the equivalent residues in the mammalian SLC6 family form the binding sites for its substrates (Beuming *et al.*, 2006; Rudnick, 2006; Henry *et al.*, 2007). An overview of the equivalent residues is shown in Table 7. Interestingly, LeuT has been crystallized also together with TCAs as well as with two SSRIs, fluoxetine and sertraline, for which LeuT displays low affinity. The structures showed a binding site for TCAs and SSRIs in the LeuT, located in the extracellular vestibule (S2) (Singh *et al.*, 2007; Zhou *et al.*, 2007; 2009). On the basis of these observations and mutagenesis of the corresponding site in SERT, it was proposed that the high-affinity binding site of TCAs and SSRIs is also located at the putative S2 site in SERT (Zhou *et al.*, 2007; 2009). However, other studies have suggested that both SSRIs (S-CIT, fluoxetine and sertraline) and TCAs (CMI, imipramine, and amitriptyline) are classical competitive inhibitors and that their primary, high-affinity, binding site is located in the substrate binding pocket (S1) (Barker *et al.*, 1998; Henry *et al.*, 2006; Andersen *et al.*, 2009; Sarker *et al.*, 2010; Sinning *et al.*, 2010). Similarly, there is strong evidence that DAT inhibitors such as cocaine and benztropines have their high-affinity binding site in the central substrate binding cavity (Beuming *et al.*, 2008; Bisgaard *et al.*, 2011).

Table 7

Residues involved in substrate binding in the SLC6 family

Transporter LeuT	α-NH_2 A22 (O)	α-NH_2 F253 (O)	α-NH_2 T254 (O)	α-NH_2 S256 (Oγ)	α-COOH L25 (N)	α-COOH G26 (N)	α-COOH Y108 (OH)	Side chain V104	Side chain F253	Side chain F259	Side chain S355	Side chain I359
GAT1	[A]	[F]	[S]	[G]	L	G	Y	L	F	L	S	T
GAT2	[I]	[F]	[S]	[A]	L	G	Y	L	F	L	S	C
GAT3	[I]	[F]	[S]	[A]	L	G	Y	L	F	L	S	C
BGT1	I	F	S	A	L	G	Y	L	F	Q	S	C
TauT	[F]	[F]	[S]	[A]	L	G	Y	L	F	L	S	E
CT1	[A]	[F]	[S]	[A]	L	G	Y	C	F	L	S	G
NET	A	F	S	G	[L]	[A]	[Y]	V	F	F	S	G
DAT	A	C	S	G	[L]	[A]	[Y]	V	C	F	S	G
SERT	A	F	S	G	[L]	[G]	[Y]	I	F	F	S	G
GlyT1	A	F	S	A	L	G	Y	I	F	W	T	L
GlyT2	A	F	S	S	L	G	Y	I	F	W	T	T
B^0AT3	A	F	S	S	L	G	Y	I	F	F	T	T
PROT	C	F	S	G	L	G	Y	V	F	F	S	F
IMINO	A	F	S	G	L	G	Y	L	F	F	S	N
NTT4	S	F	A	G	L	G	Y	V	F	F	S	T
NTT5	S	L	N	G	P	S	F	L	L	L	S	I
$ATB^{0,+}$	A	F	S	S	L	G	Y	V	F	W	S	S
B^0AT1	S	F	S	S	L	G	Y	V	F	F	S	N
B^0AT2	C	F	A	G	L	G	Y	V	F	F	S	T

Alignment of corresponding residues in the peptide sequences of SLC6 family transporters with critical residues in the high-resolution structure of LeuT (Yamashita *et al.*, 2005). The first line indicates the interaction with the substrate molecule (α-NH_2, α-amino group; α-COOH, α-carboxy group; side chain, side chain of substrate). GABA, taurine and creatine do not have α-amino groups; while monoamines do not have α-carboxy group. The corresponding residues are listed in brackets. Atoms involved in binding are given in parentheses.

Conclusion

Transporters of the SLC6 family are involved in a wide variety of pathological conditions. The structure of the bacterial transporter LeuT allows the generation of homology models, which will help in the design of new inhibitors, targeting specific SLC6 transporters. The pharmacology of the monoamine transporters is highly developed, but the biomedical relevance of other transporters in this family is less well explored.

Conflict of interest

The authors do not have a conflict of interest.

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