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REVIEW

Vesicular monoamine transporters (VMATs): From discovery to therapeutic targets

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Vesicular monoamine transporters (VMAT) are proteins essential for life. They play central roles in neurotransmission and hormone storage and are targets of neuroactive drugs. This retrospective chronicles the decades-long journey of studying VMAT, from early biochemical explorations to the recent surge of structural information. We delve into the serendipitous discoveries, methodological innovations, and collaborations that enhanced our understanding of monoamine storage and synaptic transmission. Importantly, this narrative highlights that a degree of initial "optimistic ignorance"—a failure to fully appreciate the system's complexity—can, paradoxically, be a driving force in scientific exploration. Our early, naïve assumption about VMAT's abundance, while ultimately an oversimplification, was instrumental in launching the project to identify, purify, and characterize the protein. An unprecedented flood of cryo-EM structures now provides invaluable molecular insights into the molecule's intricate workings, synergistically complementing functional studies and offering the crucial framework needed to mechanistically link structure to its function.

Vesicular monoamine transporters (VMATs) actively sequester monoamines such as serotonin, dopamine, histamine, adrenaline, and noradrenaline from the cytosol. These monoamines are stored in synaptic vesicles at nerve terminals or in storage organelles found in platelets, enterochromaffin cells, chromaffin cells, and other similar cells. This storage process is crucial for allowing regulated release of neurotransmitters into the synaptic cleft or to appropriate targets throughout the human body (Fig. 1) (Njus et al., 1981; Johnson, 1988; Schuldiner et al., 1995; Weihe and Eiden, 2000; Edwards, 2007).

The past year has been marked by a dramatic surge of groundbreaking publications, revealing 33 cryo-EM structures of human VMATs in diverse states, encompassing both liganded and unliganded forms (Table S1) (Dalton et al., 2023; Pidathala et al., 2023; Im et al., 2024; Schuldiner and Forrest, 2024; Wang et al., 2024; Wu et al., 2024a; Ye et al., 2024; Wei et al., 2025; Ye et al., 2025). Analysis of these structures not only updates our understanding but also provides in-depth insights into the inner workings of these transporters. It presents opportunities for developing improved therapeutics and a deeper understanding of a transporter that plays an essential role in synaptic transmission.

VMATs are essential for life. In humans, mutations in VMATs can lead to developmental delay, dystonia, Parkinsonism, and increased mortality, underscoring the critical role of these transporters in neurotransmission (Rosenthal et al., 2025). VMATs are also a target for treating hypertension, psychotic agitation, psychostimulant abuse, and hyperkinetic movement disorders, such as Huntington's disease-related chorea (Rosenthal et al., 2025).

Tracing the scientific evolution of VMATs, from Carlsson's pioneering work to the current high-resolution structural insights, offers historical value and continues to define our understanding of molecular neurotransmission. Reviewing the origins of VMAT research is vital for placing the current high-resolution mechanistic understanding in context. The early work on bioenergetics, kinetics, and pharmacology of VMAT provides the necessary background to interpret today's structural breakthroughs and motivates the next generation of discovery. Tracing this journey, from Carlsson's early work to the present, demonstrates the enduring value of scientific exploration. I now find myself with the time to reflect on over 50 years of VMAT history, a story closely intertwined with the work of my own laboratory and our collective fascination with the chromaffin cells in the adrenal medulla.

Chromaffin cells

Chromaffin cells are responsible for the fight-or-flight reaction, where a surge of blood adrenaline increases its concentration up to 300-fold. These cells produce large amounts of the hormone and store it in chromaffin vesicles through a process mediated by VMAT (initially termed chromaffin granule amine transporter, CGAT). Two crucial substances synthesized in the cytosol must

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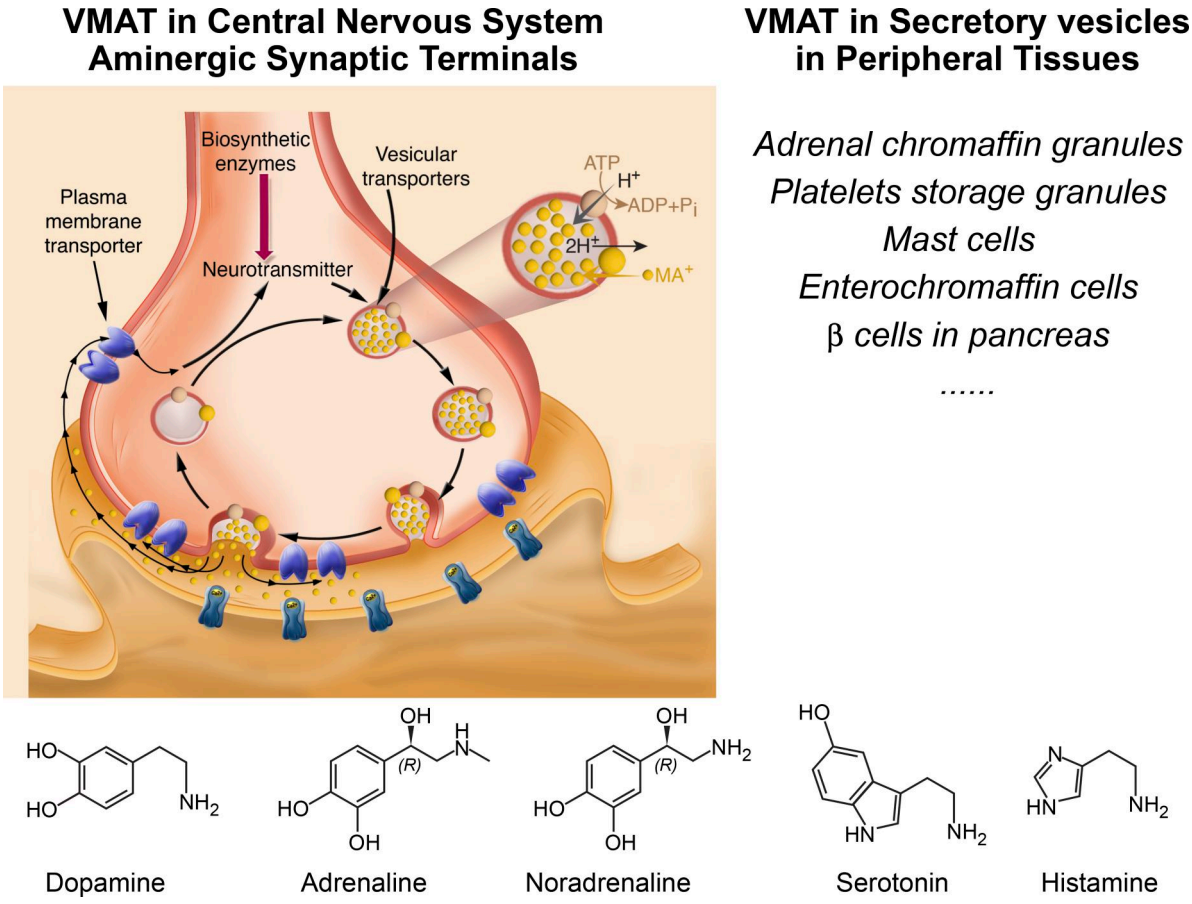

Figure 1. **VMATs mediate the loading of monoamine neurotransmitters into synaptic vesicles and storage organelles.** Monoamine neurotransmitters are synthesized in the cytosol and subsequently packaged into synaptic vesicles at nerve terminals or into storage organelles found in platelets, enterochromaffin, chromaffin, and other similar cells. When an action potential triggers calcium influx, these synaptic vesicles fuse with the presynaptic membrane, releasing neurotransmitters into the synaptic cleft. There, they bind to postsynaptic receptors, transmitting the signal. Signal termination is rapidly achieved by retrieval of the neurotransmitters, primarily mediated by specific Na^+-coupled plasma membrane transporters. This crucial packaging of monoamines into vesicles is mediated by VMATs, specifically VMAT1 and VMAT2. VMAT1 is predominantly expressed in neuroendocrine cells, while VMAT2 is found throughout the central and peripheral nervous systems.

be transported into the chromaffin vesicles during the process of adrenaline synthesis. VMAT transports dopamine into the vesicles where it is converted into noradrenaline, which then exits the vesicle to be converted into adrenaline in the cytoplasm and then transported by VMAT back into the vesicles for storage. Inside the vesicles, the concentration of adrenaline is at least 25,000 times greater than in the cytosol, indicating the effectiveness of the transport mechanism (Carmichael and Winkler, 1985).

In 1962, Arvid Carlsson and his colleagues at the University of Göteborg and Norman Kirshner of the Duke University School of Medicine made a significant breakthrough in understanding the nature of the transport mechanism (Carlsson et al., 1962; Kirshner, 1962; Carlsson, 2001). They demonstrated that isolated chromaffin vesicles can accumulate and store adrenaline from a dilute solution.

Their discovery that accumulation occurred only when magnesium ions and ATP were included in the solution was a crucial early clue, paving the way for further research into the bioenergetics of the transport mechanism. The significance of these findings extended far beyond the immediate transport process. This seminal research, coupled with subsequent investigations into dopamine and other neurotransmitters, had a profound and pivotal impact on fields such as neurology and psychiatry. Indeed, it was this very line of inquiry—particularly the unequivocal demonstration of the role of catecholamines—that decisively settled the long-standing debate concerning whether electrical stimuli ("the sparks") or chemical compounds ("the soup") served as the primary transmitting agents for signals in the central nervous system (Valenstein, 2005). This culminated in Arvid Carlsson being awarded the Nobel Prize in Physiology or Medicine in 2000, which he shared with Paul Greengard and Eric Kandel for their discoveries concerning signal transduction in the nervous system (Carlsson, 2001).

Bioenergetics and my involvement with VMAT

My involvement with VMAT began after I attended a lecture at the late Roche Institute of Molecular Biology, where I trained with the legendary Ron Kaback. Those were the opening years

of the "chemiosmotic wars," a period of intense controversy in mitochondrial and chloroplast research. The debate centered on whether oxidative phosphorylation was driven by elusive high-energy chemical intermediates or by the transmembrane (TM) electrical and chemical potentials proposed by Mitchell (Mitchell, 1976; Rottenberg, 1986; Nicholls and Ferguson, 2013). The discussion around protons, proton gradients, and vectorial reactions began to gain traction among a general biochemical audience. New methods for measuring pH and TM potentials in cells and internal organelles have become increasingly accessible (Nicholls and Ferguson, 2013). As a result, many research groups with expertise in these techniques expanded their focus beyond pure mitochondrial and chloroplast bioenergetics, delving into the mechanistic details of transport processes in bacteria, animal cells, plant cells, and their organelles.

My doctoral studies with Mordhay Avron, a superb scientist and mentor at the Weizmann Institute, prepared me for this work, as I learned how to measure proton gradients and how chloroplasts utilize these gradients to synthesize ATP. I then applied these techniques in Kaback's lab to study the mechanisms of lactose transport in the bacterium *Escherichia coli*. I was preparing to open my lab in Jerusalem and considering possible projects with medical significance. The lecture mentioned above, given by Harvey Pollard, then at the NIH, presented one of many attempts to understand the role of ATP in adrenaline transport into granules. I hypothesized that a classical chemiosmotic mechanism could explain the results presented, where an ATP-driven pump in the granule membrane pumps protons into the granule lumen, creating a driving force, and the amine transporter exchanges luminal protons for cytoplasmic amine, thereby using the energy of ATP hydrolysis to accumulate amine inside the granule. I decided to delve into this project during my independent research career in Jerusalem, aiming to deepen our understanding of these mechanisms.

I was not alone in this endeavor; during this period, the entire field of bioenergetics was experiencing a transformative phase driven by the pioneering insights of the Mitchellian revolution. This paradigm shift, initiated by Peter Mitchell's groundbreaking proposal of the chemiosmotic theory, fundamentally changed our understanding of how energy transduction occurs across biological membranes (Mitchell, 1976; Rottenberg, 1986; Nicholls and Ferguson, 2013). Building on these revolutionary concepts, at least half a dozen other research groups worldwide harnessed the techniques and ideas developed during this era—such as measurements of proton gradients, membrane potential analyses, and the use of ionophores—to explore a variety of transport processes beyond mitochondria, including vesicular systems (Njus et al., 1981; Johnson, 1988; Schuldiner et al., 1995). In Jerusalem, our team applied these bioenergetic principles to dissect the mechanisms of catecholamine transport into chromaffin granules, integrating the emerging understanding of proton motive force-driven transport into our experimental design. This collective endeavor, rooted in the innovative spirit of the Mitchellian revolution, provided new tools and perspectives that fueled our progress in understanding VMAT and related transporters.

Thanks to a wealth of contributions from various laboratories, including ours, it soon became clear that the ATP requirement described by Carlsson over a decade earlier was indirect. An H^+-translocating V-type ATPase, embedded in the granule membrane, harnesses the energy from ATP hydrolysis to actively pump protons (H^+) into the granule lumen. This process generates a substantial electrochemical gradient across the vesicular membrane. This gradient has two key components: a pH difference (ΔpH), more acidic by about 2 pH units inside the vesicle compared with the cytoplasm, and a small membrane potential ($\Delta\psi$), positive inside. The resulting force driving protons from the lumen to the cytoplasm is used to accumulate catecholamines (such as dopamine, norepinephrine, and epinephrine) by coupling downhill proton efflux to uphill catecholamine influx (Njus et al., 1981; Johnson, 1988; Schuldiner et al., 1995). In this classical antiport mechanism, the VMAT protein acts as a molecular seesaw, alternately translocating two protons down their electrochemical gradient (from inside the vesicle to the cytoplasm) while moving catecholamines up their concentration gradient (from the cytoplasm into the vesicle). This antiport is energetically favorable because the movement of protons down their gradient provides the energy needed to concentrate the catecholamines against their gradient, effectively trapping them within the vesicle for subsequent release (Fig. 1).

The very beginning

As I mentioned above, the project in my lab took advantage of the techniques and approaches I developed while studying proton gradients in chloroplast bioenergetics during my doctoral studies. Later, as part of my postdoctoral training, I investigated symporters in *E. coli*, with a particular focus on the lactose transporter. My experience with living organisms at the time was limited to mincing lettuce to prepare chloroplasts and growing bacteria to prepare membranes.

It took me some time to learn how to dissect adrenal glands and extract chromaffin granules. To gather fresh material, we visited the slaughterhouse with an ice-filled portable cooler. We persuaded the person in charge of cow dissection to provide us with the adrenal glands, which were not intended for sale as food. This arrangement benefited us and the workers who received a small payment as a token of our gratitude. One skill I had to master while waiting for the glands is dissociation. I had to learn to become numb to death and suffering, but I was very young, and the idea that this study could lead to exciting findings immunized me. For several years, I took the task of collecting the glands on myself because I did not want to expose my young students to the views of the abattoir. It was also a practical decision, as I was the only one with a car, an excellent 1960 VW Beetle that only sometimes started on cold mornings. The number of students using the glands increased, and the decision to purify the transporter using the old, good, classical biochemistry, the only available method at the time, necessitated the use of more and more glands. Luckily, at this point, I recruited a new student who had a car, lived close to the slaughterhouse, and willingly volunteered to collect the glands before

coming to the lab. He succeeded in making his job easier once he convinced the dissection worker that his presence was not necessary during the entire gland collection process and that he could collect the cooler after a couple of hours.

The next challenge was gland dissection, which we quickly mastered. At the height of our production period, there were three or four of us together to extract the gland's medulla and prepare the chromaffin granules. The granules store catecholamines alongside acidic proteins (chromogranins), neuropeptides, and ATP (Carmichael and Winkler 1985). Preparation of membrane vesicles ("ghosts") by osmotic lysis of the granules to remove the endogenous catecholamines was essential to work out the bioenergetics and as a first step in the purification of the transporter (Schuldiner et al., 1978).

The aim was to quickly freeze the vesicles so that we could go home before it was too late and start working the day after. Luckily, vesicles properly frozen at −80°C were stable for months and were used for initial kinetic, bioenergetic, and pharmacological studies (Schuldiner et al., 1994). Yael Stern Bach, now a Professor at the Hebrew University Medical School, along with other lab members, used the vesicles as starting material for purification of CGAT, which is now known as VMAT2.

Early mechanistic studies

These vesicle preparations proved essential for our early studies on the kinetics and pharmacology of the then named CGAT. VMAT's pharmacology provided essential tools to dissect specific steps in the transport cycle. The pharmacology of VMAT differs from that of plasma membrane amine transporters with high-affinity inhibition by reserpine and tetrabenazine (TBZ) but not by cocaine or antidepressants. Reserpine is an indole alkaloid, an antipsychotic, and an antihypertensive drug. However, with the introduction of many new classes of medications over the past two decades, its use has declined substantially, and it is now used mainly in veterinary medicine (see also Slim et al., 2011; Weir, 2020).

The discovery of reserpine enabled several vital lines of scientific inquiry, including the mechanism of dopamine storage and release in the central nervous system, as well as the generation of animal models of Parkinson's disease (Carlsson, 2001; Duty and Jenner, 2011).

TBZ, on the other hand, is used for the symptomatic treatment of hyperkinetic disorders associated with Huntington's disease and Tourette's syndrome (Paleacu, 2007).

Detailed kinetic studies of TBZ binding conducted mainly by Jean-Pierre Henry's group in Paris in the 1980s provided essential insight to the concept that reserpine and TBZ bind to two different conformations of VMAT (Scherman and Henry, 1984). The availability of these two ligands proved crucial in developing tools to dissect the VMAT transport mechanism.

Reserpine is a potent competitive inhibitor of both VMAT1 and VMAT2, and its mechanism of action is unique. Early studies demonstrated that reserpine binding to chromaffin granule membranes is dependent on ATP (Weaver and Deupree, 1982). This ATP dependence reflects the generation of an electrochemical H^+ potential by the granule ATPase, as evidenced by the inhibition of ATP-induced binding by protonophores. My longtime friend and excellent scientist, Gary Rudnick, was on sabbatical in my lab, and together we showed that the energetics of reserpine binding to the bovine adrenal biogenic amine transporter suggest that H^+ ion translocation converts the transporter to a form that binds reserpine practically irreversibly. Reserpine binding is less dependent than amine transport on the pH gradient, suggesting that translocation of fewer H^+ ions is required to expose the high-affinity site than is necessary for net transport (Rudnick et al., 1990). The model proposed in these studies was published 35 years ago and has been validated numerous times, including in the recently published structures as discussed below.

Purifying membrane proteins

To gain a deeper molecular perspective, we sought to purify the transporter and reconstitute its activity into proteoliposomes. By isolating the protein from the complexities of the native membrane, we aimed to determine its primary sequence; this information was essential for designing the oligonucleotides required to finally clone the gene. Purifying a functional membrane protein in the 1980s was an extremely challenging task, a stark contrast to the relative ease of today's methods. What may seem trivial now was monumental at the time. The choice of detergent, for example, was critical, requiring a delicate balance: it needed to effectively solubilize the protein from the lipid bilayer without denaturing it or stripping away essential lipids as are necessary for its activity. We gained valuable insights from a fruitful and enjoyable decade-long collaboration with Baruch Kanner, a, then young, new recruit to the Medical School that trained in Racker's lab, one of the pioneers in membrane protein biochemistry. Racker's lab had successfully purified components of the mitochondrial respiratory chain, establishing key techniques for the field (Racker, 1979). While there were many reports of purified functional membrane proteins, they all exhibited activities such as ATP hydrolysis, redox reactions, and ligand binding—activities that could be readily assayed to track protein integrity during purification.

In contrast, ion-coupled transporters, such as those we studied, had no activity that could be measured after solubilization, and presented a much more complex challenge. To measure their activity, they had to be carefully reconstituted into highly specialized liposomes. These liposomes had to be not only of defined lipid composition but also capable of maintaining stable ion gradients, often pH or sodium gradients, crucial for driving transport. This reconstitution process demanded meticulous attention to detail and the use of specific detergents, often nonionic detergents like octylglucoside or dodecylmaltoside, that could be removed entirely without disrupting the fragile liposome membrane or compromising protein function. Despite these hurdles, thanks to the dedication of my excellent students who were willing to spend long, frustrating hours in front of ion exchange, hydrophobic, and size exclusion columns, we successfully purified several functional bacterial and

mammalian transporters (Stern-Bach et al., 1990; Taglicht et al., 1991; Pinner et al., 1992; Yerushalmi et al., 1995). At the time of our successful purification and functional reconstitution of VMAT2, NhaA, NhaB, EmrE, and others, the field of ion-coupled transporter research with purified, reconstituted proteins was still in its infancy.

Nowadays, protein production methods such as those using insect cells, yeasts, bacteria, tissue culture, and cell-free expression systems have rendered previously labor-intensive procedures largely unnecessary. Protein tagging, with techniques like His-tag or FLAG-tag affinity purification, has dramatically simplified the purification processes. It is now possible to achieve what used to take my excellent students 4–5 years of hard work in a matter of weeks.

The role of serendipity and failure

Serendipity and failure have played a crucial role in shaping scientific discovery. Often, unexpected findings or setbacks lead to significant breakthroughs. One such example is the decision to purify VMAT2, which illustrates how ignorance and naivety can sometimes yield surprising results.

After reading only part of the relevant literature, I mistakenly believed that VMAT was the primary protein in the chromaffin granule membrane. This assumption arose from my perception that the only role of these organelles is to store adrenaline and noradrenaline. Additionally, I was aware of the published success in purifying the erythrocyte band 3, a vital component of the erythrocyte cell membrane that constitutes about 25% of its surface (Steck, 1978). Consequently, similar to the success with band 3, we would only need to eliminate some minor impurities to obtain a pure VMAT. However, 5 years later, we slowly realized that VMAT comprised <1% of the chromaffin granule membrane protein (Stern-Bach et al., 1990). It took me almost a decade to understand what others had already established: the chromaffin granule is a complex organelle responsible not only for the storage and secretion of adrenaline but also for the steps of its synthesis and the storage and secretion of peptides and proteins (Carmichael and Winkler, 1985; Maneu et al., 2023).

In my laboratory, Yael Stern-Bach successfully purified the vesicular amine transporter from bovine adrenal medulla using the potent inhibitor reserpine. Reserpine binds noncovalently, yet virtually irreversibly, to these transporters. By labeling trace amounts of the transporter in bovine chromaffin granules with ^{3}H-reserpine, she utilized this tracer to guide the purification of two ∼80-kD proteins that differed in isoelectric point. Crucially, she then successfully demonstrated the functional reconstitution of one of these isoforms in liposomes, a critical step toward mechanistic studies (Stern-Bach et al., 1990).

Once VMAT was purified, we obtained partial sequences of the N terminus, and we attempted to use this information to identify and clone the gene that encodes it (Stern-Bach et al., 1992). Despite our efforts, we failed due to a combination of a lack of experience, insufficient funding to purchase high-quality cDNA libraries, and difficulties in establishing effective collaborations. After many efforts, it turns out that, as with many libraries at the time, the only cDNA library we obtained was missing the 5′ terminal sequence, and the primers we designed were, therefore, useless.

At that time, and again by chance, I was lucky to meet Robert Edwards, who was then at UCLA. Robert, a talented scientist as well as a neurologist and expert in Parkinson's, succeeded in cloning the gene coding for resistance to MPP^{+}, a Parkinsonian toxin. It turned out that this gene coded for VMAT, as MPP^{+} is a substrate of VMAT (Liu et al., 1992a; Liu et al., 1992b). VMAT confers resistance by sequestering the toxin into acidic subcellular compartments and away from its target. The cDNA sequence predicted a novel protein with 12 TM domains, similar to transporters in the major facilitator superfamily (MFS). At the time, this protein exhibited no primary sequence similarity to known plasma membrane sodium dependent neurotransmitter transporters or, indeed, to other mammalian proteins. This suggested that the vesicular amine transporter defined a novel mammalian gene family. The N-terminal half of the protein displayed weak but significant sequence similarity to several bacterial antibiotic resistance proteins. This connection was particularly interesting because these bacterial proteins transport antibiotics out of the cell, driven by the proton electrochemical gradient, a process topologically and energetically equivalent to transport into an intracellular organelle. Thus, the vesicular amine transporter displays striking physiological, energetic, pharmacological, and structural similarities to this class of ancient bacterial detoxification systems, strongly suggesting an evolutionary lineage (Schuldiner et al., 1995).

In a classical example of parallel discovery, work was proceeding simultaneously across the continent. While the breakthrough in Edwards lab at UCLA was unfolding, Erickson, Eiden, and Hoffman at the NIH on the East Coast independently identified the same VMAT gene using expression cloning in mammalian cells (Erickson et al., 1992). This parallel discovery quickly led to the identification of two distinct genes coding for two isoforms: VMAT1 and VMAT2, each displaying characteristic differences in tissue distribution, substrate affinity, and inhibitor sensitivity (Erickson and Eiden 1993; Peter et al., 1995). Human VMAT1 is primarily expressed in neuroendocrine cells and peripheral tissues like the adrenal medulla, while hVMAT2 serves as the major neural isoform, found throughout all central nervous system aminergic neurons. Although VMAT1 generally exhibits a somewhat higher affinity for most substrates, particularly histamine, it is notably poorly inhibited by TBZ (Peter et al., 1994).

This expanding family of vesicular neurotransmitter transporters soon welcomed new members. The gene coding for the vesicular acetylcholine transporter (VAChT), responsible for transporting acetylcholine into synaptic vesicles at cholinergic synapses, was identified shortly thereafter (Erickson et al., 1994; Peter et al., 1994). A more recent addition to this family, now collectively known as the SLC18 family, is the vesicular polyamine transporter, which transports polyamines like spermine and spermidine and is broadly expressed in the brain and peripheral tissues (Hiasa et al., 2014).

I spent a short sabbatical with Ron Kaback in 1991, during which I was fortunate to assist Robert's team with some essential functional characterizations of the protein (Liu et al., 1992a;

Schuldiner et al., 1993). Armed with the newly identified gene, I returned to my lab in Jerusalem. We finally had the tools to move from classical biochemistry to molecular genetics. Using the VMAT2 plasmid, we launched a series of site-directed mutagenesis studies to map out the functional domains of the protein. By combining these molecular tools with our established purification protocols, we were able to reach a much deeper level of mechanistic insight.

Powerful tools and beautiful structures

While these studies have provided important insights into the mechanism of proton-monoamine antiport by VMATs, many questions remained at this point, particularly relating to the binding sites of the various substrates and inhibitors, as well as the conformational changes that connect lumen- and cytoplasmic-facing states. Addressing these questions required structural models or structural data for conformations, such as the occluded and ligand-bound states, complemented by functional studies to uncover the conformational dynamics occurring during neurotransmitter storage into vesicles.

We were fortunate to begin collaborating with Dr. Lucy Forrest at this stage. This partnership enabled us to explore our questions using homology models, which we supplemented with biochemical methods to reveal the conformational dynamics involved in the storage of neurotransmitters in vesicles. Homology modeling proved instrumental in advancing our understanding of VMAT2's structure, providing a crucial framework that guided many of our biochemical investigations. An initial model was constructed using LacY as a structural template (Vardy et al., 2004). Approximately a decade later, updated and more sophisticated models emerged (Yaffe et al., 2013; Yaffe et al., 2014). These second-generation models, developed through aligning VMAT2 with a broader array of homologous bacterial MFS transporter structures, offered a significantly more accurate representation of the critical pore-lining helices and the substrate-binding cavity. While our working homology model cannot supersede the elegant cryo-EM structures recently published, it served for over a decade as an invaluable framework for our experimental planning and mechanistic understanding (Fig. 2) (Yaffe et al., 2013; Yaffe et al., 2018). Like most MFS transporters, VMAT consists of 12 putative TM segments organized into two lobes of six-helix bundles. A feature distinguishing VMAT proteins from other drug transporters and multidrug transporters of the MFS is a long loop between TM segments TM1 and TM2, which contains glycosylation sites (not shown in the figure). The distinct sequence similarity between VMATs and bacterial multidrug transporters that allowed us generating the homology models is also reflected in their functional promiscuity. Unlike the large family of plasma membrane transporters, which are fine-tuned for high selectivity, VMATs are capable of sequestering a variety of monoamines. Ultimately, what is stored within the vesicle depends on the specific neurotransmitters produced and supplied by the host cell.

Gates and conformational dynamics

The alternating access model requires the movement of elements, often referred to as "gates," on both the cytoplasmic and lumenal sides. These gates on each face of the membrane, separate the substrate-binding site from the cytoplasm and lumen, that open and close to facilitate substrate movement across the membrane. MFS transporters typically form these gates by residues from the N- and C-terminal domains. Considering the organization of the two bundles in MFS transporters, such gates should feature residues in the N and C domains that meet in some, but not all, of the conformations in the transport cycle.

In VMAT2, a unique pharmacological arsenal offered a rare insight into the role of the proton gradient and linked proton binding to conformational changes. As mentioned above, reserpine provides information on the substrate-binding site and its accessibility, as its binding is dramatically accelerated by the imposition of a proton gradient (lumen acidic), suggesting that the proton gradient facilitates a conformational change that unveils the substrate-binding site. On the other hand, the binding of TBZ, a noncompetitive inhibitor of VMAT2, is independent of the proton gradient.

Our first insight into the cytoplasmic gate emerged unexpectedly during extensive mutagenesis studies focused on TM helix 11 (TM11), where we systematically replaced several residues with cysteine (Yaffe et al., 2016). Specifically, characterization of the Y423C replacement (in rat VMAT2) revealed unforeseen and complex effects on the binding of both TBZ and reserpine.

To understand these effects, it is crucial to recall the distinct binding properties of these inhibitors. In wild-type rVMAT2, reserpine, the most potent transport inhibitor, inhibits TBZ binding only at very high concentrations (Scherman and Henry 1984; Yaffe et al., 2016). This apparent paradox stems from differing assay conditions: Reserpine's inhibitory effect on transport is typically observed when a proton gradient is present (conditions that enable its binding and transport activity), whereas TBZ-binding assays often lack this gradient, thus preventing reserpine binding. Conversely, TBZ, capable of binding even without a pH gradient, effectively blocks the binding of reserpine or transportable substrates to the wild-type protein.

Intriguingly, the Y423C mutation significantly increased the sensitivity of TBZ binding to reserpine inhibition. The mutations at position 423 likely induce a conformational change within the protein, which alters the accessibility of the reserpine-binding site. This alteration, in turn, allows reserpine to bind more readily, even at low concentrations. Furthermore, this specific mutation appears to disrupt TBZ's normal ability to prevent reserpine binding, either by altering the conformation of the binding site itself or by directly interfering with TBZ's binding mechanism (Yaffe et al., 2016).

The homology models developed in a fruitful and enjoyable collaboration with Dr. Lucy Forrest provided a powerful tool to identify additional residues involved in what we determined to be part of the cytoplasmic gate. The model of the lumen-facing conformation predicts a set of interactions located at the cytoplasmic face of the transporter, connecting TM helices 5 (R218, M222) and 11 (Y419, Y423) (ratVMAT2 numbering) (Fig. 2 C).

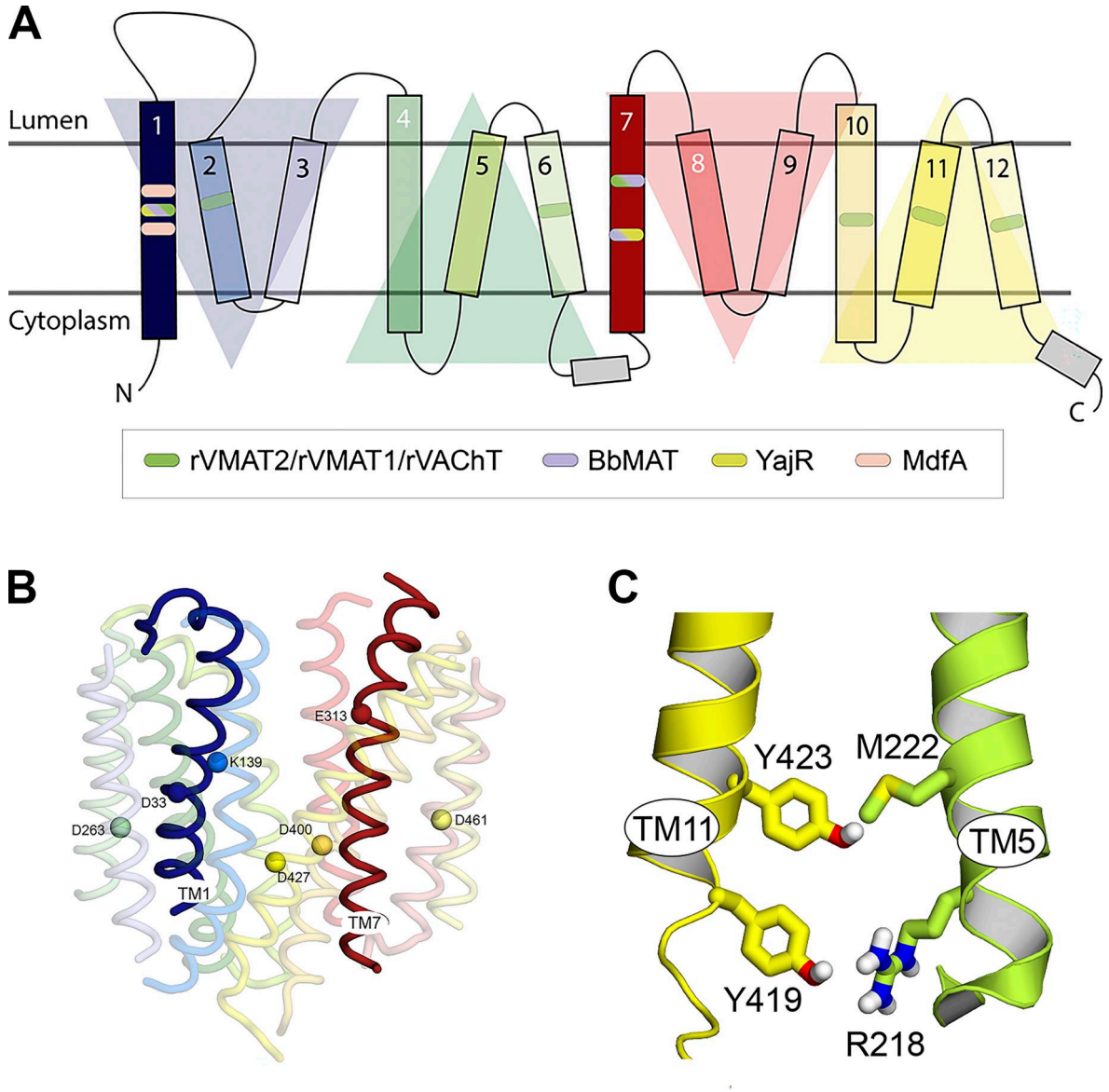

Figure 2. **The evolution of VMAT2 structural models.** Homology modeling proved instrumental in advancing our understanding of VMAT2's structure, providing a crucial framework that guided many of our biochemical investigations. An initial model was constructed using LacY as a structural template. Approximately a decade later, an updated and more sophisticated model emerged (Yaffe et al., 2013). This second-generation model, developed through aligning VMAT2 with a broader array of homologous MFS transporter structures, offered a significantly more accurate representation of the critical pore-lining helices and the substrate-binding cavity. **(A)** TM topology of antiporters from the DHA1 family. The structure of the bacterial multidrug transporter YajR and the homology model of BbMAT were aligned to locate the conserved membrane embedded charged residues, which are indicated as ellipses, based on the color-coding shown below. Transparent triangles indicate the location of three-helix structural repeats in the N-terminal (blue and green) and C-terminal halves (pink and yellow) of the MFS fold. **(B)** Model of rVMAT2 in the lumen-facing conformation indicating the location of membrane embedded charged residues in rVMAT2, and colored by TM helix, with the N-terminal half in shades of blue and green and the C-terminal half in shades of red and yellow (rVMAT2 numbering). **(C)** Gating residues. Magnification of the cytoplasmic domain of TM5 and TM11. Residues contributing to the cytoplasmic gate are shown as sticks (rVMAT2 numbering). Adapted from Yaffe et al. (2018).

Mutating any of these four residues generated proteins that bind reserpine at fast rates, independent of the presence of a proton gradient, suggesting that in these mutants, the reserpine-binding site is "constitutively" accessible. Further characterization revealed that while mutants in positions 222 or 423 can still bind TBZ and transport serotonin, mutating residues Y419 and R218 abolish both transport and TBZ binding but not reserpine binding. We, therefore, concluded that the interactions between TM5 and TM11 contribute to the cytoplasmic gate needed for proper equilibrium between the inward- and outward-facing conformations. Specifically, the interactions at the cytoplasmic gate stabilize the lumen-facing conformation, which appears to be the resting state conformation of the transporter. Weakening these interactions by mutagenesis shifts the conformational equilibrium and increases the fraction of VMAT2 in the cytoplasmic-facing conformation.

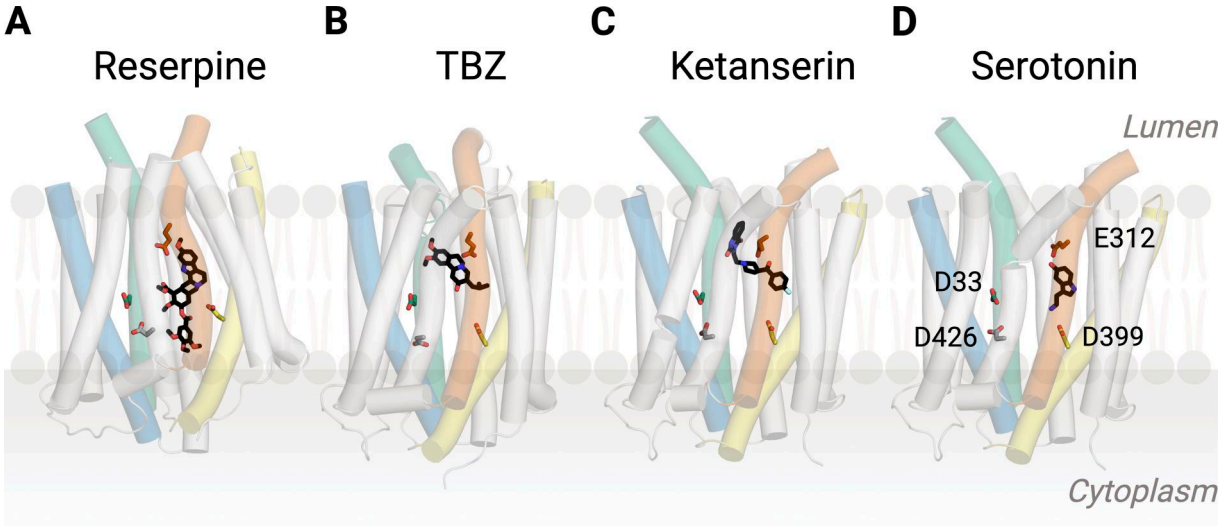

Figure 3. **Structures of human VMAT2 reveal different inhibitor-binding profiles.** Since October 2023 until the time this review was written, 25 cryo-EM structures of human VMAT2 have been deposited in the PDB (Table S1). **(A–D)** Structures have been determined in the presence of several inhibitors, three of them shown here: (A) reserpine, (B) TBZ, (C) ketanserin, and various substrates (D, a structure with serotonin as an example), shown as sticks. Key acidic residues are also shown as sticks (hVMAT2 numbering). The structures reflect distinct conformational states: open to the cytoplasm (A), completely occluded (B), and open to the lumenal side (C and D). Pathway opening involves relative rocker switch movements of the N- and C-terminal halves of the transporter. The pathways on either side of the transporter involve membrane-spanning helices from pseudosymmetric repeated elements, highlighted by the positions of the first helix of each repeat (TM 1 in green, TM4 in blue, TM7 in orange, and TM10 yellow). Figures created with PyMol for PDB identifiers 8T6A, 8T69, 8JT9, and 8JSW, respectively, and compiled with biorender.com (Adapted from Schuldiner and Forrest [2024]).

The results supported that reserpine and the substrate bind to the cytoplasmic-facing conformation, while TBZ binds to the lumen-facing conformation noncompetitively. The findings indicate that the two inhibitors demonstrate conformational selectivity, which, to my knowledge, has been reported for only three other transporters: the mitochondrial ATP–ADP exchanger (Klingenberg, 2008), the human erythrocyte glucose transporter (Deng et al., 2015), and the serotonin transporter (Jacobs et al., 2007).

Beyond molecular models

The past 20 mo have witnessed a remarkable flood of structural data, providing beautiful high-resolution snapshots of VMATs in various conformations and powerfully demonstrating the synergy between structural and functional studies. As noted earlier, 25 cryo-EM structures of human VMAT2 and 8 of human VMAT1, encompassing diverse liganded and unliganded states, have been deposited in the PDB bank since October 2023 (Table S1). As observed in all MFS structures to date, the VMAT2 structures comprise two lobes of six membrane-spanning helices. These core regions are relatively small and lack features required for cryo-EM image processing. Most groups tackled this challenge by adopting a fusion protein strategy that effectively enlarges the particle size. One group study capitalized on the serendipitous finding that reserpine, at very high, submillimolar concentrations added prior to solubilization, induces VMAT1 dimerization.

The structures reflect distinct conformational states: open to the cytoplasm (Fig. 3 A), completely occluded (Fig. 3 B), and open to the lumenal side (Fig. 3, C and D). Pathway opening involves relative "rocker switch" movements of the N- and C-terminal halves of the transporter. The pathways on either side of the transporter are formed by membrane-spanning helices from four pseudosymmetric repeated elements, highlighted by the positions of the first helix of each repeat (TM 1 in green, TM4 in blue, TM7 in orange, and TM10 in yellow).

Most VMAT1 and VMAT2 structures with serotonin or other substrates bound, including the inhibitor ketanserin and the neurotoxin MPP^+, adopt a lumenal-open conformation. These structures are very similar to the lumenal facing apo structure and most likely represent a state prior to substrate release. The binding pocket itself is located approximately halfway across the membrane, forming a central, amphipathic "side-pocket" on the C-terminal domain side, intricately surrounded by TM helices TM5, TM7, TM8, TM10, and TM11. Within this environment, the substrate's amine group typically interacts with acidic residues.

The structures confirm the critical roles and locations of four previously identified acidic residues: D33, E313, D400, and D427 (Fig. 2—rVMAT2 numbering). While E313 and D400 directly interact with the amine group of the substrates, D33 and D427 are notably involved in a broad hydrogen bond network spanning both the N- and C-terminal lobes, suggesting their possible role as key proton carriers. Though the precise mechanism by which the binding of two protons facilitates the necessary conformational change remains to be fully elucidated, the high conservation of D33 across MFS transporters underscores its fundamental importance. Moreover, the role of D33 protonation in shifting the conformational equilibrium is clearly demonstrated in a D33N mutant by both reserpine binding kinetics and differential accessibility to MTS reagents (Yaffe et al., 2016). The intricate and fascinating details of VMAT's interactions with

various substrates and inhibitors, beyond this general overview, are discussed in depth in the original publications. These studies provide valuable insights into the concept of multiple drug recognition and are crucial for the future development of new, clinically relevant inhibitors.

The reserpine- and TBZ-bound structures confirm predictions from functional studies: Reserpine and TBZ stabilize VMAT2 in distinct conformations. Specifically, the TBZ-bound state reveals VMAT2 in an occluded lumen-facing conformation, while the reserpine-bound state captures it in a cytoplasmic-facing conformation (Fig. 3, B and A, respectively).

Capturing the reserpine-bound state proved particularly challenging, as the absence of a proton gradient disfavors reserpine interaction, necessitating innovative approaches. Several labs successfully employed different strategies: (1) Mutagenesis-enabled binding: Pidathala et al. (2023) and Wang et al. (2024) introduced specific VMAT2 substitutions (Y418S and Y422C, respectively; corresponding to Y419S and Y423 in bovine VMAT2) described above, known to enable reserpine binding even without a proton gradient (Pidathala et al., 2023; Wang et al., 2024). (2) Irreversible binding exploitation: Wu et al. (2024a) leveraged reserpine's practically irreversible binding by adding the inhibitor to cells prior to solubilization and purification, effectively "trapping" the bound state (Wu et al., 2024a). (3) Fortuitous dimerization: The reserpine induced nonphysiological dimer of VMAT1 allowed the simultaneous capture of both the ligand-free and reserpine-bound conformations, as the reserpine-occupied protomer adopted a cytoplasmic-facing state while its partner remained lumen-facing (Ye et al., 2024).

In the reserpine-bound structure, the N and C domains create a pathway with a large vestibule toward the cytoplasm (Fig. 3 A). This pathway connects the central cavity with the cytoplasm, rendering the cavity fully accessible to the cytoplasmic solvent and allowing ligands to reach the center of the transporter, about halfway across the membrane. Reserpine occupies the pathway in a way that suggests that it would compete with substrate binding competitively. Notably, the reserpine and TBZ sites overlap only minimally, providing two entirely different frameworks for drug development.

All studies of VMAT2 reported structures in the presence of TBZ, whose binding site is capped on the lumenal side in large part by the lumenal end of TM helices 1 and 7, which adopts a distinct conformation (Fig. 3 C, orange). As expected from the ability of TBZ to inhibit reserpine binding, the transporter is also fully occluded on the cytosolic side when bound to TBZ. The studies agree on the location of the TBZ ligand, and they demonstrate the importance of identified interactions using mutagenesis. Identifying the structural basis for VMAT1 and VMAT2-specific differences remains a challenge. Despite the determination of the hVMAT1 structure, a clear explanation for its distinct specificity has not yet emerged (Ye et al., 2024). It is suggested that the structural flexibility of the TM regions, along with variations in the substrate-binding pocket likely drive the differing TBZ sensitivities observed between the isoforms. Additional studies should provide a foundation for development of drugs with similar noncompetitive profile to TBZ.

Interestingly, the location of ketanserin and serotonin within the binding pocket are remarkably similar. Given that ketanserin is known to be a competitive inhibitor of TBZ binding, these observations present two distinct possibilities for interpreting the structural differences between their respective ligand-bound states and the TBZ-bound state: Induced occlusion: Upon binding to the lumenal conformation (a site shared with ketanserin and other substrates), TBZ may induce a distinct conformational change that leads to its partial occlusion within the transporter, effectively trapping it. Differential release: Alternatively, the "occluded" binding site observed for TBZ might be accessible, at least partially, from the lumenal side, allowing TBZ to bind and become kinetically trapped there. In contrast, substrates and other inhibitors like ketanserin proceed to a pre-release conformation before dissociating.

Mechanistic model and future outlook

In summary, the journey of VMAT research has been marked by significant advancements, from its earliest biochemical characterization to the recent transformative breakthroughs in structural biology. These discoveries have collectively paved the way for a deeper understanding of VMAT's pivotal role in neurotransmission and have opened new avenues for the development of innovative therapeutic approaches. Our scientific odyssey began almost 50 years ago, and we are now delighted to witness a surge of renewed interest in VMAT, vividly demonstrated by the abundance of published cryo-EM structures of VMAT1, VMAT2, and their highly homologous counterpart, VAChT (Ma et al., 2025). This period represents an enlightening encounter of structure and function, where high-resolution structural data provide an invaluable molecular glimpse into the inner workings of these molecules, while decades of functional studies offer the essential background to mechanistically link observed structure with biological function.

Building on this powerful convergence of structural and functional insights, we can now articulate a comprehensive mechanistic model for the VMAT transport cycle (Fig. 4) (Yaffe et al., 2016). VMAT operates as an antiporter, harnessing the electrochemical energy from the downhill movement of two protons to drive the uphill translocation of a single substrate molecule. This complex process involves the transporter cycling through at least three distinct conformations: cytoplasm-facing, occluded, and lumen-facing. At its core, the model posits that the transporter's resting state, in the absence of a proton electrochemical potential, is the lumen-facing apo conformation (see Table S1 for PDB IDs of various conformations in the scheme). The generation of the transporter's high-affinity conformation for its substrate, a higher-energy state, is critically dependent on H^+ translocation. The energy released from this proton flux is directly utilized by the transporter, contributing to the binding energy for specific ligands such as reserpine.

Our extensive early research, coupled with the exquisite sensitivity of the reserpine binding assay, provided critical tools for dissecting the role of the first proton. We found that the transition to the cytoplasmic-facing conformation is energetically unfavorable, as evidenced by the time required for

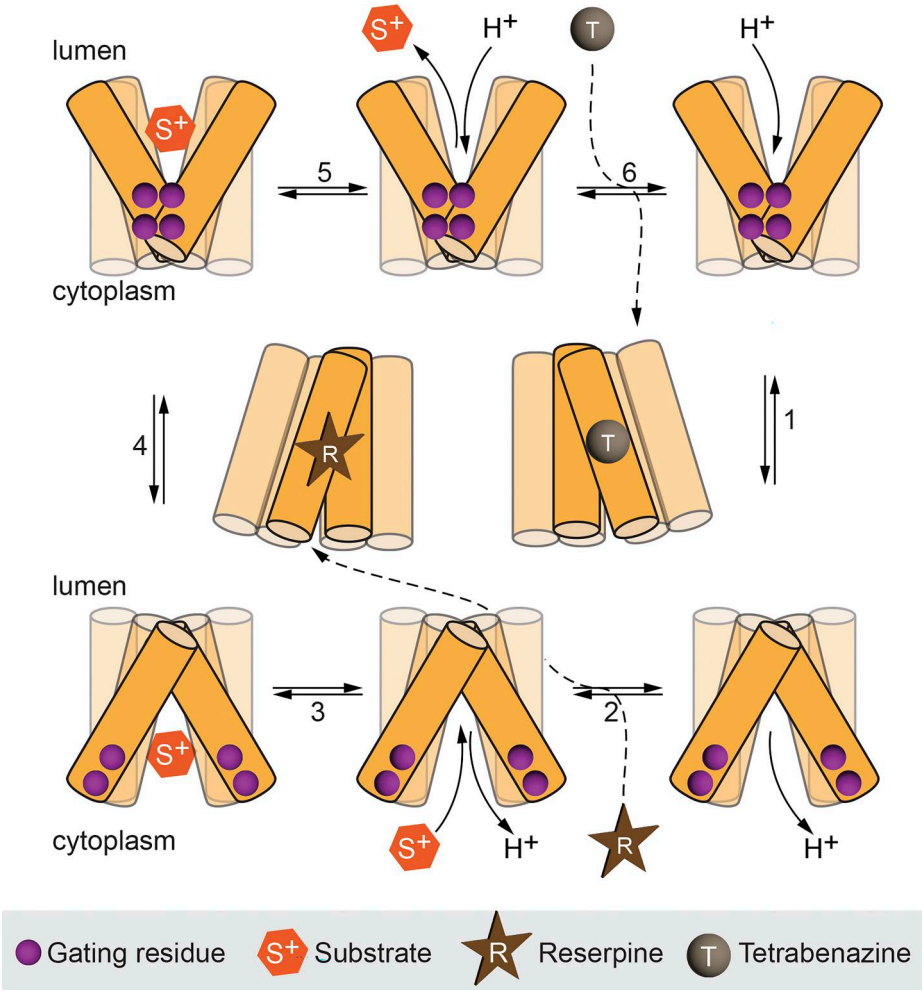

Figure 4. **The evolution of a mechanistic model: from 1990 to 2025.** For simplicity, only six TMs are shown. The cycle is assumed to involve six steps (numbered). In the absence of a proton gradient, the dominant population is of the lumen-facing conformation, as indicated by the transporter's ability to bind TBZ but not reserpine. Upon acidification of the lumen, binding of protons enables the conformational switch to the cytoplasm-facing conformation (step 1), whereas binding of substrate enables the change to the lumen-facing conformation (step 4). Binding of TBZ locks the transporter in a conformation that appears incompatible with substrate binding and is therefore presumably not cytoplasm facing (shown as an off-cycle state connected to step 6). Binding of reserpine also locks the transporter in a dead-end conformation, but reserpine binding competes with substrate binding, and therefore the reserpine-bound conformation is presumably cytoplasm facing (shown as an off-cycle state connected to step 2). We propose that the second proton is needed for release of the substrate in the lumen (step 5). Residues contributing to the cytoplasmic gate are shown as purple circles. Adapted from Yaffe et al. (2016).

reserpine binding. Acidification of the lumen, however, triggers a conformational change via protonation of a key residue, an event crucial to the process (Fig. 4, step 1). Based on bioenergetic considerations and supported by the work described above, we concluded that one proton is sufficient for this initial transition, which opens the cytoplasmic gate and facilitates access to the substrate-binding site—a key first step in the transport process (Rudnick et al., 1990).

The new structures now vividly confirm the locations for four acidic residues previously identified as critical for proton binding: D33, E312, D399, and D426 (hVMAT2 numbering) (Fig. 3). These residues are hypothesized to act as proton sensors or components of a proton relay, facilitating rapid, efficient proton transfer, which is essential for coupling proton and substrate transport. Specifically, D33, a residue highly conserved in proton-coupled MFS transporters, and E312 appear to be irreplaceable, suggesting their direct and critical involvement in substrate binding and proton translocation (Yaffe et al., 2013). In contrast, D399 and D426 form part of a more flexible network that interacts directly with some substrates, a feature elegantly visualized in the recently published structures. While single mutations of these latter residues can eliminate transport, secondary mutations have been shown to restore activity, indicating their role in a more complex network required for substrate binding and either proton passage or conformational changes (Yaffe et al., 2013). The opening of the cytoplasmic gate is a necessary step for the generation of the substrate- and reserpine-binding site. Importantly, the only substrate-bound cytoplasmic-facing structure was obtained with a protein bearing mutations in the gate, a strategy identical to that used to obtain reserpine-bound structures (Table S1, PDB ID 8XO9) (Wu et al., 2024b).

Cryo-EM structures have since elegantly illuminated and confirmed how inhibitors exploit these conformational states. TBZ locks the transporter in an occluded or lumen-facing conformation, rendering it unable to bind substrate. Its binding is independent of the proton gradient, suggesting it can bind either before or after the protonation event needed to generate the cytoplasmic-facing conformation (Fig. 4, step 6). Conversely, reserpine traps the transporter in a dead-end state by stabilizing the cytoplasmic-facing conformation (Fig. 4), where it directly competes with substrate binding (Fig. 4, step 2).

Despite these advancements, a key piece of the VMAT puzzle remains elusive: the precise contribution of the second proton. While the first proton's role in initiating the conformational switch is relatively well-defined, the precise path of the second proton and its detailed contribution to the conformational transitions that drive substrate translocation against a substantial electrochemical gradient require further elucidation. For instance, after substrate binds and its amino group interacts directly with E312, the conformational change returns the transporter to the acidic lumen-facing state (Fig. 4, step 4); the second proton's role may be needed for the subsequent release of the substrate into the lumen, where its concentration is very high (Fig. 4, step 5). We still lack detailed information on the subsequent release of both protons to the cytoplasmic side: does this occur before, concomitantly with, or after the binding of either the neurotransmitter or reserpine (Fig. 4)? Precisely how the interplay between the four identified carboxylates, the substrate, and the binding of the second proton orchestrates the complete transport cycle is a complex and compelling area for continued investigation.

Our current hypothesis regarding the two protons is schematically presented in Fig. 4. In this proposed model, one proton (termed "the first") binds to the already protonated, acidic lumen-facing VMAT, initiating the isomerization (step 1) and rendering VMAT doubly protonated. Following this, one of the two protons is released upon exposure of the cavity to the higher pH in the cytosol before reserpine binding. This proton release is critical and accounts for the conclusion that only one proton is directly involved in facilitating reserpine binding. The "second" proton, in this scenario, may be released upon substrate binding that interacts directly with E312, enabling isomerization back to the lumen-facing conformation. Subsequently, a proton then binds to the charged transporter, facilitating substrate release. Addressing these outstanding questions will undoubtedly lead to further breakthroughs, refining our mechanistic understanding and potentially unlocking new strategies for pharmacological intervention in monoamine-related disorders.

In summary, the journey of VMAT research has been marked by significant advancements, from early biochemical characterization to recent breakthroughs in structural biology. These discoveries have paved the way for a deeper understanding of VMAT's role in neurotransmission and the development of innovative therapeutic approaches. Our journey began almost 50 years ago, and we are delighted to see the surge of interest in VMAT, as demonstrated by the abundance of published cryo-EM structures of VMAT1, VMAT2, and the highly homologous VAChT. We are now witnessing the enlightening encounter of structure and function. While structure provides us with an invaluable molecular glimpse into the inner workings of the molecule, functional studies provide the necessary background to link the structure with its function.

Online supplemental material

Table S1 shows Protein Data Bank accession IDs for VMAT structures available at the time of manuscript submission.

Data availability

This manuscript presents no original data. It is based entirely on previously published research, with all sources cited accordingly. Given the high volume of VMAT structures currently deposited in the Protein Data Bank, we have itemized the relevant PDB IDs in the supplementary table.

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Supplemental material

Provided online is Table S1. Table S1 shows Protein Data Bank accession IDs for VMAT structures available at the time of manuscript submission.