

Biogenic Amine Transporters

Key References

Amara, S.G., et al., Molecular physiology and regulation of catecholamine transporters., in *Catecholamines: Bridging Basic Science with Clinical Medicine, Adv. Catecholamines in Pharmacology*, Vol. **42**, eds. Goldstein, D.S., Eisenhofer, G., McCarty, R., pp.164-168 Academic Press, Inc., San Diego (1998).

Blakely, R.D. and Bauman, A.L., Biogenic amine transporters: regulation in flux., *Curr. Opin. Neurobiol.*, **10**, 328-336 (2000).

Blakely, R.D., et al., Regulation of antidepressant-sensitive serotonin transporters. in *Neurotransmitter Transporters: Structure, Function, and Regulation*. ed. Reith, M.E.A., pp. 29-72 Humana Press, Inc., NJ (1997).

Fon, E.A. and Edwards, R.H., Molecular mechanisms of neurotransmitter release., *Muscle & Nerve*, **24**, 581-601 (2001).

Lui, Y. and Edwards, R.H., The role of vesicular transport proteins in synaptic transmission and neural degeneration., *Annu. Rev. Neurosci.*, **20**, 125-156 (1997).

Meltzer, P.C., et al., 2-Carbomethoxy-3-aryl-8-oxabicyclo[3.2.1]octanes: Potent non-nitrogen inhibitors of monoamine transporters., *J. Med. Chem.*, **401**, 2661-2673 (1997).

Owens, M.J., et al., Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites., *J. Pharmacol. Exp. Ther.*, **283**, 1305-1322 (1997).

Roman, D.L., et al., Interactions of antidepressants with the serotonin transporter: A contemporary molecular analysis., *Eur. J. Pharmacol.*, **479**, 53-63 (2003).

Rudnick, G., Ion-coupled neurotransmitter transport: thermodynamic vs. kinetic determinations of stoichiometry. in *Methods in Enzymology: Neurotransmitter Transporters*, Vol. 296, ed. Amara, S.G., pp. 233-247, Academic Press, Inc., San Diego (1998).

Sitte, H.H., et al., Sodium-dependent neurotransmitter transporters: Oligomerization as a determinant of transporter function and trafficking., *Mol. Interv.*, **4**, 38-47 (2004).

Tatsumi, M., et al., Pharmacological profile of antidepressants and related compounds at human monoamine transporters., *Eur. J. Pharmacol.*, **340**, 249-258 (1997).

Torres, G.E., et al., Plasma membrane monoamine transporters: Structure, regulation and function., *Nat. Rev. Neuroscience*, **4**, 13-25 (2003).

Overview

Following vesicular release, the biogenic amine neurotransmitters, norepinephrine, dopamine and serotonin, are removed from the extracellular space by selective and pharmacologically distinct transport proteins. These transporters, abbreviated NET, DAT and SERT, respectively, are of particular clinical interest because they are the molecular targets for many antidepressants as well as drugs of abuse such as cocaine and the amphetamines. The molecular cloning of NET, DAT and SERT revealed that these transporters arise from single genes with homology to members of a larger sodium-dependent transporter gene family. Structural features common to these transporters are a 12 transmembrane-spanning domain structure with intracellular amino- and carboxy-tails that has been largely confirmed by site-specific labeling techniques. Multiple potential phosphorylation sites exist on intracellular domains, supporting evidence of kinase-mediated regulation of these transporters. In addition, amphetamines and other substrates for biogenic amine transporters appear capable of regulating transporter cell surface expression perhaps via activity-dependent processes, thereby establishing both endogenous and pharmacological regulatory mechanisms. Further regulation of biogenic amine transporter function may come from the formation of homo-oligomers or the associations with other membrane proteins to form heterooligomers. As our understanding of these protein-protein interactions evolve, significant efforts will be focused on elucidating the structural requirements for these interactions and how drugs may influence associations to regulate function.

The availability of cDNAs for NET, DAT and SERT has permitted detailed pharmacologi-

cal study of each protein in heterologous expression systems. Interestingly, both NET and DAT transport norepinephrine and dopamine with relatively high affinity; thus, distinction between NET and DAT relies upon sensitivity to transporter antagonists and anatomical localization. Whereas NET is inhibited by the tricyclic antidepressants, including desipramine and nortriptyline as well as more selective drugs such as nisoxetine, DAT is relatively insensitive to these agents, but is potently inhibited by GBR-12909 and GBR-12935, compounds that demonstrate lower affinity for NET. SERT shows the greatest substrate selectivity with >1000-fold higher affinity for serotonin as compared to the other biogenic amines. SERT is most potently inhibited by the highly prescribed class of selective serotonin reuptake inhibitors (SSRIs) that include paroxetine, fluoxetine, and sertraline. All of the biogenic amine transporters are inhibited with approximately equal potency by cocaine, suggesting commonality among the transporters with regards to cocaine recognition. With regards to general structure-activity relationships for biogenic amine transporter ligands, studies have consistently demonstrated the importance of substrate and antagonist amine groups for high-affinity interactions. However, a novel class of high-affinity transporter ligands has been described that lack amine groups, challenging the requirement for amine-containing structures and suggesting new directions in the design and synthesis of biogenic amine transporter ligands.

By comparison to the plasma membrane monoamine transporters, the vesicular monoamine transporters (VMATs), while retaining a predicted 12 transmembrane-spanning domain structure, are members of a separate proton-dependent gene family

of transporters with distinct pharmacological sensitivity. This family of vesicular neurotransmitter transporters also contains the vesicular acetylcholine transporter. The two VMATs, VMAT-1 and VMAT-2, have been cloned and demonstrate broad substrate recognition for monoamine neurotransmitters. The major differences between VMAT-1 and VMAT-2 are in their tissue localization, with VMAT-1 primarily found in endocrine cells and VMAT-2 localized to neuronal tissues. Pharmacologically, VMAT-2 is inhibited by reserpine and tetrabenazine, whereas VMAT-1 is relatively insensitive to inhibition by tetrabenazine. In general, these vesicular transporters are thought to play a major role in packaging neurotransmitters into distinct secretory vesicles in preparation for subsequent exocytotic release, thus controlling quantal size of each release event. The important role that these transporters play in neurotransmission has led to their implication in psychiatric and neurodegenerative disorders and fueled interest in developing selective pharmacological agents targeted to these transport proteins.

Biogenic Amine Transporters

CURRENTLY ACCEPTED NAME	Dopamine transporter (DAT) (D209)	Norepinephrine transporter (NET)	Serotonin transporter (SERT) (S1943)	Vesicular monoamine transporters (VMATs)
STRUCTURAL INFORMATION	620 aa (human)	617 aa (human)	630 aa (human)	525 aa (human VMAT-1) 514 aa (human VMAT-2)
UPTAKE INHIBITORS	GBR-12909 (D052), ^a Nisoxetine (N151), ^a GBR-12935 (G9659), ^a Indatraline (Lu-19-005) (I119), Bupropion (B102), Amfonelic acid (D044), BTCP (B138), Mazindol (M2017), Nomifensine (N1530), β-CFT (WIN 35,428) (C124), β-CPT (WIN 35,065-2) (C156), β-CIT (RTI-55), GYKI 52895 (G120), 4',4''-Difluoro-3α-diphenyl- methoxytropone (D205), 4'-Chloro-3α-diphenylmethoxy- tropone (C207), Cocaine (C5776)	Citalopram (C7861), ^a Tomoxetine (T7947), ^a Desipramine (D3900), Nortriptyline (N7261), Protriptyline (P8813), Imipramine (I7379), Xylamine, Nomifensine (N1530), Mazindol (M2017), Amoxapine (A129), Indatraline (Lu-19-005) (I119), Reboxetine (R6527), Maprotiline (M9651), Duloxetine, Venlafaxine, Cocaine (C5776)	Reserpine (R0875), 6-Nitroquipazine (Q109), ^a Paroxetine (P1372), ^a Sertraline (S6319), ^a Fluoxetine (F132), ^a Clomipramine (C7291), Imipramine (I7379), Alaproclate (A164), Trazodone (T6154), Zimelidine (Z101), Indatraline (Lu-19-005) (I119), Fluvoxamine (F2802), Venlafaxine, β-CIT (RTI-55), Nefazodone (N5536), LY-367,265 (L2411), Duloxetine, Cocaine (C5776)	Tetrahydrobenzazine
TISSUE EXPRESSION	Brain	Brain, adrenal gland, placenta	Brain, platelet, GI tract, placenta	Brain, endocrine cells, adrenal gland, GI tract, mast cells
PHYSIOLOGICAL FUNCTION	Regulates the temporal and spatial actions of dopamine by removing them from the extracellular space	Regulates the temporal and spatial actions of norepinephrine by removing them from the extracellular space	Regulates the temporal and spatial actions of serotonin by removing them from the extracellular space	VMATs package neurotransmitters into secretory vesicles in preparation for exocytotic release, thus controlling quantal size of each release event
DISEASE RELEVANCE	Parkinson's disease, drug abuse, schizophrenia	Affective disorders, attention-deficit/hyperactivity disorder (ADHD), drug abuse, neuropathic pain	Affective disorders, anxiety disorders, neuropathic pain, drug abuse	Hypertension, Parkinson's disease, drug abuse

Abbreviations

BTCP: N-[1-(1-Benzo[β]thien-2-ylcyclohexyl)]piperidine

β-CFT: 2β-Carbomethoxy-3β-(4-fluorophenyl)tropane

β-CIT: 2β-Carbomethoxy-3β-(4-iodophenyl)tropane

β-CPT: 2β-Carbomethoxy-3β-phenyltropane

GBR-12909: 1-[2-[bis(4-Fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine

GBR-12935: 1-(2-[Diphenylmethoxy]ethyl)-4-[3-phenylpropyl]-piperazine

GYKI 52895: 1-(4-Aminophenyl)-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine

LY-367,265: 1-[2-[4-(6-fluoro-1H-indol-3-yl)-3,6-dihydro-1(2H)-pyridinyl]ethyl]-5,6-dihydro-1H,4H-[1,2,5]thiadiazolo[4.3.2-ij]quinoline-2,2-dioxide

FOOTNOTES

^a Best characterized, selective inhibitors for each transporter.