A Synopsis on the Role of Tyrosine Hydroxylase in Parkinson's Disease

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Abstract: Parkinson's disease (PD) is a common chronic progressive neurodegenerative disorder in elderly people. A consistent neurochemical abnormality in PD is degeneration of dopaminergic neurons in substantia nigra pars compacta, leading to a reduction of striatal dopamine (DA) levels. As tyrosine hydroxylase (TH) catalyses the formation of L-dihydroxyphenylalanine (L-DOPA), the rate-limiting step in the biosynthesis of DA, the disease can be considered as a TH-deficiency syndrome of the striatum. Problems related to PD usually build up when vesicular storage of DA is altered by the presence of either α-synuclein protofibrils or oxidative stress. Phosphorylation of three physiologically-regulated specific sites of N-terminal domain of TH is vital in regulating its kinetic and protein interaction. The concept of physiological significance of TH isoforms is another interesting aspect to be explored further for a comprehensive understanding of its role in PD. Thus, a logical and efficient strategy for PD treatment is based on correcting or bypassing the enzyme deficiency by the treatment with L-DOPA, DA agonists, inhibitors of DA metabolism or brain grafts with cells expressing a high level of TH. Neurotrophic factors are also attracting the attention of neuroscientists because they provide the essential neuroprotective and neurorestorative properties to the nigrostriatal DA system. PPAR-γ, a key regulator of immune responses, is likewise a promising target for the treatment of PD, which can be achieved by the use of agonists with the potential to impact the expression of pro- and anti-inflammatory cytokines at the transcriptional level in immune cells *via* expression of TH. Herein, we review the primary biochemical and pathological features of PD, and describe both classical and developing approaches aimed to ameliorate disease symptoms and its progression.

Keywords: Parkinson disease, tyrosine hydroxylase, dopamine, α-synuclein, neuroprotection, neurotrophic factors, L-DOPA.

INTRODUCTION

Parkinson's disease is a chronic progressive neurodegenerative disorder. First identified by James Parkinson in 1817, who wrote an essay on the shaking palsy for the description of disease. Currently, it represents the second most common neurodegenerative disorder, after Alzheimer's disease [1, 2], and affects approximately 6 million people worldwide [3]. Patients exhibit a range of clinical symptoms, with the most common affecting motor function, including the development of resting tremor, rigidity, akinesia, bradykinesia and postural instability. However, and non-motor symptoms, like dementia, depression, visual hallucination and autonomic dysfunction, are also common [4, 5].

PD classically involves the degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNC), leading to a reduction of striatal dopamine (DA) levels that are critical in the coordination and control of motor activity [6]. The enzyme, tyrosine hydroxylase (TH) [EC 1.14.16.2] sometimes known as tyrosine 3-monooxygenase) catalyses the formation of L-DOPA, the rate-limiting step in the biosynthesis of DA, thereby directly linking PD with TH. L-DOPA is a precursor for DA synthesis, which, in turn, is a precursor for both norepinephrine (noradrenaline) and

epinephrine (adrenaline). The loss of ability to optimally synthesize catecholamines is an important step in the progression of PD and other neurodegenerative diseases [7]. Indeed, early loss of TH activity followed by a decline in TH protein is considered to contribute towards DA deficiency and phenotypic expression in PD [7, 8].

The prevalence of PD varies among ethnic and geographic groups around the world, being particularly low in China and high in Argentina [9]. In general, however, its highest prevalence is found in Caucasians, and in men versus women; nevertheless, across numerous studies prevalence and annual incidence appear to increase continuously into very old age. Albeit that a number of studies have reported that rural versus urban living is a risk factor for PD, whereas others have not, it is clear that geographic 'disease belts' of high prevalence/incidence exist [10], and this nonrandom disease distribution reasons compellingly for an environmental impact on the pathogenesis of PD. However, the main etiology of the disease has yet to be identified, albeit occupational and genetic factor, in addition to environment, seem to play important roles [10-12]. In the light of this introduction, research elucidating underlying mechanisms and towards the development of new therapies for PD has become a highly active area within neuroscience and continuous progress warrants frequent analysis and review.

TH is present in all dopaminergic cells and catalyzes the formation of L-DOPA, a deficiency in TH is a hallmark of PD [13]. This enzyme is a highly specific, non-heme iron,

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tetrahydrobiopterin-dependent protein that catalyzes the conversion of tyrosine to L-DOPA, and represents the rate-limiting step in the biosynthesis of catecholamines (CA) functioning as hormones and neurotransmitters both in invertebrates and vertebrates [14]. In mammals, TH is found in diverse tissues comprising the central and sympathetic nervous system: brain, adrenal medulla and other peripheral sympathetic neurons [15-17]. It is considered as a cytosolic protein, however some investigators have also reported it as a membranous fraction [18, 19]. The physiological significance of this fraction remains to be elucidated, but it has been proposed to be associated with the coupling of synthesis and storage of neurotransmitters into their synaptic vesicles [20]. A largely unexplored and interesting field has hence emerged in relation to TH binding to the synaptic vesicle membrane [21]. TH is found in the neuro-endocrine system, mainly in brain and in chromaffin cells of adrenal medulla, predominantly in the cytoplasm [22]. The emergence of simpler organisms as animal models to study neurological functions and development, epitomized by *D. melanogaster* or *Caenorhabditis elegans*, opens the possibility of exploring these new roles of DA, adrenaline and nor adrenaline [23-25].

CHEMISTRY OF TH IN PD *VIA* DA BIOSYNTHESIS

A primary problem in PD is insufficient DA, whatever the cause may be. DA is generated within dopaminergic neurons *via* the following pathway (Fig. **1**).

The initial step is catalyzed by the enzyme, TH. Within the full reaction of L-DOPA formation from L-tyrosine, a coenzyme THFA (tetrahydrofolic acid) and ferrous ions are necessary [26]. The activity of TH is often as low as 25% of healthy age-matched controls in parkinsonism, and in severe cases can fall as low as 10%. This suggests that one or more key elements essential for the formation of L-DOPA likely is in insufficient quantity in PD. The second step for DA generation involves the enzyme DOPA decarboxylase (EC 4.1.1.28 known also as aromatic L-amino acid decarboxylase), in pyridoxal phosphate is essential. The activity of the enzyme varies in accord with the amount of pyridoxal phosphate present [26].

DA AS AN ENDOGENOUS TOXIN

DA neurons of the nigrostriatal pathway are a primary site of neuronal degeneration in PD, whereas neuronal alterations observed in PD are not restricted to DA neurons only [27]. The presence of mitochondrial dysfunction and oxidative stress in these highly metabolic neural cells, does not fully explain the heightened vulnerability of dopaminergic neurons. The fact that substantia nigra DA neurons degenerate in PD to a greater extent than other neurons might be due, in part, to the presence of DA itself [28]. The unique chemical structure of DA has the potential to form an endogenous toxin that may contribute further to mitochondrial dysfunction and oxidative damage, accelerating dopaminergic cell death in PD in response to oxidative insult [29]. Under normal conditions, DA is synthesized from tyrosine *via* L-DOPA. DA, once generated by the enzymatic activities of TH and DOPA decarboxylase, is rapidly stored at a high millimolar concentration and stabilized at a low pH within synaptic vesicles following uptake by the vesicular monoamine transporter, thereby limiting its cytoplasmic concentration [30]. Cytoplasmic (non-vesicular) DA is subject to oxidation, on exceeding physiological concentrations, *via* monoamine oxidase type A (MAO-A) and aldehyde dehydrogenase (ADH) to generate non-toxic 3,4-dihydroxyphenylacetic acid and hydrogen peroxide. It can be additionally sequestered into lyposomes where it can be oxidized to neuromelanin (NM).

Problems can potentially develop when vesicular storage of DA is altered by the presence of α-synuclein protofibrils, aberrant oxidative stress and weak base compounds [31], resulting in elevated cytoplasmic DA levels. Under such circumstances secondary oxidative pathways can be triggered that divert the typical non-toxic metabolic sequence and NM formation to allow the generation of the intermediates, such as dopamine-*o*-quinone and dopaminochrome, which can subsequently generate further metabolites, such as leukodopamineochrome-*o*-semiquinone that are considered powerful endogenous neurotoxins consequent to an ability to reduce oxygen to a superoxide radical anion, giving rise to redox cycling and producing acute toxicity [26].
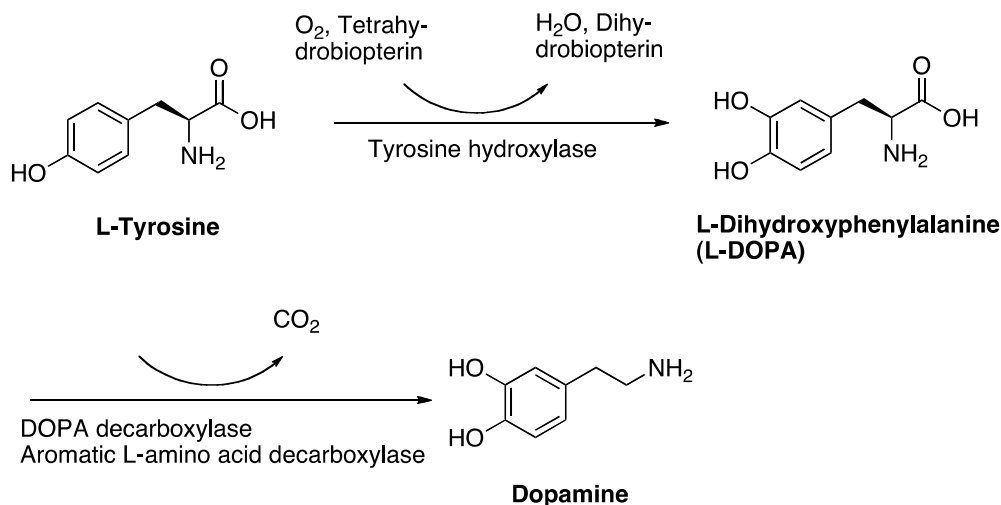

Fig. (1). Generation of DA from L-tyrosine.

INVOLVEMENT OF TH IN THE PATHOGENESIS OF PD AT GENETIC LEVEL

The identification of three genes and several additional loci linked with inherited forms of PD signifies that it is not a single disorder. The analyses of structure and function of these gene products implicates the significant role of protein aggregation in dopaminergic neurons of the substantia nigra as the common mechanism leading to neurodegeneration in all known forms of PD. The three specific genes identified thus far are *α-synuclein, Parkin* and *ubiquitin C terminal hydrolase*, which are either closely implicated in the appropriate functioning of the ubiquitin-proteasome pathway or are degraded by this protein-clearing machinery of cells [32]. The biochemical and molecular cascades characterized from genetic studies in PD may provide novel targets for curative therapies [33].

STRUCTURE AND MOLECULAR GENETICS OF TH

Mammalian TH is a highly homologous oligomeric protein that are composed of four subunits, having three well-differentiated domains for each subunit [34]. These domains include a N-terminal regulatory domain that varies in length depending on the enzyme. In the case of TH4, the central catalytic domain is composed of some 280 residues and the C-terminal oligomerization domain contains 45-50 residues [15]. The N-terminal domain of TH is quite complex, with 4 to 5 phosphorylation sites [35]. These multiple phosphorylation sites have been associated to the tight regulation of this enzyme, by controlling either kinetic activity and/or binding to protein partners [22, 36]. The boundary between the regulatory and the catalytic domain is located in the region of residues 165-179, and the last 50 residues in the C-terminal constitute the oligomerization domain [37].

The activity of TH is hence regulated by site-specific phosphorylation of three physiologically-regulated sites in the central nervous system, namely Ser19, Ser31 and Ser40 [38], which impact the function of TH. It has been reported that the phosphorylation of Ser19 does not directly influence TH activity [39] but increased phosphorylation of Ser40 can augment TH activity within a certain threshold and is associated with elevated DA turnover in neurodegenerative disorders [36, 40-42]. Ser31 phosphorylation alone can increase L-DOPA biosynthesis. Salvatore *et al*. [40] reported that Ser31 or Ser40 phosphorylation modulates DA availability and is necessary to define the molecular basis for DA dependent behaviors.

The human TH gene (hTH) is located on chromosome 11p15.5, spanning approximately 8 kilo bases and containing 14 codifying exons [43]. It codes for four different isoforms, created by alternative splicing (hTH1-4), albeit that later studies have revealed that more mRNA species may be expressed within the cell [44-46]. The hTH1-4 isoforms are expressed in same tissue, although in different proportions and share similar kinetic properties, with hTH1 being the more active and abundant form [47]. The physiological significance of the TH isoforms remains to be fully elucidated and remains an interesting area of current research [48]. Crystal structure analyses of catalytic and tetramerization domains of TH have revealed a novel alpha-helical basket that supports catalytic iron and a $40A^0$ long anti-parallel coiled coil, which forms the core of tetramer. The catalytic iron is located $10A^0$ below the enzyme surface in a $17A^0$ deep active site pocket and is coordinated by the conserved residues His331, His336 and Glu376. The structure provides a basis for understanding the effect of point mutations in TH [37, 49].

ROLE OF α-SYNUCLEIN IN THE REGULATION OF TH ACTIVITY AND DA BIOSYNTHESIS

PD is characterized by the loss of DA containing neurons projecting from the substantia nigra to the dorsal striatum [50, 51]. A key role for α-synuclein has been postulated, albeit with largely undetermined function in the pathogenesis of PD [52-55]. α-Synuclein is a major component of Lewy bodies, a neuropathological hallmark of PD [56-59]. This small and highly conserved protein is enriched in presynaptic terminals and is implicated in both normal as well as pathogenic brain function [60-64]. Whereas the physiological function of α-synuclein remains to be fully characterized [63-65]. Several studies have established that interaction of α-synuclein and TH is of functional relevance to dopaminergic neurotransmission and the pathophysiology of PD [49, 66, 67]. Indeed, α-synuclein gene mutations and PD pathogenesis are well documented [49]. In this regard, mutations such as A53T or A30P and reduced α-synuclein expression have been reported in familial and sporadic form of PD, respectively [49, 68-70]. An interaction between α-synuclein and TH in PD pathogenesis has been confirmed by the use of immuneoprecipitation, whereby both proteins co-precipitate in brain homogenate, and immunoelectron microscopy techniques, whereby they co-localize [54]. Several cell-free studies and animal models signify that α-synuclein mutations accelerate its aggregation [49, 71, 72]. Earlier studies by Kim *et al*. [73] and Souza *et al*. [74] have revealed that α-synuclein may be a chaperone protein, based on its ability to inhibit thermally induced protein precipitation and it structural homology with 14-3-3 proteins [49, 75]. Research studies indicate that the binding and activation of TH by 14-3-3 proteins preferentially to phosphoserines [76, 77] and short-term TH activity is regulated by serine phosphorylation [78, 79]. Furthermore, 14-3-3 interacts with TH through an association with Ser19 and Ser40 and the interaction of 14-3-3 with TH Ser19 is required for its activation [49, 54, 80, 81].

Even though the function of α-synuclein remains obscure, it may bind to TH and inhibit its activity, thereby serve as a key regulator of DA synthesis. A loss of α-synuclein by its aggregation or decreased expression may selectively disrupt DA homeostasis and negatively impact DA neuronal survival [49, 54, 70]. Perez *et al*. [54] described the association of α-synuclein and inhibition of TH activity in a cell-free assay; reporting that over expression of α-synuclein in a dopaminergic cell line dramatically reduces TH activity, TH phosphorylation and DA synthesis in brain as well as in a dopaminergic cell line. Hence, elevated levels of α-synuclein, due to a specific disease or the normal ageing process could be associated with dopaminergic neuronal dysfunction through TH regulation interference [49, 63].

ENVIRONMENTAL FACTORS ASSOCIATED WITH THE ONSET OF PD

Epidemiological studies have suggested several possible environmental factors, associated with PD such as exposure to heavy metals, pesticides and farming [10, 82-85]. The mechanism(s) underpinning the actions of pesticides on the dopaminergic nigrostriatal pathway are far from understood, but the sporadic form of PD has been associated with an increased exposure to pesticides [86-88]. Furthermore, there is convincing support that several environmental chemicals, including rotenone [89], paraquat [90-92] and heptachlor [93], act directly or generate neurotoxicants that selectively induce damage to the nigrostriatal pathway and instigate PD symptoms in experimental animals. The mechanism of dopamine neuropathy induced by rotenone has been reported to be mediated selectively *via* the inhibition of mitochondrial complex I, which resulted in elevated oxidative stress. Interestingly, rotenone affected complex I uniformly throughout the brain, which results into highly selective degeneration of the nigrostriatal dopaminergic system [89]. The systemic exposure to paraquat provoked a dose-dependent loss of DA neurons in the SNC coupled to a reduction in the density of striatal DA fibers expressing TH in animal models [94-96]. Furthermore, an exposure to pesticides paraquat and maneb during early postnatal days has been reported to produce permanent and progressive lesions of the nigrostriatal DA system and enhance adult susceptibility to pesticides [92, 97]. Pesticides and metals can also produce synergistic effects on the fibrillization of α-synuclein [49, 97]. Deltamethrin, one of the most potent pyrethroid insecticides, selectively inhibits synthesis of DA while increasing the turnover of DA in the striatum. It also inhibits the activity and mRNA/protein expression of TH in striatum [97]; thereby implicating TH as a molecular target of pesticides within the nigrostriatal pathway.

A single nucleotide polymorphism (SNP) in the gene was found to be associated with PD with an early age of onset. Genetic component plays a much more important role in the pathogenesis of PD. Polymorphisms in several genes such as *α-synuclein*, *Parkin*, *UCH-L1* (ubiquitin-C terminal hydrolase-L1) and *DJ*-1 have previously been identified in PD [98, 99]. The more common, sporadic form of Parkinson's disease appears to result from an interaction between genetic and environmental factors [100]. SNP in the gene for the pro-inflammatory cytokine interleukin 6 potentiate the susceptibility to PD at early age [101]. In one study, Ohtake *et al.* (2004) suggested that mutations in β-synuclein gene may predispose to dementia with Lewy bodies in which the substitutions of amino acid from valine to methionine at codon 70 and proline to histidine at codon 123 was present. A meta-analysis of 84 association studies of 14 genes showed that polymorphisms in four genes are significantly associated with PD [102]. ATP binding cassette superfamily of transporter genes have been also reported to influence susceptibility to Parkinson's disease [103].

USE OF TH IN THE TREATMENT OF PD

A decline in DA level, formed by hydroxylation of L-tyrosine to L-DOPA by TH, is a classical pathological feature of PD patients. The most common therapeutic strategy for PD is to restore DA levels by the administration of L-DOPA, the precursor of DA, which can access brain [104] consequent to an affinity for the blood-brain barrier. However, the brain penetration of L-DOPA is limited by the presence of enzymes L-DOPA decarboxylase and monoamine oxidase within brain capillary endothelial cells, forming a "enzymatic blood-brain barrier" to limit L-DOPA passage into brain. Hence, PD therapy is enhanced by concurrent treatment with an inhibitor of L-DOPA decarboxylase. Classical pharmacological studies during 1950s initially suggested that the decline in DA levels in forebrain of experimental animals and proposed motor symptoms might be due to DA dysfunction [105]. More recent studies indicate that the intravenous/oral administration of L-DOPA minimizes PD symptoms at earlier stages [106-108]. In comparatively advanced PD patients, L-DOPA does not appear to impact disease progression, loses its potency over time and leads to non-physiological intermittent stimulation of striatal neurons that express DA receptors [109].

Therapeutic means that induce an elevation in the intrastriatal activity of TH by continuously maintaining appropriate cerebral DA concentrations are expected to palliate PD symptoms. Recently various strategies, such as neuroprotective approaches through the use of antioxidants, different neurotrophic factors, cell replacement therapies including stem cells as well as gene transfer approaches are under analysis for the treatment of PD [110-114].

TREATMENT OF PD BY GENE THERAPY

Gene therapy can be broadly described as the transfer of specific genetic material into a defined target cell for the ultimate purpose of preventing or altering a particular disease state. Strategies for the application of gene therapy techniques for PD treatment have broadened beyond classical DA replacement towards the use of neurotrophic factors. Theoretically, gene therapy in patients with PD has potential utility to replace a defective gene, introduce a potentially neuroprotective or neuro-restorative protein or to permit the physiological delivery of a deficient neurotransmitter. For example, it could be undertaken to focus the enzymes responsible for the DA biosynthesis within the affected striatum. As described earlier, the rate limiting step in DA synthesis is the conversion of L-tyrosine to L-DOPA by the enzyme TH [115], with subsequent conversion to DA by DOPA decarboxylase. Enzyme turnover is so rapid that L-DOPA levels in the brain are negligible under normal conditions. As TH is the rate-limiting enzyme in DA biosynthesis, it is a prime target for physiological regulation and hence pharmacological manipulation.

A variety of viral vectors are used in gene transfer and, based on their specific advantages and disadvantages in their ability to safely transfer genes to the cells they recognize and whether they alter the cell's DNA permanently or temporarily, have provided targeted protein expression in specific regions of the brain. Specific examples of gene therapy using viral vectors include:

ADENO ASSOCIATED VIRUS – GLUTAMIC ACID DECARBOXYLASE (AAV-GAD) GENE THERAPY

Decreased GABA input to the subthalamic nucleus (SN) results in dis-inhibition of this structure in PD. A Phase II human trial involving bilateral gene transfer of GAD into the SN has been completed using an AAV vector. The gene

transfer enhanced local synthesis of inhibitory neurotransmitter GABA in the SN and suppressed excessive activity [116].

AAV2-NEURTURIN GENE THERAPY OR CERE-120

Neurturin is a naturally occurring homologue of glial cell line-derived neurotrophic factor (GDNF). Within the basal ganglia, it promotes the sprouting and survival of dopaminergic neurons. CERE-120 is a modified form of human neurturin. Following up on an encouraging unblended Phase I trial, a Phase II double blind randomized trial was conducted in which patients received either bilateral intraputamin injection of AAV2-neurturin or sham surgery [117]. However, as assessed at 12 months, there was no difference between treatment and sham groups, with 13/38 subjects within the AAV2-neuroturin group presented serious adverse effects versus 4/20 in the sham group [112]. A further trial of neurturin, utilizing nigral and putaminal injection both, as well as a longer observation period, is currently underway in PD [118].

AROMATIC L-AMINO ACID DECARBOXYLASE GENE THERAPY

This therapy involves injecting an adenovirus vector containing DNA encoding the aromatic amino acid decarboxylase gene into the striatum of PD patients to increase local conversion of exogenous L-DOPA (administered as levodopa) to DA. The degree of conversion can then be controlled by changing the levodopa dosage. As assessed at 6 months, this gene therapy strategy proved safe, but longer-term analysis is required to define efficacy [119].

PROSAVIN

This approach utilized intrastriatal injections of a tricistronic lentivirus encoding TH, aromatic amino acid decarboxylase, and GPT cyclohydrolase [120]. These three enzymes were reported to result in an enhanced production of DA at the site of injection, but avoided excessive production in other areas where DA was not depleted.

Compared to transplantation of transfected cells, direct gene transfer is less invasive, inducing minimal disturbance of synaptic circuitry and does not involve risk of unregulated cellular proliferation or undesired delivery of cell byproducts. Several non-viral methods to deliver genes, such as liposomes or particle bombardment, have provided interesting results but often difficult to interpret [121-123]. Therefore, most direct gene transfer has been performed by means of viral vectors that serve as vehicles for transfer of genes to the central nervous system. *In vivo* gene therapy for DA replacement attempts to convert endogenous striatal cells into L-DOPA producing cells [124].

IN VIVO GENE TRANSFER OF TH

Adeno associated viral vectors (AAV), utilized to introduce the TH gene into the striatum of 6-OHDA lesioned rats, have resulted in significant behavioral recovery and were accompanied by TH immunoreactivity in striatal neurons and glia [125]. These effects were observed without signs of toxicity. During *et al.* [126] also observed similar results.

TH AND GTP CYCLOHYDROLASE

GTP cyclohydrolase is a critical enzyme in catecholamine function and is rate limiting for the synthesis of catecholamine co-factor tetrahydrobiopterin (BH4). Mandel *et al.* [127] co-transfected cells to express the genes for both TH and GTP cyclohydrolase using an AAV vector system in a rat model to achieve continuous L-DOPA delivery in the striatum. Rats that received the TH AAV vector alone produced measurable L-DOPA only after receiving exogenous BH4. Moreover, elevated L-DOPA was observed in animals that received mixed TH and GTP AAV vectors [124]. Fan *et al.* [128] reported a similar result while using transduced TH and AADC in rat striatal cells, using two separate AAV vectors.

TH, GTP CYCLOHYDROLASE AND (LEVO AROMATIC AMINOACID DECARBOXYLASE) AADC

Following the success by co-transfection of TH and AADC with two different AAV vectors, Shen *et al.* [129] attempted a triple transfection combining AAV vectors expressing TH, GTP cyclohydrolase and AADC. *In vitro* experiments showed that triple transfection results into greater DA production than double transduction with AAV-TH and AAV-AADC. Triple transfection also enhanced BH4 and DA production in the denervated striatum of parkinsonian rats and improved the rotational behavior of rats more effectively than double transfection. Their results thus indicate that GTP cyclohydrolase, in addition to TH and AADC, can be useful for effective gene therapy of PD [124].

CELL REPLACEMENT THERAPY OF PD

Several reports have suggested that injection of cells transfected with TH or dopaminergic in origin can improve PD symptoms [130-133]. In this regard, genetic manipulation and transplantation of cells with the ability to synthesize DA has become an increasingly important area of translational research in PD [134-136]. The need to maximize the early survival of newly transplanted cells to allow them the opportunity to function, integrate into the neural network and gain neurotrophic support has also been recognized [137] and is a current focus to optimize the transplantation approach with the aim of developing and optimizing an alternative or complementary strategy to L-DOPA treatment. Moreover, as yet, relatively little is known about possible modifications to cell homeostasis and the nature of cellular responses when TH is transfected into cells that normally do not express or produce only relatively low levels of this enzyme. For future research, such knowledge will be relevant for the development for truly effective cell transplantation therapy in PD.

INVOLVEMENT OF TH IN THE PATHOGENESIS OF PD *VIA* OXIDATIVE STRESS

1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) is a synthetic DA neuronal toxin that causes PD when ingested

by humans or animals [138]. It is well documented in scientific literature that the actions of MPTP are mediated, in part, by free radicals [139]. *In vivo* and *in vitro* MPTP models of PD showed that key enzymes involved in the production of reactive oxygen species (ROS)/reactive nitrogen species (RNS), are upregulated in damaged areas and contribute to DA neuronal death [140, 141]. Additionally, TNF-α and IL-1β are also reported to be increased and contribute to DA neuronal death in the MPTP model of PD [142].

Mitochondria have been shown to play a role in multiple forms of cell death in PD. Mitochondrial dysfunction in PD has been identified as a systemic deficiency of the electron transport chain Complex I activity [143]. The deficiency or partial inhibition of Complex I have been shown to result in increased mitochondrial reactive oxygen species (ROS) production and subsequently increased oxidative stress [144].

GSH is the major reducing agent in the cell and its reduction is particularly important, especially because it has been reported to be significantly decreased early in PD [145]. Mitochondrial dysfunction and oxidative stress have been increasingly implicated in the pathology associated with genetic models of familial PD [146].

TH INCREASES THE RESISTANCE OF HUMAN NEUROBLASTOMA CELLS TO OXIDATIVE INSULTS

Several studies have reported that injection into dopaminergic brain regions of a wide variety of cells tranfected with TH, such as stem cells, astrocytes, fibroblast and myoblast, can ameliorate PD symptoms in animal models [130-133]. Such action can be potentially mediated at several points, in addition to directly elevating DA levels. As described, the neurotransmitter DA under specific conditions can generate ROS spontaneously or *via* enzymatic processing, and may trigger or exacerbate dopaminergic neurodegeneration by creating potentially harmful hydroxyl radicals, which can damage proteins, lipids and nucleic acids [147-149]. Despite the potentially severe deleterious effects of high levels of ROS to cell viability, it has been proposed that low level exposure can mediate an up regulation of endogenous defense mechanisms that may protect cells against a higher oxidative burst [150]. Preconditioning by oxidative stress is reported to be similar to ischemic preconditioning, and requires the production of nontoxic amounts of ROS, which modulate signaling cascades important in the acquisition of resistance for further harmful insults [151].

Recent studies suggest that exposure to low levels of DA provides preconditioning in different models of PD in cell culture studies in which oxidative stress acts as an initiator [152-154]. Furthermore, in culture studies Franco *et al*. [155] reported that neuronal cells transfected with elevated levels of TH were more resistant than their wild-type counterparts due to the establishment of a preconditioning mechanism, resulting in an elevation in the activity of the antioxidant glutathione-metabolizing enzymes glutathione reductase and glutathione peroxidase, generated by low levels of DA exposure [155]. In accord with other studies, the transfection

of elevated TH into cells led to an elevated production of DA and its metabolites [155]. In the light of their study, they proposed that a therapy using cells expressing TH transcripts may provide multiple beneficial effects in PD-damaged brain areas: elevated DA levels to support neurotransmission and alleviate motor symptoms, as well as an improved antioxidant ability of these cells to withstand potential endogenous and exogenous physiological insults. McCormack *et al*. [156] demonstrated that specific populations of dopaminergic neurons within the brain, such as within the substantia nigra, are more vulnerable to the herbicide paraquat-induced toxicity than other dopaminergic cells within the midbrain consequent to a decreased resistance to oxidative stress. Interestingly, reduced vulnerability appeared related to presence of the calcium-binding protein, calbindin-D_{28k}, which is greater in midbrain regions and within the ventral tegmental area that are not as impacted by PD. Thus there appears to be a level of TH expression together with associated proteins in both normal and transplanted cells that confers optimal provision of DA for neurotransmission and for preconditioning against oxidative insults.

STEM CELL THERAPY FOR PD TREATMENT

It is well documented that pharmacological treatment with L-DOPA generally provides initial positive results, but gradually over the time efficacy is lost and motor complications can occur [157]. The transplantation of DA cells from human embryonic mesencephalon (7–8 weeks after conception) has been reported to improve motor function in people with advanced PD [158-160]. However, this approach has its own limitations, the least of which involves the ethics of using embryonic tissue, difficulty in obtaining it and the poor survival of the transplanted DA neurons.

Transplantation of human embryonic stem cells (hES cells) or their partially differentiated progeny, embryoid bodies leads to the development of some DA neurons and teratomas [161, 162] and can potentially be used for the treatment of patients with PD, but is not ideal [160]. Kimberley *et al*. [160] produced TH-positive cells from hES cells by co culturing them with PA6 cells (mouse stromal cells possessing stromal cell-derived inducing activity), thereby revealing that PA6 cells can cause differentiation of hES cells, as has previously been shown for both mouse and nonhuman primate ES cells [163, 164]. Importantly, the yield of TH-positive cells could be dramatically increased by co-incubation with soluble factors produced by human embryonic striatal tissue as well as by the addition of glial-derived neurotrophic factor (GDNF) [160], which has been widely shown to reduce apoptosis of dopaminergic neurons. Interestingly, GDNF increased the number as well as size of differentiating hES cell colonies and approximately doubled the number of TH-positive cells. Since numerous components of the cultures were determined to be augmented, GDNF and alike factors can therefore be considered as global survival factors that optimize the differentiation of hES and additionally amplify the expression of transcription factors important in the development of dopaminergic neurons [160]. The underlying mechanism(s) *via* which stromal cell co-cultures and related

factors promote differentiation of hES cells to TH-positive cells remains to be elucidated and represents an interesting area of ongoing research [160]. In a comparable approach, Zhou *et al.* [165] reported the efficacy of injecting adult bone marrow derived stem cells transfected with a pEGFP-C2 plasmid containing the gene for TH into the lateral ventricle for the treatment of rats with PD. Albeit more research is required to fully characterize this approach, it clearly remains a further future option in the potential utility of genetically engineered BMSCs in the treatment of PD [165].

NEUROTROPHIC FACTORS IN PD TREATMENT

Developing as well as mature neurons require the presence of neurotrophic factors to maintain their function and viability, and these, epitomized by BDNF, are very often synthesized and secreted by the target tissue. The augmentation of endogenous neurotrophic factors as well as exogenous administration of others represents a promising therapeutic strategy for PD treatment [166-169]. Among the various neurotrophic factors studied, the glial cell line-derived neurotrophic factor released by striatal neurons is more closely associated with PD [168, 170]. It is widely appreciated that glial cell line-derived neurotrophic factor is an agent necessary for maintenance of nigrostriatal DA neurons [171, 172]. In view of its role in the maintenance of DA neurons, it can be considered as a potential therapeutic target of PD treatment [173, 174]. Similarly, fibroblast growth factor 2 is a trophic factor for neurons and its potential neuroprotective actions in DA neurons have been reported [175, 176]. Involved in peripheral nerve development and regeneration, its exogenous application promotes neuronal survival and neurite outgrowth in cellular and animal models [177].

The neuroprotective effect of numerous drugs appears to be mediated, in part or in whole, *via* up regulation of endogenous neurotrophic factors and, in particular, BDNF [178] but also GDNF [179]. As a recent example among many is catalpol, an active component within a number of Chinese medicinal herbs, including *Rehmannia glutinosa*, that are widely used in treating a variety of neurodegenerative conditions in traditional Chinese medicine. Catalpol has been reported to attenuate amyloid-β peptide-induced toxicity of cholinergic neurons by elevating BDNF levels [180] and, additionally, has been reported to mitigate the toxicity of the dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), by up regulating GDNF levels [181]. The compound, thereby, elevate TH neuron number and DA levels without binding to either DA receptors or its transporter, and thus ameliorated MPTP-induced motor effects in rodents [179]. Finally, cerebral DA neurotrophic factor (CDNF), a recently discovered neurotrophic factor, has recently been reported to have neuroprotective and neurorestorative properties for the nigrostriatal DA system following administration to mice [181-183]. TH-immunoreactivity as well as the number of TH-positive cells were reportedly increased by CDNF treatment, supporting the development of CDNF-based treatment strategies for PD [181,183].

A further neurotrophic strategy in PD involves the incretin glucagon-like peptide-1 (GLP-1). This 30-amino acid endogenous insulinotropic peptide is best known for its action to regulate blood glucose levels *via* activation of the GLP-1 receptor (R) on pancreatic β-cells to induce insulin secretion. It additionally acts as a trophic agent, inducing pancreatic β-cell proliferation and neogenesis, as well as inhibiting β-cell apoptosis [184, 185]. Long-acting GLP-1R agonists, including exendin-4 (Ex-4) and liraglutide are approved and now widely used in the effective treatment of type 2 diabetes mellitus [186]; a disease that is known to be associated with an increased incidence of PD as well as of Alzheimer's disease (AD) [187]. Not only is the GLP-1R found throughout the brain but also GLP-1, exendin-4 and liraglutide, despite being peptides, readily cross the blood-brain barrier [188]. Recent studies demonstrate that both GLP-1 and exendin-4 are neurotrophic [189, 190] and increase TH expression in dopaminergic cultures [181] and, additionally, are neuroprotective and fully ameliorate TH loss, DA and metabolite reductions, as well as behavioral impairments induced by MPTP in rodents [191, 192]. Likewise, exendin-4 has been found to provide neurotrophic, neuroprotective and neuroregenerative actions in 6-hydroxydopamine and LPS animal models of PD, mitigating locomotor deficits, ameliorating neuroinflammation and elevating both TH and DA levels in dopaminergic brain regions [193-195]. Such neurotrophic/protective actions appear to translate across neuronal cell types as well neurodegenerative disorders, as GLP-1R agonists have now been reported as effective in cellular and animal models of AD, stroke, ALS and peripheral neuropathy [191, 196-202], spurring repositioning of exendin-4 in recently started clinical trials of AD, PD and peripheral neuropathy (ClinicalTrials.gov identifier: NCT01255163, NCT01174810 and NCT00855439, respectively).

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR-γ) MEDIATED TREATMENT OF PD

Neuroinflammation and the dysregulation of central and peripheral immune systems in PD have been reported in earlier studies. The specific targeting of immune functions contributing to neuroinflammation represents a promising therapeutic approach for PD treatment [203]. In this regard, peroxisome proliferator-activated receptors are members of the nuclear hormone receptor superfamily that regulate the expression of genes involved in a wide variety of biological processes. Members of the PPAR family, in particular PPAR-γ, are key regulators of immune responses, highly expressed within cells of the immune system, and play a pivotal role in microglial activation as well as in monocyte and T cell differentiation. This provides a promising strategy for the treatment of PD, which can be achieved by using PPAR-γ agonists consequent to their ability to modulate the expression of pro- and anti-inflammatory cytokines at the transcriptional level in both central and peripheral immune cells [204]. Preclinical evidence of PPAR-γ-induced neuroprotection in numerous experimental PD models has been also described [194, 195]. As an example, beneficial effects of thiazolidinediones, a class of PPAR-γ agonists, in PD treatment have been recently reviewed by Carta and colleagues [205]. As thiazolidinediones, particularly rosiglitazone and pioglitazone, are currently effectively used clinically as insulin-sensitizing agents in the treatment of

type 2 diabetes melitus [206], their repositioning to assess clinical efficacy in PD represents a feasible option. This, however, would clearly require a cautious approach in light of the recent warning on the safety of rosiglitazone in diabetes [207]; nevertheless, pioglitazone remains clinically available and has recently shown tolerability in initial clinical studies in AD [208].

ADDITIONAL AGENTS IMPACTING TH AND OF POTENTIAL IN PD

Numerous endogenous and exogenous neurotoxin pathways have been elucidated that may contribute to the pathogenesis of PD [209-212]. β-Carbolines (BCs) are endogenously produced pyridoindoles, that can be synthesized from tryptophan or tryptophan-like indolamines. Specific BCs have been found in various fruits and meats, and appear within the body. Some bear structural similarity to MPTP and have been demonstrated to induce dopaminergic neuron toxicity. In this regard, methylated BC cations, such as 2,9-dimethyl-β the -carbolinium ion (2,9-dime-BC+), have been reported to exert highly damaging actions on dopaminergic neurons; impacting mitochondrial function at the level of respiratory chain, elevating ROS, increasing caspases-3 levels and ultimately inducing a decline in cell survival [213] in a manner reminiscent of MPTP. As 2,9-dime-BC+ has been found elevated in the lumbar CSF idiopathic PD subjects and increased with the progression of the disease [214] bioactivated BC+s, might represent causative factors underlying PD. Interestingly, a different but closely related BC, 9-methyl-β-carboline (9-me-BC), has been described to be beneficial in cellular and animal models of PD by providing a plethora of potentially valuable actions [211, 215]. It has been reported to provide stimulatory effects to dopaminergic neurons by increasing the expression of TH and multiple of its transcription factors, by reducing inflammation and by lowering α-synuclein levels. These actions appeared to translate to *in vivo* in a rat MPTP model of PD, wherein dopaminergic function was restored [211, 212]; supporting the agent as potentially interesting candidate treatment for PD [211, 216]. Given the close structural resemblance between potentially detrimental BCs (e.g., 2,9-dime-BC+) and potentially valuable ones (9-me-BC) further preclinical research is warranted to ensure that potential metabolites from the latter retain valuable rather than detrimental actions prior to translation.

An agent that already appears to have effectively translated to the clinic for the treatment of PD is the sulfonamide anticonvulsant drug, zonisamide, whose mechanism of action – despite over two decade of clinical use – remains to be fully elucidated. The agent was serendipitously discovered to have beneficial actions in the treatment of both convulsive attacks and parkinsonian symptoms in a patient [217]. Zonisamide was approved in Japan [2009] as adjunctive therapy with levodopa in previously treated adult patients with PD. In animal models it appears to elevate TH levels and reduce neuroinflammation, and thereby mitigate the toxicity of MPTP [218, 219].

ESTRADIOL AND TH

The sex steroids are implicated in roles that extend well beyond reproduction alone. As examples, estrogen actions have been linked to memory and cognition, synaptic plasticity, neurogenesis and neuroprotection [211]. This, in part, may account for the lower incidence of PD in women versus men [220, 221]. Extensive evidence suggests that estradiol induces the expression of TH [222-224] and this is mediated by estrogen receptors α and β [225]. Several studies have documented the neuroprotective actions of estrogens in both cellular and animal models of PD [220, 226]. Estradiol appears to impact TH promoter activity at a transcriptional level as the promoter contains an array of response elements that are sensitive to estradiol [227], as well as an estrogen response element site [224].

The sex hormones are largely generated from the backbone of cholesterol, and it is feasible that abnormalities in cholesterol metabolism contribute to PD pathogenesis. Furthermore, instabilities in the generation of cholesterol oxidation products (oxysterols) may, likewise, contribute to PD vulnerability and onset [228, 229]. In this regard, oxysterol 27-hydroxycholesterol (27-OHC) is a major oxidized cholesterol metabolite within the circulation that has access to the brain [229]. Recent studies suggest that 27-OHC induces a loss of DA content and an elevation in α-synuclein accumulation in neuronal cells, mediated by reducing the expression of TH and increasing α-synuclein levels *via* estrogen receptors and liver X receptors [228, 229], in particular β receptors. These hence may represent potentially interesting targets *via* which TH as well as α-synuclein may be regulated to delay PD [229]. The regulation of TH by α-synuclein has been detailed at the level of phosphorylation [230, 231] and at the level of its promoter region [232].

NADH AS A TOOL IN PD TREATMENT THROUGH TH

The majority of PD treatment strategies are based on mitigating its symptoms by impacting nigrostriatal dopaminergic neurons. Largely, such approaches attempt to stimulate the natural DA production capabilities of the brain, and L-DOPA continues to be the primary choice of therapy. As L-DOPA is an endogenous intermediate catecholamine molecule its exogenous administration can induce dramatic changes in the catecholamine system, the activities of key components of which already are reduced in PD patients [233-234]. In light of this, a need was envisioned to overcome the DA deficit without altering or inhibiting key components, such as TH activity, whilst increasing L-DOPA biosynthesis.

An approach to achieve this goal was the use of the coenzyme of TH, BH4; which is reduced in PD. Therapeutic application of BH4 was considered, but it lacked beneficial clinical effects in PD [235]; a limitation potentially due to its difficulty in crossing the blood-brain barrier [234]. However, reduced nicotinamide adenine di-nucleotide (NADH) has been described to effectively boost endogenous production of DA, since NADH can indirectly supply reducing equivalents to the rate-limiting, TH-catalyzed step of DA synthesis [236]. NADH enhances BH4 by increasing a key enzyme in the BH4 pathway, quinoid H2 pteridin reductase [237]. Hence, NADH has been reported to enhance endogenous L-DOPA biosynthesis and increase in L-DOPA production to provide an improvement of the clinical

symptoms in PD patients [234]. How such treatment would compare to well established L-DOPA therapy, particularly over an extended duration, remains an important question and may warrant assessment.

CONCLUSION

There is a clear current medical need of new and effective treatment strategies for PD that impact disease progression, and go beyond the present logical and efficient strategy of L-DOPA symptomatic treatment. A number of approaches to correct or bypass the described enzyme deficiency with DA agonists, inhibitors of DA metabolism or brain grafts utilizing cells expressing a high level of TH have long remained areas of interest. Neurotrophic factors, likewise, have long attracted the attention of the neuroscientists consequent to their neuroprotective and neurorestorative properties [238, 239] by attempting to dial in on key regulators of the immune response, as epitomized by targeting PPAR-γ as well as TNF-α [240, 241]. Clearly evident in recent years is the application of cutting edge molecular biology to optimize use of viral vectors to deliver single or combinations of deficient human proteins to specific cells at defined locations. Such approaches are not only build on prior PD research as they move to assessment of clinical utility, but have valuable broad applicability to a variety of prevalent degenerative disorders in which their target may be associated with a different form of neuronal cell death in a different location within the nervous system (motor neurons in ALS, cholinergic and glutamatergic neurons in early AD). Common features between targets across diseases permit the repositioning of already developed drugs effective for one disease to be assessed in another, as epitomized by exendin-4 – an efficacious drug in type 2 diabetes mellitus [184-186, 188] presently being assessed in early efficacy studies in PD and AD. Even for drugs with a different target, such as the experimental AD agents posiphen and phenserine that lower the rate of synthesis of amyloid-β precursor protein and thereby reduces the generation of the AD toxic species amyloid-β peptide [242], the molecular mechanism *via* it is achieved (translational regulation mediated *via* an iron response element) within the 5'-untranslated region of amyloid-β precursor protein mRNA [242, 243] is present within the 5'-untranslated region of α-synuclein [244, 245] and thereby providing a basis for the assessment of these agents in PD [246, 247]. Pioglitazone represents a further potential example [248]. Whereas, the translation of drug across diseases, although based on sound science, is not invariably successful, this avenue [249-251] together with the other described basic and translational approaches are providing continuous incremental improvements in our understanding of the basis of PD and our search for targets will hopefully impact disease progression.

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CONFLICT OF INTEREST

Declared none.

ABBREVIATIONS

CDNF	=	Cerebral DA neurotrophic factor
DA	=	Dopamine
hES	=	Human embryonic stem
NTFs	=	Neurotrophic factors
NADH	=	Nicotinamide adenine di-nucleotide
PD	=	Parkinson's disease
PPAR-γ	=	Peroxisome proliferator-activated receptor gamma
TH	=	Tyrosine hydroxylase
ROS	=	Reactive oxygen species

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