Dysfunction of dopamine homeostasis: clues in the hunt for novel Parkinson’s disease therapies

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ABSTRACT Parkinson’s disease is the second most common neurodegenerative disorder and, at present, has no cure. Both environmental and genetic factors have been implicated in the etiology of the disease; however, the pathogenic pathways leading to neuronal degeneration are still unclear. Parkinson’s disease is characterized by the preferential death of a subset of neurons in the mesencephalon that use dopamine as neurotransmitter for synaptic communication. Dopamine is a highly reactive molecule that can lead to cytotoxicity if not properly stored and metabolized. Targeting any of the pathways that tightly control this neurotransmitter holds great therapeutic expectations. In this article we present a comprehensive overview of the cellular pathways that control dopamine fate and discuss potential therapeutic approaches to counteract or slow Parkinson’s disease onset and progression.—Bisaglia, M., Greggio, E., Beltramini, M., Bubacco, L. Dysfunction of dopamine homeostasis: clues in the hunt for novel Parkinson’s disease therapies. FASEB J. 27, 2101–2110 (2013). www.fasebj.org

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Although dopaminergic (DAergic) neurons constitute <1% of the total brain neurons, numerous functions are based on the activity of this class of nervous cells. DAergic pathways play a critical role in both mental and physical functions including behavior and cognition, voluntary movement, motivation and reward, inhibition of prolactin production, sleep, mood, attention, and learning (1).

Degeneration of DAergic neurons in distinct nuclei of the brain results in a severe condition, Parkinson’s disease (PD), which affects >1% of people >65 yr old. PD is characterized by a preferential degeneration of DAergic neurons in the substantia nigra (SN) pars compacta, with clinical manifestations consisting in motor dysfunctions such as tremor, postural instability, and rigidity. Due to the increase in life span of Western populations, the social effects of this incurable disease are expected to increase dramatically in the coming decades.

The aim of this review is to critically analyze the current literature to dissect the potential relationship between impairment of dopamine (DA) metabolism, i.e., synthesis, storage, reuptake, and degradation, and PD onset and progression. The correlation between molecular dysfunctions in DA metabolism and pharmacological treatments designed to counteract and/or ameliorate disease symptoms will also be discussed.

DAergic Pathways

DAergic neurons form a neuronal network, originating in the ventral tegmental area (VTA), in the SN, and in the hypothalamus (1). From these regions, DAergic neurons project axons to large areas of the brain through 4 major pathways (1).

Mesolimbic pathway

The mesolimbic pathway connects the VTA to the limbic system, including the nucleus accumbens, via the amygdala and the hippocampus. The mesolimbic pathway is important for memory and motivating behaviors. By blocking this pathway, antipsychotic drugs reduce the intense emotions caused by conditions such as schizophrenia.

Mesocortical pathway

The mesocortical pathway originates in the VTA and projects to the frontal cortex and surrounding structures. A malfunction in this pathway seems to be related...
to some of the symptoms of schizophrenia, such as hallucinations and disordered thinking.

**Tuberoinfundibular pathway**

The tuberoinfundibular pathway connects the hypothalamus to the pituitary gland, where it influences the secretion of hormones, such as prolactin.

**Nigrostriatal pathway**

The nigrostriatal pathway projects axons from the SN to the striatum (caudate nucleus and putamen) and is involved in motor control. Binding to the DA receptors, D1–5, DA reduces the influence of the indirect pathway and increases the actions of the direct pathway within the basal ganglia. Degeneration of the neurons in this pathway is associated with the trembling and muscular rigidity symptomatic of PD.

**DA AND PD**

PD, the most common neurodegenerative movement disorder, is a chronic and progressive disease. The preferential loss of DAergic neurons from the pars compacta of the SN is one of the key pathological hallmarks of PD and is responsible for the motor symptoms associated to this disorder.

As neither the cause nor the mechanism of DAergic neuronal death has been fully elucidated, drugs that can arrest, reduce, and/or delay the death of these neurons are not yet available. The current therapy to replace the loss of DA is mainly based on the administration of l-DOPA, a DA precursor that crosses the blood-brain barrier. l-DOPA therapy is initially effective in ameliorating symptoms in most patients but often loses its efficacy after several years of treatment. Complications associated with its use are involuntary movements, which occur in many patients within several years, and an unpredictable “on-off” effect in the course of treatment (2). Other adverse effects include nausea, a drop in blood pressure (hypotension), and, occasionally, psychotic symptoms (2). In addition, l-DOPA treatment requires a certain number of live DAergic neurons to convert this metabolic precursor into DA. Thus, during PD progression, the increased cell death of DA neurons leads to a lower DA synthesis during l-DOPA treatment, rendering it progressively less effective.

Even though the pathogenic origins of PD remain elusive, several clues support the role of oxidative stress in its progression. Postmortem analyses on patients with PD, compared with controls, revealed an increase in carbonyl modifications of soluble proteins (3); in 4-hydroxy-2-nonenal, a lipophilic product of lipid peroxidation (4); and in 8-hydroxydeoxyguanosine, a marker of DNA oxidation (5). Moreover, mutations in 3 genes, coding for parkin, DJ-1, and PINK1, causative for recessive familiar forms of PD, lead to mitochondrial dysfunction and oxidative damage (6, 7).

One possible mechanism responsible for the increase of oxidative stress, which could account for the preferential degeneration of DAergic neurons in PD, involves the redox reactions specific to DA. Several pathways have been identified in the oxidation of DA (8). First, at physiological pH, cytosolic DA can self-oxidize to form reactive oxygen species, which, in turn, can damage cellular components, such as lipids, proteins, and DNA. Second, the self-oxidation of DA can also generate DA-derived quinones (DAQs) that can react with cellular nucleophiles, exacerbating cytotoxicity. Indeed, DAQs have been shown to bind to cysteinyl residues of proteins both *in vitro* and *in vivo* (9–14).

From the observation described above, it appears clear that while loss of DA release in the striatum is responsible for the motor symptoms associated with PD, its cytosolic accumulation can promote DAergic neuron degeneration. In the following section, the different steps involved in the cellular homeostasis of DA will be discussed in the context of PD.

**DA METABOLISM**

Under physiological conditions, DA is synthesized in the cytoplasm by the action of tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC), and it is rapidly sequestered into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2), where it is stabilized by the low pH. Following its release into the synaptic cleft, DA reuptake rapidly ensues *via* the DA transporter (DAT). If the amount of cytosolic DA exceeds the physiological concentration, it can be metabolized *via* monoamine oxidase (MAO) and aldehyde dehydrogenase (ALDH) into the nontoxic metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). Alternatively, within astrocytes DA can be catabolized into homovanillic acid (HVA) through the combined action of catechol-O-methyltransferase (COMT), MAO, and ALDH (see Fig. 1).

**Synthesis**

Tyrosine, a nonessential amino acid, widely available in the normal diet from protein-rich sources, is the starting point for the synthesis of DA. Inside DAergic neurons, the hydroxylation of tyrosine to form l-DOPA, catalyzed by TH, is the rate-limiting step in the biosynthetic process. TH is a monoxygenase that uses tyrosine and molecular oxygen as substrates, as well as (6R)-1-erythro-tetrahydrobipterin (BH4) and a protein bound nonheme Fe(II) ion, as cofactors (15). BH4 in particular, by reducing the iron to the ferrous form and returning TH to the fully active state, is essential for the redox turnover of the protein. As BH4 synthesis depends on guanosine triphosphate cyclohydrolase I (GTPCH), this latter enzyme is indirectly acting as a limiting factor in the biosynthesis of DA. According to...
decreased GTPCH enzymatic activity results in a reduction of TH activity and DA synthesis (16). The essential role of TH and GTPCH in DA homeostasis is further evidenced by the observation that mutations of GTPCH gene, as well as homozygous missense mutations in TH gene, have been associated with DA-related phenotypes, such as Segawa’s syndrome, also called l-DOPA-responsive dystonia, and l-DOPA responsive infantile parkinsonism (17–19). Recently, a novel deletion of entire TH gene in an adult with PD has been reported suggesting that haploinsufficiency of TH could increase PD risk (20). It has also been shown that mRNA levels of TH and AADC are decreased in the SN and striatum in the PD brain (21).

An alternative hypothesis correlates TH and PD through oxidative stress. TH generates highly reactive intermediates to introduce hydroxyl groups into a phenolic ring, and it has been suggested that this could contribute to the formation of reactive oxygen species (22). A recent report by Choi et al. (23) reported that BH4 leads to generation of oxidative stress and selective DAergic neurodegeneration both in vitro and in vivo. At the same time, TH itself has been demonstrated to be target of oxidative damages. Specifically, a pronounced increase (59%) in carbonyl modifications of TH and a significant decrease in specific TH activity have been observed in the SN of aged rats compared with young animals (24). Following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice or after exposure of PC12 cells to either peroxynitrite or 1-methyl-4-phenylpyridinium (MPP+), it has also been shown that TH is a potential target for nitration, with a resulting loss of its enzymatic activity (25). With specific reference to the potential role of DA itself in the preferential loss of DAergic neurons, two independent studies have demonstrated that DA quinones covalently modify and inactivate TH (9, 26). Specifically, quinones modify TH sulfhydryl groups and convert the enzyme to a redox-cycling quinoprotein. Quinotyrosine hydroxylase causes the reduction of the transition metals iron and copper and may therefore contribute to Fenton-like reactions and oxidative stress in neurons (9).

Taken together, these studies point to a link between TH activity and pathology of DAergic systems in the human brain. TH inactivation, and consequent DA synthesis failure, could represent an early pathological event in PD etiology. If this is the case, it follows that the TH gene is a promising target for developing treatment strategies against motor dysfunction or to slow down the progression of PD, based on correcting or bypassing the enzyme deficiency. In this context, TH and GTPCH gene replacement therapy offers potential treatment for PD. Although many different viral platforms have been developed with the aim of facilitating gene transfer into humans, based on the criteria of safety, stability of gene expression, and transduction of
specific cellular targets, only adeno-associated virus (AAV) and lentivirus vectors are currently exploited in clinical trials for PD (27). Most individuals that have been exposed to AAV through environmental exposure develop a minimal immune response when the host encounters the latter as a vector (28). Lentiviruses have the advantage of being able to package a larger genetic cargo, allowing the transfer of more or larger genes, but there are more concerns of a proinflammatory and infectious response from the host. For these reasons, lentivectors have been genetically engineered to be self-inactivating (28). As a proof of concept, Mandel et al. (29) used AAV vectors injected into the striatum to overexpress both TH and GTPCH to achieve continuous l-DOPA delivery in a rat model of PD. Rats that received the TH-AAV vector alone produced measurable l-DOPA only after receiving exogenous BH4, while elevated l-DOPA levels were observed in animals that received mixed TH- and GTPCH-AAV vectors. Although a direct connection between AAV and PD has not been established, an alternative strategy to increase DA concentration would be to inject a vector encoding the AADC gene into the striatum of patients with PD to increase local conversion of exogenous administered l-DOPA. Studies on nonhuman primates demonstrated that gene therapeutic delivery of human AADC via AAV vector technology restored the ability of the striatum to convert l-DOPA into DA and abolished PD-like behavior in MPTP-based models of the disease (30, 31). In addition to increasing DA synthesis in surviving DAergic neurons, it has been suggested that the direct transduction of non-DA striatal neurons could provide a new source of DA (28). Thus, the conversion to DA is regulated by oral administration of l-DOPA and it does not require healthy SN DAergic neurons. This last aspect is particularly appealing, as at the onset of symptoms, ~60% of SN DAergic neurons have already been lost (32). In another PD model, 6-hydroxydopamine-lesioned rats, it has been demonstrated that cotransduction with TH and AADC AAV vectors resulted in more effective DA production and significantly improved behavioral recovery compared with rats receiving AAV-TH alone (33). Following the success by cotransduction of TH and AADC, a further study attempted a triple transduction combining AAV vectors expressing TH, GTPCH, and AADC (34). This treatment resulted in a greater DA production than double transduction with TH and AADC. Triple transduction also enhanced BH4 and DA production in the denervated striatum of parkinsonian rats and improved the rotational behavior of rats more effectively than double transduction. A potential problem with the gene therapy described so far is that non-DAergic striatal neurons, which normally use GABA as their neurotransmitter, are transduced and coopted to produce and release DA. It is not clear whether GABAergic neurons are able to properly manage DA, i.e., to store, release and metabolize this neurotransmitter. Nevertheless, in a triple transduction study, based on a monkey model of PD, the therapy was not only safe but also provided long-term (~44 mo) improvement without evidence of l-DOPA-induced dyskinesias (35).

It is worth mentioning that gene therapy-based studies are not restricted to animal models of PD but are also being tested in phase I and II clinical trials (see ref. 34 for a review). A clinical trial by Oxford BioMedica (Oxford, UK) was designed to test the effect of transferring TH, GTPCH, and AADC genes into striatal neurons via a multicistronic lentiviral vector. Two different doses were evaluated, and although experiments were carried out in a very small number of patients (3 subjects/dose), both doses were well tolerated, without reported adverse effects and with improved motor function, as well as quality of life (clinical trial identifier NCT00627588).

Storage

The vesicular monoamine transporter VMAT2, a 12-transmembrane domain protein belonging to a solute carrier protein family (36), is responsible for the packaging of DA into synaptic vesicles. The transport of DA across the vesicular membrane requires a vacuolar ATPase, which transports protons (H+) from the cytoplasm into the vesicle, generating an electrochemical gradient across the vesicular membrane. This allows VMAT2 to act as an antiporter, exchanging 2 protons for a single DA molecule (36). Thus, VMAT2 is essential for reducing cytoplasmic DA accumulation after de novo synthesis and/or synaptic uptake.

Considering the role of DA oxidation in the pathogenesis of PD discussed above, VMAT2 is clearly an important determinant in DA-related toxicity and reduction of its expression or function could adversely affect DA neuron survival and function. In support of this concept, several promoter haplotypes in humans, which have been demonstrated to increase VMAT2 expression in cell cultures, are correlated with a lower incidence of PD in women (37). The effects of impaired DA storage have been well documented through both pharmacological and genetic animal models (38).

One of the first PD animal models was generated by administering the irreversible VMAT2 inhibitor reserpine (39). Systemic administration of reserpine depleted brain levels of monoamines by reducing vesicular storage and release. Parkinsonian motor signs, such as tremor, rigidity, and hypokinesia, developed in response to this and were attenuated by administration of l-DOPA, as well as of other classes of pharmacological agents used in the treatment of PD (40). More recently, pharmacological inhibition of VMAT2 by ketanserin was used to test the hypothesis that the accumulation of DA in the cytosol renders DAergic cells vulnerable to BH4 (41). Ketanserin causes enhancement of BH4-induced damage and increased lipid peroxidation and protein bound quinone in DAergic cells. As cells that have been depleted of DA exhibited no significant damage after exposure to ketanserin and BH4, the drug effect appears to be mediated by increased cyto-
solic concentration of DA, indicating that VMAT2 plays a protective role against BH4-induced oxidative stress by sequestering DA. Amphetamine and its derivatives have been shown to perturb cytosolic catecholamine homeostasis by promoting the collapse of the pH gradients in synaptic vesicles and preventing VMAT2-mediated uptake of DA (42). In differentiated PC12 cells, exposure to methamphetamine (100–500 μM) led to significant cell death that could be attenuated by the overexpression of VMAT2 (43). Furthermore, in primary postnatal rat ventral mesencephalic cultures with down-regulated levels of VMAT2, the same investigators found enhanced cytoplasmic DA levels correlated to a drastic reduction in the number and length of TH-positive neurites (43). Of interest, tetrabenazine, another specific inhibitor of VMAT2 function (44) that is currently used to treat hyperkinetic movement disorders, such as chorea associated with Huntington’s disease (45) and tics in Tourette’s syndrome (46), leads to parkinsonism as side effect (see ref. 47 for a review), highlighting the importance of a fully functioning DA storage system.

Animals genetically engineered to express reduced levels of VMAT2 have also been important in demonstrating the importance of vesicles in DA handling and neuroprotection from DA-induced oxidants. Mice expressing 50% of normal VMAT2 exhibit a significant reduction in striatal DA accompanied by a more pronounced methamphetamine-induced DAergic toxicity compared with wild-type mice (48). While mice with complete deletion of VMAT2 gene do not survive for more than a few days (49), a transgenic mouse line expressing ~5% of wild type VMAT2 protein levels survives into adulthood (50), providing a unique opportunity to examine the effect of disruption of vesicular storage on an aging DA system. Using this model, Caudle et al. (51) reported that disruption of DA storage by reduction of VMAT2 expression leads to an age-dependent degeneration of nigrostriatal DA neurons, similar to that seen in PD. In addition, as a consequence of the age-associated decrease in striatal DA, mice exhibited a significantly reduced locomotor activity and the deficits were abolished after administration of L-DOPA. Despite lower levels of striatal DA, mice with a 95% reduction in VMAT2 expression exhibit an age-dependent increase of cysteinyl catechols, as well as of other markers of oxidative and nitrosative stress, such as protein carbonyls and 3-nitrotyrosine (51), illustrating the importance of correct neurotransmitter compartmentalization.

Considering the pivotal role of VMAT2, which is the only transporter that moves cytosolic DA into synaptic vesicles for storage, pharmacologically enhancing DA sequestration by VMAT2 may be a strategy for treating PD. Accordingly, pramipexole and apomorphine, two DA D2/D3 agonists used to treat PD, may also exhibit neuroprotective properties toward DAergic neurons (52, 53). Looking for the mechanisms underlying the neuroprotective effects, it has been demonstrated that both these molecules increase vesicular DA uptake, so that this mechanism has been suggested to be important for the neuroprotection.

Reuptake

After DA release into the synaptic cleft, one of the key activities regulating the effect of the neurotransmitter is its reuptake into the presynaptic neuron. Central to this process is the action of the DA transporter DAT, which regulates both intensity and duration of DA signaling. DAT is a 12-transmembrane domain glycoprotein that belongs to a family of Na⁺Cl⁻-dependent transporters (54).

A possible involvement of DAT in PD emerged in the early 1980s when a group of drug abusers manifested permanent PD-like symptoms after the accidental consumption of the neurotoxin MPTP (55). MPTP toxicity was then found to be related to the DAT-mediated accumulation of its active metabolite MPP⁺ into DAergic neurons (56) where it induces mitochondrial respiration deficit, oxidative stress, and energy failure (57).

Epidemiologic studies have implicated a number of different pesticides, such as paraquat, organochlorines, and carbamates, in the etiology of PD (see ref. 58). Considering that MPTP has a chemical structure close to that of paraquat, this raises the possibility that, by analogy with MPTP, the toxicity of environmental toxicins is dependent on their interaction with DAT. In such a manner, DAT might play a contributory role in some cases of idiopathic PD. Nevertheless, it must be pointed out that while some studies have reported that inhibition of DAT protects against paraquat-induced toxicity, suggesting a role for DAT in the cellular uptake of paraquat (59), other studies report that paraquat is not a substrate of DAT (60). The vulnerability of distinct subgroups of DAergic neurons observed in PD is directly correlated with basal levels of DAT protein expression and, in particular, with the glycosylated form of DAT (61). For example, DAergic neurons of the SN express higher levels of glycol-DAT, transport DA more efficiently, and are more affected than ventral tegmental neurons (61). Furthermore, the DAergic neurons surviving in PD midbrain at time of death exhibit lower DAT gene expression per cell than the DAergic cells in control subjects (62). In agreement with this hypothesis, it has been demonstrated that mice lacking DAT are protected against methamphetamine-induced DAergic neurotoxicity (63), highlighting the essential role of DAT. Genetic variability in the DAT locus has also been described as risk factor for PD depending on exposure to pesticide: Kelada et al. (64) reported on the combined effect of DAT genetic variability and occupational pesticide exposure. A second, population-based case-control study of PD conducted in a California population heavily exposed to pesticides replicates and extends previous evidence for an association between DAT variants and PD and highlights possible interactions of disease-associated DAT susceptibility alleles and pesticide exposure. In the latter study, the investigators estimated residential pesticide exposure using a geo-
The combination of blood level of inhibitors of peripheral AADC, which increases the peripheral AADC in the metabolism of the administered dose (68). Recognition of the role of AADC remains the most effective treatment for PD. Even with the brain after oral administration, however, is divided into presynaptic terminals in the striatum, reducing the is then decarboxylated to DA by AADC and released by DAT, while the second, tolcapone, which does not cross the blood-brain barrier. However, its liver toxicity has led to the withdrawal of tolcapone in the European Union and Canada, while in the United States, its use has been restricted (68).

Two COMT inhibitors have been used to treat PD. The first, entacapone, does not cross the blood-brain barrier and increases the bioavailability of l-DOPA by inhibiting peripheral COMT. The second, tolcapone, is the only currently available COMT inhibitor able to cross the blood-brain barrier. However, its liver toxicity has led to the withdrawal of tolcapone in the European Union and Canada, while in the United States, its use has been restricted (68).

In contrast to orally administered l-DOPA, the metabolism of endogenous DA mainly occurs in the cytoplasm of the same cells in which DA is synthesized, and most cytoplasmic DA derives from its passive leakage from synaptic vesicular storage. As extensively reviewed by Eisenhofer et al. (74), vesicular stores of DA do not exist in a static state simply awaiting a signal for exocytotic release. Instead, DA stores exist in a highly dynamic system, with passive outward leakage of DA counterbalanced by inward active transport under the control of VMAT2. The vesicular monoamine transporter avidly sequesters back into storage vesicles ~90% of the DA leaking into the cytoplasm. Nevertheless, ~10% of DA escapes sequestration and is metabolized (74).

MAO catalyzes the oxidative deamination of DA leading to the production of hydrogen peroxide and 3,4-dihydroxyphenylacetaldehyde (DOPAL). On the basis of substrate selectivity and inhibitor sensitivity, two forms of MAO were proposed and designated MAO A and B (75). Both MAO A and B are located throughout the brain in the outer membrane of mitochondria. MAO A has a higher affinity for serotonin and the inhibitor clorgyline, whereas MAO B has a higher affinity for phenylethylamine and the inhibitor deprenyl. DA, norepinephrine, epinephrine, tryptamine and tyramine are oxidized by both forms of the enzyme in most species (76). The two isoenzymes are not evenly distributed in the human brain, and the main form in the basal ganglia is MAO B (76). Immunohistochemical

Degradation

In the light of the high toxicity of the products related to the oxidative chemistry of DA, it is perhaps unsurprising that cells have developed systems that rapidly degrade free cytosolic or extracellular DA. This aspect of cellular DA metabolism becomes very relevant when considering current therapeutic approaches to PD, in particular treatment with l-DOPA.

The metabolism of orally administered l-DOPA leads to increased output of the main DA metabolites DOPAC and HVA (67), indicating that metabolism of orally administered l-DOPA in the periphery is catalyzed primarily by AADC, MAO, ALDH, and COMT. Unlike DA, l-DOPA crosses the blood-brain barrier and is then decarboxylated to DA by AADC and released by presynaptic terminals in the striatum, reducing the DAergic deficiency. The amount of l-DOPA reaching the brain after oral administration, however, is ~1% of the administered dose (68). Recognition of the role of peripheral AADC in the metabolism of l-DOPA (69) led to the demonstration that the effective dose of l-DOPA could be reduced by the coadministration of inhibitors of peripheral AADC, which increases the blood level of l-DOPA and its transfer into the brain. The combination of l-DOPA with a peripheral AADC inhibitor, unable to enter the central nervous system, remains the most effective treatment for PD. Even with this combination, only 5–10% of administered l-DOPA reaches the brain due to its metabolism by COMT (68). As a consequence, inhibitors of COMT have been used to prevent the peripheral metabolism of l-DOPA to 3-O-methyl-DOPA, thus increasing the bioavailability of l-DOPA.

COMT is required for the inactivation and metabolism of a large variety of catechol compounds including norepinephrine and epinephrine, caffeine, estrogen, dietary phytochemicals, and medicinal compounds in addition to the biogenic amines DA (68). In humans, two isofoms of COMT are present, encoded by the same gene: a membrane-bound (MB) and a soluble (S) form (70). While S-COMT is particularly abundant in liver, kidney, and mammary glands (71), MB-COMT is the main form in the brain, where it has been postulated to play an important role in modulating cortical DA signaling (72). Immunohistochemical studies (73) have indicated that COMT resides predominantly in glial cells whereas in neurons COMT is missing or present only at low amounts.

Due to selective DAT expression in DAergic neurons and to the presence of a large number of specific tracers, DAT imaging is currently used in molecular imaging techniques (see ref. 66 for a review). Positron emission tomography and single photon emission tomography provide sensitive tools for quantifying the loss of nigrostriatal DAergic fibers in PD and for detecting the presence of DAergic dysfunction in asymptomatic at-risk relatives and patients with isolated tremor. Considering that the pathology of PD consists of the loss of DAergic neurons in the SN and the reduction of DAergic projections to the striatum, the main application of DAT imaging has been the quantification of the DAergic deficit in PD. In particular, it can discriminate parkinsonism associated neurodegeneration from conditions not associated with DA deficit, such as essential tremor and drug-induced, vascular, or psychogenic parkinsonism. In the clinical setting, the utility of DAT imaging is to prove the presence of parkinsonism-associated neurodegeneration in conditions having an uncertain diagnosis and warranting appropriate treatment. Moreover, the possibility of quantifying the progression of DA dysfunction has raised the question of whether imaging of DA function with DAT molecular imaging could be used as a surrogate biomarker of disease progression to assess the effect of putative neuroprotective or disease-modifying drugs.

graphic information system-based computer model rather than subjects’ self-reports eliminating bias due to differential recall (65).

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studies have shown that serotonergic neurons and astrocytes contain predominantly MAO B, whereas catecholaminergic neurons, such as cells of the SN, contain mainly MAO A (77, 78). In humans, MAO A is present before MAO B in most tissues. MAO A activity is almost at adult levels at birth, whereas MAO B activity increases severalfold with aging (79). Because MAO B is predominantly located in glial cells, the large increase in MAO B with aging may be attributable to the proliferation of these cells (76).

The use of MAO inhibitors as adjuncts to l-DOPA was considered as a treatment for PD, but such an approach with nonselective inhibitors was abandoned because of the so-called “cheese effect.” This effect occurs when tyramine and other sympathomimetic amines, which are found in fermented foods such as cheese, enter the circulation and potentiate sympathomimetic cardiovascular activity by releasing norepinephrine (80). At present, selegiline and rasagiline are the two MAO inhibitors clinically used to treat motor symptoms in PD as monotherapy and in combination with l-DOPA and a decarboxylase inhibitor (81). The selective MAO B inhibitor l-deprenyl (selegiline) was first investigated as an adjuvant to l-DOPA on the basis of the following considerations: the levels of MAO B are increased in the brains of patients with PD as a consequence of gliosis; the human basal ganglia has higher MAO B than MAO A activity; and DA is equally well metabolized by both isoenzymes in humans (82). There is now overwhelming evidence that selegiline has beneficial effects on motor fluctuations, as shown in many clinical studies, as recently summarized by Hauser (83). Rasagiline is another selective and irreversible MAO-B inhibitor, 5 times more potent than selegiline. It is well tolerated, has beneficial effects on quality of life parameters, is beneficial in early and late stages of PD, and is safe when combined with all other PD-relevant therapies, including COMT inhibitors (81). Despite some suggestion of a possible disease-modifying effect for rasagiline, in 2011 the U.S. Food and Drug Administration concluded that the evidence was not compelling enough to support the proposed expanded indication for rasagiline in slowing of clinical progression of PD (84).

As previously indicated, the end products of MAO enzymatic activity are hydrogen peroxide and DOPAL, both toxic to DAergic neurons. In particular, as exhaustively reviewed by Marchitti et al. (85), aldehydes, including DOPAL, form adducts with various cellular nucleophiles, resulting in impaired cellular homeostasis, dramatically reduced enzyme activity, and DNA damage. DOPAL accumulation and impaired detoxification have been hypothesized to play a role in the pathogenesis of PD (74, 86). Accordingly, it was recently reported that patients who died with PD had an elevated DOPAL-to-DOPAC ratio in caudate and putamen (87). DOPAL is primary detoxified by ALDH, a gene superfamily that contains 19 putatively functional genes and 3 pseudogenes (88). Cytosolic ALDH1 and mitochondrial ALDH2 have been reported to be present in various human brain areas, including striatum and SN (89). ALDH1 is highly and specifically expressed in human SN and VTA DAergic neurons, with an almost complete absence of this gene in neighboring non-DAergic cells (90). In brains from patients with PD, markedly lower expression of ALDH1 mRNA was found in surviving neurons in the SN, compared with non-PD controls. In contrast, ALDH1 expression was not significantly decreased in TH- or DAT-positive neurons in the VTA in most patients with PD (90). Based on these data, it is conceivable that reduced ALDH1 expression in the SN could also contribute to the development of PD. Subsequent to a reduced ALDH1 expression, the accumulation of DOPAL could be potentially toxic to DAergic neurons and could render these neurons more susceptible to aldehyde toxicity and degeneration. Two different groups have recently tested this hypothesis. In the first study, an aldhl null mouse model was used. While some alterations were observed in DA release in response to KCl stimulation, the absence of ALDH1 does not appear to negatively affect the initial growth and development of SN DAergic neurons nor affect their survival (91). However, the mice analyzed were 8–12 wk old, an age that might not be sufficient for the induction of Parkinson’s-related phenotypes. In the second study, aldhl−/− and aldhd2−/− mice were generated and analyzed at 3 different time periods, corresponding to 5–8, 12–14, and 18–27 mo (92). Loss of these two enzymes resulted in a decline in locomotor function, loss of TH immunoreactive neurons in the SN, and reductions in monoamines and metabolites in the neostriatum.

In light of their fundamental role in detoxifying cells from toxic aldehydes, ALDH enzymes could represent a promising target of pharmacological therapy directed to contrast PD progression. Recently, it has been found that Alda-1, a compound originally identified as an activator of ALDH2, can also activate ALDH1 (93). The same investigators carried out a virtual screen of ~20,000 compounds and tested the top 21 hits in a DOPAL inactivation assay with ALDH1. This led to the identification of an activator as well as two inhibitors (93). These findings represent an attractive starting point for developing compounds that may have utility in restoring the metabolism of DOPAL in PD.

**CONCLUSIONS**

PD is currently incurable, and there is a concerted effort from within the research community to develop pharmacological treatments that result in a slower progression of symptoms and an improved quality of life for patients. The slow onset and progression of this disorder make it difficult to test working hypotheses for the ethiopathogenetic mechanisms leading to neuronal loss and thence to the macroscopic pathological manifestations. In this review, we have shown that impairment of DA metabolism, based on both genetic and biological evidence, has precise cause-effect relation-
ships that play an important part in the disease process. A holistic view of the different levels at which the normal functions of DAergic neurons can be affected by DA metabolism will undoubtedly help in designing and optimizing therapeutic treatments.

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