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Protective Actions of the Vesicular Monoamine Transporter 2 (VMAT2) in Monoaminergic Neurons

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Abstract Vesicular monoamine transporters (VMATs) are responsible for the packaging of neurotransmitters such as dopamine, serotonin, norepinephrine, and epinephrine into synaptic vesicles. These proteins evolved from precursors in the major facilitator superfamily of transporters and are among the members of the toxin extruding antiporter family. While the primary function of VMATs is to sequester neurotransmitters within vesicles, they can also translocate toxicants away from cytosolic sites of action. In the case of dopamine, this dual role of VMAT2 is combined—dopamine is more readily oxidized in the cytosol where it can cause oxidative stress so packaging into vesicles serves two purposes: neurotransmission and neuroprotection. Furthermore, the deleterious effects of exogenous toxicants on dopamine neurons, such as MPTP, can be attenuated by VMAT2 activity. The active metabolite of MPTP can be kept within vesicles and prevented from disrupting mitochondrial function thereby sparing the dopamine neuron. The highly addictive drug methamphetamine is also neurotoxic to dopamine neurons by using dopamine itself to destroy the axon terminals. Methamphetamine interferes with vesicular sequestration and increases the production of dopamine, escalating the amount in the cytosol and leading to oxidative damage of terminal components. Vesicular transport seems to resist this process by sequestering much of the excess dopamine, which is illustrated by the enhanced methamphetamine neurotoxicity in VMAT2-deficient mice. It is increasingly evident that VMAT2 provides neuroprotection from both endogenous and exogenous toxicants and that while VMAT2 has been adapted by eukaryotes for synaptic transmission, it is derived from phylogenetically ancient proteins that originally evolved for the purpose of cellular protection.

Keywords Neuroprotection · Neurodegeneration · Dopamine · Vesicle · Vesicular monoamine transporter · MPTP · Methamphetamine

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Chemical Neurotransmission and Vesicular Transporters

Chemical neurotransmission is dependent on the release of transmitter molecules from the presynaptic neuron into the synaptic cleft. Neurotransmitters can be divided into two broad classes: peptides and small molecules. The small molecule neurotransmitters are that synthesized by cytosolic enzymes must be properly loaded by a transporter into the exocytotic vesicle before an action potential signals imminent release [1, 2]. There are several families of vesicular transporters that package a diverse array of neurotransmitters such as glutamate, gamma aminobutyric acid (GABA), glycine, acetylcholine, histamine, serotonin, epinephrine, norepinephrine, and dopamine [3–6]. The transporters have specific structural and biophysical characteristics that determine affinity for a particular chemical as well as the energy source for translocation.

Monoamines such as serotonin, epinephrine, norepinephrine, and dopamine are packaged by two different 12 transmembane domain proteins known as vesicular monoamine transporters (VMATs), which are part of the larger solute carrier (SLC) protein family [7]. VMAT1 (SLC18A1) is located predominately in the periphery and serves to sequester not only epinephrine and norepinephrine but also serotonin in the melatonin synthesizing cells of the pineal gland [8, 9]. VMAT2 (SLC18A2) is present in the periphery in specialized places such as the platelets, Beta cells of the Islets of Langerhans, and the histaminergic enterochromaffin-like cells of the gastric mucosa [10–14]. However, VMAT2 is largely confined to the central nervous system where it packages dopamine, serotonin, norepinephrine, epinephrine, and histamine, where it critical for neuronal health [15–20].

Regulation of Monoamine Loading

Vesicular transporters require energy to sequester neurotransmitters and, despite the differences in chemical structure among transmitters themselves, their transporters either use an exchange-diffusion of protons or the electrical gradient to package them [21]. In the case of the VMAT1 and VMAT2, two protons are exchanged for one molecule of monoamine transmitter [22, 23] (see Fig. 1).

Protons are present in the vesicles due to the action of a phylogenetically ancient protein known as the V-type ATPase [24, 25]. The action of this protein is thought to underlie the acidification of all metazoan endocytic compartments, including vesicles, lysosomes, and the Golgi bodies [26, 27]. It has a high degree of homology to the bacterial F_0F_1 ATPase, which translocates protons to produce the mechanical energy to rotate a flagellum as well as bacteriorhodopsin, which uses light energy to translocate protons [28, 29]. Antibiotic compounds, such as the bafilomycins, can inhibit proton translocation by V-type ATPase proteins [30].

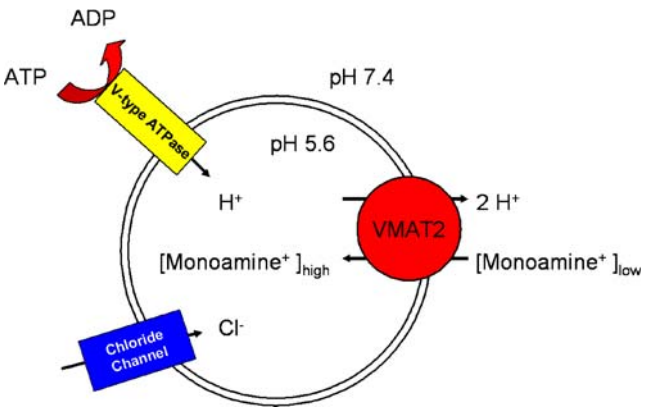

Fig. 1 Monoamine loading by vesicular transporters. The V-type ATPase uses the energy of *ATP* hydrolysis to translocate protons into the vesicular lumen. This action provides the potential energy for monoamine storage. Chloride ions brought in by channel proteins depolarize the vesicular membrane to prevent a repulsive force on positively charged monoamines. Vesicular monoamine transporters are antiporters that use the pH gradient to allow two protons to exit the vesicle while simultaneously translocating a single monoamine molecule into the vesicle. Single vesicle capacity is hypothesized to be up 20,000 molecules and have a transmitter concentration several orders of magnitude above cytosolic levels [4]

The V-type ATPase requires the hydrolysis of ATP to translocate protons into an endocytic compartment. In the case of VMATs, it has been shown that several glycolytic enzymes are present on the vesicles themselves to provide this energy. These enzymes, such as glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase, may use glycolytic products to locally generate ATP for the V-type ATPase [31]. The resulting proton gradient across the vesicular membrane is estimated to be 2 to 2.4 pH units below the more neutral pH 7.4 of the cytosol and can be dissipated experimentally using proton ionophores such as carbonylcyanide para-trifluoromethoxyphenylhydrazone, carbonylcyanide meta-chlorophenylhydrazone, antibiotic nigericin (from *Streptomyces hygroscopicus*), as well as high concentrations of bases such as ammonium chloride [32, 33].

Chloride channels also determine the loading of monoamines in vesicles by coordinating chloride flux with acidification [34]. This has an obvious function of maintaining charge balance across the membrane, but there is evidence that chloride itself modulates the function of the V-type ATPase [35, 36]. This has implications for the transmitter phenotype of the vesicle, as monoamine vesicles need to maintain a high ΔpH for exchange-diffusion and a neutral or negative membrane potential to prevent repulsion of the positively charged monoamines. In contrast, glutamate vesicles have a positive membrane potential to aid in the translocation of the negatively charged transmitter. Genetic deletion of chloride channels known to be present on synaptic vesicles and endosomes in the brain, such as ClC-3 and ClC-7, lead to impaired acidification and neurodegeneration [37, 38].

Phylogenetic Origins of VMATs

The proton gradient created by V-type ATPases allows the function of the toxin extruding exporter (TEXAN) family in both prokaryotes and eukaryotes [39]. The mammalian VMATs contain sequence homology and functional characterisitics to the major facilitator superfamily (MFS) of drug resistance transporters, such as the bacterial antiporter TetA which exchanges tetracycline for protons [40]. These MFS proteins remove perceived toxins from the cytosol and

into the culture medium in prokaryotes and into endocytic compartments in mammals.

The cnidarians, such as anemonae and hydrae, are the forerunners to all metazoan life on earth and possess a simple neural net that communicates using chemical neurotransmission. Intriguingly, there is evidence that monoamines such as serotonin, octopamine, norepinephrine, and dopamine are used by these animals; therefore, they probably possess an early form of a VMAT [41]. Additionally, *Caenorhabditis elegans*, a nematode, expresses a VMAT-like protein called CAT-1 that regulates serotonin and dopamine storage [42]. This system of monoamine neurotransmission is thought to have first evolved roughly 600 Ma ago and is dependent on transport protein motifs which even more ancient.

Although vesicular monoamine transporters are antiporters, they have functional homology to lactose permease (LacY), a cotransporter found in *Escherichia coli*, which translocates one sugar and one proton into the periplasm together [43]. Additionally, there exists functional homology to the *E. coli* glycercol 3-phosphate (G3P) antiporter, which exchanges G3P for inorganic phosphate [44]. Furthermore, sequence and structural homology has been detected between VMAT2 and HSmdr, a multidrug resistance protein from *Halobacterium salinarium*, which is a halophilic achaeon found in the Dead Sea [45]. Thus, it is clear that these types of proteins are basic to survival not only in eukaryotes and bacteria but also among archaea, the first domain of life to appear on earth.

Role of VMAT2 in Dopamine Neurotransmission

In mammals, dopamine is a critical mediator of behavior and behavioral states such as voluntary movement, arousal, motivation, reward, and reinforcement of natural rewards such as food, water, and reproductive activities. Dopamine neurotransmission is also involved in the pathology of mental disorders such as drug addiction and schizophrenia and neurodegenerative disorders like Parkinson's disease. These ascribed functions are generally attributed to four main pathways which utilize dopamine in the brain.

The mesolimbic pathway, which projects from the ventral tegmental area (VTA) in the midbrain to an anterior structure called the nucleus accumbens, is thought to mediate natural and artificial reward and reinforcement [46, 47]. Another pathway that originates in the VTA is known as the mesocortical, which sends projections to the frontal cortices and modulates cognitive processes such as learning as well as those that go awry in schizophrenia [48, 49]. Also originating in the midbrain, the substantia nigra pars compacta (SNpc) has terminal fields in the striatum which make up the nigrostriatal pathway. In Parkinson's disease, the cell bodies of the SNpc die, depriving the basal ganglia of dopamine input and leading to classic signs of this bradykinetic movement disorder [50, 51]. Lastly, release of prolactin from the pituitary gland is controlled by the hypothalamic dopamine cells of the tuberoinfudibular pathway, which, among other functions, can provide negative feedback on gonadal hormone synthesis [52, 53]. All these phenomena are mediated by dopamine release from presynaptic neurons, which must be properly loaded into vesicles before an action potential signals release.

Vesicular monoamine transporters not only package dopamine into synaptic vesicles but they are also connected to a cycle of production, packaging, release, and degradation of transmitter (see Fig. 2). In mammalian dopamine neurons, tyrosine is transported into the cell via a large neutral amino acid (LNAA) L-system transporter where it is oxidized by tyrosine hydroxylase (TH) to yield 3,4-dihydroxyphenylalanine (L-DOPA) [54, 55]. Aromatic amino acid decarboxylase (AADC) removes the carboxylic acid moiety from L-DOPA to yield dopamine [56]. Newly synthesized dopamine is then sequestered into vesicles by VMAT2 [17]. Dopamine that is not immediately packaged gets degraded by monoamine oxidase (MAO) to 3,4-dihydroxyphenylacetaldehyde (DOPAL), and further oxidized by aldehyde dehydrogenase to 3,4-dihydroxyphenylacetate (DOPAC) [57, 58]. Dopamine-loaded vesicles are taken to the plasma membrane by motor proteins and interact with a series of protein complexes that dock and then prime the vesicle for membrane fusion [59].

An action potential signals calcium influx, leading to a brief fusion of the vesicle with the plasma membrane and exocytosis of neurotransmitter [2, 60]. Dopamine floods into the synaptic space, where it binds to receptors, gets degraded into 3-methoxytyramine (3-MT) by catechol-*O*-methyl-transferase (COMT), or can be taken back into the neuron via the dopamine transporter (DAT) [61, 62]. The combined action of MAO and COMT on dopamine yield one of its most commonly measured extracellular metabolites, homovanillic acid (HVA) [63, 64]. The neuron maintains a balance of neurotransmitter production, recycling, and degradation that is reflected in the ratio of a metabolite to neurotransmitter.

During exocytosis, the number of transmitter molecules released when a vesicle fuses with the plasma membrane is known as the quantal size. This value is determined by the concentration of neurotransmitter in the vesicle and, in dopamine neurons, is regulated by VMAT2 activity [65, 66]. For example, it was observed that viral-mediated overexpression of VMAT2 in a catecholaminergic cell line nearly quadrupled quantal size [19]. Furthermore, providing more substrate to VMAT2, such increasing dopamine production by administering the precursor L-DOPA, also leads to a greater quantal size [67]. Variations in quantal

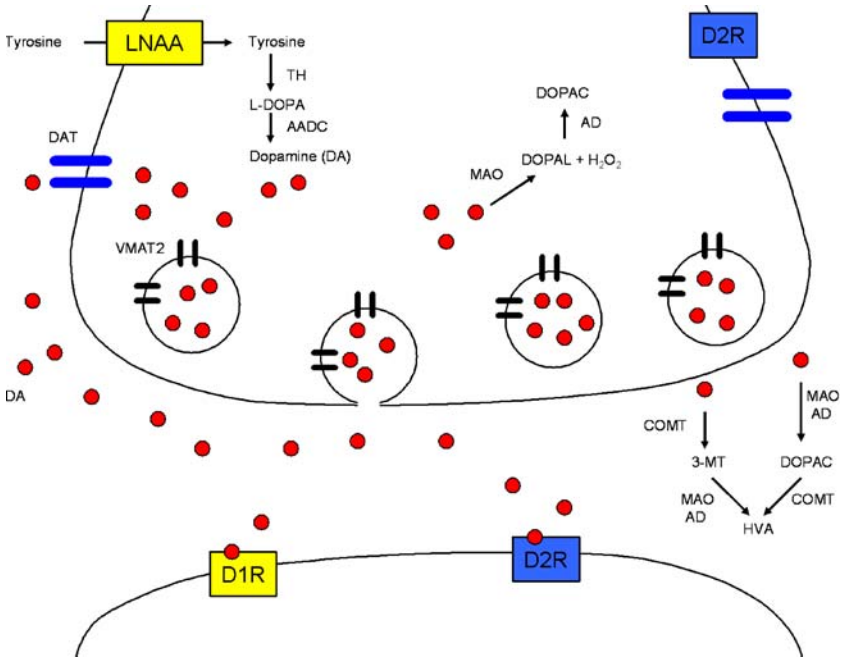

Fig. 2 Cycle of dopamine in the neuron terminal. Tyrosine is transported into the neuron by the *LNAA* L-system transporter and then oxidized by tyrosine hydroxylase (*TH*) to yield 3,4-dihydroxyphenylalanine (*L-DOPA*). *AADC* removes the carboxylic acid moiety from *L-DOPA* to yield dopamine (*DA*). Newly synthesized DA is then sequestered into vesicles by *VMAT2*. *DA* loaded vesicles are taken to the membrane and are made ready for release. An action potential signals leads to fusion of the vesicle with the plasma membrane and exocytosis of neurotransmitter. *DA* rushes into the synaptic space where it binds to receptors on the pre- or postsynaptic neurons, gets recycled into the presynaptic neuron via the dopamine transporter (*DAT*), or is degraded. Intracellular dopamine degradation by monoamine oxidase (*MAO*) yields 3,4-dihydroxyphenylacetaldehyde (*DOPAL*), that is further oxidized by aldehyde dehydrogenase (*AD*) to 3,4-dihydroxyphenylacetate (*DOPAC*). Extracellular dopamine gets degraded by a two step process: Catechol-*O*-methyl-transferase (*COMT*) activity yields 3-methoxytyramine (*3-MT*) from dopamine and *HVA* from *DOPAC*, while extracellular *MAO* converts *DA* to *DOPAC* and *3-MT* in *HVA*

size, due to vesicular transport and the kinetics of vesicle fusion with the plasma membrane (i.e., kiss-and-run or full-collapse), can alter the total amount of transmitter released into the synapse [65, 66, 68, 69]. The synaptic neurotransmitter concentration is important because it directly affects the cumulative responses of the dopamine receptors to the transmitter [70].

Since VMAT2 is responsible for dopamine storage, it is positioned to be a major regulator of dopamine receptor sensitivity. Inhibition of vesicular dopamine transport results in decreased synaptic dopamine, increased postsynaptic sensitivity to agonists, and altered patterns of gene expression in postsynaptic cells [71, 72]. When VMAT2 is genetically reduced by half, normal dopamine receptor physiology is altered such that the locomotor response to amphetamine is elevated, but it can no longer induce a place preference [73]. Additionally, a reduction in vesicle storage affects the presynaptic dopamine receptors as well. When VMAT2 expression in mice is reduced by 90%, dopamine autoreceptors are sensitized, and this imbues quinpirole, a D2/3 agonist, with an approximately threefold increase its normal potency [74]. Furthermore, similar to pharmacological inhibition of VMAT2, these mice have no change in dopamine receptor expression but exaggerated postsynaptic signal transduction responses [75]. From these experiments, it is clear that alterations in synaptic vesicle storage of dopamine and other monoamine transmitters profoundly affect physiology and behavior.

VMAT2 is known to influence the sensitivity of dopamine autoreceptors, but these receptors can also alter vesicular transport by changing the subcellular distribution of VMAT2 [76]. Several dopaminergic agonist drugs, such as pramipexole, a Parkinson's disease therapeutic, as well as the aforementioned quinpirole and apomorphine exhibit this ability [77, 78]. Indirect agonists, such as drugs that block DAT activity and increase extracellular dopamine, can also redistribute VMAT2. These include attention deficit hyperactivity disorder and antidepression therapeutics, like methylphenidate (Ritalin) and bupropion (Wellbutrin), respectively, as well as drugs of abuse such as cocaine [79–81]. Due to the fact that D2 autoreceptor activation can attenuate the rate of transmitter release, it is speculated that this pause could allow vesicle recycling to increase the number of vesicles. These vesicles would have VMAT2 and, thus, contribute to the elevation of vesicular transport. Since this is a short-term consequence of receptor

stimulation, it is unknown if long-term exposure to these drugs can permanently increase vesicular transport.

Pharmacological Inhibition of VMAT Activity

Pharmacology has made the greatest strides in defining the physiology of a system when a specific inhibitor is available, and the best known inhibitor of vesicular monoamine uptake is reserpine. Derived from the Indian snakeroot plant *Rauwolfia serpentina*, reserpine has been used historically to treat diverse conditions such as insanity, snake bites, and fevers [82, 83]. It was first used clinically in the USA to control hypertension by reducing peripheral stores of monoamines and, thus, decreasing heart rate and vasoconstriction [84].

Reserpine is one of the most important drugs in the history of psychopharmacology as its behavioral effects have led to theories of monoamine control of mood and behavior. While depletion of peripheral monoamines accounted for the reduction in hypertension, patients exhibited signs of depression while taking the drug [85, 86]. Since that time, the clinical condition of depression has been inextricably linked to monoamine levels in the brain, and most modern pharmacological treatments for depression aim to modulate the transmission of monoamines, especially serotonin and norepinephrine [87, 88]. By serendipity, the discovery of reserpine, along with iproniazid (a monoamine oxidase inhibitor antidepressant orginially tested against tuberculosis) and chlorpromazine (an antiemetic that was found to reduce symptoms of psychosis), enabled the psychopharmacological revolution in the treatment of psychiatric disorders.

Reserpine inhibits both VMAT1 in the periphery and VMAT2 in peripheral tissues and the brain [89]. It binds very tightly at low concentrations near the monoamine recognition site and prevents substrate transport, presumably by occlusion [90]. The association of reserpine with VMAT2 is slightly more potent when a vesicle is acidified, and this binding is considered irreversible due to slow dissociation [91]. This irreversible inhibition means new transporter must be made and excess reserpine metabolized before monoamine storage can normalize [92].

Tetrabenazine, a specific inhibitor of VMAT2 function, binds at a different site on the protein from reserpine, and this binding is not affected by the proton gradient [90, 93]. Like reserpine, it has a very high affinity for VMAT2, but it dissociates more easily and, thus, is not considered an irreversible inhibitor [94]. It is used clinically to treat disorders such as Tourette's, hemiballismus, and motor dysfunctions associated with Huntington's disease, in addition to its use as a VMAT2 positron emission tomography ligand [95–98]. A related compound that binds at the tetrabenazine site on VMAT2 is ketanserin [99, 100]. While ketanserin inhibits VMAT2, it is more often identified as a serotonin receptor (5-HT 2_A) antagonist and has some antihypertensive properties [101]. A radioactively labeled ketanserin analog, 7-azido-8-ketanserin, was used to identify VMAT2 on immunoblots before antibodies were available [102, 103].

Lobeline, derived from tobacco, has been classified as a nicotinic agonist, but is also a high affinity inhibitor of VMAT2 [104]. It binds at the tetrabenazine site, is not a transport substrate, and leads to a reduction in transmitter stores [105, 106]. Due to its ability to reduce transmitter stores without increasing extracellular dopamine, lobeline, and several of its analogs are being investigated as a possible therapeutic against methamphetamine abuse [107–110].

Amphetamine and amphetamine analogs (such as methamphetamine) have several pharmacological actions including the inhibition of vesicular uptake. Similar to reserpine, amphetamine, and some analogs have been used, unsuccessfully, to treat depression [111, 112]. Unlike reserpine, they are psychostimulants that increase the extraneuronal concentrations of catecholamines in the central as well as peripheral nervous systems. As amphetamines inhibit VMAT2 in the micromolar range, they are three orders of magnitude less potent than reserpine or tetrabenazine. Similar to the case of tetrabenzine, stereochemistry of amphetamine analogs is important for inhibition potency [113, 114]. For example, *S*-(+)-amphetamine is approximately five- to tenfold more potent at inhibiting transmitter uptake by VMAT2 than *R*-(−)-amphetamine. This is due to the fact that amino acid precursors to neurotransmitters are all made from the S form; thus, the VMAT2 binding pocket has evolved to accept neurotransmitters in this spatial configuration [115]. Amphetamines are among the most easily synthesized psychoactive chemicals, and due to this, they have a long history of commercial as well as illicit use and manufacture.

Some industrially manufactured chemicals, which have become environmental contaminants, can inhibit VMAT2 at physiologically relevant concentrations. Organochlorine compounds in particular, which are widely dispersed in the environment and slow to degrade, are a cause for concern. Polychlorinated biphenyls (PCBs), which were largely banned in 1978 but prior to then were used extensively as lubricants and dielectric material, prevent VMAT2 from transporting monoamines [116]. When administered to mice, PCBs reduce the level of VMAT2 expression in the nigrostriatal dopamine system, which can possibly lead to a dysfunction of transmitter storage similar to the beginning of Parkinson's disease [117, 118].

Cloning of VMATs

Recently, cloning of the VMAT genes has allowed a more detailed view of the physiology of vesicular transport activity. For example, expression of VMAT proteins in heterologous systems is critical to delineating the binding and mechanisms of action of pharmacological VMAT inhibitors. Additionally, transgenic animal models have helped define the role of vesicular transport in neurophysiology and neurotoxicology. Similar to the discoveries of the mood altering effects of reserpine and iproniazid, the cloning of VMATs has benefited greatly from accidental discoveries that have made pivotal contributions to medical research.

Meperidine (Demerol) is a synthetic analgesic with morphine-like subjective effects and can even substitute for cocaine due to its ability to block DAT [119]. A related compound, 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), has the same chemical formula but with an ester bond in the opposite orientation. Several batches of MPPP were synthesized and injected by a chemistry graduate student in 1976, who subsequently developed Parkinsonian symptoms [120, 121]. A few years later, in 1982, several people in southern California injected adulterated MPPP and also presented with Parkinsonian symptoms shortly thereafter. It was determined that a contaminant of MPPP synthesis, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), caused these effects [122]. This pivotal revelation has made MPTP one of the standard models of rodent and nonhuman primate Parkinson's disease research.

MPTP, however, is not the actual neurotoxin; it is the parent compound of the neurotoxic metabolite 1-methyl-4-phenylpyridinium (MPP^+; also known as the herbicide Cyperquat) [123, 124]. It was observed that adrenal chromaffin cells, which normally release the catecholamines epinephrine and norepinephrine, could accumulate MPP^+ and also exhibited a greater resistance to its toxic effects than other cell types [125, 126]. A cDNA was isolated from these cells which encoded a vesicular transport protein and could confer protection against MPP^+ when transfected into more vulnerable cells [127]. It was later demonstrated that the protection against MPP^+ was abolished by pretreatment with reserpine, again suggesting a neuroprotective role of vesicular transport [128]. It was noted that messenger transcripts for this protein were detected in the periphery and was originally named chromaffin granule amine transporter. This protein is now recognized as the VMAT1. To date, no laboratories have reported on the effects of genetic deletion of VMAT1.

When determining the expression of VMAT1 in different tissues, a sequence with high similarity was detected in the brain [127]. It was later identified as MAT by a research group at the National Institutes of Health. The mRNA for MAT was located in the monoaminergic regions of the brain stem corresponding to dopamine, norepinephrine, and serotonin producing cell bodies [129]. This transporter, which would eventually become known as VMAT2, required the generation of a proton gradient by the V-type ATPase to translocate monoamines, and the monoamine transport activity was found to be directly inhibited by reserpine and tetrabenazine. Genetic deletion of VMAT2 was accomplished by several research groups which all reported that a complete lack of this protein resulted in the mutant animals dying within days after birth [17, 73, 130]. It was also reported that VMAT2 heterozygote knockout mice were more susceptible to MPTP neurotoxicity, suggesting that vesicular sequestration of toxins occurs in the brain as well as peripheral tissues [73, 131]. See Fig. 3.

The Role of VMAT2 in Neuroprotection

Given the phylogentic orgins of vesicular monoamine transporters as toxin extruding proteins, it is fitting they have a postulated role in neuroprotection. VMAT1 was isolated based on its ability to prevent cellular damage by MPP^+, the neurotoxic metabolite of MPTP [127, 132]. Cells expressing VMAT2 also display this ability [133]. VMAT2 has other hypothesized functions in protecting neurons, such as preventing neurotransmitter oxidation and free radical damage [134, 135]. Futhermore, VMAT2 activity opposes the neurotoxic actions of amphetamine, which appears to be an extreme case of uncontrolled neurotransmitter oxidation [115, 136, 137]. The neuroprotective mechanisms of VMAT2 versus these potentially toxic challenges are explored herein.

Vesicular Transport Attenuates MPTP Neurotoxicity

MPP^+ causes cell death by the blockade of Complex I of the mitochondrial electron transport chain, derailing energy production, and creating oxygen radicals [138, 139]. VMAT1 and VMAT2 oppose this action by transporting MPP^+ into the vesicle as if it were a neurotransmitter and prevent its buildup in the cytosol after MPTP administration [125, 140]. Since MPP^+ enters dopaminergic cells via the dopamine transporter and VMAT2 sequesters it in vesicles, the ratio of DAT to VMAT2 function is postulated to be a key determinant of vulnerability to MPP^+ [141, 142]. Regional differences in this ratio may explain the characteristic pattern of neuronal loss in nonhuman primates treated with MPTP. Dorsal striatal projections of the putamen and caudate nucleus express greater DAT relative to VMAT2 and are more readily lost, whereas terminals of

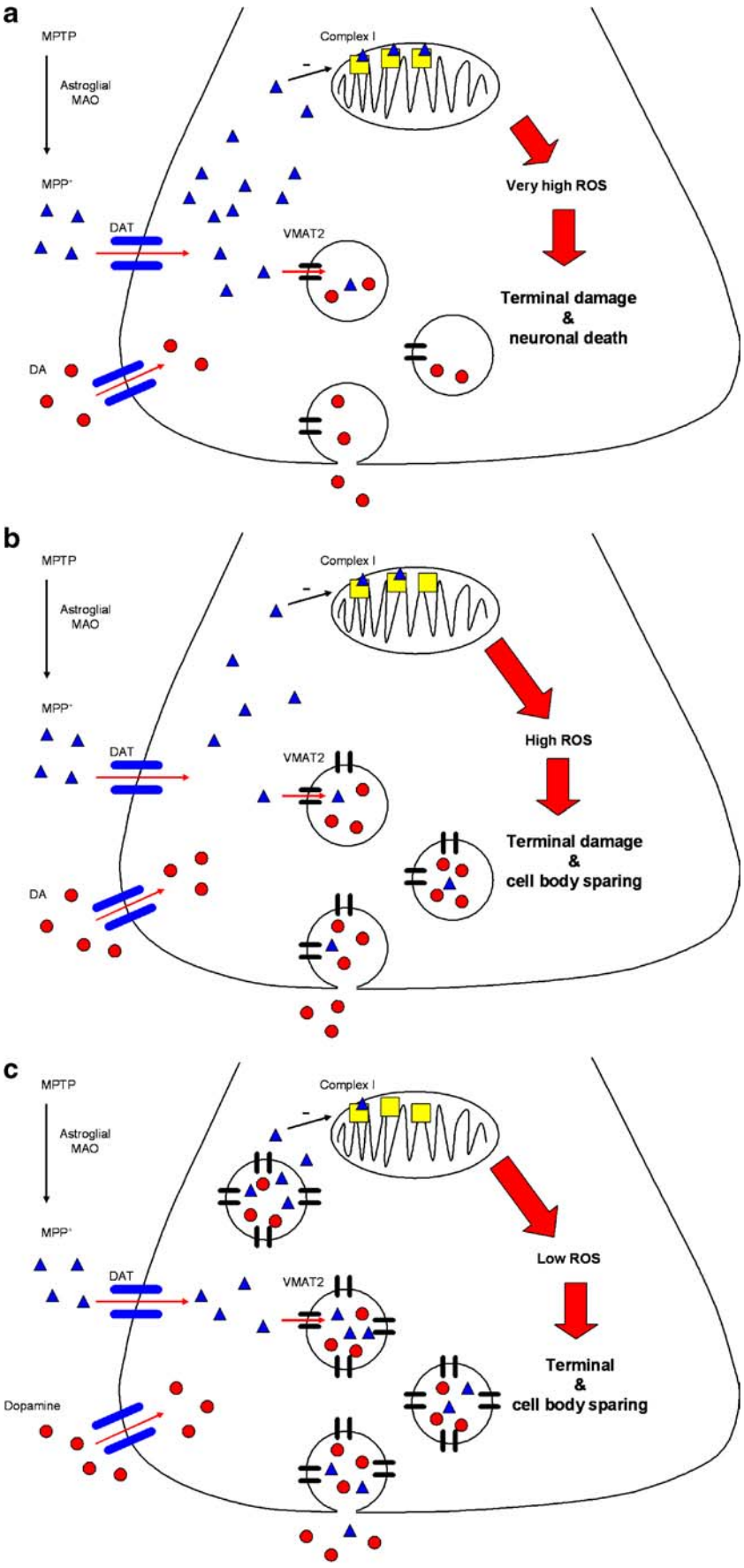

Fig. 3 Metabolic activation of MPTP and vesicular storage of MPP^+. Conditions of low (**A**), normal (**B**), and high (**C**) expression of VMAT2 illustrate differential neuronal reactions to identical quantities of *MPTP*. *MPTP* is converted to MPP^+ by monoamine oxidase (*MAO*) located in astroglial cells. MPP^+ diffuses into the extracellular space where it is transported by the dopamine transporter (*DAT*) into the neuron. Once in the cytosol, MPP^+ can be taken up by the mitochondria. MPP^+ binds to Complex I of the electron transport chain, creating inefficiency in oxygen reduction and the generation of oxidative stress such as superoxide anions and hydroxyl radicals. If these oxidative species overwhelm the antioxidant capacity of the neuron, cell death ensues. However, if MPP^+ is sequestered by the vesicular monoamine transporter 2 (*VMAT2*), it is prevented from reaching the mitochondria and will eventually be metabolized and excreted. *DAT* activity is held constant in across these scenarios

the nucleus accumbens have less DAT relative to VMAT2 and exhibit sparing after MPTP administration [141, 143].

MPTP administration has been shown to be more efficient at killing dopamine neurons in mice than rats, despite greater MPP^+ formation and uptake in rat striatum [144–146]. It was found that rat synaptic vesicles have twice the maximal uptake velocity of MPP^+ as mouse synaptic vesicles because they have approximately twice the amount of VMAT2 [147]. The same research group then confirmed that blocking VMAT2 in rats caused MPTP to be more toxic in this species, indicating the resistance conferred by VMAT2 was indeed counteracting the massive influx of MPP^+ [148]. These were critical demonstrations that vesicular storage can determine the neurotoxicity of an exogenous toxicant and that increased VMAT2 activity can be protective in vivo.

The protective role of vesicular toxicant sequestration has also been conspicuous in its absence, as demonstrated by the genetic reduction of VMAT2 levels. Mice that express half the normal amount of VMAT2 show greater damage to the dopamine terminals in the striatum after MPTP, with potentiated losses of both dopamine and DAT [131]. Damage is not confined to neuron terminals, as twice as many cell bodies are lost in the midbrain of VMAT2 heterozygous mice after MPTP, despite beginning with the same number of cells as wild-type mice [73]. This was a further indication that altering vesicular sequestration of exogenous toxicants can regulate their neurotoxicity.

Several studies suggest the DAT to VMAT2 ratio can be altered without genetic manipulations. The ratio can be changed by pramipexole, a dopamine receptor agonist that transiently increases vesicular uptake and is protective against MPTP [149]. One intriguing study suggests that this ratio can be altered by the subjective experience of animals. Mice given environmental enrichment such as exercise, learning activities, and social interaction exhibited a decreased ratio of DAT to VMAT2 and reduced neurotoxicity of MPTP [150]. The environment, however, can also contribute negatively to the DAT to VMAT2 ratio. Mice developmentally exposed to the pesticides deltamethrin (a pyrethroid) or dieldrin (an organochlorine) have increased DAT expression relative to VMAT2 and exhibit greater MPTP induced dopamine loss [151]. It has also been suggested that the organochlorine pesticide heptachlor and its epoxide metabolite, which indirectly increases DAT activity and directly decreases VMAT2, respectively, would enhance vulnerability of dopaminergic cells to MPP^+ neurotoxicity [152].

Curiously, the *tottering* mouse mutant, derived from a voltage-gated calcium channel defect, has elevated VMAT2 due to an increase in striatal norepinephrine innervation [153]. MPP^+ can utilize the norepinephrine transporter to access these neurons, and it is possible that they act as a sink to store excess MPP^+. Consequently, these mice display remarkable resistance to the neurodegenerative effects of MPTP administration, possibly due to increased vesicular storage of MPP^+ in noradrenergic neurons [154]. However, tottering mice have enhanced levels of norepinephrine, and there is also evidence that high extracellular norepinephrine concentrations may be protective against MPTP neurotoxicity [155].

Vesicular Transport and Neurotransmitter Toxicity

The use of monoamine transmitters can be considered an occupational hazard for neurons. Due to their chemical structures, dopamine, norepinephrine, and serotonin have the capability to auto-oxidize and create oxidative stress (see Fig. 4). Furthermore, cytosolic metabolism of monoamines exacts a cost from a neuron's antioxidant capacity, as MAO activity leads to the formation of two other toxic compounds: aldehydes and peroxides. In order to prevent transmitter oxidation, monoamines synthesized in the cytosol or recycled from the extracellular space must be rapidly transported into synaptic vesicles. Thus, in monoamine neurons, an additional function of VMAT2 is to protect the cells from the toxicity of their own neurotransmitters.

Dopamine oxidation is considered clinically important, as antioxidant balance is more precarious in substantia nigra neurons, and this has been hypothesized to contribute to neurodegenerative disorders such as Parkinson's disease and progressive supranuclear palsy [156–159]. Dopamine possesses a catechol moiety that, in the slightly basic pH of the cytosol, auto-oxidizes into a quinone. Dopamine quinones can adduct to macromolecules or, after conversion to a semiquinone radical by NADPH P450 reductase, can react with molecular oxygen to produce superoxide and hydroxyl radicals. Catechol quinone formation can also further reduce the antioxidant capacity of neurons by adduction to the cysteine residue of glutathione, a peptide antioxidant. The glycine and glutamate residues of conjugated glutathione are subsequently cleaved, leaving cysteine conjugated catechols such as 5-cysteinyl-DOPA, 5-cysteinyl-dopamine, and 5-cysteinyl DOPAC [160]. These compounds are considered markers of excess cytosolic catechol concentrations and oxidative stress [135, 161–163]. Additionally, cysteinyl catechols are directly neurotoxic in culture and have been implicated in dopamine neuron death in Parkinson's disease [164, 165].

In addition to the context of neurodegenerative disorders, there is evidence that pharmacological inhibition of vesicular uptake promotes the formation of cysteinyl catechols and the generation of oxidative stress in the brain. By inhibiting VMAT2, reserpine promotes the

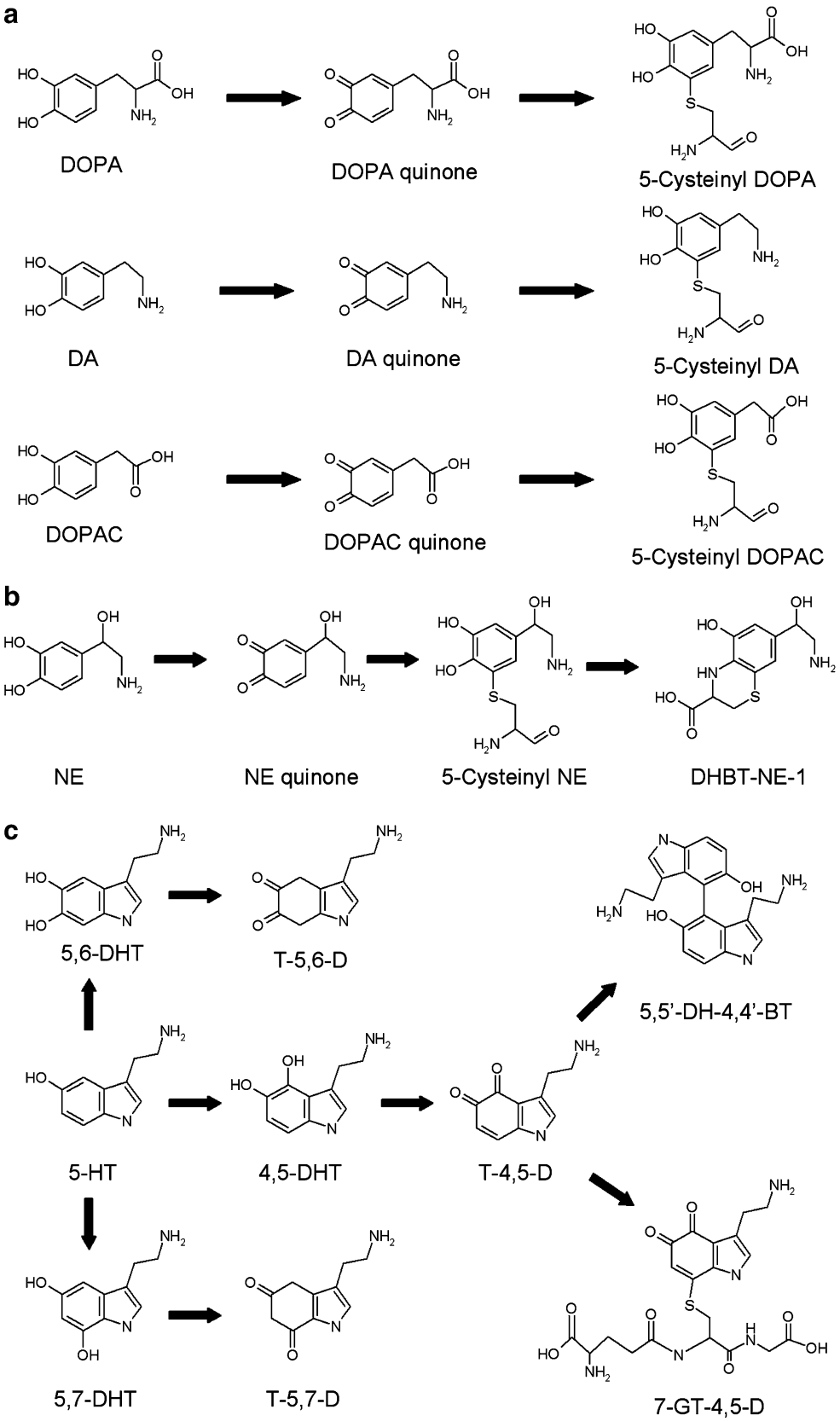

Fig. 4 Catechol and indole oxidation products are neurotoxins. **A** Dopamine (*DA*), its precursor (*DOPA*), and metabolite (*DOPAC*) can auto oxidize to quinones. These catechol quinones can be adduced to glutathione and free cysteine to form 5-cysteinyl derivatives. **B** Norepinephrine (*NE*) can auto oxidize to a quinone, which, like dopamine, can be adduced to glutathione and free cysteine; *5-cysteinyl NE* can cyclize to yield 7-(1-hydroxy-2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1, 4-benzothiazine-3-carboxylic acid (*DHBT-NE-1*). **C** Serotonin oxidation can generate several dihydroxy products, such as *4,5-DHT*, *5,6-DHT*, and *5,7-DHT*. These dihydroxy products can auto-oxidize to tryptamine diones (*T-4,5-D*, *T-5,6-D*, *T-5,7-D*). Two molecules of T-4,5-D can react to form 5,5′-dihydroxy-4,4′-bitryptamine (5,5′-DH-4,4′-BT). T-4,5-D can also be adduced to glutathione, creating 7-*S*-glutathionyl-tryptamine-4,5-dione (*7-GT-4,5-D*)

formation of quinones and 5-cysteinyl dopamine [166, 167]. Also within the dopamine-rich striatum, two studies have shown that reserpine administration leads to a massive increase in oxidized glutathione [168, 169]. Consistent with these observations, a proposed mechanism of reserpine-induced orofacial dyskinesia in rats is the generation of oxidative stress that damages the dopaminergic terminals normally preventing this behavior [170]. While reserpine is more often used due to commercial availability, prolonged tetrabenazine administration leads to a reduction in dopamine neurons in the substantia nigra; the authors hypothesize that this is due to cytosolic dopamine build up and subsequent oxidative stress [171]. Additionally, genetic (A30P alpha-synuclein) and environmental contaminant (PCB and rotenone) models of degeneration show a dependence on dopamine for toxic effect [172–174]. These studies strongly suggest that VMAT2 activity prevents high levels of cytosolic dopamine, which can lead to oxidative stress and, if not halted, neuronal damage and death.

Genetic alteration of VMAT2 levels offers further evidence of neuroprotective action against cytosolic dopamine and neurodegeneration. VMAT2 deficient mice (95% reduction in protein levels) exhibit elevated cysteinyl catechols despite lower levels of striatal dopamine, illustrating the importance of neurotransmitter compartmentalization. These mice also show an age-dependent acceleration of other markers of oxidative and nitrative stress such as protein carbonyls and 3-nitrotyrosine. The excessive oxidative damage experienced by these mice over time leads to destruction of the vulnerable dopamine neurons in the substantia nigra and emphasizes the role VMAT2 as neuroprotective protein [135]. While an animal model with genetic overexpression of VMAT2 has not been made, VMAT2 promoter haplotypes which alter expression have been identified in humans. Several of these polymorphisms increase VMAT2 expression in cell culture and negatively correlate with the incidence of Parkinson's disease in women [175].

Norepinephrine-producing neurons that project from the locus coeruleus (LC) also degenerate in Parkinson's disease, and the LC terminal fields in the hippocampus are damaged in Alzheimer's disease and dementia with Lewy bodies [176–178]. The degeneration of LC neurons may be similar to dopamine neurons because improperly sequestered norepinephrine can oxidize and form cysteinyl conjugates like dopamine [179, 180]. Further oxidation of cysteinyl norepinephrine creates compounds that inhibit the mitochondrial electron transport chain, causing ATP deprivation and possibly neurodegeneration [181]. Neuronal damage, however, is not always the result of cytosolic catecholamine oxidation. Dopamine and norepinephrine neurons are darkly pigmented in part because the oxidation of cytosolic DOPA is an early, common step in neuromelanin synthesis [137]. Neuromelanin has a matrix of oxidized catechol polymers thought to sequester catechol quinones and reactive metals, preventing them from causing oxidative damage [182, 183]. Overexpression of VMAT2 in cell culture results in a reduction of neuromelanin formation, supporting the hypothesis that oxidized dopamine is needed for its synthesis [184]. While it has been demonstrated that severely reduced VMAT2 leads to increased dopamine oxidation and neuronal damage, it is unknown if this increases neuromelanin content, nor is it known if too much VMAT2 would negate the protective effect of neuromelanin.

Cytosolic indole toxicity is also a possibility in neurons with insufficient VMAT2 activity. Oxidation of serotonin (5-hydroxytryptamine) has been linked to experimental and clinical neurodegeneration. While the indole structure of serotonin is less susceptible to quinone formation, the addition of a hydroxyl group creates the possibility for further oxidation to an ortho- or metaquinone, causing adduction to macromolecules and terminal destruction [185]. Injection of 5,6-dihydroxytryptamine or 5,7-dihydroxytryptamine destroys serotonin neurons, and it has been suggested that serotonin can be oxidized to these compounds in vivo [186–188]. Experimental evidence suggests that in Alzheimer's disease and disorders of copper storage, serotonin oxidation produces 4,5-dihydroxytryptamine which further oxidizes to compounds that inhibit acetylcholine esterase and cause oxidative stress [189–192]. Furthermore, the production of neurotoxic oxidation products of cytosolic serotonin has been proposed as a possible mechanism of methamphetamine neurotoxicity in these neurons [187, 191, 193].

While speculative in nature, another possible mechanism of neuroprotection by VMAT2 is revealed by the fact that monoamines can react with aldehydes under physiological conditions to form toxic compounds [194–196]. These aldehydes are derived from intracellular metabolism of alcohols and, thus, are present in the cytosol where they can react with unsequestered monoamines via a Pictet–Spengler condensation. Combinations of aldehydes and catecholamines produce tetrahydroisoquinolines (TIQs) and those of aldehydes and indoleamines yield beta carbolines (BCs). While no research has yet explored this idea, it is hypothesized that vesicular transport of monoamines may influence the rate of TIQ and BC formation in synaptic terminals by controlling the levels of cytosolic monoamines exposed to reaction with aldehydes (See Fig. 5).

Tetrahydroisoquinolines are naturally occurring derivatives of catecholamines or phenylethylamines; thus, their formation may be regulated in part by the cytosolic concentrations of these precursors. The controlled formation of these TIQs may be critical to neuronal health, as many have suspected or proven neurotoxic actions. Due to

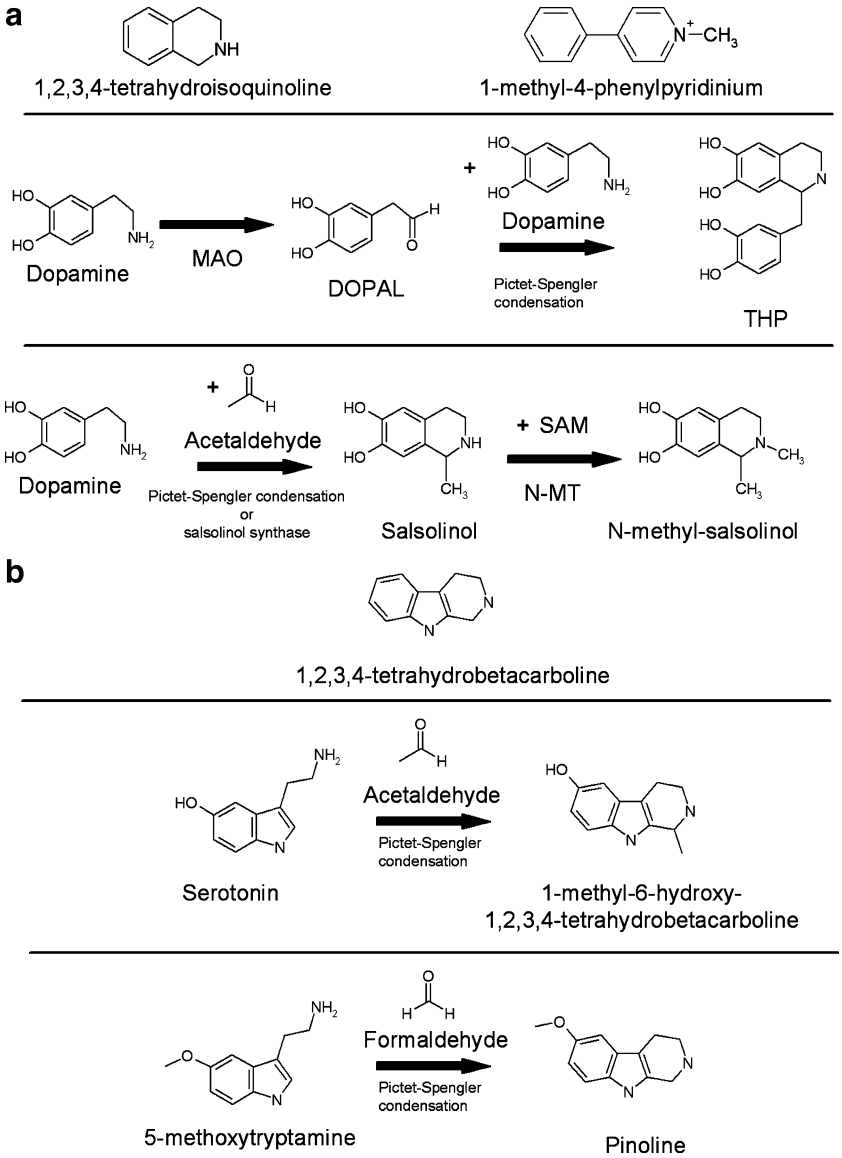

Fig. 5 Condensation of monoamines and aldehydes. Catecholamines react with aldehydes to form tetrahydroisoquinoline derivatives, depicted in (**A**). MPP+ is included for comparison. Tetrahydropapaveroline (*THP*) is formed from the condensation of DOPAL, the immediate product of MAO, and dopamine. Salsolinol is synthesized from acetaldehyde and dopamine. Nonspecific *N*-methyltransferases produce *N*-methyl-salsolinol, a neurotoxic agent. Indoleamines condense with aldehydes to create beta carbolines, shown in (**B**). Structure of tetrahydrobetacarboline is shown for comparison. Serotonin reacts with acetaldehyde to form the neurotoxic product, 1-methyl-6-hydroxy-1,2,3,4-tetrahydrobetacarboline. 5-methoxy-tryptamine, derived from the action of 5-hydroxyindole-*O*-methyltransferase and serotonin, reacts with formaldehyde to yield pinoline, a suggested antioxidant

this possibility, some TIQs are postulated to contribute to substantia nigra degeneration in idiopathic Parkinson's disease [197–202].

Tetrahydropapaveroline (THP) is a TIQ formed via condensation of dopamine with its MAO metabolite, DOPAL [203]. Physiologically, THP can inhibit DAT-mediated dopamine uptake in vitro and is readily detectable in the brain of rats after ethanol administration [204]. It is an interesting thought experiment that the levels of THP in humans could be manipulated by therapeutics that block the formation of DOPAL (MAO inhibitors) or decrease the oxidation of DOPAL to DOPAC (aldehyde dehydrogenase inhibitors; disulfiram for the treatment alcoholism).

If VMAT2 activity does affect the synthesis of harmful TIQs, it is hypothesized that it may be able to provide some measure of neuroprotection against ethanol. Ethanol ingestion promotes the formation of TIQs due to its oxidized metabolite, acetaldehyde, which may be formed from the actions of alcohol dehydrogenase, catalase, or cytochrome P450 (CYP2E1) and react with dopamine to yield salsolinol by the same Pictet–Spengler reaction as THP [203, 205–207]. While not a popular assertion, salsolinol is hypoth-

esized to be an endogenous neuromodulator; this is because an enzyme in the brain (so-called salsolinol synthase) has been shown to produce *R*-salsolinol enantioselectively from achiral dopamine [208–210]. However, *N*-methyltransferases enzymes can act salsolinol it to create *N*-methyl-salsolinol, which has been demonstrated to be neurotoxic to SH-SY5Y neuroblastoma cells [211]. By a similar pathway, dopamine and formaldehyde can react to yield the putative toxicants norsalolinol and *N*-methyl-norsalsolinol [212]. Norsalsolinol has been shown in vitro to be transport substrate for plasmalemmal and vesicular transporters, suggesting a potential for a protective effect of VMAT2 similar to that for MPP^+ [213, 214]. While not constituting proven disease mechanisms, these TIQs are hypothesized to impact the etiology of several disorders including addiction, attention-deficit hyperactivity disorder and Parkinson's disease, possibly by interfering with dopamine production, storage, or degradation [212, 215–219].

Beta carbolines are pharmacologically active condensation products of indoleamines such as tryptophan, tryptamines, or other indolealkylamines with metabolic or dietary aldehydes [196, 220]. BCs commonly occur in plants and are thought to contribute hallucinogenic properties in spiritual concoctions, such as the South American shaman preparation, *ayahuasca* [221]. BCs have a wide variety of pharmacological effects including the inhibition of plasmalemmal dopamine transport, inhibition of MAO, and, perhaps the most unusual, inverse agonism of $GABA_A$ receptors at the benzodiazepine site [222, 223]. Like some TIQs, it has been thought that BCs impact addiction and neurodegeneration: in vitro, BCs have been shown to act as plasmalemmal and vesicular uptake inhibitors of serotonin and dopamine, and, in vivo, can reduce striatal serotonin and dopamine concentrations, and lead to bradykinesia [224–226]. Conversely, one BC, pinoline, has been postulated to confer protective effects from oxidative stress [227].

The normal physiology of TIQs and BCs is still largely undefined and, while no studies are currently published that link TIQ or BC production and vesicular monoamine uptake, it is possible that VMAT2 function is a determining factor in endogenous TIQ and BC production. Given the studies showing the capability of TIQs and BCs to cause dysfunction of monoamine systems in the brain, it would be interesting to investigate the influence of VMAT2 on these processes.

VMAT2 Resists the Neurotoxicity of Amphetamine and Methamphetamine

The therapeutic benefits of amphetamine have had a long commercial history, even though its varied pharmacological actions were not identified until comparatively recently. Amphetamine was first synthesized in 1887 but was not marketed commercially in the USA until 1932. Benzadrine, an amphetamine inhaler manufactured by Smith, Kline, and French and made famous by students desiring supplementation-induced studiousness, was widely available until it was made prescription-only in 1939. It was not, however, classified as a controlled substance until 1946 and amphetamine, due to its now famous stimulant properties, were widely used by American soldiers in World War II and beyond. Amphetamine is currently in clinical use, under the brand names Dexadrine and Adderall, to treat conditions of arousal such as narcolepsy and attention deficit hyperactivity disorder. Methamphetamine, an N-methylated analog, also possesses stimulant and anorectic properties and is currently available as a weight loss agent known as Desoxyn or Syndrox. Amphetamine and methamphetamine are classified as Schedule II controlled substances by the US Food and Drug Administration that can only be purchased by prescription and used under strict surveillance. However, methamphetamine can still be purchased over-the-counter as an inhaled decongestant (Vick's; Proctor and Gamble, Cincinnati, OH, USA) as the product only contains the much less potent R-(−) isomer. Recently, the ease with which methamphetamine can be illicitly manufactured led to the restricted sale of its most common precursor, the nasal decongestant pseudoephedrine (Sudafed; Ortho-McNeil, Raritan, NJ, USA) in the USA. Despite more than 70 years and the myriad uses for amphetamine and its analogs, its pharmacological mechanisms are not yet fully understood.

It is known that amphetamine releases monoamines into the synapse and thereby provides psychostimulant action, but questions remain how exactly this is accomplished. In dopamine neurons, it is known that DAT mediates amphetamine access into the cytosol, where it can directly inhibit VMAT2 [228, 229]. Amphetamine then enters the synaptic vesicles, possibly by VMAT2 transport or membrane diffusion. Once inside the vesicle, amphetamine induces the release of stored dopamine into the cytosol by an unknown mechanism but may involve reverse transport by VMAT2 or another, as yet unidentified, transport protein [115, 230]. It has been hypothesized that weak bases, such as amphetamine (pKa of 9.9), are protonated in the acidic vesicle, thus, depleting the driving force for bringing dopamine into the vesicle from the cytosol and inducing vesicular dopamine efflux [231, 232]. Secondly, the synthesis of DOPA, a dopamine precursor, is enhanced, while the metabolism of dopamine by MAO is suppressed by methamphetamine [115, 233, 234]. The high concentration of cytosolic dopamine is then subject to release via DAT reversal, with amphetamine possibly inducing a channel mode which causes rapid efflux into the synapse

[235]. The behavioral effects are thought to derive from the extracellular dopamine whereas the neurotoxic effects are considered dependent on the intracellular dopamine [136, 236].

The best supported neurotoxic mechanism of amphetamine and methamphetamine in dopamine neurons involves the mishandling of increased cytosolic neurotransmitter. It is known that these chemicals promote efflux of dopamine into the cytosol and inhibit VMAT2 [115, 228, 237]. Several reports suggest that, after methamphetamine administration, sequestration of dopamine into vesicles could be impaired two ways apart from direct inhibition of the transporter: by oxidative damage to VMAT2 and trafficking of the protein to nonvesicular compartments [238–240]. Additionally, vesicular uptake capacity is strained further as monoamine oxidase is inhibited and tyrosine hydroxylase is activated by methamphetamine, thus ceasing the intracellular degradation of dopamine and promoting the production of L-DOPA and dopamine in the cytosol [233, 234]. Thus, the combined mechanism of release of vesicular dopamine from the vesicles, reduction in VMAT2 activity, and the enzyme-modulating effects of methamphetamine cause a massive increase in cytosolic dopamine. When degradation and vesicular sequestration are impaired, then cytosolic dopamine auto oxidizes and leads to oxidative stress [241]. This oxidative damage can cause the loss of terminal marker proteins and neurodegeneration [242–245].

Genetic reductions in VMAT2 have yielded additional evidence for the hypothesis that cytosolic dopamine causes amphetamine neurotoxicity. Mice, expressing only half the normal amount of VMAT2, are more sensitive to methamphetamine induced striatal dopamine and DAT depletion but curiously show less evidence of extracellular free radical production [246]. Mice that express only 5–10% of the normal amount of VMAT2 have severely exaggerated toxic responses to methamphetamine including exacerbated DAT and TH loss, as well as potentiated oxidative stress and astrogliosis compared to heterozygotes and wild-types [243]. This phenomenon strongly suggests that proper compartmentalization of the transmitter within the neuron is more critical for terminal health, despite the reduced extracellular radical formation. Once methamphetamine causes a surge in cytosolic dopamine, the clearance into the vesicles with lowered VMAT2 is too slow to prevent oxidation of the transmitter and thus intraneuronal oxidative stress and terminal damage are greater. The critical concept is that despite the exact mechanism by which methamphetamine may cause dopamine terminal damage, it is clear that having less VMAT2 increases its in vivo neurotoxicity [243] (see Fig. 6).

VMAT2 knockout mice do not survive more than a few weeks after birth, but midbrain cultures from these mice have provided some insight into the dopamine mechanism of methamphetamine neurotoxicity. Removal of the vesicular storage capability from the neurons means that the dopamine content is reduced by 97% compared to cultures of neurons with full expression of VMAT2. However, even in neurons devoid of VMAT2 and with low dopamine content, methamphetamine is able to cause the formation of high amounts of oxidative stress traceable to dopamine oxidation [233]. Therefore, it seems that vesicular storage of dopamine is not required for methamphetamine neurotoxicity but that cytosolic dopamine oxidation still occurs to

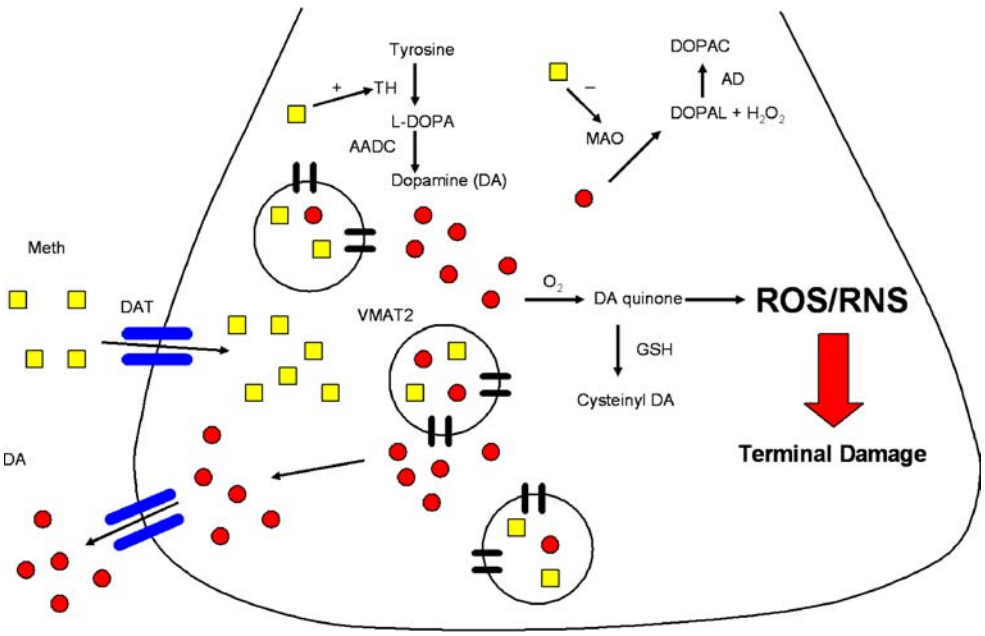

Fig. 6 Role of cytosolic dopamine in methamphetamine neurotoxicity. Methamphetamine is a substrate for the dopamine transporter (*DAT*) and is taken into the neuron terminal where it gains access to the vesicular interior, possibly by VMAT2 transport. Dopamine is released from the vesicle into the cytosol while methamphetamine inhibits monoamine oxidase (*MAO*) and activates tyrosine hydroxylase, enhancing dopamine synthesis and inhibiting its degradation. Cytosolic dopamine can then be resequestered by VMAT2, released into the synapse by reverse transport of DAT or oxidized to neurotoxic quinones. Quinones create oxidative stress and can cause degeneration of the terminal

a greater extent than in neurons with full expression of VMAT2. This further suggests that the enzymatic the actions on methamphetamine on enzymes are critical for an increase in cytosolic dopamine and its mechanism of neurotoxicity.

In vivo and in vitro studies have shown that methamphetamine is more neurotoxic to dopamine neurons with reduced or absent VMAT2, but, compared to the relatively straightforward mechanism of MPTP, it is less clear how vesicular transport is protective in this case. Several studies have demonstrated that the function of VMAT2 is severely compromised by methamphetamine's promotion of oxidative and nitrative stress; therefore, it is less able to efficiently transport dopamine into the vesicle [231, 238, 239, 247]. Reports indicate that VMAT2 function in the dopamine neurons is depressed up to 75% for 24 h and that radioligand binding is reduced for up a week after methamphetamine exposure [239, 248]. Judging by the experimental evidence, methamphetamine seems to leave the vesicular pH gradient at least partially intact as well as some functional VMAT2, which must be providing a protective effect; otherwise the observed terminal damage would not depend on VMAT2 expression. The total amount of vesicular dopamine transport capacity and, therefore, the capacity of the functional fraction after methamphetamine

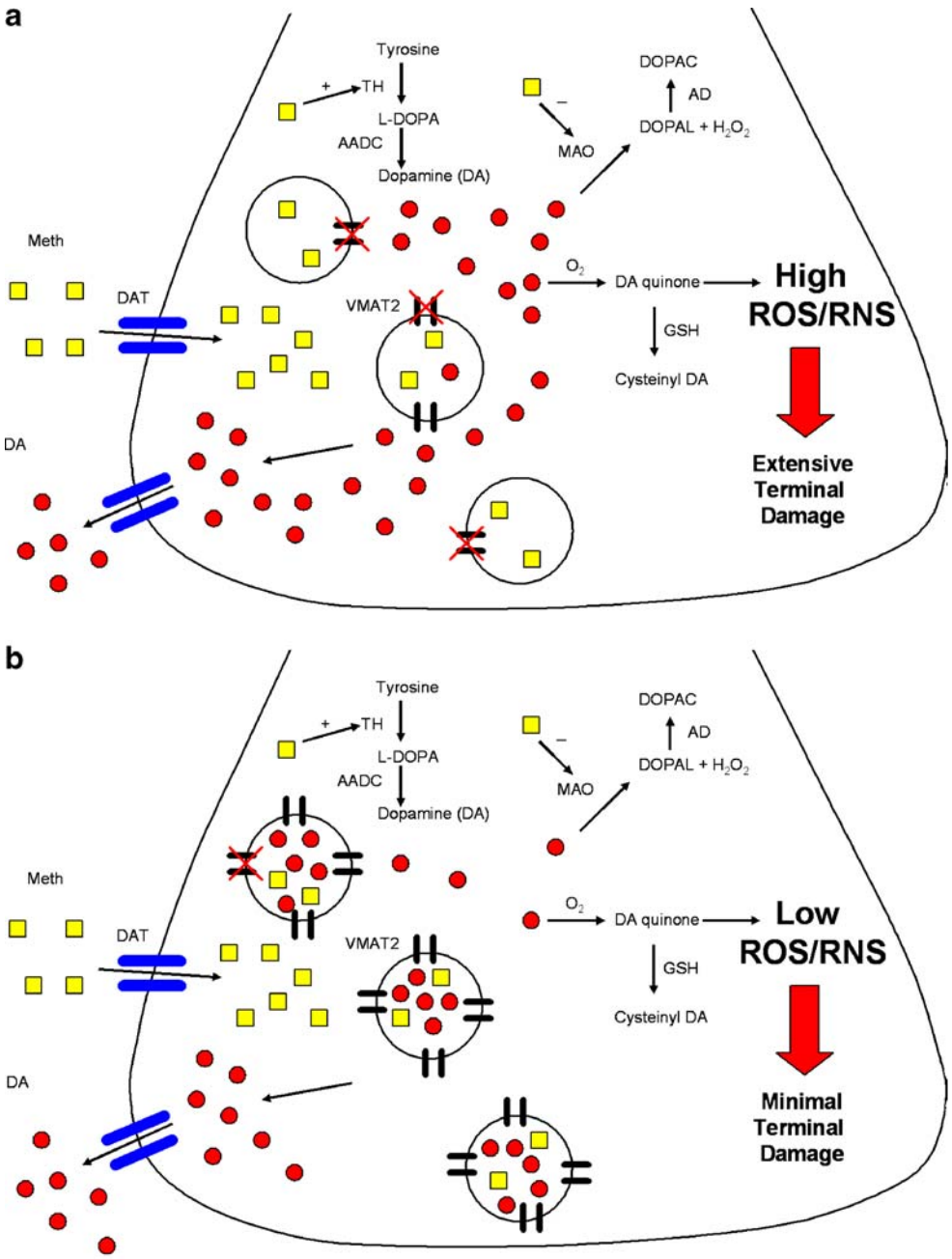

Fig. 7 Function of VMAT2 in methamphetamine neurotoxicity. Methamphetamine is suggested to initially decrease vesicular uptake by a percent of total function. The fraction of functional VMAT2 remaining determines extent of cytosolic dopamine increase and oxidative damage after methamphetamine. This is illustrated in conditions of low (**A**) and high (**B**) VMAT2 expression

exposure most likely mediate the differences in neurotoxicity among mice with genetically altered VMAT2 levels. This is illustrated by comparing the increasing striatal degeneration after methamphetamine among wild-type mice, animals that express 50% VMAT2, and those that express only 5–10% VMAT2 [243]. Neurons with low VMAT2 expression may also contribute to a feed-forward exacerbation of oxidative damage: if the vesicles cannot take up vesicle-released and newly synthesized cytosolic dopamine, this allows more dopamine oxidation which could further damage the small pool of VMAT2 protein.

It was recently reported that increasing VMAT2 expression reduces apoptosis after methamphetamine exposure and decreasing VMAT2 rendered cultured midbrain neurons more vulnerable to dopamine toxicity [249]. Additionally, increasing the expression of VMAT2 in vivo is able to mitigate the oxidative stress and gliosis caused by methamphetamine [250]. These converging lines of evidence suggest that elevating the expression of functional VMAT2 in dopamine neurons in vivo will defend against spikes in cytosolic dopamine after administration of amphetamine or methamphetamine (see Fig. 7).

Conclusions

VMAT2 is an excellent example of a phylogenetically ancient protein being adapted for other physiological uses. It is hypothesized that forerunners of VMAT2 were able to effectively transport several types of toxic compounds and, eventually, some of these came to be used as intercellular signaling molecules. The protective function of vesicular sequestration of these potentially toxic compounds is a reminder that an important method by which biological systems evolve is differential use and modification of readily available components.

Similar to the flexibility inherent in related bacterial and archaeal multidrug transporter proteins in the TEXAN and MFS groups, the vesicular monoamine transporters are able to recognize and translocate compounds that are manmade. MPP^+ is an example of a synthetic chemical that is transported, and its toxicity attenuated, by VMAT2. Pyridine motifs, like the one in MPP^+, often occur in nature: in plant-derived alkaloid toxicants such as nicotine (from *Nicotiana tabacum*, tobacco, also the source of the VMAT2 inhibitor lobeline) and ricinine (from *Ricinis communis*, castor bean), as well as in essential vitamins like nicotinic acid (vitamin B_3). Similarly, while methamphetamine is synthetic, it is structurally related to phenylethylamine, the backbone of oxidizable neurotransmitters such as dopamine and norepinephrine. Methamphetamine also has structural similarity and comparable monoamine releasing action to the naturally occurring yet potentially toxic chemical tyramine [251, 252]. Thus, it may be said that in mammalian nervous systems, vesicular monoamine transporters are still part of the family business of recognizing and translocating toxic and potentially toxic molecules away from the vulnerable cytosol.

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