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RESEARCH HIGHLIGHT

Packaging monoamine neurotransmitters

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Vesicular monoamine transporters mediate the loading of monoamine neurotransmitters into synaptic vesicles. Recent studies by four groups report the structural basis of monoamine transport and the inhibition mechanism of VMAT2.

Monoamine neurotransmitters such as serotonin, dopamine, epinephrine, and norepinephrine play critical roles in pain, mood, cognition, sleep, emotions, and reward behaviors. These neurotransmitters are synthesized in the cytosol and packaged into synaptic vesicles (Fig. 1a). The vesicular monoamine transporter 1 and 2 (VMAT1 and VMAT2) mediate the transport of monoamines into intracellular synaptic vesicles from the cytosol.[1] VMAT1 is expressed in neuroendocrine cells whereas VMAT2 is expressed in the central and peripheral nervous system. These transporters belong to the major facilitator superfamily and utilize the proton gradient across the vesicular membrane for transport. For each monoamine transported into the vesicle, the transporter antiports two protons from the vesicle lumen to the cytosol. However, the absence of structural investigations limits the mechanistic understanding of transporter function. Recently, four groups independently reported the structures of VMAT2 in various conformations using cryo-electron microscopy (cryo-EM), thereby providing critical insights into the mechanisms of substrate transport and drug inhibition.[2–5]

Structure determination of small membrane proteins is challenging due to the lack of fiducials, low signal-to-noise ratio, and relatively higher signal from micelles. To overcome imaging challenges, these studies utilized fusion-based strategies to incorporate fiducials for particle alignment. Fusion tags were incorporated on one of the termini, and a binding partner was either fused on the other termini or added externally which enabled high-resolution structure determination of VMAT2 in various conformations. The VMAT2 structure is comprised of twelve transmembrane (TM) helices that are divided into the N-domain (TM1–6) and C-domain (TM7–12) which are arranged in a pseudosymmetric pattern. The translocation path is lined by helices TM1, TM4, and TM5 of the N-domain and helices TM7, TM8, TM10, and TM11 of the C-domain. The interaction between translocation path helices modulates the transporter conformation.

To investigate the substrate recognition and transport mechanism, cryo-EM structures of VMAT2 in complex with serotonin were determined in either lumen-open or cytosol-open conformation.[3–5] These structures provided snapshots into the transport cycle by representing substrate binding and pre-release states. In the pocket, substrates are stabilized by hydrophobic interactions with V232 and I308 of VMAT2 and potentially form salt-bridge interactions with E312. Residues V232 and I308 are mutated to leucine and valine, respectively, in VMAT1. These mutations might impact the stability of substrates in VMAT1 and may explain the differences observed in the binding affinity of substrates between transporters. Interestingly, histamine shows a significant preference for VMAT2 over VMAT1.[6] The lack of hydroxyl groups in histamine might have implications for the binding and transport activity.[7] However, further studies are required to delineate the mechanism of histamine recognition and transporter preference.

Reserpine, a competitive inhibitor of VMAT2, was approved for the treatment of hypertension but has since been designated as second-line therapy due to various side effects. In the cryo-EM structures of reserpine-bound VMAT2, reserpine occupies the central binding site that overlaps with the serotonin binding site.[3–5] Reserpine binding induces rigid-body movement of C-domain helices forming a large cytoplasmic pocket that accommodates reserpine and locks the transporter in a cytosol-facing conformation. Interestingly, the reserpine-bound VMAT2 structure revealed the presence of a lateral gate near the cytoplasmic half that presents an opportunity for hydrophobic molecules to diffuse into the translocation path from the lipid bilayer.[4] The lateral gate might enable lipid-mediated transport regulation. However, further studies are required to investigate the role of the membrane environment in transporter regulation.

Tetrabenzine (TBZ) and its derivatives are used to treat chorea associated with Huntington's disease and Tardive dyskinesia. Pharmacological studies of TBZ showed that it is a selective non-competitive inhibitor of VMAT2 over VMAT1 and forms a dead-end complex halting the monoamine transport.[6,8] The high-resolution cryo-EM reconstruction of the TBZ-bound VMAT2 demonstrated that TBZ binding rearranges the translocation path helices, thus locking VMAT2 in an occluded conformation, and consequently blocking the transport.[2–5] An intriguing feature of the TBZ–VMAT2 complex is the unwinding of TM2 and TM7 to close the luminal side of the vestibule. Specifically, residue W318, located at the luminal end of TM7, folds back onto the luminal opening upon TM7 unwinding and forms the proposed dead-end complex. Alanine substitution at W318 decreases the TBZ binding and substrate transport, thereby highlighting its important role in drug binding and luminal gating. Additionally, mutating the binding pocket residues of VMAT2 to the corresponding VMAT1 residues decreases the TBZ binding and therefore provides insights into TBZ's selective inhibition of VMAT2 over VMAT1.

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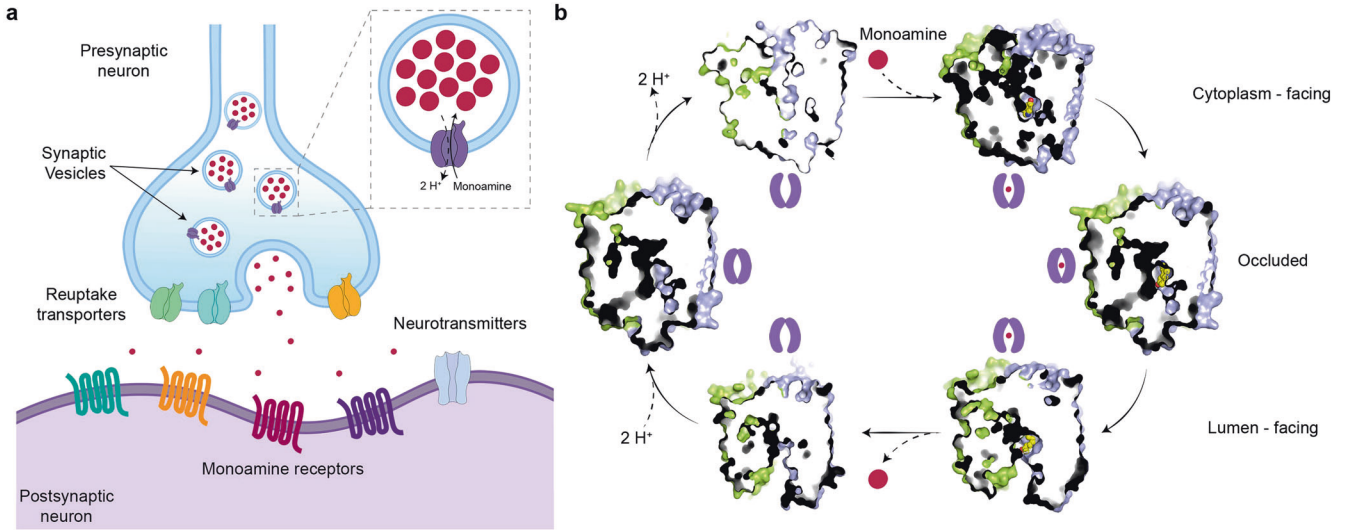

Fig. 1 Monoamine neurotransmission and vesicular monoamine packaging. a Schematic representation of monoamine neurotransmission at synapses. The close-up depicts a synaptic vesicle with a monoamine transporter and packaged monoamines. **b** Transport cycle of vesicular monoamine transporter. VMAT2 structures of lumen-facing apo (PDB ID: 8WLJ), lumen-facing serotonin-bound (PDB ID: 8WLM), cytosol-facing serotonin-bound (PDB ID: 8T6B), and occluded TBZ-bound (PDB ID: 8T69) were used as representative states during the transport cycle. The apo cytosol-facing and occluded states were obtained by removing ligands from cytosol-facing serotonin-bound and occluded TBZ-bound structures, respectively. Similarly, the serotonin-bound occluded form was obtained by replacing TBZ with serotonin.

In the absence of a ligand, VMAT2 obtains a lumen-facing conformation.[3] This conformation may represent the low-energy resting state of the transporter and underscores the importance of proton antiport during monoamine transport (Fig. 1b). To initiate the transport, proton binding drives the VMAT2 into a cytosol-facing conformation where the monoamines bind at the central pocket. The rearrangement of translocation path helices leads to the transition of the transporter into an occluded conformation. The unwinding of TM2 and TM7 helices facilitates the opening of the lumen gates. Substrates are released into the lumen, and the transporter is reset into a cytosol-facing conformation that is driven by a proton gradient primed for the next transport cycle.

Recent advances in structure prediction methods have created a new revolution in large-scale structural-omics for proteome-wide analysis.[9] However, it is important to remember that these predictions are simply predictions and may or may not be correct.[10] Therefore, experimental investigations to obtain high-resolution structures are still required to identify conformational changes, binding pocket architectures, and protein–protein/ligand interactions. For instance, in the VMAT2–TBZ complex structure, Wang et al. noted that while the occluded conformation resembles the AlphaFold2-predicted structure, the unwinding of TM2 and TM7 to block the lumen exit of the vestibule was unique to the experimental structures.[3] Integrative studies combining experimental, theoretical, and computational techniques are thus paramount to understanding the physiological functions of biomolecules.

Overall, these studies provided fundamental insights into packing monoamines into synaptic vesicles. These insights open the next avenue of research for identifying new and better inhibitors of VMAT2 and investigating the structural basis of transport inhibition by drugs of abuse, such as amphetamine and methamphetamine. Also, VMAT2 is used as an imaging marker for Parkinson's disease (PD). These structures can inform the rational design of improved binding tracers for early diagnosis and tracking disease progression in PD patients. Furthermore, VMAT2's transport activity regulation by G-proteins is important for maintaining monoamine homeostasis between vesicles and the cytosol.[11] However, the molecular basis of regulation remains elusive. Thus, these studies represent a preview of exciting future research on vesicular transporters.

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