Published in final edited form as:
Curr Neurol Neurosci Rep. 2013 July ; 13(7): 362. doi:10.1007/s11910-013-0362-3.

Vesicular Integrity in Parkinson's Disease

Shawn P. Alter,
Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Gina M. Lenzi,
Department of Biochemistry, School of Medicine, Emory University, Atlanta, GA, USA

Alison I. Bernstein, and
Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Gary W. Miller
Department of Neurology, School of Medicine, Emory University, Atlanta, GA, USA. Department of Pharmacology, School of Medicine, Emory University, Atlanta, GA, USA. Department of Environmental Health, Rollins School of Public Health, Claudia Nance Rollins Bldg, Room 8007, 1518 Clifton Road, NE, Atlanta, GA 30322, USA

Gary W. Miller: gary.miller@emory.edu

Abstract

The defining motor characteristics of Parkinson's disease (PD) are mediated by the neurotransmitter dopamine (DA). Dopamine molecules spend most of their lifespan stored in intracellular vesicles awaiting release and very little time in the extracellular space or the cytosol. Without proper packaging of transmitter and trafficking of vesicles to the active zone, dopamine neurotransmission cannot occur. In the cytosol, dopamine is readily oxidized; excessive cytosolic dopamine oxidation may be pathogenic to nigral neurons in PD. Thus, factors that disrupt vesicular function may impair signaling and increase the vulnerability of dopamine neurons. This review outlines the many mechanisms by which disruption of vesicular function may contribute to the pathogenesis of PD. From direct inhibition of dopamine transport into vesicles by pharmacological or toxicological agents to alterations in vesicle trafficking by PD-related gene products, variations in the proper compartmentalization of dopamine can wreak havoc on a functional dopamine pathway. Findings from patient populations, imaging studies, transgenic models, and mechanistic studies will be presented to document the relationship between impaired

Correspondence to: Gary W. Miller, gary.miller@emory.edu.

Conflict of Interest Shawn P. Alter declares that he has no conflict of interest.
Gina M. Lenzi declares that she has no conflict of interest.
Alison I. Bernstein declares that she has no conflict of interest.
Gary W. Miller declares that he has no conflict of interest.

Compliance with Ethics Guidelines
Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

This article is part of the Topical Collection on *Movement Disorders*

vesicular function and vulnerability of the nigrostriatal dopamine system. Given the deleterious effects of impaired vesicular function, strategies aimed at enhancing vesicular function may be beneficial in the treatment of PD.

Keywords

Dopamine; Vesicle; Transport; VMAT2; Parkinson's disease

Introduction

The disease James Parkinson first described in 1817 as the *Shaking Palsy*, which now bears his name, is the second most common neurodegenerative disorder. Parkinson's disease (PD) is defined by its motor symptoms, tremor, rigidity, bradykinesia, but is, in fact, multifaceted and is associated with nonmotor symptoms as well, including autonomic, cognitive, and neuropsychiatric disturbances. The motor symptoms of PD are in all likelihood due to deficits in synaptic dopamine transmission, while the nonmotor symptoms may involve other neurotransmitters. PD pathology is characterized by deposition of α-synuclein-positive Lewy bodies in susceptible neurons and neuronal degeneration. However, Lewy pathology and neurodegeneration do not follow the same pattern of progression. Lewy bodies are, early on, deposited in the dorsal motor nucleus of the brainstem and spread in an ascending manner throughout the nervous system, but neurodegeneration is limited to specific areas. The dopaminergic neurons of the substantia nigra pars compacta (SNpc), which innervate the dorsal striatum, are preferentially destroyed in the course of the disease. Notably, cell loss is also observed in the locus ceruleus, in sympathetic neurons of the peripheral nervous system (releasing norepinephrine), and the dorsal raphe (releasing serotonin) [1, 2].

The key role for vesicular function in PD was first hinted at over 50 years ago. In 1952, reserpine, an alkaloid isolated from the Indian snakeroot *Rauwolfia serpentine*, was introduced to Western medicine and widely prescribed for its antihypertensive and antipsychotic properties. Despite the beneficial effects of reserpine, side effects include depression, gastric dysmotility, and extrapyramidal symptoms, "resembling a complete Parkinson Syndrome" [3]. While the molecular target of reserpine would not be identified for decades, investigators noted that reserpine depleted dopamine in biological tissue and caused parkinsonism in rats [4, 5]. Arvid Carlsson found that administration of the dopamine precursor, L-3,4-dihydroxyphenylalanine (levodopa), rescued the parkinsonian phenotype in rats [6]. Soon after, Ehringer and Hornykiewicz discovered that striatal dopamine deficiency was responsible for Parkinsonian motor deficits [7] and in 1961, Birkmayer and Hornykiewicz intravenously administered levodopa to a PD patient for the first time with marked success [8]. An oral form of levodopa was approved for the treatment of PD in 1970.

Storage of neurotransmitters relies on the function of synaptic vesicles; disrupting vesicular function results in decreased release and increased cytoplasmic neurotransmitter, which is toxic to neurons. The toxicity of cytoplasmic dopamine, in particular, has been studied extensively and results from oxidative stress stemming from excessive autoxidation and

enzymatic deamination. Similar mechanisms of toxicity have been studied in the norepinephrine system, and to a lesser extent, the serotonin system. For the purpose of this review, we will focus on the vesicular storage of dopamine and ways it is disrupted in PD. We will both review evidence that defective vesicular function may be a determinant of PD pathogenesis and examine clinical indices of vesicular function and their potential as biomarkers for disease progression.

Synaptic Vesicle Function

Vesicles consist of spherical lipid bilayers and a vast array of proteins that mediate their various functions. Combined biochemical and genomic approaches have led to the identification of dozens of integral vesicular proteins involved in these activities, as well as biomolecules that are transiently associated with vesicles throughout their lifecycle [9–13]. The composition and size of synaptic vesicles vary with respect to their neuronal phenotype and cargo [14]. Synaptic vesicles transport and store a variety of neurotransmitters including acetylcholine, amino acids (including GABA and glycine, which are inhibitory and glutamate, which is excitatory), biogenic amines (including DA, NE, and serotonin), and neuropeptides. By concentrating neurotransmitters into discrete packages for subsequent release, synaptic vesicles are essential for neurotransmission. The vast majority of neurotransmitters within a neuron are found stored within vesicles. As such, vesicles must sequester transmitters against a concentration gradient, using specialized transporters. Within monoaminergic neurons, the type II vesicular monoamine transporter (VMAT2; *SLC18A2*) preferentially transports 1 cytosolic monoamine into the vesicle in exchange for release of 2 protons into the cytosol [15]. This process is dependent on an electrochemical gradient generated by the vacuolar ATPase, which transports protons into the vesicle, acidifying the vesicle lumen and providing substrate for antiport by VMAT2 [15, 16].

Formed in the Golgi apparatus, vesicles are transported to the synaptic terminal via transient interactions between cytoskeletal and vesicular proteins [17]. At the synaptic terminal, vesicles exist in distinct pools of various stages in the vesicle cycle; movement between these pools is dynamically regulated by vesicular proteins that respond to neuronal activity [18]. A subset of vesicles, comprising the readily releasable pool (RRP), dock at the active zone of the presynaptic membrane awaiting calcium influx. Calcium induces vesicles to fuse with the presynaptic membrane and release their contents by exocytic release (Fig. 1a) [18]. Closely associated with the RRP is the larger reserve pool, and collectively these make up the recycling pool. Most distal to the terminal is the resting pool [19]. From electrophysiological and morphological observations in cultured hippocampal neurons, investigators have estimated that there are 6–8 vesicles in the RRP, 17–20 in the reserve pool, and 180 in the much larger resting pool [20–23]. Docking of RRP vesicles is mediated by interactions between vesicular and plasmalemmal proteins of the SNARE complex [24], including synaptotagmins, which are calcium-sensing proteins that initiate membrane fusion during exocytosis [25, 26]. With prolonged stimulation, vesicles in the reserve pool are recruited to the RRP. Vesicles fused to the plasma membrane have several possible fates, including reuse by so-called “kiss-and-stay” (immediate reuse) and “kiss-and-run” (trafficking to the reserve pool) mechanisms [18, 27, 28]. Vesicle components remaining in the plasma membrane are recycled or degraded by endosomes.

Proteins, such as LRRK2 and α-synuclein, which are implicated in PD, may disrupt vesicular trafficking and function. Mutations in the *LRRK2* gene (encoding the leucine-rich repeat kinase 2), account for the highest proportion of genetic cases of PD [29]. LRRK2 has a functional kinase domain with a multitude of cellular phospho-targets that may alter vesicular activities [30]. In addition, the circularized beta-propeller structure of the WD-40 domains in LRRK2 has been shown to interact with cytoskeletal and vesicular proteins. Mutations in LRRK2 may disrupt vesicular trafficking and endosomal recycling of vesicular proteins (Fig. 1e) [30].

The physiological role of α-synuclein (encoded by *SNCA*) remains elusive, though its genetic and pathological role in PD is well established [31–33]. Monomeric α-synuclein has been shown to reversibly bind and adjoin vesicle membranes [34]. Based on this and its presynaptic localization, it is hypothesized that its endogenous function is to regulate vesicular trafficking between the active zone and cytoplasmic pools. Combining transgenic models of α-synuclein overexpression and deletion, Scott and Roy observed that α-synuclein regulates the size of the recycling pool in cultured hippocampal neurons because overexpression decreased the recycling pool size and deletion increased its size and enhanced intersynaptic vesicular trafficking [35•]. Rats overexpressing human α-synuclein have reduced dopamine vesicle density and correlative reductions in motor activity [36•]. In the context of pathogenesis, fibrilization of α-synuclein forms the primary structural component of Lewy bodies, and toxic α-synuclein protofibrils form as intermediates in the fibrilization process [37]. Oxidized dopamine covalently binds α-synuclein, stabilizing these protofibril intermediates [38] (for a biophysical review of this subject, see [39]). In vitro, oligomeric α-synuclein can also disrupt SNARE complex formation [40]. Furthermore, α-synuclein protofibrils have been shown to permeabilize vesicle membranes, which would facilitate leakage of vesicular dopamine into the cytoplasm and ablate the vesicle electrochemical gradient [41]. These processes may synergistically interact in PD (Fig. 1d) and, in part, explain the vulnerability of dopamine neurons in PD.

Cytoplasmic Dopamine Toxicity and VMAT2

Despite the essential role of dopamine to life processes, it is neurotoxic if vesicular storage is disrupted. Under normal conditions, low levels of dopamine are present in the cytosol following synthesis from DOPA, plasmalemmal transport by the dopamine transporter (DAT), and vesicular leak [42]. Cytosolic dopamine is metabolized by enzymatic deamination or broken down by autoxidation, producing reactive, harmful products. Efficient transport of dopamine by VMAT2 prevents accumulation of these toxic byproducts. Thus, the relative expression of DAT to VMAT2 has been suggested as a determinant of neuronal vulnerability in PD [43, 44].

Enzymatic deamination of dopamine occurs at the mitochondria by monoamine oxidase (MAO), forming DOPAL, a toxic aldehyde intermediate, as well as H_2O_2 [45]. DOPAL is reactive, and it readily forms adducts to cytosolic proteins. Alternatively, it may autoxidize, generating reactive oxygen species [46]. The toxicity of DOPAL has been thoroughly studied in vitro [47] and has been shown to cause neurodegeneration in mice [48]. Relative

DOPAL formation is elevated in the striata of postmortem PD brains [49]. Intraneuronally, DOPAL is preferentially detoxified by aldehyde dehydrogenase to form DOPAC [45].

While autoxidation of cytosolic dopamine occurs readily in the alkaline environment of the cytosol, dopamine is stable in the acidic environment of the vesicle. Autoxidation is particularly deleterious because it yields a reactive dopamine quinone, as well as superoxide, hydrogen peroxide, and a hydroxyl radical. Furthermore, the dopamine quinone itself is highly reactive and forms cysteinyl adducts [50], disrupting the function of target proteins and potentially DNA [51]. Another fate of cytosolic monoamines that is not necessarily toxic is the formation of neuromelanin, the dark pigment that gives the SNpc its color. Notably, neuromelanin is also present in the LC, where oxidation of dopamine and NE also occurs. Neuromelanin forms in the acidic lysosomal compartments, which contain VMAT2 and are capable of monoamine transport. Within the confines of these organelles, dopamine quinones may instead polymerize, forming neuromelanin, [52]. However, neuromelanin biosynthesis from autoxidized dopamine is prevented in cultured nigral rat neurons overexpressing VMAT2, suggesting that the ability to properly store dopamine in vesicles is critical in this process [53].

Numerous studies have shown that unregulated cytosolic dopamine is neurotoxic [50, 54–56]. Mice expressing DAT on non-dopaminergic striatal neurons that lack VMAT2 are able to take up dopamine but not store it in vesicles; these mice exhibit motor deficits and profound striatal neurodegeneration, accompanied by markers of increased dopamine oxidation [57]. In vitro experiments have also demonstrated that relative vulnerability of dopamine neurons in PD may be mediated by levels of cytosolic dopamine [58]. Additionally, transgenic mice with altered expression of VMAT2 (Fig. 1c) have illustrated how crucial vesicular storage of dopamine is to the integrity of the nigrostriatal system. Vesicular function is essential and VMAT2 knockout mice die soon after birth. Heterozygotes develop normally, but have increased sensitivity to amphetamine-induced locomotion, susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity [59–61], and levodopa induced death of SNpc neurons [58]. VMAT2 hypomorphic mice (~5 % wildtype expression; VMAT2LO) have emerged as a mouse model of PD [62–64, 65•]. These mice develop normally, but undergo progressive nigrostriatal degeneration, α-synuclein accumulation, as well as motor and nonmotor symptoms of PD [63, 64]. They also show markers of oxidative stress, including cysteinyl-catechol adducts. Notably, neurodegeneration occurs only when these mice express α-synuclein [63]. VMAT2LO mice exhibit levodopa-correctable motor deficits, including reduced stride-length and locomotor activity, as well as nonmotor symptoms of PD, including anosmia, gastric dysmotility, and depressive and anxiety-like behaviors. In contrast, PACAP38, a neuropeptide shown to increase VMAT2 expression, attenuates methamphetamine-induced neurotoxicity in mice, reduces markers of oxidative stress and neuroinflammation, and suggests that increase VMAT2 protects against oxidative stress [66].

In addition to VMAT2's protective role against cytoplasmic dopamine toxicity, it also plays an important role in neuroprotection against exogenous toxicants that may damage dopamine neurons [16, 67]. The classic dopaminergic toxicant, MPTP, was serendipitously but tragically discovered to induce chronic parkinsonism in humans after intravenous

administration [68]. After crossing the blood-brain barrier, MPTP is metabolized by glial monoamine oxidase to form the toxic metabolite, MPP+, which is then transported into neurons by the dopamine transporter [69–72]. MPP+ causes neurotoxicity by inhibiting mitochondrial respiration and triggering cell death. MPP+ is also a substrate for VMAT2 and can be sequestered away from sites of action. In fact, VMAT2 was initially cloned for its ability to confer resistance to MPP+ toxicity and is evolutionarily related to bacterial toxin extruding antiporters (TEXANS) [73–75]. In support of this, heterozygote VMAT2 knockout mice have heightened sensitivity to MPTP toxicity [61]. It is conceivable that VMAT2 expression may modulate the effects of other exogenous toxicants involved in PD pathogenesis [59, 76].

Pharmacologic Blockade of VMAT2

Carlsson and colleagues' observation that reserpine induced a parkinsonian phenotype that was correctable with levodopa was a launching point for pharmacotherapy in PD [6, 77]. Subsequent experimentation revealed that the effects of reserpine were akin to sympathectomy, in that reserpinized subjects were still responsive to the inotropic and pressor effects of direct-acting sympathomimetics or adrenergic receptor agonists (directly acting sympathomimetics, eg, norepinephrine, epinephrine, neosynephrine), but abolished the effects of indirectly acting sympathomimetics including the phenethylamines, amphetamine, ephedrine, and tyramine [78, 79]. It was later realized that reserpine and the aforementioned indirectly acting sympathomimetics all function by inhibiting VMAT2 (Fig. 1b).

Amphetamine (AMPH) and the substituted AMPH derivative methamphetamine (METH) are catecholamine releasers in the peripheral and central nervous systems. The rewarding effect of striatal dopamine release is largely responsible for the abuse potential of this class of drugs. When abused, AMPH and METH are capable of exerting profound dopaminergic toxicity [84]. AMPH and METH share the same mechanism of action and similar pharmacokinetic and pharmacodynamic properties [80, 81]. AMPH and METH mediate dopamine release by competing for vesicular uptake of dopamine by VMAT2. Furthermore, they are weak bases and may disrupt the electrochemical gradient of the vesicle by accepting free lumenal protons [81]. The increased level of cytosolic dopamine in turn facilitates non-exocytic dopamine release into the synaptic terminal by efflux via the dopamine transporter [81].

Administration of METH in animals causes striatal denervation, including loss of dopamine transporter expression, gliosis, and autophagic vacuolization [82–84]. In rats, this pathology is associated with cysteinyl dopamine adducts, suggesting that dopamine oxidation is indeed involved in METH toxicity [85]. This toxicity is relevant in humans; striatal dopamine transporter loss in METH-treated baboons is comparable with losses in human METH users who reported using similar doses of METH [86]. METH-induced striatal DAT loss in humans is not as severe as losses observed in PD, but it has been shown to persist in patients 3 years after quitting, suggesting long-term damage that may sensitize one to PD [87]. Recently, a retrospective statewide study in California found that patients admitted to the hospital for an AMPH or METH related incident were significantly more likely to develop

PD than matched patient controls admitted for appendicitis (hazard ratio=1.75) [88]. In contrast, the same study showed that patients admitted to the hospital for cocaine-related incidents did not have an increased likelihood of developing PD. Because cocaine inhibits DAT and also increases dopamine levels in the synaptic cleft, these findings support the notion that PD risk may stem from cytosolic dopamine toxicity. In support of this notion, our lab has shown that VMAT2 expression in mice inversely correlates with their susceptibility to METH-induced dopaminergic neurotoxicity [66, 89].

Toxicological Blockade of VMAT2

Epidemiological evidence supporting an environmental connection to PD is compelling [90–99]. As we and others have recently reviewed, several classes of compounds have been associated with PD pathology, especially halogenated persistent organic pollutants (POPs) [16, 100, 101]. Halogenated POPs represent a broad class of environmental toxicants that include organochlorine insecticides, polychlorinated biphenyls (PCBs), and brominated flame retardants. Higher levels of many organochlorines and PCBs have been reported in postmortem brain tissue and serum of PD patients [102–106] and epidemiological studies have linked the compounds to PD [91]. Mechanistic studies into these compounds suggest that many exert selective toxicity to dopaminergic neurons, inhibit synaptosomal and vesicular uptake, and cause oxidative stress [107–125]. Taken together, these data suggest that many compounds epidemiologically linked to PD act by impairing vesicular function and increasing oxidative stress.

Genetic Variability of SLC18A2 in PD

Given the role of VMAT2 in regulating cytosolic dopamine toxicity, mutations affecting the function or expression of the transporter (Fig. 1c) might be expected to affect susceptibility to PD. Genetic mutations in the coding region of *SLC18A2* are extremely rare, reflecting the essential nature of VMAT2 function in an evolutionary context. A recent report identified a crippling movement disorder in members of a consanguineous Saudi Arabian family carrying a recessive mutation in the coding region *SLC18A2* resulting in a P387L substitution in the fifth luminal loop of VMAT2 [126•]. Affected individuals developed infantile-onset parkinsonism, severe cognitive impairment, mood disturbance, and autonomic dysfunction. Heterozygote parents were unaffected by the disorder, but were clinically depressed, consistent with a reduction in brain monoamines. The homozygous patients exhibited reductions in CSF and urinary monoamines, but increased monoamine metabolites. The investigators found that P387L mutation virtually abolished VMAT2 function in transfected cells. Treatment with the dopamine receptor agonist pramiprexole dramatically improved symptoms in all patients, though efficacy appeared to correlate inversely with age. Other coding mutations have been identified in humans, though they are very rare and have not been associated with neurological outcomes [127, 128].

While *SLC18A2* has very little variability in the coding regions, the gene has a large and highly polymorphic promoter sequence (17.4 kb) [127, 129]. This high variability results in many low frequency haplotypes and renders genetic risk assessment difficult. Thus, studies of *SLC18A2* haplotype and PD risk have been inconclusive [127, 130]. However, analyses

of the functional consequences of promoter polymorphisms have provided useful information. Glatt and colleagues [131] screened the most prevalent promoter haplotypes in an American population and identified those that increased transcriptional activity of *SLC18A2* in vitro. Collectively, gain-of-function haplotypes were found to be protective against risk of PD in women; the effect was particularly robust in women homozygous for gain-of-function haplotypes (odds ratio= 0.37). In a more recent study in Italy, investigators identified 2 SNPs within the promoter that conferred protection against PD, presumably by increasing VMAT2 expression [132•]. The variability of the *SLC18A2* promoter suggests that genetic and epigenetic influences may contribute to variable VMAT2 expression and risk of PD within populations.

VMAT2 Imaging in PD

Positron emission tomography (PET) ligands of striatal dopamine terminal markers have been used to monitor the progression of PD and response to therapeutic treatments. VMAT2 has emerged as a reliable marker of striatal innervation in PD [76, 133, 134]. High-affinity radioligands for DAT and the fluorinated DOPA analog, ^{18}F-DOPA, have been utilized for PET scans, but their binding substrates were found to be modulated by levodopa and dopamine agonists. For instance, changes in DAT binding by β-CIT in response to pramipexole (increased binding) and levodopa (reduced binding) had been mistakenly interpreted as changes in striatal innervation, when in fact DAT membrane expression is modified by these therapies [135, 136]. ^{18}F-DOPA PET signal does not reliably correlate with striatal innervation because expression of AADC, for which ^{18}F-DOPA is a substrate, increases in response to neuronal damage [137–140]. In contrast to DAT, VMAT2 expression is not modified by the PD therapeutics levodopa or selegiline [138, 141]. Bohnen and colleagues demonstrated that striatal C11-dihydrotetrabenazine binding correlated reliably to clinically rated (UPDRSIII) motor deficits in PD patients [133]. Another VMAT2 radioligand, F-18-AV-133 (a tetrabenazine derivative), is also now used to study nigrostriatal degeneration [142, 143]. While VMAT2 radioligand binding conveniently correlates to nigrostriatal denervation, it does not provide direct evidence of vesicular function or VMAT2 activity.

VMAT2 as a Peripheral Biomarker of PD

Studies examining VMAT2 in the human periphery may provide insight into how to measure central VMAT2 activity in PD. VMAT2 mediates the storage and release of norepinephrine in postganglionic neurons of the sympathetic nervous system. Sympathetic denervation commonly occurs in PD; orthostatic hypotension is a comorbid feature, affecting 30 % of PD patients [144, 145]. In vivo imaging of sympathetic denervation in PD patients is accomplished with radiolabeled ligands for both the norepinephrine transporter (expressed at the plasma membrane of sympathetic neurons) and VMAT2 [146–150]. Sympathetic denervation may occur prior to the onset of motor symptoms of PD, with noradrenergic denervation of the heart paralleling the dopaminergic denervation of the striatum [149, 151].

A novel approach pairing analysis of myocardial ^{18}F-DA uptake and arterial ^{18}F-DOPAC formation can provide an in vivo index of vesicular activity [152]. ^{18}F-DA uptake is dependent on the presence of intact noradrenergic terminals, and MAO metabolism of ^{18}F-DA to ^{18}F-DOPAC is in competition with vesicular uptake [153]. Thus, one can determine both the extent of cardiac noradrenergic innervation (by PET imaging) and the relative uptake activity in vesicles (by measuring relative 18 F-DOPAC formation) with these combined methods. Goldstein and colleagues performed this analysis in patient cohorts including those with PD with orthostatic hypotension, pure autonomic failure (a Lewy body disease featuring orthostatic hypotension without parkinsonism), multiple systems atrophy (a non-Lewy body synucleinopathy that clinically resembles PD), and healthy controls. The authors concluded that myocardial vesicular function was impaired only in patients with Lewy body diseases (PD with orthostatic hypotension and in pure autonomic failure), consistent with the effects of pathogenic α-synuclein in disrupting vesicular function [39, 154].

VMAT2 is also present in platelets and may serve as a peripheral biomarker of monoaminergic vesicular function. Platelets express proteins involved in the storage and metabolism of serotonin, including the serotonin transporter (SERT), VMAT2, and monoamine oxidase. These blood cells have been used as models of serotonin neurons [155]. Profiles of imipramine binding to platelet SERT have been used as peripheral biomarkers of serotonergic neurotransmission in the brain [156–160]. Similarly, platelet VMAT2 levels may serve as a peripheral biomarker of central VMAT2 expression. Alterations in VMAT2 dihydrotetrabenazine binding in platelets have been linked to cases of depression, juvenile behavior disorders, and schizophrenia [161–169]. Platelet *SLC18A2* mRNA levels may also be predictive of PD. In 39 PD patients vs 39 healthy control subjects, the relative quantity of *SLC18A2* mRNA in platelets was decreased by 23 % in the PD patients [170]. Therefore, assessing platelet expression of VMAT2 may prove useful as a noninvasive biomarker of PD risk.

Conclusions

We have summarized ways in which alterations in vesicular storage of dopamine influences neuronal viability and susceptibility to neurodegeneration in PD. All disruptions of vesicular storage discussed have the overall effect of increasing cytosolic dopamine. In sporadic PD, it is conceivable that a combination of genetic and epigenetic factors decrease levels of functional VMAT2 while environmental exposure to compounds that alter vesicular function may have this same effect. Therefore, measuring vesicular uptake capacity in human patients may represent a useful measure of PD risk. Furthermore, interventions aimed at increasing vesicular function may be beneficial in the treatment of PD and may even be neuroprotective. These warrant a closer look in controlled trials.

Acknowledgments

P01 ES016731, T32 ES012870, T32 GM008602, and T32 DA015040.

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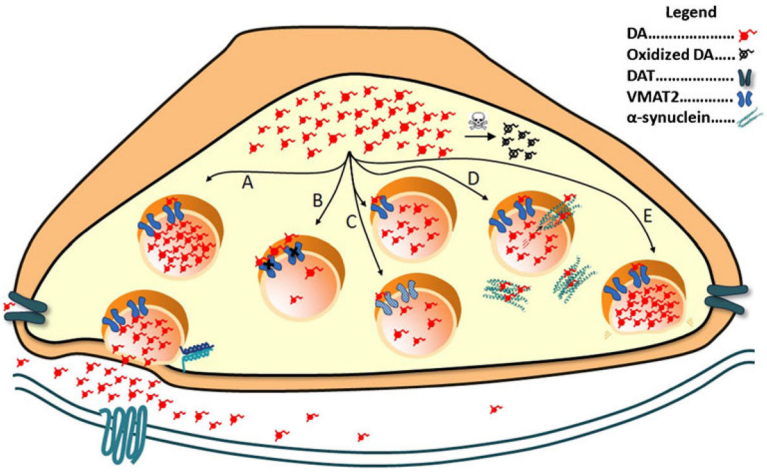

Fig. 1.

Mechanisms of vesicular disruption in Parkinson's disease. After synthesis, dopamine is transported into vesicles. Cytoplasmic dopamine can be oxidized; in excess, this can lead to cellular injury. **a**, Vesicles in the readily releasable pool dock at the presynaptic membrane. In response to an action potential and calcium influx, vesicle membranes fuse to the plasma membrane and release their contents into the synaptic space. Dopamine is then reclaimed by the dopamine transporter (DAT) and then subsequently repackaged into the vesicle. **b**, Pharmacological inhibition of VMAT2 prevents uptake of dopamine leading to a depleted vesicle and reduced release. **c**, Genetic reduction of VMAT2 expression via promoter polymorphisms in humans or genetic manipulation in mice (upper vesicle) or VMAT2 function as noted in [126•] (lower vesicle) causes a reduction in vesicular filling and subsequent release. **d**, α-synuclein can form pores in the vesicle and cause depletion of dopamine from the vesicle or interact with cytoplasmic dopamine to form toxic species. **e**, Altered trafficking of the vesicle to the presynaptic membrane has been proposed to occur in the presence of overabundant α-synuclein or mutated LRRK2