Molecular Neurobiology

ISSN0893-7648/98/16(3): 285–309/$14.25

Tyrosine Hydroxylase and Parkinson's Disease

Jan Haavik* and Karen Toska

Department of Biochemistry and Molecular Biology, University of Bergen, N-5009 Bergen, Norway

Abstract

A consistent neurochemical abnormality in Parkinson's disease (PD) is degeneration of dopaminergic neurons in substantia nigra, leading to a reduction of striatal dopamine (DA) levels. As tyrosine hydroxylase (TH) catalyses the formation of L-DOPA, the rate-limiting step in the biosynthesis of DA, the disease can be considered as a TH-deficiency syndrome of the striatum. Similarly, some patients with hereditary L-DOPA-responsive dystonia, a neurological disorder with clinical similarities to PD, have mutations in the TH gene and decreased TH activity and/or stability. Thus, a logical and efficient treatment strategy for PD is based on correcting or bypassing the enzyme deficiency by treatment with L-DOPA, DA agonists, inhibitors of DA metabolism, or brain grafts with cells expressing TH. A direct pathogenetic role of TH has also been suggested, as the enzyme is a source of reactive oxygen species (ROS) in vitro and a target for radical-mediated oxidative injury. Recently, it has been demonstrated that L-DOPA is effectively oxidized by mammalian TH in vitro, possibly contributing to the cytotoxic effects of DOPA. This enzyme may therefore be involved in the pathogenesis of PD at several different levels, in addition to being a promising candidate for developing new treatments of this disease.

Index entries: Neurodegeneration; dopamine; iron; oxidant stress; mutations; pathogenesis; etiology; gene therapy; transplantation.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that is clinically characterized by tremor, bradykinesia, rigidity, and loss of postural reflexes (Strange, 1992). It is generally believed that the major symptoms of PD are caused by a striatal dopamine (DA) deficiency, secondary to degeneration of nigrostriatal dopaminergic neurons and possibly a decreased DA-biosynthetic capacity in the surviving cells. Although the DA loss is most pronounced, norepinephrine, serotonin, and melanin pigments are also decreased, whereas cholinergic activity seems to be increased (Ehringer and Hornykiewicz, 1960). Although the cause of PD still is unknown, a number of different hypotheses for its etiology have been

*Author to whom all correspondence and reprint requests should be addressed.

presented. This review focused on alterations in the function of tyrosine hydroxylase (TH; EC 1.14.16.2), which is the rate-limiting enzyme in the biosynthesis of DA, and its importance for the pathogenesis and treatment of PD.

Etiology of Neurodegenerative Disorders

The selective loss of specific neurons in the central nervous system (CNS) is a characteristic feature for PD and other common neurodegenerative disorders, such as Alzheimer's disease, Huntington's chorea, and amyotrophic lateral sclerosis. Despite intensive research efforts during recent years, fundamental questions regarding the etiology and pathogenesis of these diseases are still unresolved. Thus, the neuronal injury in neurodegenerative disorders has been linked to the possible involvement of infectious agents, exogenous toxins, genetic factors, neuronal injury caused by excitatory amino acid transmitters, endogenous reactive oxygen species (ROS), or various other metabolic disturbances, including lack of neurotrophic hormones or impaired DNA repair mechanisms (Beal, 1995; Calne and Takahashi, 1991; Youdim and Riederer, 1997). In a variant form of these hypotheses, PD is considered to be partly the result of an accelerated aging process (Enochs et al., 1994). Here we mainly focus on the oxidative stress hypothesis of PD (for reviews, *see* Jenner, 1991; Bondy, 1992; Coyle and Puttfarcken 1993; Ebadi et al., 1996) and its relationship to recently discovered genetic factors in this disease, whereas the reader is referred to general reviews for a discussion of the other theories (Poirier et al., 1991; Evans, 1993).

The Oxidant Stress Hypothesis of Parkinson's Disease

All aerobic cells are constantly exposed to oxidant stress, although certain cell types have metabolic characteristics that can be considered as particular "risk factors" for the development of oxidative injury. For several reasons, catecholaminergic neurons may belong to this category. First of all, neurons consume large amounts of dioxygen which is used in mitochondrial respiration for the generation of ATP and conservation of the neuronal energy balance. "Leakage" along the mitochondrial electron chain causes the formation of ROS, such as H_2O_2 and $^{\bullet}O_2^-$. These compounds can either directly, or by the formation of the extremely reactive hydroxyl radical ($^{\bullet}OH$), cause oxidative damage to cellular macromolecules (proteins, lipids, carbohydrates, and nucleic acids) (Beal, 1995). $^{\bullet}OH$ may be generated spontaneously, but its formation is markedly increased in the presence of redox-active metal ions, in particular by the reaction between Fe(II) and H_2O_2 (The Haber-Weiss/Fenton reaction). Superoxide ($^{\bullet}O_2^-$) has a more restricted reaction pattern than $^{\bullet}OH$ and reacts mainly with proteins that contain transition metals (Gutteridge, 1994). Such reactions have been found to accelerate lipid peroxidation (found in SN *pars compacta* in PD; Hirsch, 1994) and can also oxidize amino acids with subsequent loss of protein function.

Other metabolic features that are characteristic of catecholaminergic neurons and neuroendocrine cells include the synthesis, release, and metabolism of DA, noradrenaline, and adrenaline. Several of the enzymes involved in these reactions are thought to produce ROS as byproducts of their activity.

Monoamine Oxidase (MAO)

The mitochondrial outer membrane enzyme monoamine oxidase (MAO), catalyzes the deamination of catecholamines (e.g., DA) and generates one equivalent of H_2O_2 per equivalent of catecholamine oxidized (Sandri et al., 1990). H_2O_2 can readily dissociate over the mitochondrial membrane, react with iron(II) to form $^{\bullet}OH$, or be neutralized by the

antioxidant enzymes catalase and glutathione reductase.

Because the activity of MAO increases with age (Fowler et al., 1980; Sparks et al., 1991), this enzyme has been suggested to have a pathogenetic role in PD (Oreland, 1991). This suggestion led to the design of large multicenter clinical trials where PD patients received the MAO B inhibitor L-deprenyl (Selegiline), in an attempt to halt or reverse the progression of the disease. Because L-deprenyl inhibits the oxidative catabolism of DA, theoretically a decreased amount of toxic metabolites of DA will be formed. Although the treatment at first seemed to delay progression of the disease (*N. Engl. J. Med.*, **321,** 1364–1371), the effect has since been found only to be transient and the patients forced to return to standard treatment with L-DOPA after some time (*Arch. Neurol.* **52,** 237–245). In addition, several alternative explanations for the partial efficacy of L-deprenyl treatment in PD have been suggested. Thus, L-deprenyl may increase neurotrophic factor activity and increase the levels of bcl-2, (a regulatory protein in the apoptotic pathway), as well as factors that protect against oxidant stress (superoxide dismutase [SOD], catalase, and glutathione) (Jenner and Olanow, 1996). This correlates well with the decreased oxidative damage seen in aged rats treated with L-deprenyl (De La Cruz et al., 1996) and with its apparent effect on disorders not related to catecholamine metabolism (Wu et al., 1996). Because treatment of rats with L-deprenyl also affects TH expression, it has even been suggested that the observed effects of L-deprenyl are secondary to changes in TH activity and rate of dopamine synthesis (Haavik et al., 1997a; Goc and Stachowiak, 1994; Rodriguez et al., 1997).

Neuromelanin

It has been suggested that neuromelanin, or a compound related to this substance, is involved in the mechanisms leading to loss of dopaminergic neurons in PD, because a selective injury of dopaminergic cells with a high content of neuromelanin has been observed in several studies (Hirsch, 1993, 1994). Neuromelanin may, under normal conditions, have a cytoprotective function in the sequestration of Fe(II). However, under altered conditions, like in PD, it is thought to possess a cytotoxic role by exerting cellular damage, initiating redox reactions, and chelating and concentrating redox active transition metals, such as iron (Enochs et al., 1994).

It is well established that nonenzymatically or tyrosinase-mediated oxidation of catecholamines generates highly reactive o-quinone intermediates. These compounds can undergo cyclization and polymerization or react with cellular thiols to form neuromelanin (eumelanins and pheomelanins, respectively; Graham, 1978). However, the mechanism of the synthesis of neuromelanin in the human brain has been subject to some controversy. Whereas most investigators have concluded that its formation is initiated by the nonenzymatic oxidation of catechols and catecholamines (Barden, 1969; Rodgers and Curzon, 1975; Linert et al., 1996), the involvement of oxidative enzymes such as tyrosinase (Miranda et al., 1984), or peroxidase (Okun et al., 1971) has also been suggested. Tyrosinases are widespread in nature and are responsible for the biosynthesis of the photo protective melanin pigments that are found in external organs in higher organisms (Hearing and Jimenez, 1987). However, it is not clear whether the human SN contains significant levels of this enzyme (Miranda et al., 1984).

In contrast to tyrosinase, TH is expressed at high levels in the SN, but has been believed to possess a narrow substrate specificity, not including catechols or catecholamines (Kaufman, 1995). Therefore, the participation of TH in the oxidation of catechols and formation of neuromelanin has generally been excluded (Rodgers and Curzon, 1975; Miranda et al., 1984). However, in recent studies, the substrate

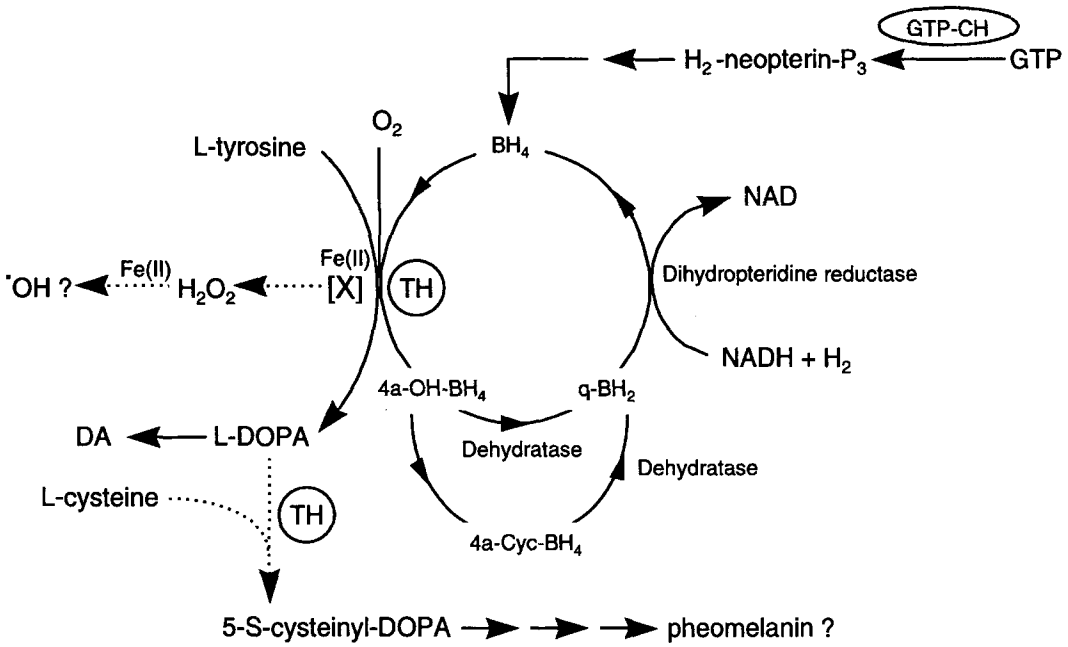

Fig. 1. Schematic presentation of the hydroxylation reaction catalyzed by tyrosine hydroxylase (TH), including a possible generation of reactive oxygen species. The cofactor (BH_4) product is released from TH as 4a-OH-BH_4, which dehydrates to the quinonoid dihydrobiopterin (q-BH_2), or forms a putative cyclic intermediate (4a-Cyc-BH_4); Almås et al., 1996. BH_4 is subsequently regenerated by the NADH-dependent enzyme dihydropteridine reductase. [X] represents an unknown intermediate. L-DOPA is converted to dopamine by DOPA decarboxylase (aromatic amino acid decarboxylase). Alternatively, some of the DOPA may be oxidized by TH to form 5-S-cysteinyl DOPA (or other thioether derivatives of DOPA). For further information, *see* text and Haavik and Flatmark, 1987; Almås et al., 1996.

specificity of TH has been investigated in more detail (Fitzpatrick, 1989; Hillas and Fitzpatrick, 1996) and found to be considerably broader than originally thought. Thus, it has recently been found that L-DOPA is an excellent substrate for rat, bovine, and human TH, (Haavik, 1997) and is converted to 5-S-cysteinyl DOPA in the presence of cysteine or to analogous thiol adducts in the presence of other thiols. This finding has raised the possibility that TH may also contribute to the formation of neuromelanin (pheomelanin) in vivo (Haavik, 1997) (Fig.1). A DOPA oxidase activity present in the cochleae of adult gerbils has also been proposed to be involved in the synthesis of neuromelanin (Benedito et al., 1997). This enzyme has many properties in common with both TH and tyrosinase, but in contrast to TH it is inhibited by 2-mercaptoethanol and is not stimulated by (6*R*)-tetrahydrobiopterin (BH_4).

Generation of ROS by Iron

Many biochemical processes are critically dependent on the cytoplasmic levels of iron(II) or iron(III), and the brain seems to have efficient mechanisms for the transport, storage, and redox shuttling of this metal (Bradbury, 1997). The accumulation of iron and possibly other transition metals in the SN of PD patients has been reported in several studies (Dexter et al., 1987, 1989; Sofic et al., 1991), whereas other investigators have not found a significantly increased iron content (Riederer et al., 1989). Iron has a potential pathogenetic

role in PD because of its ability to generate ROS and Fe(III)-melanin complexes, which subsequently can lead to oxidative stress (Linert et al., 1996; Gerlach et al., 1994). Intranigral injections of iron(II) have been shown to induce neuronal death and therefore have been suggested as a model of progressive parkinsonism (Jenner and Olanow, 1996). Recently, it has been reported that the SN of 6-hydroxydopamine (6-OHDA)-lesioned rats accumulates high concentrations of iron (He et al., 1996). However, an important question that remains unresolved is whether the increased iron concentration is an early (causal) factor or a consequence of the neurodegenerative process. As it has been shown that DA reacts spontaneously, without the necessity of MAO or metal ion catalysis, to produce stoichiometric amounts of H_2O_2 and dopaminochrome, the pathogenetic significance of both these factors has recently been questioned (Linert et al., 1996).

Biochemical Evidence for Oxidative Injury in PD

Oxidative injury manifests itself as an increased oxidation of cellular constituents (lipids and proteins) and DNA damage. Lipid peroxidation (Dexter et al., 1989) and protein damage (*see* below) have been observed in the SN of PD patients, which indicates that oxidative stress is involved in the pathogenesis of this disease. Furthermore, an age-dependent accumulation of oxidized nucleotides in mitochondrial DNA (mtDNA) (Mecocci et al., 1993) and an increased frequency of mtDNA deletion mutations in the putamen, caudate, or SN, relative to other brain regions such as the cerebellum, has been reported (Soong et al., 1992). Evidence for the involvement of hereditary mtDNA mutations in various neurological disorders (Schapira and Cooper, 1992; Egensperger et al, 1997), and an increased frequency of mtDNA deletion mutations, mainly in the genes encoding for complex I (Ikebe et al., 1990; Schnopp et al., 1996), has been found in the brain, as well as in peripheral tissues, in some PD patients. However, whether the deletion in mtDNA really contributes to the pathogenesis of PD or is purely age-related (Mann et al., 1992; Kosel et al., 1997) is not clear.

ROS Scavengers

As described above, many factors may contribute to the generation of ROS under normal as well as pathological conditions. Still, it is of equal importance to consider the scavenging of these compounds, since the cause of oxidative stress is postulated to be an imbalance between the production and the clearance of oxidative species (Kehrer, 1993). Low-molecular-weight antioxidants like vitamin E, ascorbic acid (vitamin C), carotene (vitamin A), and glutathione, directly interact with ROS, whereas enzymes such as SOD, glutathione peroxidase, and catalase convert these species to less reactive compounds. SOD and vitamin E have been found to partially protect catecholaminergic neurons against 6-OHDA-mediated injury (Albino Teixeira et al., 1991). SOD exerts its antioxidizing effect by converting superoxide to H_2O_2, which is subsequently metabolized by glutathione peroxidase or catalase (Kehrer, 1993). Still other enzymes are involved in repairing the damage on proteins, lipids, and nucleic acids.

The glial enzyme glutathione peroxidase is the major protective enzyme against accumulation of H_2O_2 (Damier et al., 1993), indicating a coupling between neurons and glia cells with regard to the clearance of toxic species. The activity of glutathione peroxidase has been reported to increase with the severity of neuronal degradation in SN (Damier et al., 1993). This suggests a protective role of glutathione peroxidase against oxidative stress and the gliosis found to surround the dopaminergic cells in PD could be a way to protect the surviving neurons. Similarly, glia also contain neurotrophic factors that are thought to protect neurons against apoptosis.

Neurotrophic Factors

The neurotrophic factors include a large group of proteins that are necessary for survival and differentiation of neural cells (Yoshimoto et al., 1995). Two such factors, brain-derived neurotrophic factor (BDNF) and glia cell-line-derived neurotrophic factor (GDNF), in particular, have been subject to much interest because of their positive effect on survival and differentiation of dopaminergic neurons (Hyman et al., 1991). Thus, both BDNF and GDNF have been shown to have neuroprotective effects in rats treated with neurotoxins like MPTP (Yoshimoto et al., 1995; Hyman et al., 1991; Tsukahara et al., 1995; Hou et al., 1996) and 6-OHDA (Fossom et al., 1992; Shults et al., 1995). Such studies have indicated that neurotrophic factors may be useful therapeutic tools, most likely in combination with other treatments in future management of neurodegenerative diseases, like PD.

An insufficient expression of these endogenous soluble proteins, may result in neuronal death or dysfunctional cells, but their direct involvement in PD has not been proven. Thus, in recent studies, no significant difference in the number of TH positive dopaminergic neurons in the SN were found between mice carrying a null-mutation for GDNF compared to wild-type mice. Also the apparent density of dopaminergic projections to the striatum was identical (Sanchez et al., 1996; Moore et al., 1996). These results indicate that GDNF is not essential for dopaminergic neurons in embryonic development. However, additional studies to determine whether a continued lack of neurotrophic factors will have any effects on dopaminergic neurons later in life, need to be done (Granholm et al., 1997).

Is Tyrosine Hydroxylase a Source of Oxidant Stress?

The apparent correlation between the extent of neuronal damage and the regional distribution of catecholamines, neuromelanin, iron, and so on, has been presented as evidence supporting a causal relationship between these findings. Although such arguments must be supplemented by more direct evidence, a similar correlation can also be found for other biochemical markers of catecholaminergic neurons, including TH levels. Because it employs highly reactive intermediates to introduce hydroxyl groups into the relatively inert CH-bonds in its substrates (Kappock and Caradonna, 1996), the TH system has recently been suggested to contribute to the formation of H_2O_2 and other ROS in vitro (Haavik et al., 1997a).

The arguments that support a pathogenetic role of TH can be summarized as follows: The TH cofactor BH_4 is subject to autoxidation, in a radical-mediated chain reaction generating superoxide and H_2O_2 (Blair and Pearson, 1974). TH is capable of generating H_2O_2 during the uncoupled reaction in vitro. Although a small fraction (<10%) of the consumed dioxygen is converted into H_2O_2 under normal conditions, this fraction is much higher when the unnatural isomer 7-BH_4 is used as cofactor (Haavik et al., 1997). The concentration of 7-BH_4 is usually low, but significant amounts of this isomer is formed from BH_4 in patients who are deficient in pterin-4a-carbinolamine dehydratase, an enzyme involved in recycling of BH_4 (*see* Fig. 1; Adler et al., 1992). TH contains redox-active non-heme iron (Meyer Klaucke et al., 1996). Using an indirect liquid chromatography/electrochemical assay, a significant increase (40%) in $^{\bullet}OH$ formation from H_2O_2 and Fe(II) was observed in the presence of stoichiometric amounts of TH (Haavik et al., 1997). In theory, the enzyme-bound iron can react with H_2O_2 and possibly superoxide to generate $^{\bullet}OH$ in a Fenton type of reaction.

Like other cellular proteins, TH is also a possible target for damaging alterations induced by ROS. The pteridine-dependent aromatic amino acid hydroxylases (TH, phenylalanine hydroxylase [PAH] and tryptophan hydroxylase [TPH]) are rapidly inactivated in vitro by exposure to H_2O_2 or high levels of O_2 (Kauf-

man, 1995; Kappock and Caradonna, 1996). This inactivation is probably caused by the reversible oxidation of enzyme-bound iron(II) and irreversible oxidative damage of essential amino acid residues by •OH or other ROS (Fink and Elstner, 1984). The extent of amino acid oxidation can be estimated by measuring the formation of carbonyl groups in the target proteins (Poirier et al., 1991).

In aged rats (24 mo), De La Cruz et al. (1996) found a significant increase (59%) of carbonyl groups of TH enzyme in SN compared to young animals (4–12 mo). This suggests that either there is an age-dependent decrease in the rate of proteolytic degradation of TH protein, or the amount of TH protein damaged by ROS is really higher in aged rats. In addition, a decrease in specific TH activity was observed, which implies the formation of inactive TH enzyme. These alterations were mainly seen in the SN, whereas the striatum seemed to be relatively unaffected by this oxidative damage. Thus, the SN seems to be more vulnerable to oxidative damage than other brain regions. Interestingly, TH in intact PC12 cells is also inactivated by peroxynitrite, a compound that has been implicated as a mediator for the oxidative injury in neurodegenerative disorders (Ischiropoulos et al., 1995). Although it has not been proven that oxidative inactivation of TH is involved in the pathogenesis of PD in humans, it is interesting to note that the TH content (and DA biosynthetic capacity) also seems to be decreased in the surviving nigral neurons (McRitchie et al., 1997).

TH's ability to generate ROS, together with the fact that some of these species are very short lived and therefore mainly exert a local effect, suggest that some of the oxidative damage to TH could be generated by the TH system itself. However, as it is very difficult to separate the possible pathogenetic effects of the TH system from the effects of catecholamines and other components of catecholaminergic neurons, this hypothesis is difficult to test. It has been suspected that treatment with high doses of L-DOPA can accelerate the progression of PD, because of the cytotoxic effects of this amino acid (Youdim and Riederer, 1997). If the intracellular levels of DOPA are equal to or higher than the tyrosine concentration, the DOPA oxidase activity of TH could be significant and theoretically contribute to the cytotoxicity of DOPA (Haavik, 1997).

Genetic Factors

The genetic contribution to the etiology of PD has been controversial. Although most PD cases seem to be sporadic, many theories suggesting both environmental and genetic factors, or a combination of these, have been put forward. Early searches for a genetic contribution to PD using twin studies did not reveal significantly higher rates of concordance in monozygotic than dizygotic pairs. However, more recent studies have concluded that in certain populations genetic factors play a substantial etiological role (for a review, *see* Vieregge, 1994), and indications for an autosomal dominant inheritance of PD in some large multi case families have been reported (Degl'Innocenti et al., 1989; Golbe et al., 1990). Recently, genetic markers on chromosome 4q21-q23 were found to be linked to PD phenotype in a large Italian kindred with autosomal dominant PD (Polymeropoulos et al., 1996) and the patients were shown to have a single nucleotide change at position 209 in the α-synuclein gene (Polymeropoulos et al., 1997). This corresponds to a missense mutation (Ala53Thr) in this presynaptic protein, which is thought to be involved in synaptic plasticity. Interestingly, rats and zebra finches normally have this amino acid change in their homologs to the human α-synuclein gene, apparently without any harmful effects. As the mutation was found in all except one of the patients, and was not present in more than 300 human control chromosomes, it was suggested that α-synuclein is involved in the pathogenesis of PD in this family. One possible dominant negative disease mechanism would involve the formation of insoluble aggregates of the mutant protein (Polymeropoulos et al., 1997).

Although mutations in the α-synuclein gene probably accounts for a small proportion of PD cases, this finding illustrates the similarities between PD and familial forms of other mammalian neurodegenerative disorders, where at least a dozen different mutant proteins have been assigned a role in the pathogenesis (Heintz and Zoghbi, 1997; Warner, 1994). In most cases in which the biological roles of the proteins have been identified, they normally function as receptors, ion channels, or enzymes. A particularly instructive example of the latter involves the SOD1 gene which is mutated in some cases of familial amyotrophic lateral sclerosis (*Nutr. Rev.* **51,** 243–245).

In theory, different alterations in the properties of SOD can have various detrimental effects on neurons. Thus, mutations leading to an elevated activity of SOD1 will increase the metabolism of superoxide and the formation of H_2O_2. On the other hand, if the SOD activity is decreased, superoxide will accumulate, also having potential neurotoxic effects. Gain of function mutants with an altered spectrum of substrates and reaction products can also be imagined. However, apart from these special cases, the best established mechanism of cellular injury in neurodegenerative disorders seems to be the abnormal accumulation of insoluble aggregates of the mutant proteins, as observed for familial Alzheimers disease, prion-related diseases, and, more recently, trinucleotide repeat diseases such as Huntington's chorea (Davies et al., 1997; Scherzinger et al., 1997).

Dystonia and Atypical PD

Dystonia refers to a heterogenous group of movement disorders of unknown etiology, where some forms have many clinical similarities to PD. The primary dystonias can be divided into various clinical subtypes that have been mapped to several chromosomal loci, including 9q34 (Orelius et al., 1989), 14q22.1-22.2 (Ichinose et al., 1994), 17q21 (Wijker et al., 1996), and Xq21 (Kupke et al., 1992). Segawa's syndrome (hereditary progressive dystonia with marked diurnal fluctuation) or DOPA-responsive dystonia (DRD), which accounts for 5–10% of primary dystonia of childhood and adolescence, is characterized by a dramatic response to low doses of L-DOPA (Nygaard et al., 1988). As some DRD patients have reduced levels of biopterin and respond to treatment with BH_4 (Fink et al., 1989), it has been proposed that DRD is caused by a disturbance in the synthesis of DA, possibly secondary to an impaired synthesis of BH_4 (Fink et al., 1988) (Fig.1). A neurochemical and neuropathological study of an autopsied case of DRD revealed a remarkable reduction in DA levels and TH activity in the striatum, leading the investigators to suggest that DRD may be caused by a disturbed DA synthesis or arborization of striatal DA terminals (Rajput et al., 1994). Consistent with this, Ichinose et al. (1994) reported that several patients with autosomal dominant DRD were heterozygous for mutations in the gene for GTP cyclohydrolase I (GTP-CH), the enzyme that catalyses the first and rate-limiting step in the biosynthesis of BH_4. They also showed that the chromosomal location of GTP-CH corresponded to the DRD locus (14.q.22.1-14.22.2). Later, several additional GTP-CH mutations have been detected in patients with autosomal dominant DRD in different populations (Bandmann et al., 1996a). However, recent studies on sporadic cases of DRD have indicated that mutations in the GTP-CH gene are not likely to be responsible for the development of parkinsonism or dystonia in patients without a positive family history of DRD (Bandmann et al., 1996b).

As the DRD patients were heterozygous for the GTP-CH mutations, and affected females more frequently than males, it has been speculated that the mutations could have a dominant negative effect, and that the clinical outcome is also determined by other unknown factors. The apparently selective loss of dopaminergic neuronal function has been explained by relative low levels of GTP-CH expression in dopaminergic neurons (Kapatos et al., 1997), the high rate of DA production in the SN pars compacta,

and the high K_m value of BH_4 for TH, relative to other pteridine dependent enzymes, such as PAH and TPH (Segawa, 1996). However, the last explanation is not completely satisfactory, considering the wide and overlapping ranges of K_m values for these enzymes reported in the literature. In contrast to the relatively mild phenotype associated with the dominant form of GTP-CH mutations, patients who are homozygous for GTP-CH deficiency have a severe clinical picture, dominated by atypical malignant phenylketonuria (PKU) (Ichinose et al., 1994; Thöny and Blau, 1997).

TH Mutations in Humans

Based on observations suggesting an impaired DA synthesis and beneficial effects of BH_4 therapy in DRD, it was suggested that TH is a likely candidate gene in this disease (Bartholome, 1983). Indeed, shortly after the discovery of GTP-CH mutations in patients with DRD, Lüdecke et al. reported a homozygous missense mutation (Q381K) in the conserved exon 11 (catalytic domain) of TH in two siblings with autosomal recessive DRD (Lüdecke et al., 1995). Subsequent expression of the mutant protein and activity measurements in vitro showed a reduced V_{max} and an increased K_m for tyrosine, indicating a causal relationship between the TH mutation and the metabolic defect of the patients (Lüdecke et al., 1995; Knappskog et al., 1995). Furthermore, examination of the X-ray structure of truncated rat TH (*see below*), has shown that this mutation may lead to local movement in an α-helix close to the active site (Goodwill et al., 1997). Subsequently, two other mutations in exon 5 (L205P and R202H) have been shown to be associated with a severe form of DRD. Preliminary studies of the L205P mutant protein, expressed in eukaryotic and prokaryotic cells, showed a reduced stability and V_{max} in vitro (Lüdecke et al., 1996). This was expected from the structural modeling, as this mutation probably disrupts an α-helical structure (Goodwill et al., 1997).

In contrast to the documented role of TH in the etiology of familial DRD, studies in patients with sporadic or familial PD have suggested that the TH locus is not a major genetic determinant (Plante Bordeneuve et al., 1994; Gasser et al., 1994). Although PD is different from DRD in that no signs of dopaminergic neurodegeneration appears in the latter (Rajput et al., 1994), the clinical features of the two syndromes are overlapping. Thus, DRD typically presents with dystonia in the lower limbs in childhood, but may produce an akinetic-rigid syndrome in middle and old age (Bandmann et al., 1996b), and the patients with mutations in exon 5 of TH had a clinical picture consistent with severe DRD or juvenile PD. Still, it is presently not clear how suitable the DRD model is for the study of all aspects of PD etiology and comparisons between the two syndromes must obviously be done with caution.

In contrast to the small number of disease-related mutations that have been detected in the TH gene, the PAH locus mutation database has registered more than 300 different mutations and polymorphisms, making it one of the best-characterized human genes (Scriver et al., 1996). As TH and PAH are functionally and structurally homologous proteins (Erlandsen et al., 1997a), and have a similar DNA composition (Dahland Mercer, 1986), the mutation rates of the two genes are likely to be similar. The frequency of heterozygous carriers or homozygous patients with mutant genes should therefore be comparable. Thus, it has long been suspected that the main reasons for this large difference in observed mutation frequencies is the existence of efficient biochemical screening programs for PKU and that many mutations in the TH gene are incompatible with life in the homozygous form. In accordance with this notion, it has been found that all the DRD associated TH mutants characterized so far have some residual activity, i.e., corresponding to the mild forms of hyperphenylalaninemia associated with PAH mutations (Lüdecke et al., 1996; Goodwill et al., 1997). In analogy with TH, very few PAH mutations have been found among non PKU

hyperphenylalaninemia patients. Furthermore, inactivation of both TH alleles in mice results in >90% lethality caused by cardiovascular failure between embryonic days 11.5 and 15.5 (Kobayashi et al., 1995; Zhou et al., 1995).

From studies on other diseases, it is clear that mutations in a single gene may be associated with several different clinical phenotypes. As discussed above, different mutations in enzymes such as SOD or TH may either lead to loss of function, or to the gain of new functions with various consequences for the host cells. In particular, active site mutations in TH could either lead to loss of activity or affect the reaction coupling, increasing ROS formation and causing oxidative damage. In addition to its importance in extrapyramidal motor disorders, the TH gene has particularly been implied in the pathogenesis of psychiatric disorders, such as manic depressive illness and schizophrenia. Whereas several studies have concluded that the TH gene is not a major locus in these diseases, a recent two-locus admixture analysis of patients with affective disorder showed that a locus at or near TH influenced disease susceptibility in some pedigrees (Smyth et al., 1997). Furthermore, a DNA polymorphism in the first intron of the TH gene appears to be associated with disturbances of the catecholaminergic system in schizophrenia (Thibaut et al., 1997). These recent findings illustrate that it is premature to exclude the involvement of TH in the pathogenesis of psychiatric disorders (Mallet, 1996).

The Tyrosine Hydroxylase Reaction

TH is a non-heme iron protein that uses one molecule of dioxygen to hydroxylate its amino acid and tetrahydropterin substrates to hydroxy-amino acids and 4a-hydroxy-tetrahydropterins, respectively (Kappock and Caradonna, 1996; Nagatsu, 1995). As the 4a-hydroxy-tetrahydropterin subsequently dehydrates and is regenerated by the NADH-dependent enzyme dihydropteridine reductase (Hearing and Jimenez, 1987; Haavik and Flatmark, 1987), it is frequently termed a co-factor for the pteridine-dependent hydroxylases (Fig.1). BH_4 is the most abundant of the unconjugated tetrahydropterins in mammalian tissues and is considered to be the natural tetrahydropterin substrate for these enzymes, although many different tetrahydropteridines and pyrimidine analogs will function as substrates (Kappock and Caradonna, 1996).

TH Structure

The active site structure and catalytic mechanism of the aromatic amino acid hydroxylases have been investigated by kinetic and spectroscopic techniques, as well as by site-directed mutagenesis. In particular, the metal-binding sites in TH and PAH have been studied by UV-visible, electron-paramagnetic resonance, resonance Raman, Mössbauer, ^{1}H-nuclear magnetic resonance, and extended X-ray-absorption fine-structure spectroscopy (Meyer Klaucke et al., 1996; Ramsey et al., 1996). These studies indicate that the active site iron(II) in TH is solvent exposed and is hexa-coordinated to two histidine residues and several oxygen ligands, such as water or carboxylate residues. The histidines were tentatively identified as His331 and His336 (numbering corresponding to the rat TH sequence), based on experiments using site-directed mutagenesis (Ramsey et al., 1995). The iron is necessary for catalytic turnover, and the tetrahydropterin and amino acid substrates bind close to the iron(II) center, but probably without a direct coordination to the metal (Martinez et al., 1993).

Recently, the three-dimensional structure of the homotetrameric C-terminal domain of rat TH (including the active site) has been studied by X-ray crystallography (Goodwill et al., 1997), providing a structural basis for many of the observed properties of this enzyme. As the truncated form of the enzyme has full catalytic activity, it was assumed that the active site structure was identical to that of the full-length enzyme. Each monomer contains 14 α-helices

(49%) and 8 β-strands (9%) and can be subdivided into an active site domain (residues 155–456) and a tetramerization domain (residues 457–498). The active site is formed by a 17-Å deep crevice in the center of the C-terminal domain. This study showed that the iron is placed 10 Å below the enzyme surface, in a flexible, solvent exposed position, coordinated to His331, His336, Glu376, and at least two water molecules. The iron-coordination geometry has many similarities to that of other non-heme iron proteins, such as the extradiol-cleaving dioxygenase 2,3-dihydroxybiphenyl 1,2-dioxygenase (Han et al., 1995). In addition, a truncated form of human PAH has recently been crystallized (Erlandsen et al., 1997b) and X-ray studies have shown a similar active-site structure as reported for TH, although different amino acid residues may be involved in substrate binding (Erlandsen et al., 1997a). While the crystal structure and spectroscopic studies indicated the presence of 5- and 6-coordinate iron in TH, respectively, recent Mössbauer spectroscopic studies (Meyer Klaucke et al., 1996) have indicated that the iron environment probably is flexible, as the sixth iron ligand probably is a loosely associated water molecule (Schünemann et al., manuscript in preparation). The X-ray structure has also identified the position of a number of the known substitution mutants in human TH and PAH (*see above*). Hopefully, it will be possible to crystallize at least some of the disease-related mutants and subject them to X-ray crystallographic analysis, in order to verify their effects on the tertiary and quaternary structure of the enzymes. Similarly, structural studies on the intact enzymes, including their N-terminal regulatory domains, should provide further insight into their function.

Iron Dependency of TH

The cellular concentration of iron is critical for the function of a number of iron-dependent biochemical processes, including the biosynthesis of catecholamines (Bradbury, 1997). Recently, the four isoforms of human TH and rat TH have been expressed in *E. coli* and purified in large quantities (Kaufman, 1995; Haavik et al., 1991). From these studies, it has been concluded that the enzyme activity is dependent on iron(II), which binds to the protein in stoichiometric amounts with a K_D in the micromolar range (Kaufman, 1995; Haavik et al., 1991; Haavik et al., 1992). As TH is completely dependent on Fe(II), and it appears to be a direct relationship between the iron concentration and the enzyme activity, activation of TH by Fe(II) has even been suggested as a biochemical assay for measuring free iron concentrations in cellular extracts (Tangerås, unpublished observations). If the enzyme really is dependent on micromolar levels of free intracellular Fe(II), such high iron levels may also constitute a risk factor for oxidative stress in the catecholaminergic neurons by other mechanisms (*see above;* Haavik et al., 1997).

Regulation of TH

The regulation of tyrosine hydroxylase activity has been most thoroughly studied in the peripheral nervous system, e.g., in sympathetic ganglia and adrenal medulla. These studies have shown that TH is regulated by different mechanisms in response to changes in the environment and to neuronal and hormonal stimuli. The short-term regulation includes posttranslational modifications, such as enzyme phosphorylation and inhibition by catecholamines, whereas long-term regulation is executed via transcriptional modifications and possibly alterations in enzyme stability (Hearing and Jimenez, 1987; Kappock and Caradonna, 1996; Gahn and Roskoski, 1995; Kumer and Vrana, 1996; Goldstein, 1995).

The feed-back inhibition of TH by catecholamines was reported more than 30 yr ago (Nagatsu et al., 1964). More recently, this inhibition has been shown to be related to the high affinity of catechols and catecholamines for the active-site iron in TH and other aromatic amino acid hydroxylases (Andersson et al.,

1988; Almås et al., 1992; Michaud Soret et al., 1995). The catecholamines trap the active-site iron in the Fe(III) state, inhibiting the enzyme, but also seems to protect TH from inactivation (Kumer and Vrana, 1996; Okuno and Fujisawa, 1991), possibly by preventing its interaction with dioxygen, H_2O_2, or other oxidants. Although catecholamines bind to the active site, the N-terminal domain of TH is involved in regulation of TH activity by feedback inhibition. Thus, truncated forms of rat and human TH, lacking 52-157 of the N-terminal amino acids are reported to be less susceptible to catecholamine inhibition and to have similar or higher specific activities than the intact enzymes (Ota et al., 1996; Moffat et al., 1997; Ribeiro et al., 1993; Daubner et al., 1993). Although some of the truncated enzyme forms prepared so far have suffered from a decreased stability *in vitro*, such mutants may be considered candidates for developing "improved" TH forms more suitable for gene therapy (*see below*). The pronounced inhibition of TH in the presence of an excess of DA has lead to the suggestion that intermittent inhibition of DA synthesis may contribute to the "on-off effects" observed on treatment of PD patients with L-DOPA (Haavik et al., 1990).

Phosphorylation of TH

The N-terminal sequence of mammalian TH contains several serine residues that are known to be phosphorylated by a variety of protein kinases, i.e., Ser8, Ser19, Ser31, and Ser40. The kinase specificities can be summarized as follows: Ser8 is phosphorylated in vitro by a protein kinase related to the cyclin-dependent protein kinases, Ser19 by calmodulin-dependent protein kinase-II (CaM kinase II) and by a MAP kinase-activated protein kinase-2 (MAPKAPK-2; Sutherland et al., 1993). Ser31 is phosphorylated by mitogen-activated protein kinase (MAPK). The four hTH isoforms vary with regard to inserts in the N-terminal domain that result in different amino acid sequences immediately after the amino acid Ser31. This means that although Ser31 of all the isoforms are phosphorylated by MAPK, the in vitro phosphorylation rates differ (Sutherland et al., 1993). Ser40 is phosphorylated in vitro by CaM kinase-II, cAMP-dependent protein kinase (PKA), protein kinase C (PKC), MAPKAPK-1, and MAPKAPK-2. Interestingly, the regulation of TH by phosphorylation and catecholamine binding are related phenomena, as phosphorylation of Ser40 is known to increase the K_i for catecholamine inhibition (Kumer and Vrana, 1996; Almås et al., 1992; Daubner et al., 1992). Using site-directed mutagenesis, Ser40 has been replaced by other amino acids, in order to determine to which degree phosphorylation of this serine contributes to the activation of TH. Replacement of Ser40 with alanine (Daubner et al., 1992), leucine, or tyrosine (Wu et al., 1992) resulted in a loss of phosphorylation ability by PKA, and a constitutively activated enzyme, with a decreased K_m for BH_4 and an increased K_i for DA. The same phenomenon was observed by deletion of the 40 N-terminal amino acids of TH (Walker et al., 1994). This implies that Ser40 in the intact enzyme inhibits TH activity and that the inhibition is reversed by phosphorylation with PKA. In cell-culture studies, the phosphorylation of Ser19, Ser31, and Ser40 is increased by agonists that stimulate TH activity and catecholamine synthesis (Haycock, 1993; Thomas et al., 1997; Haavik et al., 1997b).

In addition to its regulation by catecholamines and phosphorylation, which are likely to have a physiological significance, the enzyme activity can also be modulated by a variety of different compounds in vitro, such as heparin, phospholipids, and polyanions (for a review, *see* Kumer and Vrana (1996). Association of the effectors with the enzyme are believed to be through electrostatic interactions. It remains to be established whether these mechanisms are involved in the regulation of TH *in situ*.

Stress-Activated Protein Kinases

Neuronal cell death in the neurodegenerative disorders occurs by apoptosis, and an ini-

tial oxidative injury may be considered as a trigger for the apoptotic signaling pathway (Whittemore et al., 1994). The intracellular signals leading to apoptosis involve several protein kinases that are activated by physiological stimuli or by cellular injury. Recent work has defined two cascades activated preferentially by inflammatory cytokines, as well as by a wide variety of cellular stresses, such as UV radiation, hyperosmolarity, heat stress, oxidative stress, and so on. One pathway converges on the extracellular-regulated kinase (ERK) subfamily known as the stress-activated protein kinases (SAPKs, also termed Jun N terminal kinases, JNKs; for reviews, *see* Cano and Mahadevan, 1995; Woodgett et al., 1996), whereas the second pathway recruits the p38 kinases. It has been proposed that these cascades signal cell-cycle delay, cellular repair, or apoptosis in many cell types, as well as activation of immune and reticuloendothelial cells (Kyriakis and Avruch, 1996; Kummer et al., 1997). However, it has also been argued that the SAPK activation and some of these biological responses may represent parallel events that are not causally related.

SAPKs have recently been characterized in PC12 cells and chromaffin cells, where they seem to be involved in TH regulation. Thus, the TH activity is increased by different cellular stress-stimuli including sodium arsenite, osmotic shock and UV radiation (Thomas et al., 1997; Haavik et al., 1997b). The effects of such stimuli are transmitted via SAPK-2, which activates the MAPKAPK-2, which subsequently phosphorylates Ser19 in TH, resulting in activation of the enzyme (Fig. 2). Interestingly, in intact cells, this kinase specifically increases the phosphate content of Ser19, whereas the purified enzyme phosphorylates both Ser19 and Ser40. This may be because of the rapid dephosphorylation of Ser40 in chromaffin cells. It is not known whether these kinases are involved in the pathogenesis of PD, but it is interesting to note that TH has been reported to be in an activated and possibly phosphorylated state in the SN of PD patients (Mogi et al., 1988).

Long-Term Regulation

The long-term regulation of TH can also be mediated by phosphorylation mechanisms. Hormones (e.g., glucocorticoids), drugs (e.g., cocaine), or second messengers such as cAMP increase TH transcription (Kumer and Vrana, 1996). cAMP activates cAMP- dependent protein kinase (PKA), which phosphorylates and activates cAMP-responsive element binding proteins (CREB and/or c-*jun*). Binding to response elements in the TH-gene promoter region then induces transcription of the gene (Kim et al., 1993; Stachowiak et al., 1994). Studies on PC18 cells, stimulating the TH-gene promoter activity by carbachol, showed that the response was mainly mediated by transcription factors interacting with the CRE site, but that the AP1 site, though to a smaller extent, also participated in the response to carbachol (Chen et al., 1996).

Alternative Splicing

Alterations in TH expression by alternative splicing of TH-mRNA were first discovered in humans, resulting in at least four different isoforms of TH (Grima et al., 1987). Using isoform-specific antibodies, it has been possible to measure the relative abundance of all these forms in the human brain (Lewis et al., 1993). Alternative splice forms have recently also been found in rat (Laniece et al., 1996) and in *Drosophila melanogaster* TH mRNA (Birman et al., 1994). This diversity of TH isoforms and their differences in activity and distribution have suggested that alternative splicing could be an additional mechanism for the regulation of TH activity and control of catecholamine synthesis. Recently, Dumas et al. discovered additional splice variants of human TH (hTH) mRNA in postmortem adrenal medulla (AM) at a similar level as the previously known hTH3 and hTH4 isoforms (Dumas et al., 1996). However, the new isoforms were not detected in SN. In patients suffering from progressive

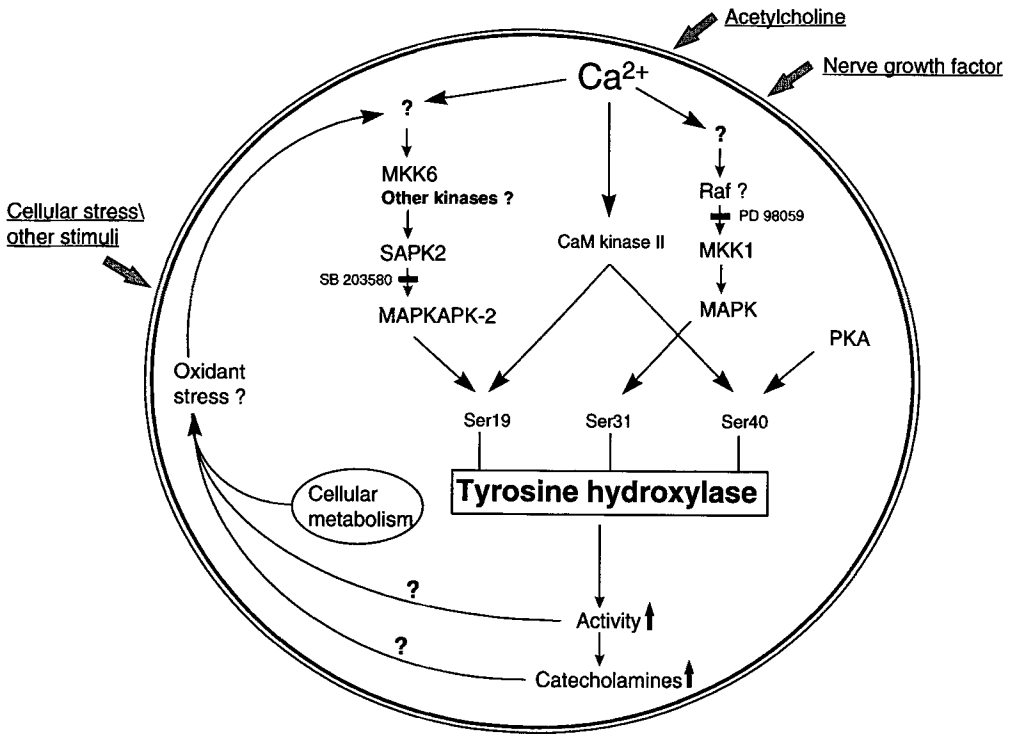

Fig. 2. Relationship between the activation of tyrosine hydroxylase by cellular stresses and by acetylcholine (bovine chromaffin cells or PC12 cells). The figure is showing a situation in which oxidative injury can activate TH and increase TH activity, which can further increase oxidative injury. The inhibitors PD 98059 and SB 203580 bind specifically to MAP kinase kinase 1 (MKK1) and SAPK2, respectively. For abbreviations and further details *see* the text and (Wu et al., 1992).

supranuclear palsy, an increase from 4–6% of total hTH mRNA in controls to 11–34% in patients of these alternatively spliced TH mRNA species were observed. Whether the increase was caused by mRNA degradation caused by the stress associated with the disease or is causally involved, remains to be established. Furthermore, it is not yet known how efficiently these novel mRNA species are translated and the putative novel TH proteins have not been characterized.

TH mRNA Stability

The long-term regulation of TH activity also involves posttranscriptional mechanisms (Kumer and Vrana, 1996; Tumer et al., 1996). For instance, a limited availability of oxygen (hypoxia), has been found to increase both the rate of TH gene transcription and TH-mRNA stability in carotid bodies (Czyzyk Krzeska et al., 1992) and oxygen-sensitive PC12 cells (Czyzyk Krzeska et al., 1994). The increased stability correlated with an enhanced binding of a cytoplasmic protein to a sequence in the 3′ untranslated region of TH-mRNA which could serve as a protection of mRNA, degradation by nucleases (Czyzyk Krzeska et al., 1997).

The Potential of TH in the Treatment of PD

The common treatment strategies for PD usually consist of supplying substances that

compensate for the DA and TH deficiency in dopaminergic neurons. Such treatments include the administration of L-DOPA, DA agonists, and/or inhibitors of DA metabolism. As L-DOPA is converted to DA, it can, to some extent, compensate for the DA deficiency. However, it has also been speculated that exogenous L-DOPA is a source of ROS formation (Walkinshaw and Waters, 1995). In theory, this problem may be avoided by using stabile DA agonists instead of, or in addition to the L-DOPA therapy. Treatment with L-DOPA and agonists is successful in suppressing many of the symptoms found in PD, but after a few years the effect of the treatment gradually becomes ineffective either because of the loss of efficacy or the appearance of adverse side effects. This may be related to the progressive nature of the degeneration of dopaminergic neurons or to a possible injury caused by the treatment. Currently, much effort is being devoted to developing alternative treatment strategies that should be able to delay, halt, or even reverse the progression of the disease. The importance of TH in the formation of DA and the problems associated with the conventional treatment of PD, has led to the suggestion of restoring the TH activity by cell transplantation or gene therapy.

Surgical Treatment

In the past, several different surgical procedures have been tried in PD with moderate degree of success. More recently, implantation of DA-producing tissues such as fetal nigral grafts into the striatum of patients with PD, has given some promising results (reviewed in Olanow et al., 1996). The reason for placing the graft in striatum and not in SN, is the apparent inability of nigral grafts to grow axons for long distances in the adult host (Mendez et al., 1996). Also, considering the clinical application, severe surgical complications have been seen to follow nigral transplantation (Yoshimoto et al., 1995). Although these grafts have been found to survive for extended periods of time, the patient's own cells continue to degenerate. In theory, the transplant could be thought to substitute for the affected cells because the fetal tissue is able to reinnervate the surrounding tissue of the host (Mendez et al., 1996). However, the method is not very practical in use, it has a very low efficiency, corresponding to a 90–95% loss of the dopaminergic cells during the transplantation procedure (Sofic et al., 1991), and the dopaminergic neurons constitute only a few percent of the cell population. This is a problem because it means that a large amount of tissue is needed, whereas the supply of material is limited. To improve the fraction of surviving cells during transplantation, various approaches are being followed, such as inhibiting the generation of free radicals by addition of lazaroids (Nakao et al., 1994), or inhibiting apoptosis. Thus, the injection of glial cell-line-derived neurotrophic factor (GDNF; Wang et al., 1996) or infusion of brain-derived neurotrophic factor (BDNF; Yurek et al., 1996) has been successful in improving both survival, growth, and function of fetal graft implanted in rat PD models (Rosenblad et al., 1996), but many practical and ethical issues must be resolved before treatment with fetal tissue can be offered as a routine procedure in humans.

Ex Vivo and In Vivo Gene Therapy

Genes can be transferred into a host cell using different vectors, including plasmids or recombinant viruses that contain targeting sequences and/or systems for regulated expression of the gene (for a review, *see* Miller and Vile, 1995). As TH probably is a rate-limiting factor for DA biosynthesis both under normal conditions and in PD, the introduction of the TH gene has been a logical choice for gene therapy in PD. Using ex vivo gene therapy of PD, one can maintain DA or L-DOPA producing cells in vitro for intracerebral grafting. This approach makes it possible to characterize and optimize the expression and activity of TH and the synthesis of DA before transplantation, and

TH expression can be restricted to a specific group of cells. In addition to TH, several other genes have the potential of being efficient in the treatment of PD, e.g., cells modified to express neurotrophic factors have been found to partially reverse PD symptoms induced by 6-OHDA lesions in rats (Levivier et al., 1995; Horellou et al., 1990; Wolff et al., 1989).

Direct intra cerebral gene transfer, which involves infection and expression of genes in neurons of the mature CNS, has given promising results using viral vectors in rats. In particular, recombinant adenoviruses (Riederer et al., 1989; Horellou et al., 1994) and adeno-associated viruses (Kaplitt et al., 1994) have shown a high efficiency and relatively low cytotoxicity when using low viral titers (Slack and Miller, 1996; Fisher et al., 1996). The advantage of using the adenovirus-based system for gene therapy is not only its high efficiency, but also its ease of manufacture. The system has been successful in TH transfection experiments, as measured by a decrease in apomorphine-induced rotational behavior in rats (Horellou et al., 1994; Freese et al., 1996). Herpes simplex virus type 1 (HSV-1) is another vector that has been promising for TH transfection in rats, efficiently expressing human TH mRNA with a subsequent release of L-DOPA (During et al., 1994). This treatment caused a significant amelioration of symptoms in 6-OHDA-lesioned rats for a period of 6 mo to 1 yr. However, the HSV-1 approach has been associated with infections of the host tissue that preclude its use in humans (reviewed by Glorioso et al. 1995).

Choice of Cell Type for Gene Therapy

For treatment of PD using gene therapy it is desirable to find donor cells that have the ability to reconstruct the damaged neural circuits, have a long-term survival after transplantation, are suitable for genetic manipulations, and are readily available and inexpensive. Fibroblasts have the advantage of being easily obtainable and, easy to culture (Ishida et al., 1996), and make it possible to use the patient's own cells (autografts) avoiding some of the immunological problems (e.g., histoincompatibility; Björklund, 1993). Wolff et al. (1989) showed that when rats lesioned with 6-OHDA received fibroblasts infected with a retroviral vector containing the rat TH cDNA, reduction of abnormal behavior was obtained. The grafts were able to survive for 2 mo and continued to express TH, and produce and release L-DOPA during this period. However, fibroblasts do not seem to synthesize adequate levels of BH_4. To overcome this deficit, fibroblasts have been transfected with the genes for both TH and GTP-CH (Bencsics et al., 1996). These cells were found to possess a higher TH activity than cells without the double transfection. However, the survival of the fibroblast transplants were limited, declining 4–8 wk after grafting (Fisher et al., 1991). Also, fibroblasts are not capable of restoring synaptic circuits and problems with tumor formation are obvious because of the tumorigenic nature of these cells (Horellou et al., 1990). A way to obviate the latter problem could be to isolate the cells in a capsule prior to transplantation. This would also permit the use of animal cells, and the capsule could be removed if problems should occur.

Cells of neural origin like glia cells, astrocytes, or fetal mesencephalic cells are suitable for transplantation in the CNS, in that they contain many of the features needed for successful implantation (Lundberg et al., 1996). However, because these are nondividing cells that are difficult to culture and transfect, approaches such as using immortalized neural cells (Anton et al., 1994) and retrovirus transfection are needed to transfect with TH. The use of retroviruses has the advantage of allowing easy transfection and high expression levels of the transfected gene, but as with immortalized cells, the possibility of tumor formation and virus infection makes a direct injection into the human brain very risky. In attempts to generate neural stem cell lines that have not been modified with transforming or immortalizing genes, a number of growth factors have been used to stimu-

late cell proliferation (for recent reviews, *see* Baetge, 1993; Snyder et al., 1997).

Primary astrocytes transduced with retrovirus containing the human TH gene have been grafted into rat striatum (Lundberg et al., 1996). The grafted astrocytes seemed to migrate into the surrounding striatum, expressing TH and secreting high levels of L-DOPA. In this experiment virus-producing cells were killed by a mitomycin-C treatment in culture for 3-4 wk prior to grafting, but this approach can also reduce the viability of the cells. Unfortunately, the system showed an in vivo downregulation of the TH gene, resulting in a transient production of the enzyme (Lundberg et al., 1996).

Future Directions

Even if gene therapy has given some promising results, extensive work still remains to be done before a routine clinical application in PD is possible. A major problem with this strategy appears to be the downregulation of the TH transgene in the transplant, which leads to decreased expression efficiency. The TH protein is relatively unstable, in particular when expressed in nonneuronal cells (Wu and Cepko, 1994). The duration of expression thus seems to be dependent on the cell type. In addition, the choice of delivery system (vector) and the nature of transgene are also important factors. These problems may be solved by developing more efficient vectors for transfection with improved promoters and using new forms of TH with higher specific activity and, in particular, increased stability in vivo. The aromatic amino acid hydroxylases are probably catabolized via ubiquitin-dependent pathways (Døskeland and Flatmark, 1996). Possibly, the rate of degradation may be modified, in order to design new enzyme forms that are more suitable for gene therapy.

It is clear that disturbances in DA synthesis and TH function play important roles in the pathogenesis of PD. Whether TH is an innocent bystander, i.e., that the enzyme deficiency only is secondary to cell loss, or if TH also has a more direct role in promoting neuronal injury is not clear. Still, gene therapy and/or transplantation with cells expressing active TH continues to be a promising treatment strategy for severe PD. Therefore, an increased knowledge about the TH structure, function, and regulation will be important for understanding the etiology and pathogenesis, as well as the treatment of this important neurodegenerative disease.

Acknowledgments

The authors are grateful for helpful suggestions from our colleagues at the Department of Biochemistry and Molecular Biology and other institutions. This work was supported by the Research Council of Norway.

References

(1989) Effect of deprenyl on the progression of disability in early Parkinson's disease. The Parkinson Study Group. *N. Engl. J. Med.* **321,** 1364–1371.

(1993) Mutations in the copper- and zinc-containing superoxide dismutase gene are associated with "Lou Gehrig's Disease". *Nutr. Rev.* **51,** 243–245.

(1995) Cerebrospinal fluid homovanillic acid in the DATATOP study on Parkinson's disease. Parkinson Study Group. *Arch.Neurol.* **52,** 237–245.

Adler C., Ghisla S., Rebrin I., Haavik J., Heizmann C. W., Blau N., Kuster T., and Curtius H. C. (1992) 7-substituted pterins in humans with suspected pterin–4a–carbinolamine dehydratase deficie[illegible] Mechanism of formation via non–enzym transformation from 6–substituted pterins. *E Biochem.* **208,** 139–144.

Albino Teixeira A., Azevedo I., Martel F., and wald W. (1991) Superoxide dismutase par prevents sympathetic denervation by 6-hyd dopamine. *Naunyn Schmiedebergs Arch. Ph col.* **344,** 36–40.

Almås B., Le Bourdelles B., Flatmark T., Ma and Haavik J. (1992) Regulation of recom human tyrosine hydroxylase isozymes b cholamine binding and phosphorylation

ture/activity studies and mechanistic implications. *Eur. J. Biochem.* **209,** 249–255.

Andersson K. K., Cox D. D., Que L. Jr., Flatmark T., and Haavik J. (1988) Resonance Raman studies on the blue-green-colored bovine adrenal tyrosine 3-monooxygenase (tyrosine hydroxylase). Evidence that the feedback inhibitors adrenaline and noradrenaline are coordinated to iron. *J. Biol. Chem.* **263,** 18,621–18,626.

Anton R., Kordower J. H., Maidment N. T., Manaster J. S., Kane D. J., Rabizadeh S., Schueller S. B., Yang J., Edwards R. H. and et al. (1994) Neural-targeted gene therapy for rodent and primate hemiparkinsonism. *Exp. Neurol.* **127,** 207–218.

Baetge E. E. (1993) Neural stem cells for CNS transplantation. *Ann. N.Y. Acad. Sci.* **695,** 285– 291.

Bandmann O., Daniel S., Marsden C. D., Wood N. W., and Harding A. E. (1996a) The GTP-cyclohydrolase I gene in atypical parkinsonian patients: a clinico-genetic study. *J. Neurol. Sci.* **141,** 27–32.

Bandmann O., Nygaard T. G., Surtees R., Marsden C. D., Wood N. W., and Harding A. E. (1996b) Dopa-responsive dystonia in British patients: new mutations of the GTP-cyclohydrolase I gene and evidence for genetic heterogeneity. *Hum. Mol. Genet.* **5,** 403–406.

Barden H. (1969) The histochemical relationship of neuromelanin and lipofuscin. *J. Neuropathol. Exp. Neurol.* **28,** 419–441.

Bartholome K. (1983) Deficiency of tyrosine hydroxylase or tryptophan hydroxylase: a possible cause of two hypothetical metabolic diseases. *Acta Paediatr. Scand.* **72,** 921–922.

Beal M. F. (1995) Aging, energy, and oxidative stress in neurodegenerative diseases. *Ann.Neurol.* **38,** 357–366.

Bencsics C., Wachtel S. R., Milstien S., Hatakeyama K., Becker J. B., and Kang U. J. (1996) Double transduction with GTP cyclohydrolase I and tyrosine hydroxylase is necessary for spontaneous synthesis of L-DOPA by primary fibroblasts. *J. Neurosci.* **16,** 4449–4456.

Benedito E., Jimenez C. C., Perez D., Cubillana J. D., nez C. J., Meyer-zum G. A., Lozano ia B. J. (1997) Melanin formation in is catalyzed by a new tyrosine inetically and structurally different se. *Biochim. Biophys. Acta* **1336,** 59–72. gan B., Anzivino M., and Hirsh J. vel and major isoform of tyrosine in Drosophila is generated by alter- processing. *J. Biol. Chem.* **269,**

Björklund A. (1993) Neurobiology. Better cells for brain repair. *Nature* **362,** 414–415.

Blair J. A. and Pearson A. J. (1974) Kinetics and mechanism of autooxidation of the 2- amino-4-hydroxy-5,6,7,8-tetrahydrobiopteridines. *J. Chem. Soc. Perkin. Trans.* **II,** 80–88.

Bondy S.C. (1992) Reactive oxygen species: relation to aging and neurotoxic damage. *Neurotoxicology* **13,** 87–100.

Bradbury M.W.B. (1997) Transport of iron in the blood-brain-cerebrospinal fluid system. *J. Neurochem.* **69,** 443–454.

Calne D. B. and Takahashi H. (1991) The origin of idiopathic Parkinsonism, in *Parkinson's: How to Proceed Today in Treatment* (Rinne, U. K., Nagatsu T., and Horowski R., eds.) Medicom EW, Bussum, pp. 3–9.

Cano E. and Mahadevan L. C. (1995) Parallel signal processing among mammalian MAPKs. *Trends.Biochem.Sci.* **20,** 117–122.

Chen Y., Best J. A., Nagamoto K., and Tank A. W. (1996) Regulation of tyrosine hydroxylase gene expression by the m1 muscarinic acetylcholine receptor in rat pheochromocytoma cells. *Brain Res. Mol. Brain Res.* **40,** 42–54.

Coyle J.T. and Puttfarcken P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science* **262,** 689–695.

Czyzyk Krzeska M. F., Bayliss D. A., Lawson E. E., and Millhorn D. E. (1992) Regulation of tyrosine hydroxylase gene expression in the rat carotid body by hypoxia. *J. Neurochem.* **58,** 1538–1546.

Czyzyk Krzeska M. F., Furnari B. A., Lawson E. E., and Millhorn D. E. (1994) Hypoxia increases rate of transcription and stability of tyrosine hydroxylase mRNA in pheochromocytoma (PC12) cells. *J. Biol. Chem.* **269,** 760–764.

Czyzyk Krzeska M. F., Paulding W. R., Beresh J. E., and Kroll S. L. (1997) Post-transcriptional regulation of tyrosine hydroxylase gene expression by oxygen in PC12 cells. *Kidney Int.* **51,** 585–590.

Dahl H. H. and Mercer J. F. (1986) Isolation and sequence of a cDNA clone which contains the complete coding region of rat phenylalanine hydroxylase. Structural homology with tyrosine hydroxylase, glucocorticoid regulation, and use of alternate polyadenylation sites. *J. Biol. Chem.* **261,** 4148–4153.

Damier P., Hirsch E. C., Zhang P., Agid Y., and Javoy Agid F. (1993) Glutathione peroxidase, glial cells and Parkinson's disease. *Neuroscience* **52,** 1–6.

Daubner S. C., Lauriano C., Haycock J. W., and Fitzpatrick P. F. (1992) Site-directed mutagenesis of serine 40 of rat tyrosine hydroxylase. Effects of dopamine and cAMP-dependent phosphorylation on enzyme activity. *J. Biol. Chem.* **267,** 12639–12646.

Daubner S. C., Lohse D. L., and Fitzpatrick P. F. (1993) Expression and characterization of catalytic and regulatory domains of rat tyrosine hydroxylase. *Protein Sci.* **2,** 1452–1460.

Davies S. W., Turmaine M., Cozens B. A., DiFiglia M., Sharp A. H., Ross C. A., Scherzinger E., Wanker E. E., Mangiarini L., and Bates G. P. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90,** 537–548.

De La Cruz C.P., Revilla E., Venero J. L., Ayala A., Cano J., and Machado A. (1996) Oxidative inactivation of tyrosine hydroxylase in substantia nigra of aged rat. *Free Radic. Biol. Med.* **20,** 53–61.

Degl'Innocenti F., Maurello M. T., and Marini P. (1989) A parkinsonian kindred. *Ital. J. Neurol. Sci.* **10,** 307–310.

Dexter D. T., Carter C. J., Wells F. R., Javoy Agid F., Agid Y., Lees A., Jenner P., and Marsden C. D. (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* **52,** 381–389.

Dexter D. T., Wells F. R., Agid F., Agid Y., Lees A. J., Jenner P., and Marsden C. D. (1987) Increased nigral iron content in postmortem parkinsonian brain [letter]. *Lancet* **2,** 1219–1220.

Dexter D. T., Wells F. R., Lees A. J., Agid F., Agid Y., Jenner P., and Marsden C. D. (1989) Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. *J. Neurochem.* **52,** 1830–1836.

Døskeland A. P. and Flatmark T. (1996) Recombinant human phenylalanine hydroxylase is a substrate for the ubiquitin-conjugating enzyme system. *Biochem. J.* **319,** 941–945.

Dumas S., Le Hir H., Bodeau Pean S., Hirsch E., Thermes C., and Mallet J. (1996) New species of human tyrosine hydroxylase mRNA are produced in variable amounts in adrenal medulla and are overexpressed in progressive supranuclear palsy. *J. Neurochem.* **67,** 19–25.

During M. J., Naegele J. R., O'Malley K. L., and Geller A. I. (1994) Long-term behavioral recovery in parkinsonian rats by an HSV vector expressing tyrosine hydroxylase. *Science* **266,** 1399–1403.

Ebadi M., Srinivasan S.K., and Baxi M.D. (1996) Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog. Neurobiol.* **48,** 1–19.

Egensperger R., Kosel S., Schnopp N. M., Mehraein P., and Graeber M. B. (1997) Association of the mitochondrial tRNA(A4336G) mutation with Alzheimer's and Parkinson's diseases. *Neuropathol. Appl. Neurobiol.* **23,** 315–321.

Ehringer H. and Hornykiewicz O. (1960) Verteilung von noradrenalin und dopamin (3-hydroxytyramin) in gehirn des menchen und ihr verhalten bei erkrankungen des extrapyramidalen systems. *Klin.Wochenschr.* **38,** 1236–1239.

Enochs W. S., Sarna T., Zecca L., Riley P. A., and Swartz H. M. (1994) The roles of neuromelanin, binding of metal ions, and oxidative cytotoxicity in the pathogenesis of Parkinson's disease: a hypothesis. *J. Neural Transm. Park. Dis. Dement. Sect.* **7,** 83–100.

Erlandsen H., Fusetti F., Martinez A., Hough E., Flatmark T., and Stevens R. C. (1997a) Crystal structure of the catalytic domain of human phenylalanine hydroxylase reveals the structural basis for phenylketonuria. *Nature Struct. Biol.* **4,** 995–1000.

Erlandsen H., Martinez A., Knappskog P. M., Haavik J., Hough E., and Flatmark T. (1997b) Crystallization and preliminary diffraction analysis of a truncated homodimer of human phenylalanine hydroxylase. *FEBS Lett.* **406,** 171–174.

Evans P.H. (1993) Free radicals in brain metabolism and pathology. *Br. Med. Bull.* **49,** 577–587.

Fink J. K., Barton N., Cohen W., Lovenberg W., Burns R. S., and Hallett M. (1988) Dystonia with marked diurnal variation associated with biopterin deficiency. *Neurology* **38,** 707–711.

Fink J. K., Ravin P., Argoff C. E., Levine R. A., Brady R. O., Hallett M., and Barton N. W. (1989) Tetrahydrobiopterin administration in biopterin-deficient progressive dystonia with diurnal variation. *Neurology* **39,** 1393–1395.

Fink R. M. and Elstner E. F. (1984) Studies on the possible mechanism of inactivation of phenylalanine hydroxylase by destructive oxygen species. *Z. Naturforsch. C.* **39,** 734–737.

Fisher K. J., Kelley W. M., Burda J. F., and Wilson J. M. (1996) A novel adenovirus-adeno- associated virus hybrid vector that displays efficient rescue and delivery of the AAV genome. *Hum. Gene Ther.* **7,** 2079–2087.

Fisher L. J., Jinnah H. A., Kale L. C., Higgins G. A., and Gage F. H. (1991) Survival and function of intrastriatally grafted primary fibroblasts geneti-

cally modified to produce L-dopa. *Neuron* **6,** 371–380.

Fitzpatrick P. F. (1989) The metal requirement of rat tyrosine hydroxylase. *Biochem. Biophys. Res. Commun.* **161,** 211–215.

Fossom L. H., Sterling C. R., and Tank A. W. (1992) Regulation of tyrosine hydroxylase gene transcription rate and tyrosine hydroxylase mRNA stability by cyclic AMP and glucocorticoid. *Mol. Pharmacol.* **42,** 898–908.

Fowler C. J., Wiberg A., Oreland L., Marcusson J., and Winblad B. (1980) The effect of age on the activity and molecular properties of human brain monoamine oxidase. *J. Neural Transm.* **49,** 1–20.

Freese A., During M. J., Davidson B. L., Gennarelli T. A., Kaplitt M. G., Flamm E. S., and Snyder P. J. (1996) Transfection of human lactotroph adenoma cells with an adenovirus vector expressing tyrosine hydroxylase decreases prolactin release. *J. Clin. Endocrinol. Metab.* **81,** 2401–2404.

Gahn L. G. and Roskoski R. Jr. (1995) Thermal stability and CD analysis of rat tyrosine hydroxylase. *Biochemistry* **34,** 252–256.

Gasser T., Wszolek Z. K., Trofatter J., Ozelius L., Uitti R. J., Lee C. S., Gusella J., Pfeiffer R. F., Calne D. B., and Breakefield X. O. (1994) Genetic linkage studies in autosomal dominant parkinsonism: evaluation of seven candidate genes. *Ann. Neurol.* **36,** 387–396.

Gerlach M., Ben Shachar D., Riederer P., and Youdim M. B. (1994) Altered brain metabolism of iron as a cause of neurodegenerative diseases? *J. Neurochem.* **63,** 793–807.

Glorioso J. C., DeLuca N. A., and Fink D. J. (1995) Development and application of herpes simplex virus vectors for human gene therapy. *Annu. Rev. Microbiol.* **49,** 675–710.

Goc A. and Stachowiak M.K. (1994) Bovine tyrosine hydroxylase gene-promoter regions involved in basal and angiotensin II-stimulated expression in nontransformed adrenal medullary cells. *J. Neurochem.* **62,** 834–843.

Golbe L. I., Di Iorio G., Bonavita V., Miller D. C., and Duvoisin R. C. (1990) A large kindred with autosomal dominant Parkinson's disease. *Ann. Neurol.* **27,** 276–282.

Goldstein M. (1995) Long- and short-term regulation of tyrosine hydroxylase. In *Psychopharmacology: The Fourth Generation of Progress* (Bloom F. E. and Kupfer D. J., eds.), pp. 189–195. Raven Press, Ltd., New York.

Goodwill K. E., Sabatier C., Marks C., Raag R., Fitzpatrick P. F., and Stevens R. C. (1997) Crystal structure of tyrosine hydroxylase at 2.3 Å and its implications for inherited neurodegenerated diseases. *Nature Struct. Biol.* **4,** 578–585.

Graham D. G. (1978) Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol. Pharmacol.* **14,** 633–643.

Granholm A. C., Srivastava N., Mott J. L., Henry S., Henry M., Westphal H., Pichel J. G., Shen L., and Hoffer B. J. (1997) Morphological alterations in the peripheral and central nervous systems of mice lacking glial cell line-derived neurotrophic factor (GDNF): immunohistochemical studies. *J. Neurosci.* **17,** 1168–1178.

Grima B., Lamouroux A., Boni C., Julien J. F., Javoy-Agid F., and Mallet J. (1987) A single human gene encoding multiple tyrosine hydroxylases with different predicted functional characteristics. *Nature* **326,** 707–711.

Gutteridge J.M. (1994) Biological origin of free radicals, and mechanisms of antioxidant protection. *Chem. Biol. Interact.* **91,** 133–140.

Haavik J. (1997) L-DOPA is a substrate for tyrosine hydroxylase. *J. Neurochem.* **69,** 1720–1728.

Haavik J. and Flatmark T. (1987) Isolation and characterization of tetrahydropterin oxidation products generated in the tyrosine 3-monooxygenase (tyrosine hydroxylase) reaction. *Eur. J. Biochem.* **168,** 21–26.

Haavik J., Almås B., and Flatmark T. (1997a) Generation of reactive oxygen species by tyrosine hydroxylase: a possible contribution to the degeneration of dopaminergic neurons? *J. Neurochem.* **68,** 328–332.

Haavik J., Thomas G., Sutherland C., and Cohen P. (1997b) Activation of tyrosine hydroxylase by phosphorylation. A comparison of different protein kinases and phosphorylation sites, in *Chemistry and Biology of Pteridines and Folates* (Pfleiderer W. and Rokos H., eds.), 11th International Symposium, Blackwell Science, Berlin pp. 509–514.

Haavik J., Le Bourdelles B., Martinez A., Flatmark T., and Mallet J. (1991) Recombinant human tyrosine hydroxylase isozymes. Reconstitution with iron and inhibitory effect of other metal ions. *Eur. J. Biochem.* **199,** 371–378.

Haavik J., Martinez A., and Flatmark T. (1990) pH-dependent release of catecholamines from tyrosine hydroxylase and the effect of phosphorylation of Ser-40. *FEBS Lett.* **262,** 363–365.

Haavik J., Martinez A., Olafsdottir S., Mallet J., and Flatmark T. (1992) The incorporation of divalent metal ions into recombinant human tyrosine

hydroxylase apoenzymes studied by intrinsic fluorescence and 1H-NMR spectroscopy. *Eur. J. Biochem.* **210,** 23–31.

Han S., Eltis L. D., Timmis K. N., Muchmore S. W., and Bolin J. T. (1995) Crystal structure of the biphenyl-cleaving extradiol dioxygenase from a PCB-degrading pseudomonad. *Science* **270,** 976–980.

Haycock J. W. (1993) Multiple signaling pathways in bovine chromaffin cells regulate tyrosine hydroxylase phosphorylation at Ser19, Ser31, and Ser40. *Neurochem. Res.* **18,** 15–26.

He Y., Thong P. S., Lee T., Leong S. K., Shi C. Y., Wong P. T., Yuan S. Y., and Watt F. (1996) Increased iron in the substantia nigra of 6-OHDA induced parkinsonian rats: a nuclear microscopy study. *Brain Res.* **735,** 149–153.

Hearing V. J. and Jimenez M. (1987) Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation. *Int. J. Biochem.* **19,** 1141–1147.

Heintz N. and Zoghbi H. (1997) α-synuclein—a link between Parkinson and Alzheimer diseases? *Nat. Genet.* **16,** 325–327.

Hillas P. J. and Fitzpatrick P. F. (1996) A mechanism for hydroxylation by tyrosine hydroxylase based on partitioning of substituted phenylalanines. *Biochemistry* **35,** 6968– 6975.

Hirsch E. C. (1994) Biochemistry of Parkinson's disease with special reference to the dopaminergic systems. *Mol. Neurobiol.* **9,** 135–142.

Hirsch E.C. (1993) Does oxidative stress participate in nerve cell death in Parkinson's disease? *Eur. Neurol.* **33 Suppl 1,** 52–59.

Horellou P., Brundin P., Kalen P., Mallet J., and Björklund A. (1990) In vivo release of dopa and dopamine from genetically engineered cells grafted to the denervated rat striatum. *Neuron* **5,** 393–402.

Horellou P., Vigne E., Castel M. N., Barneoud P., Colin P., Perricaudet M., Delaere P., and Mallet J. (1994) Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease. *Neuroreport.* **6,** 49–53.

Hou J. G., Lin L. F., and Mytilineou C. (1996) Glial cell line-derived neurotrophic factor exerts neurotrophic effects on dopaminergic neurons in vitro and promotes their survival and regrowth after damage by 1-methyl-4-phenylpyridinium. *J. Neurochem.* **66,** 74–82.

Hyman C., Hofer M., Barde Y. A., Juhasz M., Yancopoulos G. D., Squinto S. P. and Lindsay R. M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350,** 230–232.

Ichinose H., Ohye T., Takahashi E., Seki N., Hori T., Segawa M., Nomura Y., Endo K., Tanaka H., Tsuji S., and et al (1994) Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. *Nat. Genet.* **8,** 236–242.

Ikebe S., Tanaka M., Ohno K., Sato W., Hattori K., Kondo T., Mizuno Y., and Ozawa T. (1990) Increase of deleted mitochondrial DNA in the striatum in Parkinson's disease and senescence. *Biochem. Biophys. Res. Commun.* **170,** 1044–1048.

Ischiropoulos H., Duran D., and Horwitz J. (1995) Peroxynitrite-mediated inhibition of DOPA synthesis in PC12 cells. *J. Neurochem.* **65,** 2366–2372.

Ishida A., Yamashiro K., Mukawa J., and Hasegawa M. (1996) Regulation of L-DOPA production by genetically modified primary fibroblasts transfected with retrovirus vector system. *Cell Transplant.* **5,** S5–S7.

Jenner P. (1991) Oxidative stress as a cause of Parkinson's disease. *Acta Neurol. Scand. Suppl.* **136,** 6–15.

Jenner P. and Olanow C.W. (1996) Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* **47,** S161–170.

Kapatos G., Hirayama K., and Stegenga S. (1997) Clinical relevance and molecular mechanisms of heterogenety in tetrahydrobiopterin biosynthesis within monoaminergic neurons. *Pteridines* **8,** 64.

Kaplitt M. G., Leone P., Samulski R. J., Xiao X., Pfaff D. W., O'Malley K. L., and During M. J. (1994) Long-term gene expression and phenotypic correction using adeno- associated virus vectors in the mammalian brain. *Nat. Genet.* **8,** 148–154.

Kappock T. J. and Caradonna J. P. (1996) Pterin-dependent amino acid hydroxylases. *Chem. Rev* **96,** 2659–2756.

Kaufman S. (1995) Tyrosine hydroxylase. *Adv. Enzymol. Relat. Areas. Mol. Biol.* **70,** 103–220.

Kehrer J. P. (1993) Free radicals as mediators of tissue injury and disease. *Crit. Rev. Toxicol.* **23,** 21–48.

Kim K. S., Park D. H., Wessel T. C., Song B., Wagner J. A., and Joh T. H. (1993) A dual role for the cAMP-dependent protein kinase in tyrosine hydroxylase gene expression. *Proc. Natl. Acad. Sci. USA.* **90,** 3471–3475.

Knappskog P. M., Flatmark T., Mallet J., Lüdecke B., and Bartholome K. (1995) Recessively inherited L-DOPA-responsive dystonia caused by a point

mutation (Q381K) in the tyrosine hydroxylase gene. *Hum. Mol. Genet.* **4,** 1209–1212.

Kobayashi K., Morita S., Sawada H., Mizuguchi T., Yamada K., Nagatsu I., Hata T., Watanabe Y., Fujita K., and Nagatsu T. (1995) Targeted disruption of the tyrosine hydroxylase locus results in severe catecholamine depletion and perinatal lethality in mice. *J. Biol. Chem.* **270,** 27,235–27,243.

Kosel S., Egensperger R., Schnopp N. M., and Graeber M. B. (1997) The 'common deletion' is not increased in parkinsonian substantia nigra as shown by competitive polymerase chain reaction. *Mov.Disord.* **12,** 639–645.

Kumer S. C. and Vrana K. E. (1996) Intricate regulation of tyrosine hydroxylase activity and gene expression. *J. Neurochem.* **67,** 443–462.

Kummer J. L., Rao P. K., and Heidenreich K. A. (1997) Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. *J. Biol. Chem.* **272,** 20,490–20,494.

Kupke K. G., Graeber M. B., and Muller U. (1992) Dystonia-parkinsonism syndrome (XDP) locus: flanking markers in Xq12-q21.1. *Am. J. Hum. Genet.* **50,** 808–815.

Kyriakis J. M. and Avruch J. (1996) Protein kinase cascades activated by stress and inflammatory cytokines. *Bioessays* **18,** 567–577.

Laniece P., Le-Hir H., Bodeau-Pean S., Charon Y., Valentin L., Thermes C., Mallet J., and Dumas S. (1996) A novel rat tyrosine hydroxylase mRNA species generated by alternative splicing. *J. Neurochem.* **66,** 1819–1825.

Levivier M., Przedborski S., Bencsics C., and Kang U.J. (1995) Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J. Neurosci.* **15,** 7810–7820.

Lewis D. A., Melchitzky D. S., and Haycock J. W. (1993) Four isoforms of tyrosine hydroxylase are expressed in human brain. *Neuroscience* **54,** 477–492.

Linert W., Herlinger E., Jameson R. F., Kienzl E., Jellinger K., and Youdim M. B. (1996) Dopamine, 6-hydroxydopamine, iron, and dioxygen—their mutual interactions and possible implication in the development of Parkinson's disease. *Biochim. Biophys. Acta* **1316,** 160–168.

Lüdecke B., Dworniczak B., and Bartholome K. (1995) A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome. *Hum. Genet.* **95,** 123–125.

Lüdecke B., Knappskog P. M., Clayton P. T., Surtees R. A., Clelland J. D., Heales S. J., Brand M. P., Bartholome K., and Flatmark T. (1996) Recessively inherited L-DOPA-responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene. *Hum. Mol. Genet.* **5,** 1023–1028.

Lundberg C., Horellou P., Mallet J., and Björklund A. (1996) Generation of DOPA-producing astrocytes by retroviral transduction of the human tyrosine hydroxylase gene: in vitro characterization and in vivo effects in the rat Parkinson model. *Exp. Neurol.* **139,** 39–53.

Mallet J. (1996) The TiPS/TINS lecture. Catecholamines: from gene regulation to neuropsychiatric disorders. *Trends. Pharmacol. Sci.* **17,** 129–135.

Mann V. M., Cooper J. M., and Schapira A. H. (1992) Quantitation of a mitochondrial DNA deletion in Parkinson's disease. *FEBS Lett.* **299,** 218–222.

Martinez A., Abeygunawardana C., Haavik J., Flatmark T., and Mildvan A. S. (1993) Conformation and interaction of phenylalanine with the divalent cation at the active site of human recombinant tyrosine hydroxylase as determined by proton NMR. *Biochemistry* **32,** 6381–6390.

McRitchie D. A., Cartwright H. R., and Halliday G. M. (1997) Specific A10 dopaminergic nuclei in the midbrain degenerate in Parkinson's disease. *Exp. Neurol.* **144,** 202–213.

Mecocci P., MacGarvey U., Kaufman A. E., Koontz D., Shoffner J. M., Wallace D. C., and Beal M. F. (1993) Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.* **34,** 609–616.

Mendez I., Sadi D., and Hong M. (1996) Reconstruction of the nigrostriatal pathway by simultaneous intrastriatal and intranigral dopaminergic transplants. *J. Neurosci.* **16,** 7216–7227.

Meyer Klaucke W., Winkler H., Schunemann V., Trautwein A. X., Nolting H. F., and Haavik J. (1996) Mossbauer, electron–paramagnetic-resonance and X-ray-absorption fine-structure studies of the iron environment in recombinant human tyrosine hydroxylase. *Eur. J. Biochem.* **241,** 432–439.

Michaud Soret I., Andersson K. K., Que L. Jr., and Haavik J. (1995) Resonance Raman studies of catecholate and phenolate complexes of recombinant human tyrosine hydroxylase. *Biochemistry* **34,** 5504–5510.

Miller N. and Vile R. (1995) Targeted vectors for gene therapy. *FASEB J.* **9,** 190–199.

Miranda M., Botti D., and Di Cola M. (1984) Possible genotoxicity of melanin synthesis intermedi-

ates: tyrosinase reaction products interact with DNA in vitro. *Mol. Gen. Genet.* **193,** 395–399.

Moffat M., Harmon S., Haycock J., and O'Malley K. L. (1997) L-Dopa and dopamine-producing gene cassettes for gene therapy approaches to Parkinson's disease. *Exp. Neurol.* **144,** 69–73.

Mogi M., Harada M., Kiuchi K., Kojima K., Kondo T., Narabayashi H., Rausch D., Riederer P., Jellinger K., and Nagatsu T. (1988) Homospecific activity (activity per enzyme protein) of tyrosine hydroxylase increases in parkinsonian brain. *J. Neural Transm.* **72,** 77–82.

Moore M. W., Klein R. D., Farinas I., Sauer H., Armanini M., Phillips H., Reichardt L. F., Ryan A. M., Carver Moore K., and Rosenthal A. (1996) Renal and neuronal abnormalities in mice lacking GDNF. *Nature* **382,** 76–79.

Nagatsu T. (1995) Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays. Biochem.* **30,** 15–35.

Nagatsu T., Levitt M., and Udenfriend S. (1964) Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **239,** 2910–2917.

Nakao N., Frodl E. M., Duan W. M., Widner H., and Brundin P. (1994) Lazaroids improve the survival of grafted rat embryonic dopamine neurons. *Proc. Natl. Acad. Sci. USA* **91,** 12,408–12,412.

Nygaard T. G., Marsden C. D., and Duvoisin R. C. (1988) Dopa-responsive dystonia. *Adv. Neurol.* **50,** 377–384.

Okun M. R., Patel R. P., Donnellan B., Lever W. F., Edelstein L. M., and Epstein D. (1971) DOPA compared with dihydroxyfumarate as co-factor in peroxidase- mediated oxidation of tyrosine to melanin. Histochemical studies with neutrophils, eosinophils, mast cells, melanoma cells and neurons. *Histochemie.* **27,** 331–334.

Okuno S. and Fujisawa H. (1991) Conversion of tyrosine hydroxylase to stable and inactive form by the end products. *J. Neurochem.* **57,** 53–60.

Olanow C. W., Kordower J. H., and Freeman T. B. (1996) Fetal nigral transplantation as a therapy for Parkinson's disease. *Trends. Neurosci.* **19,** 102–109.

Oreland L. (1991) Monoamine oxidase, dopamine and Parkinson's disease. *Acta Neurol. Scand. Suppl.* **136,** 60–65.

Ota A., Yoshida S., and Nagatsu T. (1996) Regulation of N-terminus-deleted human tyrosine hydroxylase type 1 by end products of catecholamine biosynthetic pathway. *J. Neural Transm.* **103,** 1415–1428.

Ozelius L., Kramer P. L., Moskowitz C. B., Kwiatkowski D. J., Brin M. F., Bressman S. B., Schuback D. E., Falk C. T., Risch N., de Leon D., and et al (1989) Human gene for torsion dystonia located on chromosome 9q32-q34. *Neuron* **2,** 1427–1434.

Plante Bordeneuve V., Davis M. B., Maraganore D. M., Marsden C. D., and Harding A. E. (1994) Tyrosine hydroxylase polymorphism in familial and sporadic Parkinson's disease. *Mov. Disord.* **9,** 337–339.

Poirier J., Kogan S., and Gauthier S. (1991) Environment, genetics and idiopathic Parkinson's disease. *Can. J. Neurol. Sci.* **18,** 70–76.

Polymeropoulos M. H., Higgins J. J., Golbe L. I., Johnson W. G., Ide S. E., Di Iorio G., Sanges G., Stenroos E. S., Pho L. T., Schaffer A. A., Lazzarini A. M., Nussbaum R. L., and Duvoisin R. C. (1996) Mapping of a gene for Parkinson's disease to chromosome 4q21-q23. *Science* **274,** 1197–1199.

Polymeropoulos M. H., Lavedan C., Leroy E., Ide S. E., Dehejia. A., Dutra A., Pike B., Root H., Rubenstein J., Boyer R., Stenroos E. S., and Chandrasekharappa S. (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* **276,** 2045–2047.

Rajput A. H., Gibb W. R., Zhong X. H., Shannak K. S., Kish S., Chang L. G., and Hornykiewicz O. (1994) Dopa-responsive dystonia: pathological and biochemical observations in a case. *Ann.Neurol.* **35,** 396–402.

Ramsey A. J., Daubner S. C., Ehrlich J. I., and Fitzpatrick P. F. (1995) Identification of iron ligands in tyrosine hydroxylase by mutagenesis of conserved histidinyl residues. *Protein Sci.* **4,** 2082–2086.

Ramsey A. J., Hillas P. J., and Fitzpatrick P. F. (1996) Characterization of the active site iron in tyrosine hydroxylase. Redox states of the iron. *J. Biol. Chem.* **271,** 24,395–24,400.

Ribeiro P., Wang Y., Citron B. A., and Kaufman S. (1993) Deletion mutagenesis of rat PC12 tyrosine hydroxylase regulatory and catalytic domains. *J. Mol. Neurosci.* **4,** 125–139.

Riederer P., Sofic E., Rausch W. D., Schmidt B., Reynolds G. P., Jellinger K., and Youdim M. B. (1989) Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J. Neurochem.* **52,** 515–520.

Rodgers A. D. and Curzon G. (1975) Melanin formation by human brain in vitro. *J. Neurochem.* **24,** 1123–1129.

Rodriguez G. J., Venero J. L., Vizuete M. L., Cano J., and Machado A. (1997) Deprenyl induces the tyrosine hydroxylase enzyme in the rat dopamin-

ergic nigrostriatal system. *Brain Res. Mol. Brain Res.* **46,** 31–38.

Rosenblad C., Martinez Serrano A., and Björklund A. (1996) Glial cell line-derived neurotrophic factor increases survival, growth and function of intrastriatal fetal nigral dopaminergic grafts. *Neuroscience* **75,** 979–985.

Sanchez M. P., Silos Santiago I., Frisen J., He B., Lira S. A., and Barbacid M. (1996) Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* **382,** 70–73.

Sandri G., Panfili E., and Ernster L. (1990) Hydrogen peroxide production by monoamine oxidase in isolated rat-brain mitochondria: its effect on glutathione levels and Ca2+ efflux. *Biochim. Biophys. Acta* **1035,** 300–305.

Schapira A. H. and Cooper J. M. (1992) Mitochondrial function in neurodegeneration and ageing. *Mutat. Res.* **275,** 133–143.

Scherzinger E., Lurz R., Turmaine M., Mangiarini L., Hollenbach B., Hasenbank R., Bates G. P., Davies S. W., Lehrach H. and Wanker E. E. (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* **90,** 549–558.

Schnopp N. M., Kosel S., Egensperger R., and Graeber M. B. (1996) Regional heterogeneity of mtDNA heteroplasmy in parkinsonian brain. *Clin. Neuropathol.* **15,** 348–352.

Scriver C. R., Byck S., Prevost L., and Hoang L. (1996) The phenylalanine hydroxylase locus: a marker for the history of phenylketonuria and human genetic diversity. PAH Mutation Analysis Consortium. *Ciba. Found. Symp.* **197,** 73–90.

Segawa M. (1996) [Segawa disease (hereditary progressive dystonia with marked diurnal fluctuation-HPD) and abnormalities in pteridin metabolism]. *Rinsho. Shinkeigaku.* **36,** 1322–1323.

Shults C. W., Kimber T., and Altar C. A. (1995) BDNF attenuates the effects of intrastriatal injection of 6-hydroxydopamine. *Neuroreport.* **6,** 1109–1112.

Slack R. S. and Miller F. D. (1996) Viral vectors for modulating gene expression in neurons. *Curr. Opin. Neurobiol.* **6,** 576–583.

Smyth C., Kalsi G., Curtis D., Brynjolfsson J., O'Neill J., Rifkin L., Moloney E., Murphy P., Petursson H., and Gurling H. (1997) Two-locus admixture linkage analysis of bipolar and unipolar affective disorder supports the presence of susceptibility loci on chromosomes 11p15 and 21q22. *Genomics* **39,** 271–278.

Snyder E. Y., Park K. I., Flax J. D., Liu S., Rosario C. M., Yandava B. D., and Aurora S. (1997) Potential of neural "stem-like" cells for gene therapy and repair of the degenerating central nervous system. *Adv. Neurol.* **72,** 121–132.

Sofic E., Paulus W., Jellinger K., Riederer P., and Youdim M. B. (1991) Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. *J. Neurochem.* **56,** 978–982.

Soong N. W., Hinton D. R., Cortopassi G., and Arnheim N. (1992) Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. *Nat. Genet.* **2,** 318–323.

Sparks D. L., Woeltz V. M., and Markesbery W. R. (1991) Alterations in brain monoamine oxidase activity in aging, Alzheimer's disease, and Pick's disease. *Arch. Neurol.* **48,** 718–721.

Stachowiak M. K., Goc A., Hong J. S., Poisner A., Jiang H. K., and Stachowiak E. K. (1994) Regulation of tyrosine hydroxylase gene expression in depolarized non-transformed bovine adrenal medullary cells: second messenger systems and promoter mechanisms. *Brain Res. Mol. Brain Res.* **22,** 309–319.

Strange P. G. (1992) Parkinson's disease. In *Brain Biochemistry and Brain Disorders* (Strange P. G., ed.) Oxford University Press, New York, pp 161–185.

Sutherland C., Alterio J., Campbell D. G., Le-Bourdelles B., Mallet J., Haavik J., and Cohen P. (1993) Phosphorylation and activation of human tyrosine hydroxylase in vitro by mitogen-activated protein (MAP) kinase and MAP-kinase-activated kinases 1 and 2. *Eur. J. Biochem* **217,** 715–722.

Thibaut F., Ribeyre J. M., Dourmap N., Meloni R., Laurent C., Campion D., Ménard J. F., Dollfus S., Mallet J., and Petit M. (1997) Association of DNA polymorphism in the first intron of the tyrosine hydroxylase gene with disturbances of the catecholaminergic system in schizophrenia. *Schizophrenia. Res* **23,** 259–264.

Thomas G., Haavik J., and Cohen P. (1997) Participation of a stress-activated MAP kinase cascade in the activation of tyrosine hydroxylase in chromaffin cells. *Eur. J. Biochem.* **247,** 1180–1189.

Thöny B. and Blau N. (1997) Mutations in the GTP cyclohydrolase I and 6-pyruvoyl- tetrahydropterin synthase genes. *Hum. Mutat.* **10,** 11–20.

Tsukahara T., Takeda M., Shimohama S., Ohara O., and Hashimoto N. (1995) Effects of brain-derived neurotrophic factor on 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in monkeys. *Neurosurgery* **37,** 733–739.

Tumer N., Brown J. W., Carballeira A., and Fishman L. M. (1996) Tyrosine hydroxylase gene expres-

sion in varying forms of human pheochromocytoma. *Life Sci.* **59,** 1659–1665.
Vieregge P. (1994) Genetic factors in the etiology of idiopathic Parkinson's disease. *J. Neural. Transm. Park. Dis. Dement. Sect.* **8,** 1–37.
Walker S. J., Liu X., Roskoski R., and Vrana K. E. (1994) Catalytic core of rat tyrosine hydroxylase: terminal deletion analysis of bacterially expressed enzyme. *Biochim. Biophys. Acta* **1206,** 113–119.
Walkinshaw G. and Waters C. M. (1995) Induction of apoptosis in catecholaminergic PC12 cells by L- DOPA. Implications for the treatment of Parkinson's disease. *J. Clin. Invest.* **95,** 2458–2464.
Wang Y., Tien L. T., Lapchak P. A., and Hoffer B. J. (1996) GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. *Cell Tissue Res.* **286,** 225–233.
Warner H. R. (1994) Superoxide dismutase, aging, and degenerative disease. *Free Radic. Biol. Med.* **17,** 249–258.
Whittemore E. R., Loo D. T., and Cotman C. W. (1994) Exposure to hydrogen peroxide induces cell death via apoptosis in cultured rat cortical neurons. *Neuroreport.* **5,** 1485–1488.
Wijker M., Wszolek Z. K., Wolters E. C., Rooimans M. A., Pals G., Pfeiffer R. F., Lynch T., Rodnitzky R. L., Wilhelmsen K. C. and Arwert F. (1996) Localization of the gene for rapidly progressive autosomal dominant parkinsonism and dementia with pallido-ponto-nigral degeneration to chromosome 17q21. *Hum. Mol. Genet.* **5,** 151–154.
Wolff J. A., Fisher L. J., Xu L., Jinnah H. A., Langlais P. J., Iuvone P. M., O'Malley K. L., Rosenberg M. B., Shimohama S., Friedmann T., and et al (1989) Grafting fibroblasts genetically modified to produce L-dopa in a rat model of Parkinson disease. *Proc. Natl. Acad. Sci. USA* **86,** 9011–9014.
Woodgett J. R., Avruch J., and Kyriakis J. (1996) The stress activated protein kinase pathway. *Cancer Surv.* **27,** 127–138.
Wu D. K. and Cepko C. L. (1994) The stability of endogenous tyrosine hydroxylase protein in PC-12 cells differs from that expressed in mouse fibroblasts by gene transfer. *J. Neurochem.* **62,** 863–872.
Wu J., Filer D., Friedhoff A. J., and Goldstein M. (1992) Site-directed mutagenesis of tyrosine hydroxylase. Role of serine 40 in catalysis. *J. Biol. Chem.* **267,** 25754–25758.
Wu R. M., Murphy D. L., and Chiueh C. C. (1996) Suppression of hydroxyl radical formation and protection of nigral neurons by l-deprenyl (selegiline). *Ann. NY Acad. Sci.* **786,** 379–390.
Yoshimoto Y., Lin Q., Collier T. J., Frim D. M., Breakefield X. O., and Bohn M. C. (1995) Astrocytes retrovirally transduced with BDNF elicit behavioral improvement in a rat model of Parkinson's disease. *Brain Res.* **691,** 25–36.
Youdim M. B. and Riederer P. (1997) Understanding Parkinson's disease. *Sci. Am.* **276,** 52–59.
Yurek D. M., Lu W., Hipkens S., and Wiegand S. J. (1996) BDNF enhances the functional reinnervation of the striatum by grafted fetal dopamine neurons. *Exp. Neurol.* **137,** 105–118.
Zhou Q. Y., Quaife C. J., and Palmiter R. D. (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* **374,** 640–643.