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Review

Leads for the development of neuroprotective treatment in Parkinson's disease and brain imaging methods for estimating treatment efficacy

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Abstract

Patients suffering from Parkinson's disease display severe and progressive deficits in motor behavior, predominantly as a consequence of the degeneration of dopaminergic neurons, located in the mesencephalon and projecting to striatal regions. The cause of Parkinson's disease is still an enigma. Consequently, the pharmacotherapy of Parkinson's disease consists of symptomatic treatment, with in particular L-dihydroxyphenylalanine (L-DOPA) and/or dopamine receptor agonists. These induce a dramatic initial improvement. However, serious problems gradually develop during long-term treatment. Therefore, a more rational, c.q. causal treatment is needed which requires the introduction of compounds ameliorating the disease process itself. The development of such compounds necessitates (1) more information on the etiopathogenesis, i.e., the cascade of events that ultimately leads to degeneration of the dopaminergic neurons, and (2) brain imaging methods, to estimate the extent of the degeneration of the dopaminergic neurons in the living patient. This is not only important for the early diagnosis, but will also allow to monitor the effectiveness of alleged neuroprotective compounds on a longitudinal base. In this paper, etiopathogenic mechanisms are highlighted along the line of the oxidative stress hypothesis and within this framework, attention is mainly focused on the putative role of glutathione, dopamine auto-oxidation and phase II biotransformation enzymes. Especially, drugs able to increase the activity of phase II biotransformation enzymes seem to elicit a broad-spectrum (neuro)protective response and look very promising leads for the development of neuroprotective treatment strategies in Parkinson's disease. New developments in brain imaging methods (single photon emission computed tomography (SPECT) and positron emission tomography (PET)) to visualize the integrity of the striatal dopaminergic neurons in humans are highlighted as well. Especially, the introduction of radioligands that bind selectively to the dopamine transporter seems to be a significant step forward for the early diagnosis of Parkinson's disease. Performing these brain imaging studies with fixed time intervals does not only create the possibility to follow the degeneration rate of the dopaminergic neurons in Parkinson's disease but also provides the opportunity to estimate therapeutic effects of putative neuroprotective agents in the individual patient.

Keywords: Parkinson's disease; Brain imaging; Neuroprotection; Oxidative stress; Treatment efficacy

1. Introduction

1.1. Symptoms and pathophysiology of Parkinson's disease

Idiopathic Parkinson's disease is a progressive neurodegenerative disorder which manifests itself by bradykinesia in combination with rigidity, tremor and a postural imbalance. In addition, autonomic nervous system dysfunctions such as micturition problems, orthostatic hypotension and seborrhea as well as subtle cognitive dysfunctions and depression occur. Parkinson's disease affects approximately 0.15% of the total population but 0.5% of people older than 50 years. The disease is neuropathologically characterized primarily by degeneration of dopamine-containing neurons in the ventral mesencephalon.

Dopaminergic neurons in the ventral mesencephalon are distributed over different cell groups, including the substantia nigra pars compacta (A9 cell group), the ventral tegmental area (A10 cell group) and the retrorubral area (A8 cell group). Recent neuroanatomical and functional studies have revealed that the dopaminergic projection is

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just one of the neuronal elements integrated in the basal ganglia-thalamocortical circuits which are involved in the regulation of motor and complex behavioral activity (Alexander et al., 1990; Groenewegen et al., 1990, 1993).

The following brief description of the functional neuroanatomy of these basal ganglia-thalamocortical circuits is useful in understanding the symptomatology of Parkinson's disease and the pharmacotherapy that is currently used.

The basal ganglia consist of four main structures: the *striatum* (caudate nucleus, putamen and nucleus accumbens), the *pallidum* (external and internal segments of the globus pallidus and ventral pallidum), the *subthalamic nucleus* and the *substantia nigra* (pars compacta and pars reticulata). The striatum is the input structure of the basal ganglia, receiving afferents, in a strict topographical way, from the entire cerebral cortex (including 'cortical-like' nuclei of the amygdala and the hippocampal formation), the midline and intralaminar thalamic nuclei, and midbrain serotonergic and dopaminergic cell groups. The output structure of the basal ganglia consists of the internal segment of the globus pallidus and the pars reticulata of the substantia nigra which project, also in a topographical manner, to different medial and ventral thalamic nuclei, the deep layers of the superior colliculus and the reticular formation of the mesencephalon. The various thalamic nuclei that are innervated by these output structures of the basal ganglia project to different cortical areas of the frontal lobe, including motor, premotor and prefrontal cortical areas.

The topography in the projections from different frontal cortical areas through the basal ganglia and the thalamus by subsequent corticostriatal, striatopallidal (or striatonigral), pallidothalamic (or nigrothalamic), and thalamocortical projections is organised in such a way that a number of parallel, functionally segregated basal ganglia-thalamocortical circuits can be discriminated. Whereas sensorimotor functions are dealt with in the circuit that originates in and returns to the (pre)motor cortex (and that at the striatal level, receives also inputs from the somatosensory cortex), other circuits are involved in complex motor/behavioral, cognitive and affective processes (Alexander et al., 1990; Bhatia and Marsden, 1994; Marsden and Obeso, 1994).

The input (striatum) and output structures (internal segment of the globus pallidus and pars reticulata of the substantia nigra) of the basal ganglia are connected with each other by means of two pathways, i.e., a 'direct' and an 'indirect' pathway. The direct pathway consists of the γ-aminobutyric acid (GABA)/substance P/dynorphin-containing striatopallidal (internal segment of the globus pallidus) and striatonigral (pars reticulata of the substantia nigra) projections. The indirect pathway is constituted by the sequence of the GABA/enkephalin-containing striatopallidal (external segment of the globus pallidus), the GABA-ergic pallido-subthalamic, and the glutamatergic subthalamo-pallidal (internal segment of the globus pallidus) and subthalamo-nigral (pars compacta of the substantia nigra) projections. At the level of the output structures of the basal ganglia, these direct and indirect pathways have opposite effects on the GABA-ergic neurons that project to the thalamic nuclei, the superior colliculus and the reticular formation. A 'balance' between these two striatal output pathways appears to be essential for the normal regulation of movement. Although recent data show a more complex picture of the distribution of dopamine receptors, it has previously been suggested that dopamine, acting through different dopamine receptors, has opposing effects on the direct and indirect pathways (Stoof and Kebabian, 1981; Alexander and Crutcher, 1990; Gerfen, 1992): via dopamine D_1 receptors a stimulatory effect on the direct pathway, whereas via dopamine D_2 receptors an inhibitory effect on the indirect pathway. The consequence of the loss of dopaminergic input to the striatum as occurs in Parkinson's disease is therefore thought to be an increase in the output from the internal segment of the globus pallidus and the pars reticulata of the substantia nigra to the thalamus (DeLong, 1990), ultimately (supposedly at about 75% depletion of striatal dopamine) resulting in a reduction of cortical activation which accounts for (most of) the Parkinsonian signs, including bradykinesia, rigidity, tremor and postural imbalance.

1.2. Symptomatic treatment of Parkinson's disease

'Restoring the balance' at the level of the output structures of the basal ganglia in order to decrease the inhibition of the thalamic nuclei can be achieved neurosurgically, by lesioning the subthalamic nucleus, a posteroventral pallidotomy and/or venterolateral thalamotomy (Marsden and Obeso, 1994). However, this therapeutic intervention is only rarely applied. In general, Parkinson's disease patients are treated pharmacologically, by suppletion and/or substitution of dopamine with the dopamine precursor L-DOPA or with dopamine receptor agonists (Wolters, 1992). In the late sixties, shortly after it became apparent that patients with Parkinson's disease were suffering from a dopamine deficit in the basal ganglia, the dopamine precursor L-DOPA (soon afterwards in combination with a peripheral decarboxylase inhibitor) was successfully given to supplete the empty dopamine stores. Currently, this treatment is still considered to be the most effective way to control the symptoms of Parkinson's disease. Unfortunately, however, long-term treatment with L-DOPA frequently results in fading of the therapeutic effect (wearing-off), in the development of serious motor side-effects such as on–off motor oscillations and dyskinesias and, although less often, in psychiatric complications. Under those conditions, increasing the dose of L-DOPA to compensate for the loss of therapeutic efficacy gives rise only to more side-effects without adding any beneficial effect. The mechanisms underlying this 'narrowing of the

therapeutic window' are still largely a matter of speculation. Lately, the hypothesis has been put forward that long-term treatment with L-DOPA might accelerate the degeneration of dopaminergic neurons by enhanced generation of cytotoxic reactive oxygen species as a consequence of dopamine and/or L-DOPA auto-oxidation (see also below).

From the 1980's onwards, the introduction of dopamine D_2 receptor agonists has extended the therapeutic armamentarium for Parkinson's disease. Although various compounds, such as lisuride, bromocriptine, 4-propyl-9-hydroxynaphtoxazine (PHNO) and pergolide have been clinically tested, it seems that bromocriptine and pergolide are now the most frequently prescribed dopaminomimetics. Both drugs have a high affinity as an agonist for the dopamine D_2 receptor. Moreover, it has been claimed that pergolide in higher concentrations displays agonistic activity at the dopamine D_1 receptor. However, given the plasma levels during therapy, it is very doubtful if this phenomenon plays any substantial role in the therapeutic effects of the compound. Although long-term treatment with dopamine D_2 receptor agonists results in less dyskinesias, the therapeutic efficacy is likewise less dramatic as compared to the initial effects of L-DOPA. Furthermore, increasing the dose of dopaminergic agonists gives rise to other serious side-effects, such as psychotic reactions. Based on the above-described insights into the clinical action of the drugs, a now generally accepted therapeutic protocol consists of the combination of a low dose of L-DOPA together with one of the dopamine D_2 receptor agonists. This treatment regime in general results in optimal control of the symptoms with fewer side-effects, at least in the early stages of the disease. Nevertheless, in the medium to long-run also this therapeutic strategy is doomed to failure.

Apart from dopamine D_2 receptors, also dopamine D_1 receptors are targets for the action of dopamine in the striatum. Especially, the (medium spiny) output neurons projecting to the internal segment of the globus pallidus, forming the so-called 'direct route', are known to express significant numbers of dopamine D_1 receptors, whereas the cells of the 'indirect route' are supposed to contain predominantly dopamine D_2 receptors (Gerfen and Young, 1988; Gerfen, 1992). Dopamine, by simultaneously facilitating the activity of the 'direct' pathway (via stimulation of dopamine D_1 receptors) and inhibiting the activity of the 'indirect' pathway (via stimulation of dopamine D_2 receptors), keeps these pathways in a delicate balance which, as noted above, is thought to be essential for the normal regulation of movement. Moreover, this concept provides also a rationale for the observed requirement of both dopamine D_1 and dopamine D_2 receptor stimulation to restore the complete repertoire of motor activity in animal models for Parkinson's disease (Waddington and O'Boyle, 1989). The fact that dopamine D_2 receptor agonists mediate therapeutic effects on their own, especially in the early phases of Parkinson's disease, could mean that in that stage residual dopamine released from surviving dopaminergic neurons is still sufficient to stimulate the 'direct' pathway (Strange, 1993). Thus, one would expect selective dopamine D_1 receptor agonists to be of additional therapeutic value later on in the course of Parkinson's disease. However, in the 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)-lesioned monkey model of Parkinson's disease, the partial dopamine D_1 receptor agonist SKF 38393, which was developed in the late seventies, failed to stimulate motor behavior (Barone et al., 1987; Bedard and Boucher, 1989). Recently, it was demonstrated that at least in primates, this compound's lack of therapeutic effect is most likely due to its very low intrinsic activity at the dopamine D_1 receptor (Pifl et al., 1992; Vermeulen et al., 1994). Subsequently, full dopamine D_1 receptor agonists became available and animal studies have now definitely proven that stimulation of dopamine D_1 receptors affects motor behavior, especially in those animals in which the nigrostriatal dopaminergic system has been lesioned (Taylor et al., 1991; Kebabian et al., 1992; Vermeulen et al., 1993). However, careful analysis of this behavioral stimulation casts doubt on the anti-Parkinsonian nature of the effects of dopamine D_1 receptor agonists. For example, the claim that dopamine D_1 receptor stimulation activates motor behavior without inducing dyskinesias cannot be generally confirmed (Blanchet et al., 1996; Andringa et al., 1998). In fact, long-term stimulation seems to induce significant dyskinesias. Another drawback of long-term treatment with dopamine D_1 receptor agonists appears to be the poorly sustained action of these drugs because of receptor desensitisation (Blanchet et al., 1996), and last but not least, the occurrence of seizures (Starr and Starr, 1993; Shiosaki et al., 1996; Andringa et al., 1999a,b). Thus, contrary to expectation, dopamine D_1 receptor stimulation, even with selective high efficacy agonists, in our opinion, will not substantially improve the pharmacotherapy of Parkinson's disease.

1.3. Urgent need for a more causal treatment

One of the mechanisms likely to underly the occurrence of wearing-off and the development of serious side-effects upon long-term treatment with dopaminergic compounds in Parkinson's disease is the progression of the pathologic process. Thus, the ongoing degeneration of dopaminergic neurons might not only prohibit efficient decarboxylation of the administered L-DOPA but could also be responsible for changes in (postsynaptic) dopamine receptor sensitivity, resulting in the aforementioned problems. Therefore, as of late, much effort has been invested in the development of neuroprotective agents that will be able slow down the degenerative process. In order to design an optimal causal treatment regime for Parkinson's disease, two lines of investigation are of crucial importance. First, in-depth knowledge about the whole cascade of events that leads to

the degeneration of the dopaminergic cells, i.e., the etiopathogenesis of Parkinson's disease, is required (Section 2), since such knowledge is indispensable for the rational development of neuroprotective agents. Secondly, in order to assess the effectiveness of such compounds in longitudinal studies, a brain imaging technique that visualizes the striatal dopaminergic innervation and is able to estimate the rate of degeneration of the dopaminergic system in the course of Parkinson's disease (Section 3) should be available.

2. Etiopathogenesis of Parkinson's disease

2.1. Oxidative stress hypothesis

As noted already, at the cellular level Parkinson's disease is characterized foremost by a loss of the neurotransmitter dopamine in the striatum due to degeneration of dopaminergic neurons located in the pars compacta of the substantia nigra (Gibb and Lees, 1991). In addition, also other neuronal systems, in particular noradrenergic neurons originating in the locus coeruleus, are affected by the disease process albeit to a substantially smaller extent (Jellinger, 1991). Despite large scale attempts at elucidation, presently the pathogenesis of Parkinson's disease remains largely enigmatic. Nevertheless, already for a number of years oxidative stress has been implicated as a major causative factor in the neuronal degeneration occurring in the Parkinsonian substantia nigra. For example, post-mortem analysis has revealed increased lipid peroxidation, superoxide dismutase activity and free iron levels in the substantia nigra of Parkinsonian patients (Jenner and Olanow, 1998). However, the earliest, reportedly even preceding the loss of dopamine, indication of oxidative stress in Parkinson's disease brains appears to be a reduction in the level of the anti-oxidant glutathione in the substantia nigra (Dexter et al., 1994). Considering these findings and the ease with which the catechol derivate dopamine oxidizes, it has been postulated that the preferential degeneration of nigral dopaminergic neurons in Parkinson's disease is related or might even be attributed to reactive oxygen species produced during dopamine breakdown. This line of thought has come to be known as the 'oxidative stress' or 'free radical hypothesis of Parkinson's disease' (Fahn and Cohen, 1992). In fact, the 'classical', i.e., monoamine oxidase-catalyzed, route of oxidative dopamine breakdown leads to the formation of the strong oxidant hydrogen peroxide. Consequently, based on the 'oxidative stress hypothesis of Parkinson's disease', it was expected that pharmacological blockade of monoamine oxidase activity would offer neuroprotection. Eventually, this led to the introduction of the monoamine oxidase inhibitor deprenyl for clinical use as an alleged neuroprotectant in Parkinson's disease (Parkinson Study Group, 1989, 1993). Unfortunately though, for a number of possible reasons, the potential to inhibit progression of the disease process in humans (solely) via blockade of monoamine oxidase until now has proven to be rather disappointing (Lees, 1995; Hagan et al., 1997). Therefore, a different approach to oxidative stress and neuroprotection in Parkinson's disease is clearly warranted.

2.2. Glutathione and the brain

There is now ample evidence suggesting that loss of glutathione in the substantia nigra is an important, if not primary, event in the pathogenesis of Parkinson's disease. Glutathione is a tripeptide containing a glutamate, a cysteine and a glycine moiety. Glutathione is present in millimolar concentrations in all cells of eukaryotic organisms, including humans. Synthesis and degradation of glutathione occur in a number of highly interactive enzymatic reactions known collectively as the 'γ-glutamyl cycle' (Meister and Anderson, 1983). Extensively studied enzymes of this cycle are γ-glutamylcysteine synthetase and γ-glutamyltranspeptidase. These enzymes are responsible for catalyzing the formation of the glutathione precursor γ-glutamylcysteine from glutamate and cysteine and the breakdown of glutathione into glutamate and cysteinylglycine, respectively. Cells use glutathione mainly as an anti-oxidant for the scavenging of organic- and inorganic hydroperoxides, such as lipid peroxides and hydrogen peroxide, and for inactivation of endogenous toxic metabolites and xenobiotics through conjugation. These reactions are catalyzed by the enzymes glutathione peroxidase and glutathione transferase, respectively (Meister and Anderson, 1983).

All components of glutathione metabolism have been shown to be present in the brain. However, the cellular localization in the brain of glutathione and related enzymes is still a matter of debate. Thus, using a rather nonspecific histochemical technique, glutathione was originally reported to be present almost exclusively in glial (astrocytic) cells (Slivka et al., 1987). This finding was supported by data obtained from cultured brain cells demonstrating a substantially higher content of glutathione in astrocytes as compared to neurons (Raps et al., 1989). In contrast, with the use of specific antibodies to glutathione on tissue sections and primary brain cell cultures, it has recently been established that apart from astrocytes, glutathione is present in substantial amounts also in neuronal fibers (Amara et al., 1994; Hjelle et al., 1994; Langeveld et al., 1996). A similarly unclear picture has emerged from studies investigating the presence of the various glutathione related enzymes in the different cell types of the brain. For example, whereas γ-glutamyl transpeptidase immunoreactivity has been detected solely in endothelial and ependymal cells, biochemical activity of this enzyme seems to be present also in other types of brain cells (Muller and Freimuller-Kreutzer, 1985; Makar et al., 1994; Philbert et al., 1995). The same sort of confounding observations have

been made regarding glutathione peroxidase and glutathione transferase (Damier et al., 1993; Makar et al., 1994; Philbert et al., 1995). In trying to explain these inconsistencies, it is important to notice that comparison of the results between the various studies is complicated by differences not only in the type(s) of animal species and experimental methods used but also in the brain areas investigated.

2.3. Glutathione and Parkinson's disease

The loss of glutathione in Parkinson's disease is reported to be restricted to the substantia nigra, the brain area most severely affected by the disease process. More important, a decreased nigral glutathione content appears to be rather specific for Parkinson's disease since other disorders involving the substantia nigra, such as multisystem atrophy, are not accompanied by such a deficit (Jenner et al., 1992; Sian et al., 1994a). Furthermore, the reduction in glutathione content is accounted for solely by the reduced form of the peptide as no changes in the level of the oxidized form have been demonstrated (Jenner et al., 1992; Sian et al., 1994a). In contrast to glutathione content, only scant information is available on the status of glutathione related enzymes in Parkinson's disease brains. The data available, however, show no consistent change in any enzyme activity (Kish et al., 1985; Sian et al., 1994b). Recently, an intriguing observation was reported demonstrating a significant increase in the activity of γ-glutamyl transpeptidase in the substantia nigra of Parkinson's disease patients (Sian et al., 1994b). Since γ-glutamyl transpeptidase, which is a cell membrane bound protein, presumably functions to increase intracellular glutathione by retrieval of extracellular glutathione (or its component amino acids), apparently in Parkinson's disease-afflicted brain, an attempt is made to compensate for the loss of intracellular glutathione by upregulation of γ-glutamyl transpeptidase activity. Together, this combination of an early decrease in cellular glutathione content, an increase in glutathione 'uptake' from the extracellular space and an absence of overt alterations in the enzymatic capacity to synthesize or use glutathione for protection against oxidative damage and/or toxic compounds suggests that the degeneration of nigral dopaminergic neurons in Parkinson's disease may be caused by a, thus far unidentified, process in which glutathione is consumed excessively. In order to possibly identify the nature of this 'glutathione consumption' process in Parkinson's disease, it is important to take into consideration that most assays used for measuring glutathione content detect only the oxidized and/or reduced form of the compound but provide no information on other major forms in which the peptide can occur, for instance, glutathione conjugates. In fact, the increasing number of studies devoted to this topic show a rise in the ratio of glutathionyl (i.e., cysteinyl) adducts of dopamine to dopamine or dopamine metabolites in both the substantia nigra and cerebrospinal fluid of Parkinson's disease patients (Fornstedt et al., 1989; Cheng et al., 1996; Spencer et al., 1998). Since these adducts are thought to be formed largely as a consequence of the interaction between glutathione and so-called dopamine–quinones which are the foremost product of the nonenzymatic, auto-oxidative breakdown of dopamine (see below), these data point towards a possible link between the early glutathione loss observed in Parkinson's disease and the process of dopamine auto-oxidation occurring in the substantia nigra.

2.4. Dopamine auto-oxidation and neuromelanin in the substantia nigra

In addition to the well-known monoamine oxidase-catalyzed enzymatic oxidation, dopamine is also able to undergo oxidative catabolism in a chain of reactions designated as dopamine auto-oxidation. During this not yet unequivocally characterized process, dopamine is oxidized first into a product named dopamine–quinone which cyclizes readily to form an indolic compound which is known under various names of which aminochrome appears to be the one used most extensively (Graham, 1978). Subsequently, this cyclic dopamine–quinone is oxidatively polymerized leading to the formation of neuromelanin, the pigment which in adult humans lends the substantia nigra its characteristic dark brown to black color (Graham, 1978; D'Ischia and Prota, 1997). The pathway for generation of the neuromelanin polymer in vivo is more complicated than described here because other factors such as metal ions and sulfhydryl agents affect the chemistry involved. Curiously, dopamine auto-oxidation apparently does not occur at the same rate in all dopaminergic neurons since the amount of intracellular neuromelanin differs markedly between dopamine-containing cells in the substantia nigra. In this context, it is important to note that neuropathological studies in Parkinson's disease have revealed that the loss of dopaminergic neurons is not distributed equally over the substantia nigra but occurs primarily in those cells heavily laden with neuromelanin granules (Hirsch et al., 1988). Recently, these data, suggesting a positive correlation between the extent of melanization and neuronal death in Parkinson's disease, gained support from the observation of a low number of melanized neurons in the substantia nigra of a population with a relatively low prevalence of Parkinson's disease (Muthane et al., 1998). Hence, in light of the evidence described in this and the previous section, it appears quite feasible to assume that the process of dopamine auto-oxidation in the substantia nigra may be directly associated to the pathogenesis of Parkinson's disease.

2.5. Dopamine auto-oxidation as a source of oxidative stress in Parkinson's disease

Whereas the functional significance of neuromelanin and its exact relation to Parkinson's disease pathogenesis

is still open to question (D'Ischia and Prota, 1997), there is now conclusive evidence to illustrate that the process of dopamine auto-oxidation involves the formation of potentially cytotoxic dopamine intermediates and constitutes a major source of oxidative stress in the human substantia nigra (Smythies and Galzigna, 1998). Thus, as described, the auto-oxidation of dopamine yields dopamine–quinones, i.e., highly reactive, electron-deficient, compounds that are able to exert cytotoxic effects in a variety of ways, for instance, by causing glutathione depletion as a result of covalent binding of the quinone to the reduced sulfhydryl residue contained in the glutathione molecule (Bindoli et al., 1992). Moreover, as shown recently, the resultant glutathionyl-dopamine adduct, although intended as a detoxified form of the dopamine–quinone, is prone to further oxidation into a highly neurotoxic benzothiazine derivative (Li et al., 1998). In addition to deleterious effects due to direct interaction with cellular constituents, dopamine–quinones are believed to be responsible for cytotoxicity also via their propensity for redox-cycling with the consequent generation of reactive oxygen species (Bindoli et al., 1992). Thus, at the expense of cellular reducing equivalents in the form of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and/or reduced nicotinamide-adenine dinucleotide (NADH) and catalyzed by quinone-reducing enzymes, in particular NADPH-cytochrome P450 reductase, the one-electron reduction of the dopamine auto-oxidation product aminochrome results in the formation of dopamine–semiquinone, a highly reactive and unstable dopamine intermediate that binds readily to cellular nucleophiles and/or reoxidizes to aminochrome with the concomitant production of superoxide radicals and hydrogen peroxide (Segura-Aguilar, 1996; Segura-Aguilar et al., 1998). In this way, aminochrome reduction elicits a cascade of reversible oxidation and reduction reactions, or so-called redox cycle, that is accompanied by excessive release of reactive oxygen species and depletion of NADPH. Via the iron (Fe^{2+})-catalyzed Haber–Weiss reaction, on their turn, the superoxide radicals and hydrogen peroxide released during aminochrome redox-cycling are able to yield the extremely toxic hydroxyl radical thereby initiating a variety of detrimental events ultimately leading to dopaminergic cell death (Segura-Aguilar, 1996). These include lipid and protein oxidation, damage to nucleic acids, aberrant redox-sensitive gene transcription, deprivation of cellular energy production and apoptosis, phenomena which have all been observed to occur in the Parkinsonian brain (Jenner and Olanow, 1998). Moreover, with respect to oxidative stress and Parkinson's disease, a (dopamine–quinone-induced) deficiency of NADPH is noteworthy in that it leads to a general reduction of cellular protective anti-oxidant capacity. Thus, while the enzyme glutathione reductase utilizes NADPH to maintain glutathione in its reduced state, reduced glutathione is required for the catalytic activity of glutathione peroxidase which, as noted before, prevents the accumulation of both hydrogen peroxide and lipid peroxides (Meister and Anderson, 1983). NADPH loss will therefore lead to an impaired reduction of oxidized glutathione by glutathione reductase with a consequent failure of scavenging toxic peroxides by glutathione peroxidase. Taken together, there appears to be ample reason to suspect that the processes underlying dopamine auto-oxidation contribute greatly to the oxidative stress and subsequent neuronal damage as observed in the substantia nigra in Parkinson's disease. Moreover, given the biochemical composition of neuromelanin and the occurrence of both the dopamine–quinone derivate 5-*S*-cysteinyl-dopamine and NADPH-cytochrome P450 reductase in human brain tissue, it is now evident that such a pro-oxidative pathway is indeed operative in vivo (Ravindranath et al., 1990; D'Ischia and Prota, 1997; Smythies and Galzigna, 1998).

2.6. Detoxication of dopamine auto-oxidation products by phase II biotransformation enzymes

Alongside the aforementioned pro-oxidant pathways, at least two anti-oxidant pathways have been implicated in the detoxication of dopamine-derived quinones. These pathways depend primarily on the presence of reduced glutathione and/or the catalytic activity of a group of enzymes that are collectively known as phase II biotransformation enzymes. Whereas phase I biotransformation enzymes, like the cytochrome P450 family of proteins, in general, increase the reactivity of their substrates and generate reactive oxygen species, phase II biotransformation enzymes, of which glutathione transferase, uridine 5′-diphosphate glucuronosyltransferase and sulfotransferase are probably the best known examples, have been shown to protect against xenobiotics and endogenous toxic metabolites by catalyzing the transformation of reactive electrophiles into more hydrophilic (inactive) conjugates that subsequently are excreted from the cell (Meyer, 1996; Wilkinson and Clapper, 1997). Originally, cellular defence against the toxicity of aminochrome and other dopamine–quinones was thought to be provided by the phase II biotransformation enzyme DT-diaphorase (NAD(P)H: quinone acceptor oxidoreductase), a flavoenzyme which, in contrast to NAD(P)H-cytochrome P450 reductase, catalyzes a two-electron reduction of aminochrome yielding dopamine–hydroquinone without the intermediate formation of free dopamine–semiquinone (Lind et al., 1982). On the condition that tissue anti-oxidant capacity, e.g., in the form of reactive oxygen species-scavenging enzymes such as superoxide dismutase and catalase, is sufficient to prevent its auto-oxidation, dopamine–hydroquinone is a redox stabile entity that lacks electrophilic reactivity and, due to the presence of hydroxyl groups at its terminal quinone moieties, fulfills the chemical requirements for further biotransformation and detoxication by, for instance, uridine 5′-diphosphate glucuronosyltransferase or sulfotransferase (Segura-Aguilar, 1996). Recently, apart from DT-di-

aphorase, μ class glutathione transferases, in particular the μ2–2 subtype, have been identified to constitute an additional protective mechanism against the toxicity of dopamine-derived quinones by catalyzing the reductive conjugation of glutathione to aminochrome. In this reaction, 4-*S*-glutathionyl-5,6-dihydroxyindole is formed which, in contrast to the glutathionyl adduct of the uncyclized dopamine–quinone, is a stable entity that is resistant to redox cycling and also amenable to further detoxication either by other phase II biotransformation enzymes or via direct cellular excretion (Baez et al., 1997; Segura-Aguilar et al., 1997). Taken together, it seems therefore that under physiological conditions, the concerted action of phase II biotransformation enzymes, most notably DT-diaphorase and glutathione transferase, provides a powerful cellular defence mechanism against the oxidative stress inherent to dopamine auto-oxidation, whereas insufficient activity of this enzyme system may lead to oxidative damage such as observed in Parkinson's disease. In this respect, it is noteworthy that while (immuno)histochemical and biochemical analysis of rat brain revealed the presence of DT-diaphorase in mesencephalic dopaminergic neurons and glial cells (Schultzberg et al., 1988; Murphy et al., 1998), molecular biological techniques have shown also the abundant expression of μ class glutathione transferases, including the μ2-2 form, in human brain structures such as the substantia nigra (Baez et al., 1997).

2.7. Phase II biotransformation enzymes as target for neuroprotection in Parkinson's disease

Apart from a putative involvement in Parkinson's disease pathogenesis (Segura-Aguilar, 1996), the pivotal role of phase II biotransformation enzymes in the inactivation of dopamine-derived cytotoxic quinones and their presence in relevant brain structures, makes these anti-oxidant proteins attractive targets for the rational development of innovative, neuroprotective agents to treat Parkinson's disease. Nevertheless, until now the therapeutic potential of manipulation of phase II enzyme activity is not widely acknowledged in neuroscience. This may at least in part be attributed to the lack of widespread information concerning the mechanisms regulating phase II enzyme expression in the nervous system. In this respect, it is of note, that besides their capability to detoxify a broad spectrum of electrophilic substances, phase II biotransformation enzymes have in common their inducibility by a large variety of structurally diverse chemicals of both natural and synthetic origin (Primiano et al., 1997; Wilkinson and Clapper, 1997). In fact, these compounds, including phenolic anti-oxidants (e.g., butylated hydroxyanisole, tea polyphenols and phytoestrogens) (Primiano et al., 1997; Wilkinson and Clapper, 1997; Wang et al., 1998 aromatic isothiocyanates (e.g., sulforaphane) (Zhang et al., 1992), nonsteroidal anti-inflammatory drugs (e.g., indomethacin, ibuprofen) (van Lieshout et al., 1998), and many others, have been shown to confer cellular protection via a coordinated upregulation of the expression of phase II enzymes, most likely mediated by activation of a so-called anti-oxidant response element present in the promotor region of the respective genes (Jaiswal, 1994). In this context, a particularly interesting and well-studied class of compounds is the dithiolethiones, a cyclic, sulfur-containing group of agents, originally described as constituents of cruciferous vegetables (Ansher et al., 1986). The dithiolethiones, of which oltipraz and anethole dithiolethione are available for clinical use in humans (Christen, 1995; O'Dwyer et al., 1996), not only increase the activity of phase II biotransformation enzymes in various cellular preparations in vitro (de Long et al., 1986; Egner et al., 1994; Maxuitenko et al., 1998), but are active also in vivo in animals and humans with only minor side-effects reported (Ansher et al., 1986; Christen, 1995; O'Dwyer et al., 1996). In addition to their effect on phase II biotransformation, dithiolethiones are especially attractive in that they boost general cellular anti-oxidant capacity by acting as regular oxidant scavengers (Christen, 1995; Khanna et al., 1998), by increasing the expression of metal-binding proteins such as ferritin (Primiano et al., 1996), and by stimulation of the enzymes responsible for the maintenance of reduced glutathione pools, in particular γ-glutamylcysteine synthetase, glutathione reductase and glucose-6-phosphate dehydrogenase (Ansher et al., 1986; Drukarch et al., 1997; Dringen et al., 1998; Khanna et al., 1998). In fact, such an additional mechanism of action has recently been shown to contribute significantly to the capacity of phase II enzyme inducers to protect against dopamine neurotoxicity (Duffy et al., 1998).

Thus, given the likely role of dopamine auto-oxidation in Parkinson's disease pathogenesis and the observation that drugs identified by their ability to increase the activity of phase II biotransformation enzymes are able to elicit a broad-spectrum (neuro)protective response, it seems plausible to conclude that such compounds are very promising leads for the development of efficacious neuroprotective treatment strategies in Parkinson's disease.

3. Brain imaging techniques to determine the integrity of the dopaminergic system and to monitor its degeneration rate

3.1. SPECT studies with [^{123}I]β-CIT, [^{123}I]FP-CIT and other dopamine transporter ligands

Possibilities to determine the integrity of the dopaminergic system in vivo emerged by the recent finding that cocaine analogues are very selective and efficient ligands for the so-called dopamine transporters, which are expressed in the striatal dopaminergic projections (Kaufman and Madras, 1991). Thus, it was proposed that labeling of the dopamine transporter in vivo with ligands that can be

visualized by brain imaging techniques could provide a valuable tool to investigate the extent of degeneration of the dopaminergic system in Parkinson's disease. Indeed, single photon emission computed tomography (SPECT) with the [^{123}I]-labeled cocaine analog 2-β-carbomethoxy-3-β-(4-iodophenyl)-tropane ([^{123}I]β-CIT) revealed a dramatic loss of striatal dopamine transporters in Parkinson's disease patients (Brücke et al., 1993; Innis et al., 1993). Likewise, SPECT studies with other cocaine analogs such as [^{123}I]2-β-carbomethoxy-3-β-(4-fluorophenyl)-*N*-(1-iodoprop-1-en-3-yl)nortropane (altropane, Fischman et al., 1998) and the [^{123}I]fluoropropyl derivative of β-CIT ([^{123}I]FP-CIT, Booij et al., 1997; Tissingh et al., 1998) demonstrated similar losses in transporter binding as with β-CIT. An important difference between altropane and FP-CIT, on the one hand, and β-CIT, on the other, is the much slower kinetics of the latter one. For instance, specific FP-CIT accumulation can reliably be measured 3 h following injection, whereas 24 h is needed to require the optimal specific/nonspecific binding ratio following the injection of β-CIT.

3.2. Monitoring the degeneration rate of dopaminergic neurons in Parkinson's disease with dopamine transporter ligands for SPECT

In light of its possible application in longitudinal studies in Parkinson's disease patients as a device to reliably monitor both the progression of the degenerative process and the effect(s) of putative neuroprotective agents, the question arises whether the [^{123}I]β-CIT SPECT procedure is sensitive enough to discriminate between early and late stage Parkinson's disease. Vermeulen et al. (1995) investigated [^{123}I]β-CIT binding in healthy controls, early stage (untreated, disease history < 2.5 years) Parkinson's disease patients and Parkinson's disease patients with a long history (> 10 years) and treated with dopaminomimetics (late stage Parkinson's disease). No differences could be detected between caudate nucleus [^{123}I]β-CIT binding in controls and early stage Parkinson's disease patients (94% of control), whereas [^{123}I]β-CIT binding in the caudate nucleus of late stage Parkinson's disease patients (35% of control) differed statistically significant from that of control and early stage Parkinson's disease patients. [^{123}I]β-CIT binding in the putamen was significantly decreased in both the early (52% of control) and late stage Parkinson's disease patients (20% of control), the binding of [^{123}I]β-CIT in the putamen being significantly more decreased in late than in early stage Parkinson's disease patients. In both early and late stage Parkinson's disease patients binding of the ligand was more reduced in the putamen than in the caudate nucleus. This differential loss of [^{123}I]β-CIT binding in putamen and caudate nucleus is in agreement with results from autopsy studies that revealed 80% decrease of dopamine concentration in the putamen and 40% decrease of dopamine level in the caudate nucleus (Kish et al., 1988; Hirsch et al., 1988) and confirms other in vivo findings of Innis et al. (1993). The fact that this differential loss can be demonstrated in vivo testifies to the power of this brain imaging method. Altogether, these results indicated that the [^{123}I]β-CIT SPECT procedure is indeed sensitive enough to discriminate Parkinson's disease patients in the early stage from those in the late stage of the disease.

In a recent study, the annual rate of decline in dopamine transporters in early stage Parkinson's disease was investigated by making two consecutive scans, 12 months apart, with [^{123}I]β-CIT and [^{123}I]FP-CIT as ligands (Booij et al., 1999). Forty six and twenty de novo patients were examined on two occasions with [^{123}I]β-CIT and [^{123}I]FP-CIT, respectively. The first SPECT-scan was made immediately after patients entered the study, whereas the second scan was made 12 months thereafter. The results showed a statistically significant decrease of 7 and 8% in binding ratios in the striatum for [^{123}I]β-CIT and [^{123}I]FP-CIT, respectively. This is a dramatic decrease in binding ratios as compared to healthy individuals in which the reported decrease amounts to less than 1% per year (van Dyck et al., 1995; Kazumata et al., 1998). Thus, using [^{123}I]β-CIT - or [^{123}I]FP-CIT SPECT, one might not only be able to monitor the rate of progression of the desease process in Parkinson's disease, but, more importantly, also to estimate in a quantitative manner the effect of neuroprotective agents on the disease process within a period of 1 year in a relatively small group of patients.

3.3. PET studies with [^{18}F]DOPA and other ligands

Besides SPECT investigations with dopamine transporter ligands, brain imaging studies of the dopaminergic deficit in Parkinson's disease in vivo have been performed using [^{18}F]DOPA positron emission tomography (PET) scanning (Brooks et al., 1990). The application of this PET-ligand is based on the assumption that [^{18}F]DOPA selectively accumulates in dopaminergic terminals in the striatal (sub)regions. However, in the early phase of Parkinson's disease, this technique reveals a less dramatic decrease in accumulation of [^{18}F]DOPA as compared to the decrease in transporter binding found with SPECT. For instance, in a series of 27 patients with early stage Parkinson's disease (Hoehn and Yahr staging scale 1.8), Morrish et al. (1995) found an average [^{18}F]DOPA accumulation of 62% of control in the putamen with PET, whereas Tissingh et al. (1998) demonstrated in a similar group of 21 patients (Hoehn and Yahr staging scale 1.8) 43% binding in the putamen by using [^{123}I]FP-CIT SPECT. Moreover, in a group of 33 early stage Parkinson's disease patients (Hoehn and Yahr staging scale 1.7), these same authors found less than 35% binding in the putamen when using [^{123}I]β-CIT SPECT (Tissingh et al., 1997). From these data, it might be concluded that dopamine transporter ligands are better compounds for the early diagnosis of Parkinson's disease

than fluoro-DOPA. This hypothesis has been underscored by a very elegant study performed recently by Itoh et al. (1999) in rats. In this study, an early to advanced stage model of Parkinson's disease was mimicked by injecting 1–10 μg of 6-hydroxydopamine into the substantia nigra. Using adjacent sections of the same animals, the binding of [^{125}I]β-CIT and the uptake of [^{14}C]L-DOPA were evaluated in the striatum 4 weeks after induction of the lesion. The results of this investigation showed a decrease in both [^{125}I]β-CIT binding and [^{14}C]L-DOPA uptake, in parallel with a decrease in dopaminergic neurons from early to advanced stage models, the decrease in L-DOPA uptake being always smaller than that of β-CIT. Thus, L-DOPA accumulation apparently underestimates the decrease in dopaminergic neurons, which indeed supports the notion that transporter ligands are better suited to establish an early diagnosis of Parkinson's disease.

In recent studies, dopamine transporter ligands have been used also for PET. For instance, the SPECT ligand FP-CIT can also be applied for PET studies, just by replacing the fluorine atom by a ^{18}F isotope. Studies with [^{18}F]FP-CIT demonstrated an age-related decline in dopamine transporter binding in normal subjects (approximately 7% per decade) as well as a significant reduction in patients with idiopathic Parkinson's disease, which correlated with disease severity (Kazumata et al., 1998). Similar results have been reported by using [^{11}C]dihydrotetrabenazine as a ligand for the monoaminergic storage vesicles (Frey et al., 1996).

3.4. Monitoring the degeneration rate of dopaminergic neurons in Parkinson's disease with [^{18}F]DOPA PET

Until now, only a few studies have investigated the rate of progression of the loss of striatal [^{18}F]DOPA metabolism in patients wit Parkinson's disease. In a group of 32 patients, Morrish et al. (1998) made two consecutive PET scans 18 months apart. The mean annual rate of deterioration in [^{18}F]DOPA accumulation varied according to striatal region, with the putamen showing the highest mean rate of progression, i.e., 9% of the initial value. This outcome compares well with a previous study from the same group (Morrish et al., 1996) in which a mean annual rate of reduction in [^{18}F]DOPA accumulation in the putamen of 12.5% per annum was obtained. Interestingly, these percentages are in close agreement with the annual decline reported in the aforementioned SPECT study with [^{123}I]β-CIT and [^{123}I]FP-CIT as ligands in early-stage Parkinson's disease (Booij et al., 1999), but are in sharp contrast to a study performed by Vingerhoets et al. (1994). These authors performed [^{18}F]DOPA PET scans on two occasions in a group of 16 patients with idiopathic Parkinson's disease 7 years apart and found an annual decline in [^{18}F]DOPA accumulation of 1.7% in patients vs. 0.3% in controls. However, from this study, it is not clear of course as to whether the percentage decline during the first year differs from that in later years, in other words, whether the progression proceeds in a linear or in an exponential way.

In conclusion, it has been clearly demonstrated that both PET and SPECT are valuable methods for the early diagnosis of Parkinson's disease. Additionally, although yet to be established definitely, both brain imaging techniques appear to offer the possibility to estimate both the rate of degeneration of the dopaminergic system in Parkinson's disease and to assess the effectiveness of (putative) neuroprotective agents.

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