Review

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Mechanisms of dopamine transporter regulation in normal and disease states

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The dopamine (DA) transporter (DAT) controls the spatial and temporal dynamics of DA neurotransmission by driving reuptake of extracellular transmitter into presynaptic neurons. Many diseases such as depression, bipolar disorder, Parkinson's disease (PD), and attention deficit hyperactivity disorder (ADHD) are associated with abnormal DA levels, implicating DAT as a factor in their etiology. Medications used to treat these disorders and many addictive drugs target DAT and enhance dopaminergic signaling by suppressing transmitter reuptake. We now understand that the transport and binding properties of DAT are regulated by complex and overlapping mechanisms that provide neurons with the ability to modulate DA clearance in response to physiological demands. These processes are controlled by endogenous signaling pathways and affected by exogenous transporter ligands, demonstrating their importance for normal neurotransmission, drug abuse, and disease treatments. Increasing evidence supports the disruption of these mechanisms in DA disorders, implicating dysregulation of transport in disease etiologies and suggesting these processes as potential points for therapeutic manipulation of DA availability.

DAT function and regulation

The neurotransmitter DA controls many functions including movement, cognition, mood, and reward. The availability of DA in the brain is modulated by DAT, a plasma membrane protein that actively translocates released transmitter from the extracellular space into the presynaptic neuron. DAT is a target for addictive drugs including cocaine, amphetamine (AMPH), and methamphetamine (METH), and for agents such as Adderall^{TM}, Ritalin^{TM}, and Wellbutrin^{TM} prescribed for the treatment of ADHD, depression, and other DA imbalance conditions. These drugs affect DAT in two ways: some, such as cocaine, bind to the protein and inhibit transport, whereas others, such as AMPH and METH, are transported and stimulate reverse transport (efflux) of intracellular DA. Both processes induce DA overflow, governing the strength of DA signaling during drug abuse and therapeutic treatments.

DAT belongs to the SLC6 family of transporters that couple inward solute transport to downhill movement of Na^+ and Cl^- [1,2]. Solute translocation occurs by an alternating access mechanism in which the protein cycles between outwardly and inwardly facing conformations that bind and release substrate on opposite sides of the membrane. The kinetics of these conformational changes and the precise structures attained establish transporter velocity and functionality, for example, the inward conformation normally releases DA to the cell interior, efflux requires binding and outward translocation of intracellular DA, and cocaine binds to the outward facing conformation [3,4]. DAT also moves ions non-stoichiometrically via channel-like mechanisms, generating currents sufficient to impact membrane potential [5,6]. Because substrate translocation occurs at the plasma membrane, the overall transport capacity of DAT is also a function of its surface density.

It is now well established that these transporter activities are regulated by mechanisms that allow neurons to modulate DA clearance rates in response to short- and long-term physiological demands [7,8]. Many of these processes are interactive or overlapping and they are affected by substrates and inhibitors. Regulation is achieved by post-translational modifications and binding-partner interactions, with additional layers of modulation imparted by cholesterol and membrane raft association. Increasing evidence indicates that many of these processes are dysregulated in DA imbalance disorders, suggesting them as factors in disease etiology and potential therapeutic targets. Here we discuss some of the most well-characterized of these mechanisms, focusing on events related to acute signaling pathways and transporter post-translational modifications, and conclude with an analysis of the processes altered in dopaminergic diseases.

Cytoplasmic domain regulatory elements

DAT comprises 12 transmembrane (TM) spanning helices with TMs 1, 3, 6, and 8 forming the substrate permeation pathway. Unwound sections of TM1 and TM6 form the core of the active site and separate the helices into functional segments (Figures 1 and 2A). Extracellular and intracellular gates above and below the active site dictate inwardly and outwardly facing conformations and control the direction of DA movement [9]. Large N- and C-terminal tails extend into the cytoplasm and contain sites for post-translational modifications, binding-partner interactions, and

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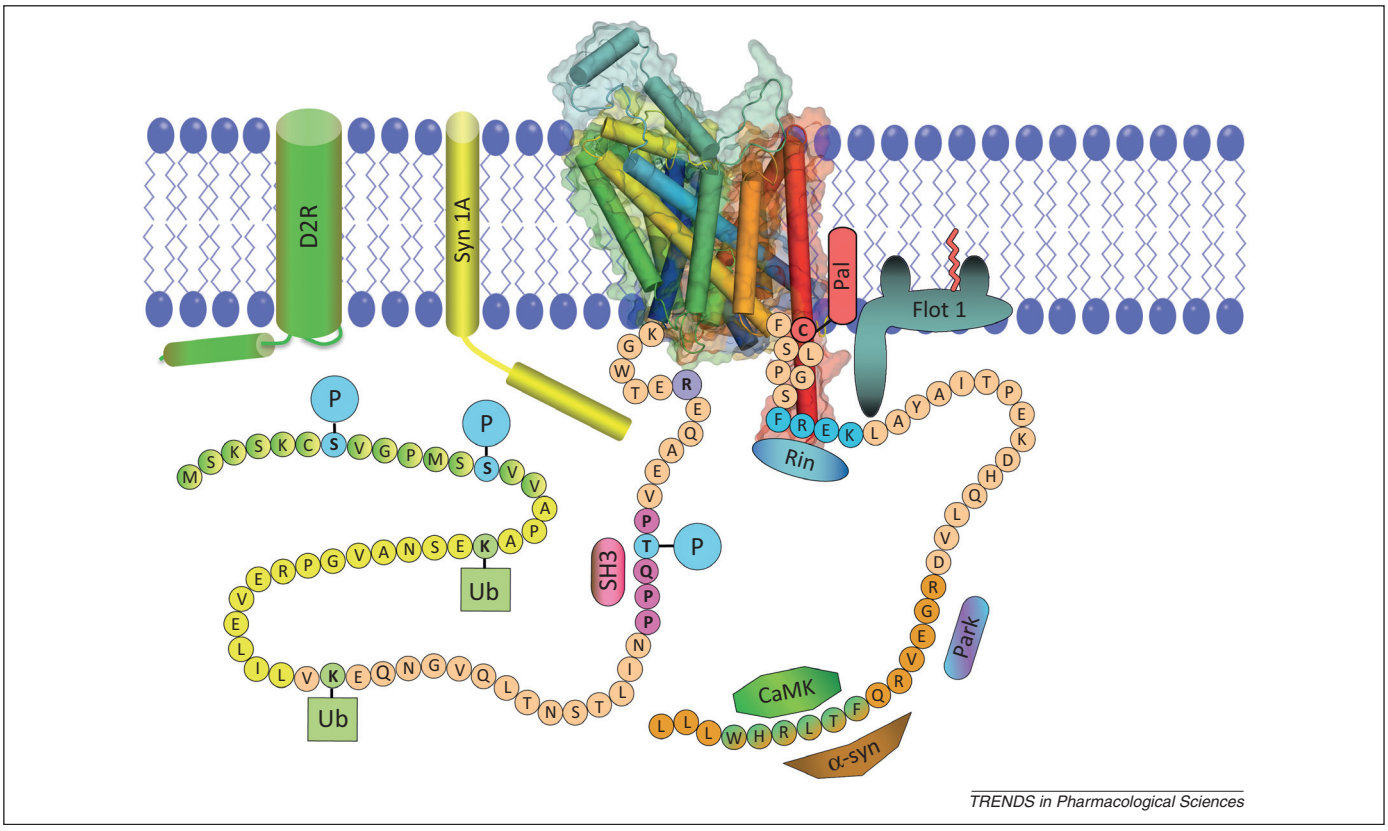

Figure 1. Regulatory elements of rat dopamine transporter N- and C-terminal domains. A 3D model of the rat dopamine (DA) transporter (DAT) based on the *Aquifex aeolicus* LeuT transporter was generated using PyMol (Schrödinger, LLC), with TM helices shown as barrels and light shading indicating semitransparent Connolly surfaces. The structure was positioned in a membrane bilayer with schematic depictions of N- and C-terminal tails extending into the cytoplasm. Post-translational modifications shown are Ser7, Ser13, and Thr53 phosphorylation (blue, P), Lys19 and Lys35 ubiquitylation (light green, Ub), and Cys580 palmitoylation (red, Pal). Motifs and sequences indicated are intracellular gate residue Arg60 (R, purple), putative Src homology domain epitope (mauve, SH3), protein kinase C (PKC) endocytosis motif (blue, FREK), and domains for interactions with Syntaxin 1A (Syn1A, yellow), D_2 DA receptor (D2R, green), Ras-like GTPase Rin 1 (Rin, blue), calcium–calmodulin-dependent protein kinase (CaMK, green), α-synuclein (α-Syn, orange) and Parkin (Park, dark blue-lavender). Flotillin 1 (Flot 1, olive green) is shown with palmitic acid modification (red line) but without a known DAT interaction site.

regulatory motifs (Figure 1). Great insights into the core structure have been obtained from homology modeling to the bacterial leucine transporter LeuT [1,10], but far less is known about the cytoplasmic domains, which are not conserved in LeuT and whose structures have not been solved. The N terminus contains an intracellular gate residue, Arg60, that stabilizes the outwardly facing form and has been implicated in control of transport by influencing the conformation of TM1a, which is thought to undergo major structural rearrangements during the outward-to-inward transition [11–13]. Less is known about the C-terminal domain or its potential impacts on TM12.

The N terminus undergoes extensive modification by phosphorylation and ubiquitylation. Phosphorylation is catalyzed by different classes of kinases on two distinct regions of the domain. The most well-studied site is a cluster of serines at positions 2, 4, 7, 12, and 13 that undergoes increased phosphorylation by protein kinase C (PKC) activation and by *in vitro* and *in vivo* exposure to AMPH and METH [14,15]. AMPH/METH-induced phosphorylation is PKC dependent, with kinase activation potentially resulting from drug-induced increases in cytosolic Ca^{2+} or reactive oxygen species [16]. Within this cluster, multiple serines are modified, but to date the only verified phosphorylation site is Ser7 [17]. The presence of these sites at the distal end of a long and potentially flexible domain suggests the possibility for regulation of binding-partner interactions, although such effects have not yet been demonstrated. The second phosphorylation site is at membrane proximal residue Thr53 [18,19]. This residue is followed by proline, making it specific for proline-directed kinases such as extracellular signal regulated kinase (ERK). Phosphorylation of proline-directed sites substantially alters protein structure by regulating *cis–trans* isomerization of the phosphoacceptor–prolyl peptide bond [20] and the location of this site suggests its potential to regulate transporter functions via impacts on TM1a or Arg60. The sequence flanking Thr53 (P–P–X–X–P) may also constitute an SH3 domain ligand for protein scaffolding [21]. Between the two phosphorylation domains is a region that undergoes ubiquitylation on lysines 19 and 35 [and on human DAT (hDAT) Lys27], catalyzed by the ubiquitin E3 ligases Nedd4-2 and Parkin [22–24]. Modification by Nedd4-2 is likely to be monoubiquitylation and is increased by PKC activation as a mechanism for stimulated endocytosis [22,25].

On the C terminus, DAT is modified by S-palmitoylation, the addition of a saturated fatty acyl moiety via a thioester bond. This occurs on Cys580 near the membrane–cytoplasm interface of TM12 and at one or more currently unknown residues [26]. Just downstream of this site is a motif at residues 587–590 (FREK) that binds the