

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).
- Barker, E.L., Blakely, R.D. "Norepinephrine and serotonin transporters: Molecular targets of antidepressant drugs." in *Psychopharmacology: The Fourth Generation of Progress*. eds. Bloom, F., Kupfer, D., pp. 321-333 Raven Press, New York (1995).
- 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).
- Danek, K. Justice, J.B. Jr. "Voltammetric studies on kinetics of uptake and efflux at catecholamine transporters." in *Methods in Enzymology: Neurotransmitter Transporters*, Vol. 296, ed. Amara, S.G., pp. 649-660, Academic Press, Inc., San Diego (1998).
- Lui, Y., 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-Carbo-methoxy-3-aryl-8-oxabicyclo[3.2.1]octanes: Potent non-nitrogen inhibitors of monoamine transporters." *J. Med. Chem.* **40**(1), 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).
- Ramamoorthy, S., Blakely, R.D. "Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants." *Science* **285**, 763-766, (1999).
- Reimer, R.J. et al. "Vesicular neurotransmitter transport and the presynaptic regulation of quantal size." *Curr. Op. Neurobiology* **8**, 405-412 (1998).
- 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).
- Saunders, C. et al. "Amphetamine-induced loss of human dopamine transporter activity: An internalization-dependent and cocaine-sensitive mechanism." *Proc. Natl. Acad. Sci. USA* **97**, 6850-6855 (2000).
- Tatsumi, M. et al. "Pharmacological profile of antidepressants and related compounds at human monoamine transporters." *Eur. J. Pharmacol.* **340**, 249-258 (1997).

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 neurotransmitter transporter gene family. Structural features common to these transporters are a putative 12 transmembrane-spanning domain structure, intracellular amino- and carboxy-tails and a large extracellular loop between transmembrane domains III and IV containing multiple N-glycosylation sites. Multiple potential phosphorylation sites exist on intracellular domains, supporting recent 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 for homeostatic control of transporter function.

The availability of cDNAs for NET, DAT and SERT has permitted detailed pharmacological 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.

Despite the availability of selective high-affinity ligands for the biogenic amine transporters, the search continues for structurally unique transporter ligands that might reveal new information regarding substrate and antagonist recognition by the transporters. For example, structure-activity studies have consistently demonstrated the importance of substrate and antagonist amine groups for interactions with the biogenic amine transporters. 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) (D-209)	Norepinephrine transporter (NET)	Serotonin transporter (SERT)	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 (D-052) ^a GBR-12935 (G 9659) ^a Indatraline (Lu-19-005) (I-119) Bupropion (B-102) Amfonelic acid (D-044) BTCP (B-138) Mazindol (M 2017) Nomifensine (N 1530) β-CFT (WIN 35,428) (C-124) β-CPT (WIN 35,065-2) (C-156) β-CIT (RTI-55) GYKI 52895 (G-120) 4',4''-Difluoro-3α-diphenylmethoxytropane (D-205) 4'-Chloro-3α-diphenylmethoxytropane (C-207)	Nisoxetine (N-151) ^a Tomoxetine (T 7947) ^a Desipramine (D 3900) Nortriptyline (N 7261) Protriptyline (P 8813) Imipramine (I 7379) Xylamine Nomifensine (N 1530) Mazindol (M 2017) Amoxapine (A-129) Indatraline (Lu-19-005) (I-119)	Citalopram (C 7861) ^a 6-Nitroquipazine (Q-109) ^a Paroxetine ^a Sertraline ^a Fluoxetine (F-132) ^a Clomipramine (C 7291) Imipramine (I 7379) Alaproclate (A-164) Trazodone (T 6154) Zimelidine (Z-101) Indatraline (Lu-19-005) (I-119) Fluvoxamine (F 2802) Venlafaxine β-CIT (RTI-55) Nefazodone	Reserpine (R 0875) Tetrahydrobenzazine

ABBREVIATIONS

BTCP: N-[1-(1-Benzo[b]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

FOOTNOTES

^a Best characterized, selective inhibitors for each transporter.