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Overexpression of Vesicular Monoamine Transporter-2 may Block Neurotoxic Metabolites from Cytosolic Dopamine: a Potential Neuroprotective Therapy for Parkinson's Disease

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Abstract

The loss of nigrostriatal dopaminergic neurons containing neuromelanin underlies the motor symptoms of Parkinson's disease. Neuromelanin accumulation into autophagic lysosomes is evidence of ongoing cytosolic dopamine stress in these neurons during normal aging. The formation of neuromelanin is likely neuroprotective, as oxidation of cytosolic dopamine to quinones and aldehydes, as reviewed here, can produce a host of neurotoxic sequela. In addition to sequestration of dopamine and its metabolites in autophagic lysosomes, the uptake of dopamine into monoaminergic neurons mediated by vesicular monoamine transporter-2 (VMAT- 2), prevents dopamine oxidation. Dopamine is stable in monoaminergic vesicles due to their low pH, and thus overexpression of VMAT-2 may provide a target for potential neuroprotective therapy in Parkinson's disease.

Dopamine Synthesis and Accumulation in Synaptic Vesicles

Dopamine is required for the normal regulation of motor activity. Dopamine is synthesized by tyrosine (4-hydroxyphe-nylalanine) conversion to L-3,4- dihydroxyphenylalanine (L-DOPA) in a reaction catalyzed by the tyrosine hydroxylase. Tyrosine hydroxylase uses one oxygen atom to hydroxylate the carbon atom at position 3 in tyrosine to produce the catechol structure (hydroxyl groups in positions 3 and 4 of L-DOPA). L-DOPA is converted to dopamine in the cytosol by aromatic L-amino acid decarboxylase, which uses pyridoxal phosphate as a cofactor [1–6]. One important feature of dopamine synthesis in neurons is the rapid uptake of cytosolic dopamine into monoaminergic vesicles catalyzed by vesicular

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monoamine transporter-2 (VMAT-2; SLC18A2), which physically and functionally interacts with enzymes responsible for DA synthesis [7].

Dopamine is stored in synaptic vesicles for neurotransmission, where it is stable due to the low pH. Dopamine uptake into monoaminergic vesicles catalyzed by VMAT-2 is coupled to an H+-ATP dependent proton pump localized in monoaminergic vesicle membranes. The pumping of protons into the monoaminergic vesicles reduces the pH, generating an environment of around pH5, where dopamine is stable and cannot oxidize to o-quinones [5–10]. Dopamine is released from synaptic vesicles during neurotransmission, and particularly in the striatum, the dopamine transporter (DAT) takes up dopamine from the extracellular space to replenish synaptic vesicle stores for subsequent rounds of neurotransmission. Interesti-ngly, DAT, VMAT-2 and synaptogyrin-3 have been suggested to form a complex that could prevent oxidation of free dopamine in the cytosol [11] [Figure. 1].

The basis for selective death of specific neuronal populations in neurodegenerative diseases remains unclear. Parkinson's disease (PD) is a synucleinopathy characterized by a preferential loss of dopaminergic neurons containing neuromelanin in the substantia nigra (SN), whereas neurons of the ventral tegmental area (VTA) that do not contain neuromelanin are quite spared until the late stage of PD. Using intracellular patch electrochemistry to directly measure cytosolic dopamine (DAcyt) in cultured midbrain neurons, we confirmed that elevated DAcyt and its metabolites are neurotoxic and that genetic and pharmacological interventions that decrease DAcyt provide neuroprotection. L-DOPA increased DAcyt in SN neurons to levels 2–3-fold higher than in VTA neurons, a response dependent on dihydropyridine-sensitive Ca^{2+} channels, resulting in greater susceptibility of SN neurons to L-DOPA-induced neurotoxicity. DAcyt was not altered by α-synuclein deletion, although dopaminergic neurons lacking α-synuclein were resistant to L-DOPA induced cell death. Thus, an interaction between Ca^{2+}, DAcyt and α-synuclein may underlie the susceptibility of SN neurons in PD, suggesting directions for multiple therapeutic targets [12].

Dopamine metabolism to quinones

While dopamine is stable inside synaptic vesicles, cytosolic dopamine can autoxidize and be neurotoxic [12]. Dopamine can oxidize to dopamine ortho(o)-quinone, which cyclizes to aminochrome with a rate of s^{-1} as dopamine o-quinone is stable at a pH lower than 2 [13, 14]. Aminochrome is the most stable o-quinone because its rearrangement to 5,6-indolequinone has a rate of 0.06 m^{-1}, and likely polymerizes after reaction with proteins to form the pigment neuromelanin [15] [Figure 2]. Dopamine oxidation, its reaction with proteins and lipids that form neuromelanin pigment inside autophagic-lysosomal organelles occurs during normal aging as neuroprotective process [16–18]. Neuromelanin accumulation in dopamine neurons of human SN can be visualized by MRI during aging, and in PD neuromelanin loss can be imaged [1, 17, 19–21]. However, o-quinones formed by dopamine oxidation can be neurotoxic, as dopamine o-quinone forms adducts with proteins including ubiquitin C-terminal hydrolase-L1, Parkinson protein 7, mortalin/GRP75/mthsp70 and actin, in cell cultures [22]. Dopamine oxidation also produces an adduct with parkin [23]. Accumulation of cytosolic or extraneuronal DA and subsequent oxidation to quinone species can induce nonspecific modifications on proteins. These processes require the presence of

redox active metals such as iron and copper, both abundant in dopaminergic brain areas. The reaction between dopamine quinone species and proteins occurs on specific amino acids according to the reactivity order, cysteine >> histidine > lysine [24].

The dopaminochrome structure is unclear and it is unknown if its structure corresponds to one of the two other o-quinones reported, that is aminochrome or 5,6-indolequinone. Dopaminochrome has absorption maxima at 303 and 479 nm [25], but the structure has not been determined by NMR, while aminochrome has absorption maxima at 280 and 475 nm and its structure has been confirmed by NMR [26]. It is therefore possible that dopaminochrome corresponds to 5, 6-indolequinone or an unidentified o-quinone.

Aminochrome is also potentially neurotoxic, due to a host of potential mechanisms, like inducing alpha-synuclein aggregation to neurotoxic oligomers [27, 28], mitochondria dysfunction by decreasing mitochondrial membrane potential and ATP levels [29–34], autophagy dysfunction [35–37], increased lysosome pH [38, 39], disruption of cytoskeleton architecture [26, 40], inhibition of axonal transport of monoaminergic vesicles to the terminals in the striatum [34], decreased dopamine release [34], neuroinflammation [38, 39], proteasome dysfu-nction [43, 44], and endoplasmic reticulum and oxidative stress [45].

The 5,6-indolequinone has also been reported to form adducts with alpha-synuclein in studies performed with NMR[15], having toxic effects similar to those of aminochrome previously described. Dopaminochrome induces neurotoxicity in cells, with a slow and progressive loss of dopaminergic neurons after intranigral injection and it has been reported to form adducts with alpha-synuclein [46–50].

Dopamine metabolism to aldehydes

Free dopamine in cytosol is degraded through oxidative deamination catalyzed by monoamine oxidase to 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is oxidized to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase-1 [5, 6] [Figure 2]. Two forms of aldehyde dehydrogenase are expressed in human SN (1 and 2), but only aldehyde dehydrogenase-1 expressed in the cytosol is decreased in PD patients [51–53] and roles for it have been proposed in the degeneration of the nigrostriatal system [54–56].

Intracerebral injection of DOPAL into the SN and the VTA induced loss of tyrosine hydroxylase positive staining [57]. DOPAL induces alpha-synuclein aggregation in vitro and in animals when injected into the SN [58]. Additionally, divalent metal ions can enhance DOPAL-induced aggregation of alpha-synuclein [59]. DOPAL induces aggregation of alpha-synuclein by generating dicatechol pyrrole adducts with lysine [60]. DOPAL-induced alpha-synuclein oligomers inhibit the formation of mature amyloid fibrils. DOPAL affects alpha-synuclein function by disturbing its interaction with lipid membranes and its role in the regulation of synaptic vesicle traffic in neurons. DOPAL-induced alpha-synuclein oligomers induce dopamine leakage in a cellular model and in an in vitro model of synaptic vesicles [61–63].

Inhibition of VMAT-2 by reserpine increases the level of DOPAL in PC12 cells, contributing to apoptosis [55].

Accumulation of cytosolic o-quinones into neuromelanin

The conversion of dopamine to neuromelanin requires the formation of dopamine o-quinone, aminochrome and 5,6-indolequinone sequentially in the cytosol. The oxidation of dopamine is catalyzed by iron, which is abundant in SN dopaminergic neurons, and is then bound into neuromelanin pigment during its biosynthesis [17]. As above, these o-quinones can be neurotoxic and induce the loss of dopaminergic neurons of the nigrostriatal system in PD if not efficiently removed. However, healthy seniors have viable dopaminergic neurons that contain high levels of neuromelanin. This apparent paradox can be explained by the existence of two enzymes that play a neuroprotective role against quinone toxicity in dopa-minergic neurons. DT-diaphorase, which is expressed in both dopaminergic neurons and astrocytes, prevents the neurotoxic effects of aminochrome by reducing aminochrome with two electrons to leukoaminochrome. Leukoaminochrome can rearrange its structure to form 5,6-dihydroxyindole, which can oxidize to one of the direct precursors of neuromelanin 5,6-indolequinone. These quinones can react with aggregated proteins to form adducts that undergo further reactions to generate neuromelanin pigment that immobilize potentially toxic dopamine-derived compounds along with metals, several proteins and lipids inside specific autolysosomes [16]. DT-diaphorase prevents aminochrome induced mitochondrial dysfu-nction [29–31], alpha-synuclein aggregation to neurotoxic oligomers [27,28], proteasome dysfunction [43], lysosome dysfunction [39], cytoskeleton architecture disruption [26] and cell death [64]. The other neuroprotective enzyme is glutathione transferase M2–2 (GSTM2), which can detoxify dopamine o-quinone and aminochro-me by conjugating these o-quinones with glutathione [65–67]. GSTM2 is produced only in astrocytes, but these cells secrete GSTM2 that dopaminergic neurons accumulate in their cytosol, protecting neurons from the neurotoxic effects of dopamine o-quinone and aminochrome [68–70].

Protective Roles of VMAT-2

VMAT-2 activity decreases the level of free cytosolic dopamine by sequestering it into synaptic and other secretory vesicles where it remains stable and can be used for neurotransmission. VMAT-2 expression has long been known to be neuroprotective, as it was originally cloned due to its role in protecting cells from MPP+ toxicity [71], and it also protects dopamine neurons from ampheta-mine neurotoxicity [72] and L-DOPA toxicity [12], presumably by decreasing cytosolic amphetamine and dopamine levels in the cytosol and preventing the formation of the toxic metabolites discussed above.

It has been suggested that the ventral SN neurons accumulate the most neuromelanin pigment because they have lower VMAT-2 expression, while the midbrain dopaminergic neurons of VTA, which produce larger amounts of dopamine, have more vesicular storage capacity for action potential-induced release of the neurotransmitter and then lower levels of neuromelanin [73]. This idea is supported by the observation that overexpression of VMAT-2 prevents neuromelanin biosynthesis [74].

Analysis of dopamine storage vesicles from the PD striatum revealed a significant decrease of VMAT-2 expression in patients’ caudate and putamen nuclei in comparison

to control brains [75]. It therefore appears plausible that overexpression of VMAT-2 in dopaminergic neurons of the nigrostriatal system could provide a gene therapy aimed at preventing dopamine oxidation-induced neurotoxicity (Figure 2). In support of this hypothesis, it was suggested that variability in VMAT-2 promoter region may reduce the risk of developing PD, so that increased VMAT-2 levels may confer protection against the disease [76]. Additionally, targeted manipulation of VMAT-2 expression in PD patients could also improve the efficacy of dopamine derived from L-DOPA administrations, increasing dopamine availability for neurotransmission in the surviving nigrostriatal neurons. Therefore, future interventions on VMAT-2 may be a viable therapeutic approach to address both dopamine deficits in neurotransmission and dopamine-derived neurotoxicity.

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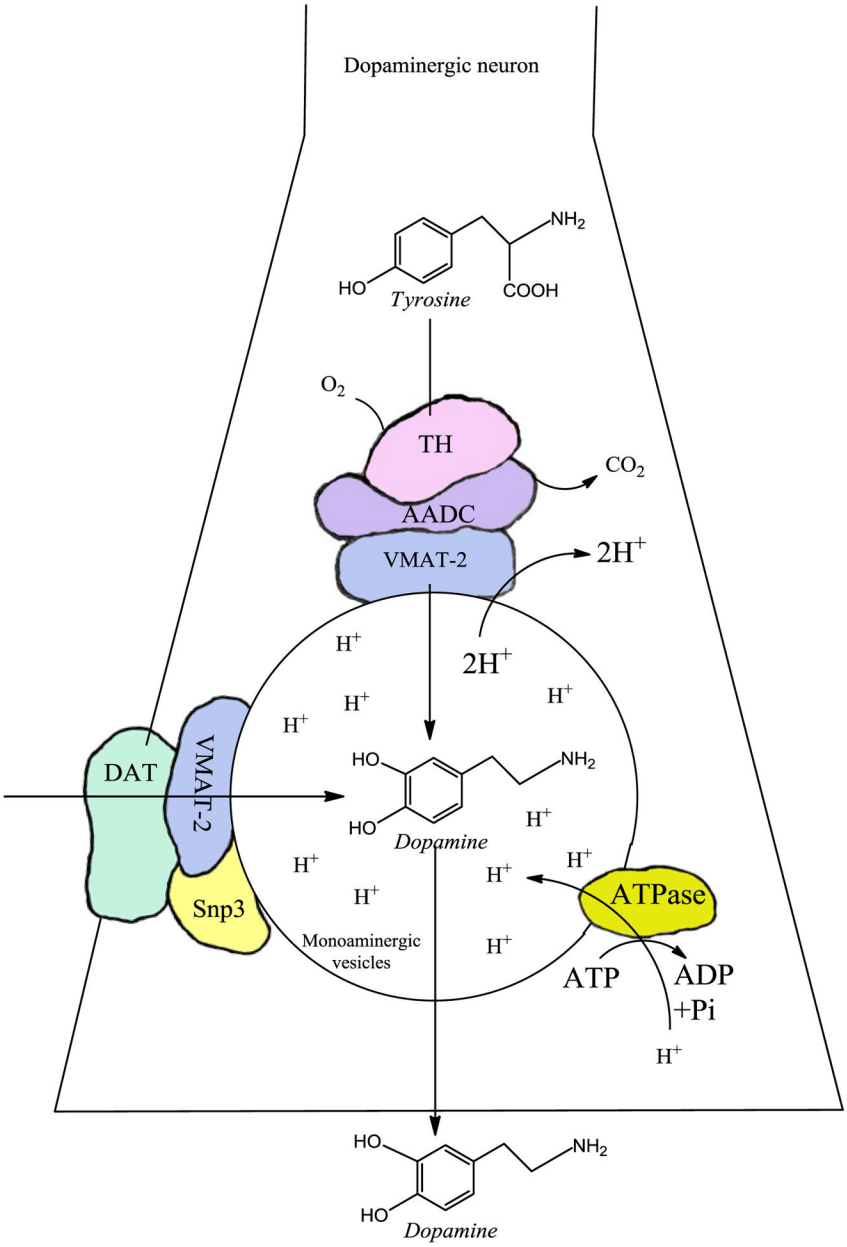

Figure 1: Dopamine synthesis and uptake.

There are two sources of dopamine in dopaminergic neurons. (i) Synthesized dopamine. Dopamine is synthesized in the cytosol of dopaminergic neurons where the amino acid tyrosine is hydroxylated by the enzyme tyrosine hydroxylase, forming L-DOPA. Subsequently, L-DOPA is converted to dopamine by the aromatic L-amino acid decarboxylase. However, these reactions do not produce free dopamine in the cytosol, since it has been reported that VMAT-2 forms a complex with the aromatic L-amino acid decarboxylase and tyrosine hydroxylase. VMAT-2, expressed on the surface of the monoaminergic vesicles, immediately transports dopamine into these vesicles. Monoaminergic vesicles have a low pH because they have an H^+-ATPase that pumps protons inward, generating an acid environment where dopamine accumulates without

risk of oxidation because it is completely stable. (ii) The other source of cytosolic dopamine is by reuptake via DAT to the cytosol. Interestingly, DAT has been suggested to form a complex with synaptogyrin-3 and VMAT-2 that immediately transports imported extracellular dopamine towards the monoaminergic vesicles, preventing the existence of free cytosolic dopamine and its autoxidation to neurotoxic o-quinones.

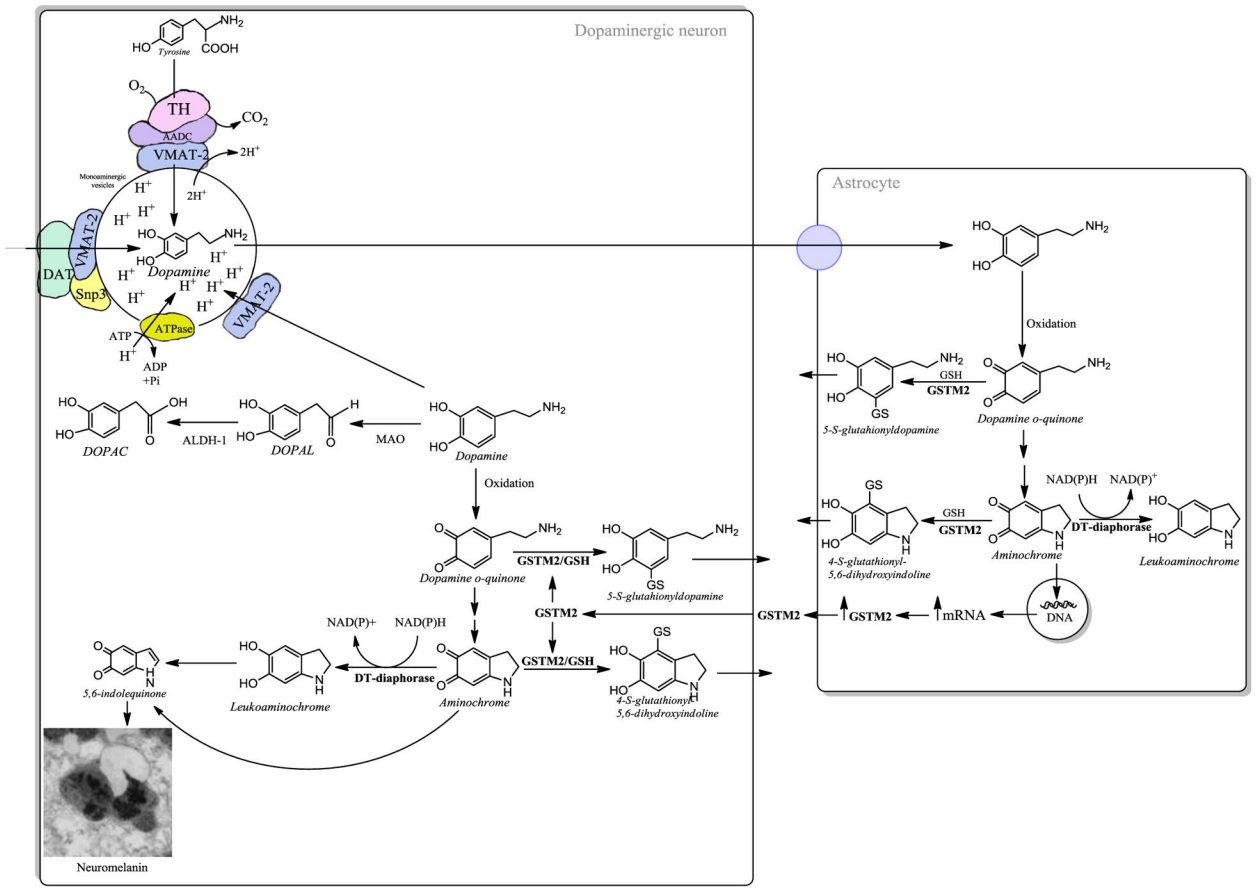

Figure 2: Neuroprotection in dopaminergic neurons.

The presence of free dopamine in the cytosol is a risk, because during the oxidation of dopamine to neuromelanin (through a multi-step biosynthetic mechanism that involves iron-catalyzed oxidation, polymerization and reaction with proteins and lipids), or in its degradation catalyzed by monoamine oxidase (MAO in the Fig.), neurotoxic o-quinones can be generated. Therefore, VMAT-2 is the first line of neuroprotection against the neurotoxic effects of dopamine oxidation. VMAT-2 protects dopaminergic neurons by transporting dopamine towards the monoaminergic neurotransmission vesicles. Dopaminergic neurons are likely exposed to dopamine-derived damage when levels of VMAT-2 are not high enough to limit free dopamine and its autoxidation occurs in the cytosol. In this case neuromelanin biosynthesis provides an additional means to limit dopamine toxicity through a neuroprotective mechanism. There are two enzymes that prevent the neurotoxic effects of dopamine o-quinone and aminochrome in dopaminergic neurons. The enzyme DT-diaphorase catalyzes the two-electron reduction of aminochrome to leukoaminochrome, then leading to formation of neuromelanin precursors and the enzyme GSTM2, which can inactivate both dopamine o-quinone and aminochrome by conjugating these o-quinones with glutathione. GSTM2 is expressed only in human astrocytes, which secrete this enzyme. Dopaminergic neurons are able to internalize GSTM2 into the cytosol, where this enzyme conjugates these o-quinones with glutathione which undergo further degradation forming other probable precursors of neuromelanin pigment.