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COMMENTARY

Drug Treatment of Parkinson's Disease

TIME FOR PHASE II

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ABSTRACT. Parkinson's disease (PD) is a neurodegenerative syndrome for which at present no cure is available; therapy consists mainly of amelioration of the symptoms with L-Dopa and/or dopamine (DA) agonists. Development of an effective causal therapy should be focussed on preventing or at least retarding the neurodegenerative process underlying the disease. At the cellular level, PD is characterized by degeneration of neuromelanin-containing dopaminergic neurons in the substantia nigra. Neuromelanin formation is the outcome of a process generally known as DA autooxidation, a chain of oxidation reactions in which highly neurotoxic DA-quinones are produced. The level of these DA-quinones, as estimated by the occurrence of their cysteinyl conjugates, is reported to be increased in the Parkinsonian substantia nigra. Hence, stimulation of pathways implicated in the detoxication of DA-quinones in the brain may provide neuroprotection in PD. Besides their inactivation through non-enzymatic antioxidants such as ascorbic acid and glutathione, DA-quinones are efficiently inactivated enzymatically by NAD(P)H:quinone oxidoreductase (NQO) and glutathione transferase(s), both of which are expressed in the human substantia nigra. The activity of these enzymes, which belong to the group of phase II biotransformation enzymes, can be up-regulated by a large variety of compounds. These compounds, including dithiolethiones, phenolic anti-oxidants, and isothiocyanates, have been shown to be active both *in vitro* and *in vivo*. Thus, considering the role of phase II biotransformation enzymes, in particular NQO and glutathione transferase(s), in the detoxication of DA-quinones, we propose that phase II enzyme inducers warrant evaluation on their neuroprotective potential in PD. BIOCHEM PHARMACOL **59**;9:1023–1031, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. Parkinson's disease; neuroprotection; quinone; Phase II biotransformation; quinone reductase; glutathione *S*-transferase

PD§ is a progressive neurodegenerative disorder characterized mainly at the cellular level by degeneration of DA-containing neurons located in a brain area known as the substantia nigra. PD affects approximately 0.5% of the population over the age of 50 years, thereby, considering the still growing number of the elderly, forming an increasing economic burden for society. Thus far, mere amelioration of PD symptoms with "classical" dopaminomimetics, in the form of the DA precursor L-Dopa and/or DA D2 receptor agonists, has been the mainstay of pharmacotherapy for this disease [1]. However, treatment with these dopaminomimetics does not mitigate progression of the disease process underlying PD, a factor that is thought to be causatively involved in the declining efficacy and occurrence of disabling side-effects upon continued pharmacotherapy with such compounds [2, 3]. Also, for later additions to the range of pharmacotherapeutics in PD, in particular glutamate receptor antagonists and the newest generation of DA agonists, despite claims based on the outcome of animal experiments, a neuroprotective effect in humans awaits confirmation [3, 4]. Thus, to optimally manage PD, there is (still) a strong requirement for drugs capable of retarding or, ideally, halting the ongoing degeneration of dopaminergic neurons in the Parkinsonian substantia nigra. This realization has led to intensive investigation of the neuroprotective potential of various agents, such as antioxidants and neurotrophic factors. Notwithstanding the reported successes with a number of such compounds in an experimental setting [3, 5–7], thus far none of these have been introduced for clinical use in PD. Here we advocate that pharmacological stimulation of the activity of certain so-called phase II biotransformation enzymes is worth considering as an alternative and feasible option for the development of neuroprotectants in PD.

OXIDATIVE STRESS AND DOPAMINERGIC CELL DEATH IN PD

Although a number of different mechanisms have been proposed, as yet the pathogenesis of PD remains largely enigmatic. Nevertheless, over the last two decades a good case has been made for a major role of oxidative stress in

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§ *Abbreviations:* DA, dopamine; GSH, glutathione; MAO, monoamine oxidase; NF-κB, nuclear factor-κB; NQO, NAD(P)H:quinone oxidoreductase; PD, Parkinson's disease; ROS, reactive oxygen species; and SOD, superoxide dismutase.

the neuronal degeneration occurring in the Parkinsonian substantia nigra. As elaborated upon by others, post-mortem analysis has revealed, for example, an early and profound loss of the antioxidant GSH, increased lipid peroxidation, increased SOD activity, and elevated free iron levels in the substantia nigra of Parkinsonian patients [7, 8]. Based on these findings, together with the apparent restriction of cell death to the DA-containing neurons in the substantia nigra and the ease with which DA was known to undergo oxidation, it was postulated that the preferential degeneration of nigral dopaminergic neurons in PD might be attributed to ROS released as by-products during "normal" oxidative DA metabolism [9]. In fact, in contrast to other metabolic pathways involving enzymes such as catechol-*O*-methyltransferase and phenolsulfotransferase, MAO-catalyzed breakdown of DA leads directly to the formation of highly neurotoxic hydrogen peroxide (Fig. 1). Consequently, reduction of the oxidant burden via pharmacological blockade of MAO activity was expected to offer neuroprotection in PD. Eventually, this line of reasoning led to introduction of the MAO inhibitor deprenyl for clinical use in PD [10]. Unfortunately, though, the potential to inhibit progression of the disease process in humans (solely) via blockade of MAO appears to be rather questionable [1, 7]. This lack of success with MAO inhibition alone might be explained by the fact that DA can form reactive metabolites also through a second oxidative pathway that has been demonstrated to be detrimental to neuronal survival, i.e. the process generally known as DA autooxidation [11, 12].

DA AUTOOXIDATION AND NEUROMELANIN IN THE HUMAN SUBSTANTIA NIGRA

Although debated for some time, it now is widely accepted that, in addition to MAO-catalyzed enzymatic oxidation, in the brain DA also undergoes oxidative catabolism in a process designated as DA autooxidation (Fig. 1). Contrary to what the term "autooxidation" suggests, DA autooxidation *in vivo* is likely to be facilitated by the presence of free metal ions (e.g. Fe^{2+}) and/or a number of enzymes, of which tyrosinase and prostaglandin H synthase are probably the best known examples [12–14]. Since the levels of both free iron and prostaglandin H synthase are increased in the Parkinsonian substantia nigra [7, 8, 15], it seems valid to assume that the rate of DA autooxidation may actually be enhanced in PD. Autooxidation of catecholamines including DA has been studied primarily in cell-free *in vitro* systems and has been shown to be a highly complex process involving the formation of a large number of intermediates that are often extremely unstable and therefore difficult to characterize [16, 17]. In contrast, only relatively little has been established as to the actual occurrence and role of the various intermediates *in vivo*. Irrespective of this uncertainty, however, in light of the data available in the literature, DA autooxidation in the human brain may be considered as a chain of reactions in which DA is oxidized first into a product named DA-quinone, which cyclizes readily to form an indolic compound described under various names, of which aminochrome or dopaminochrome appear to be the ones used most frequently [17, 18]. Subsequently, via a number of intermediate steps this cyclic DA-quinone is oxidatively polymerized, leading to the formation of neuromelanin, the pigment that in adult humans lends the substantia nigra its characteristic dark brown to black color (Fig. 1) [17–19]. Nevertheless, it should be realized that the pathway for generation of the neuromelanin polymer *in vivo* is likely to be far more complicated than has been described, since other factors, such as metal ions and sulfhydryl agents, may be involved as well [20, 21]. Curiously, DA autooxidation does not appear to occur at the same rate in all dopaminergic neurons, since the amount of neuromelanin present intracellularly differs markedly between DA-containing cells in the substantia nigra. In this context, it is of interest to note that neuropathological studies have revealed that the loss of dopaminergic neurons in PD is not distributed equally over the substantia nigra, but occurs primarily in those cells heavily laden with neuromelanin granules [22]. Recently these data, suggesting a positive correlation between the extent of melanization and neuronal death in PD, 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 PD [23]. Hence, it is tempting to assume that the sequence of events underlying neuromelanin formation in the substantia nigra, i.e. DA autooxidation, may be closely associated to the pathogenesis of PD.

DA AUTOOXIDATION AS A SOURCE OF OXIDATIVE STRESS IN PD

Whereas the functional significance of neuromelanin *per se* and its exact relation to PD pathogenesis is still open to question [19], there is now strong evidence indicating that the quinones formed during the process of DA autooxidation are likely to constitute a major source of oxidative stress as well as neurotoxicity in the substantia nigra [18]. As discussed in detail elsewhere, like other quinones of catecholaminergic origin, DA-derived quinones in general are highly reactive, electron-deficient species that may readily bind covalently to cellular nucleophiles such as DNA and reduced sulphydryl groups contained in protein cysteinyl residues and the thiol antioxidant GSH [12, 16, 24]. In this context, it is interesting to note that the level of cysteinyl conjugates of DA has been reported to be increased in the substantia nigra of Parkinsonian patients [25]. Besides toxicity due to inactivation of essential cellular constituents, the described nucleophilic attack by quinones is hazardous to cellular survival, since it ultimately may lead to lowering of GSH levels and consequent reduction of cellular antioxidant capacity (see also below). Moreover, in its turn the glutathionyl-DA adduct formed as the outcome of the interaction between GSH and DA-

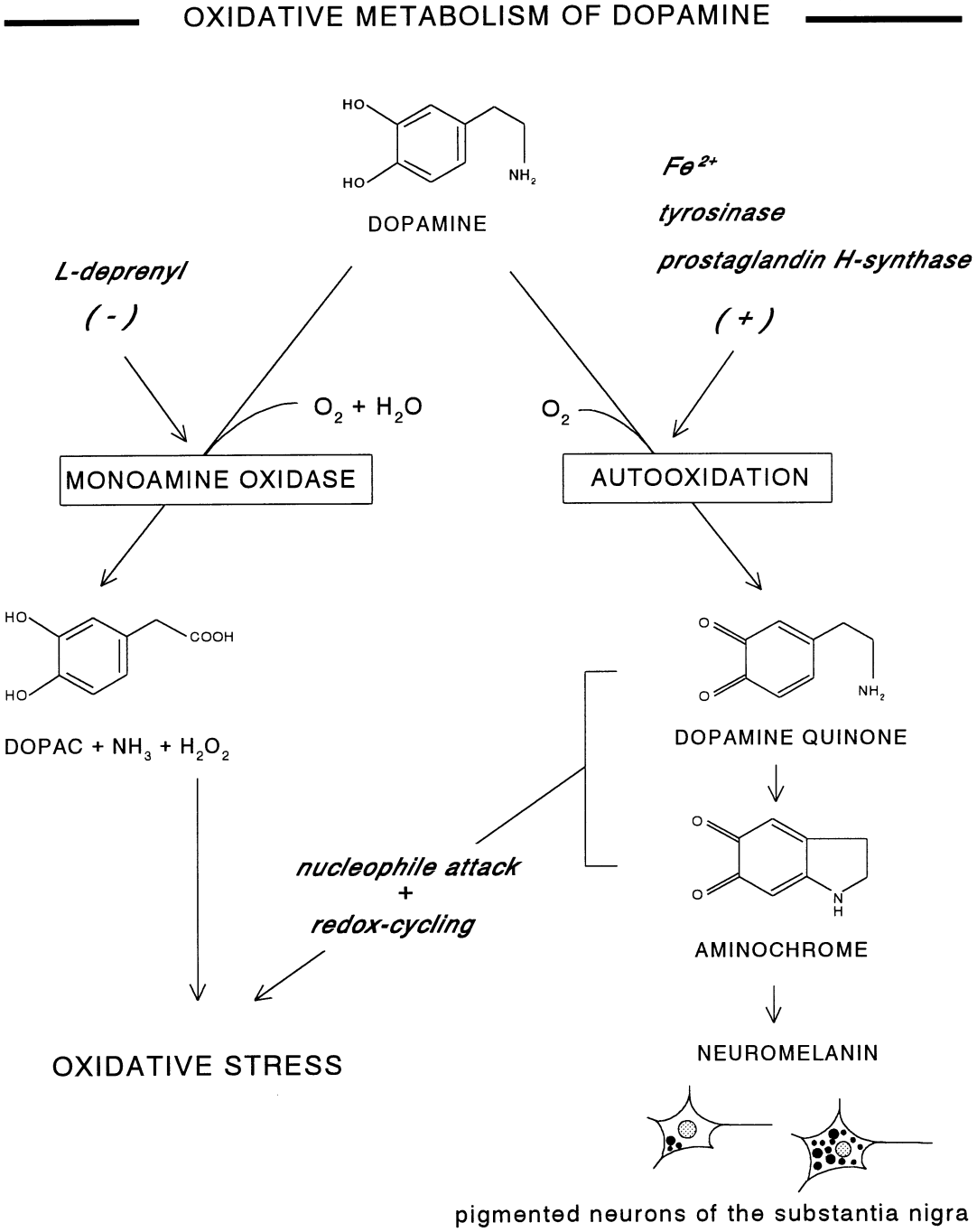

FIG. 1. Oxidative metabolism of DA in the brain. The oxidation of DA by monoamine oxidase results in the formation of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), ammonia, and hydrogen peroxide. In the process of DA autooxidation, which may be stimulated by free metal ions such as Fe^{2+} and/or enzymes such as tyrosinase and prostaglandin H synthase, DA first is oxidized to DA-quinone, which, in turn, cyclizes to form aminochrome. Following this, aminochrome will polymerize, thereby contributing to the synthesis of neuromelanin, the pigment that lends the human substantia nigra its characteristic dark color. Both pathways of oxidative DA metabolism induce oxidative stress either directly, via the release of hydrogen peroxide, or indirectly, via the action of DA-derived quinones (see also Fig. 2).

quinone has been shown to be prone to oxidation into a highly neurotoxic benzothiazine derivative [26].

More important for the mechanism of progressive neurodegeneration as observed in the Parkinsonian substantia nigra may be the propensity of DA-derived quinones for so-called redox cycling [11, 16, 18]. Although this redox-cycling may occur spontaneously, under the conditions likely to prevail in the cell it is assumed to be facilitated by the action of certain enzymes. Thus, at the expense of reducing equivalents, mainly in the form of NADPH, and catalyzed by ubiquitous cellular quinone-reductases, notably NADPH-cytochrome P450 reductase, the one-electron

reduction of the DA autooxidation product aminochrome reportedly results in the formation of a semi-quinone, i.e. a highly reactive and unstable DA intermediate that not only binds to cellular nucleophiles but also easily re-oxidizes again into aminochrome with the concomitant production of superoxide radicals [27, 28]. In this way, reduction of quinone precursors of neuromelanin is likely to elicit a cascade of reversible oxidation and reduction reactions, a redox-cycle, that is accompanied by excessive release of ROS and depletion of NADPH (Fig. 2). In their turn, the superoxide radicals released in this process may dismutate, either spontaneously or stimulated by SOD, into hydrogen peroxide, thereby facilitating the highly detrimental autooxidation of DA-semiquinone (Fig. 2) [29]. With respect to oxidative stress and PD, the NADPH deficiency resulting from the redox-cycling of DA-derived quinones is noteworthy in that it leads to a more general reduction of cellular protective antioxidant capacity. Thus, whereas glutathione disulfide reductase is dependent on NADPH to maintain GSH in its reduced state, reduced GSH is required for the catalytic activity of GSH-peroxidase, an enzyme that counteracts oxidative damage by preventing the accumulation of both hydrogen peroxide and lipid hydroperoxides [30]. Loss of NADPH, therefore, will be accompanied by a progressive lowering of reduced GSH levels, leading to inappropriate scavenging of toxic peroxides and resulting in oxidative damage. In addition, for instance via the iron (Fe^{2+})-catalyzed Haber–Weiss reaction, the superoxide radicals and hydrogen peroxide released during quinone redox-cycling are able to yield the extremely toxic hydroxyl radical, thereby initiating a variety of detrimental events culminating in dopaminergic cell death (Fig. 2) [27]. These include not only lipid and protein oxidation, oxidative damage to nucleic acids, and deprivation of cellular energy production, but also aberrant redox-sensitive gene transcription (e.g. via NF-κB) and apoptosis, phenomena that all have been observed to occur in the Parkinsonian brain [8]. Taken together, the process of DA autooxidation is likely to contribute greatly to the oxidative stress and consequent neuronal damage occurring in PD. Given the biochemical composition of brain neuromelanin and the presence of both the DA-quinone derivative 5-*S*-cysteinyl DA and NADPH-cytochrome P450 reductase in human brain tissue, it is now evident that this quinone-driven pro-oxidative pathway is indeed operative *in vivo* [18, 19, 31].

DETOXICATION OF DA AUTOOXIDATION PRODUCTS BY PHASE II BIOTRANSFORMATION ENZYMES

Alongside the aforementioned prooxidant pathway, a variety of anti-oxidant mechanisms have been implicated in the detoxication of DA-derived quinones *in vivo*. As outlined recently in this journal by Rescigno and coworkers [11], quinone reduction, leading to formation of their corresponding diphenols and/or substituted diphenols, provides the first and crucial step in the effective scavenging of these highly reactive species. This line of defense may involve a direct, non-enzymatic interaction of DA-quinones with low molecular weight reductants, in particular ascorbic acid or reduced GSH, but is likely to be controlled in a more efficient manner by the action of at least two enzymes, both belonging to the group of proteins collectively known as phase II biotransformation enzymes. In contrast to so-called phase I biotransformation enzymes such as the cytochrome P450 family of proteins, which generally are known to increase the reactivity of their substrates and to generate ROS [32], phase II biotransformation enzymes have been shown to protect against xenobiotics and endogenous toxins by catalyzing the transformation of reactive electrophiles such as DA-quinones into more stable, hydrophilic conjugates that are prone to be excreted from the cell [33, 34]. Originally, the enzymatic detoxication of DA-derived quinones was thought to rely on the phase II enzyme NQO (EC 1.6.99.2) [11, 16]. NQO, also referred to as DT-diaphorase, is a flavoenzyme that, in contrast to NADPH-cytochrome P450 reductase, reportedly catalyzes a two-electron reduction of the DA autooxidation product aminochrome, yielding a DA-hydroquinone, thereby averting formation of the extremely toxic free DA-semiquinone (Fig. 2) [35, 36]. Moreover, on the condition that cellular antioxidant capacity, especially the activity of the superoxide-scavenging enzyme SOD, is sufficient to prevent its autooxidation, DA-hydroquinone appears to be a redox-stable entity that lacks major 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 other phase II enzymes such as UDP glucuronosyltransferase(s) or sulfotransferase(s) (Fig. 2) [27, 36–38]. In the context of hydroquinone stability, it is of note that SOD expression in the human substantia nigra has been shown to be highest in melanized neurons [39]. Recently, apart from NQO, human Mu class GSH transferases (EC 2.5.1.18) have been identified as an additional protective mechanism against the toxicity of DA-quinones. More specifically, in particular the Mu 2–2 subtype was found to catalyze the reductive conjugation of GSH to aminochrome. In this reaction, 4-*S*-glutathionyl-5,6-dihydroxyindole is formed, a hydrophilic entity that is resistant to redox cycling and amenable to further detoxication either by other phase II biotransformation enzymes or via direct cellular excretion (Fig. 2) [40–42]. Taken together, by competing with one-electron transferring quinone reductases such as NADPH-cytochrome P450 reductase in the reduction of DA-derived quinones, the combined action of phase II biotransformation enzymes, most notably NQO and GSH transferase, has the potential to provide a powerful cellular defense mechanism against the oxidative stress inherent to DA autooxidation. It is noteworthy, therefore, that recent investigations in our laboratory demonstrated NQO immunoreactivity in both melanized neurons and glial cells in the human substantia nigra (Fig. 3), thereby extending earlier

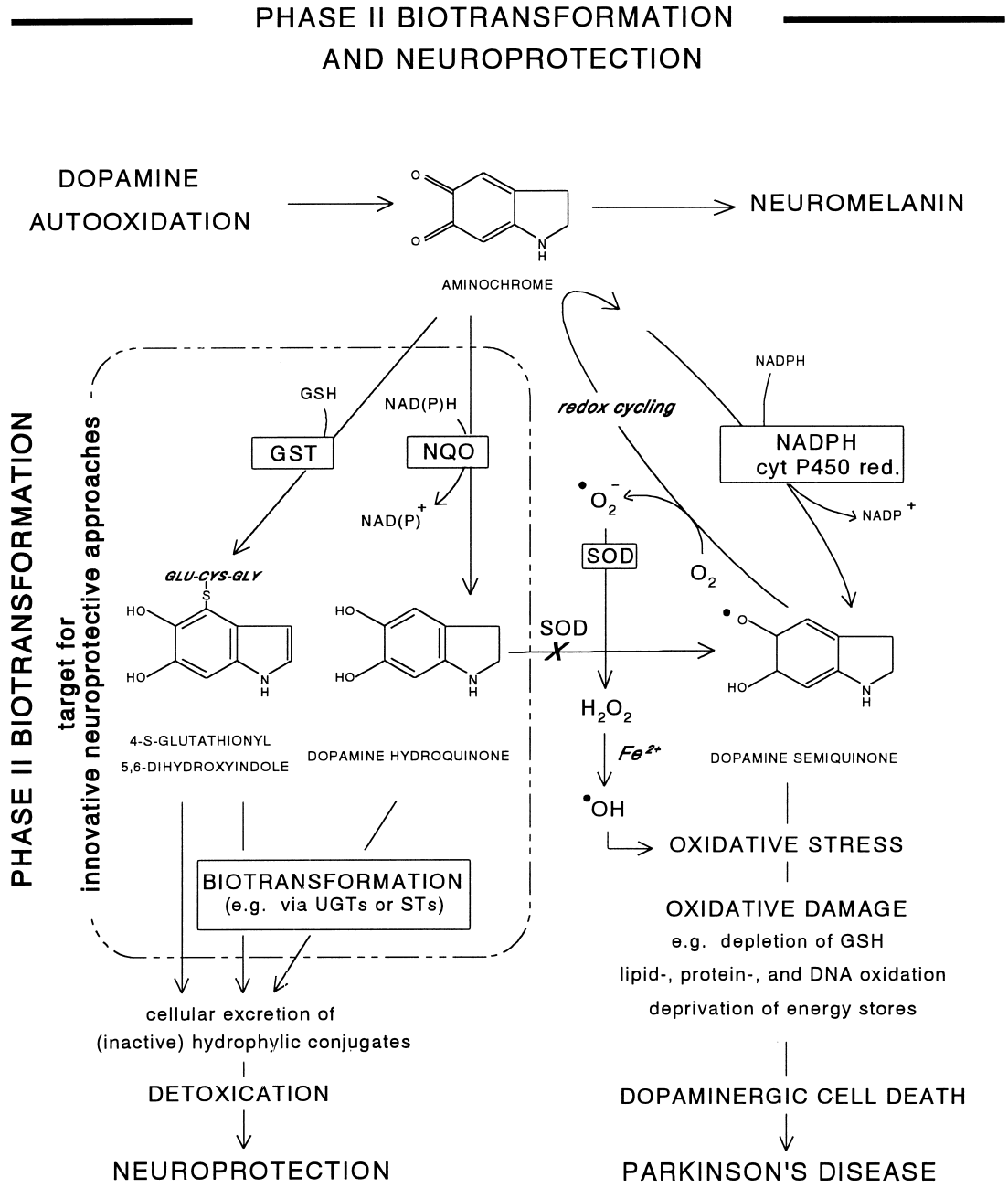

FIG. 2. Phase II biotransformation as a protective device against DA-quinone-induced neurotoxicity. The one-electron reduction of the DA-derived quinone aminochrome, as catalyzed by, for example, NADPH-cytochrome P450 reductase, elicits redox-cycling between the parent compound and its reduced semiquinone form, a process that is accompanied by the massive release of superoxide radicals. The detrimental cascade of events following the one-electron reduction of aminochrome is facilitated by the action of superoxide dismutase, which, by clearing superoxide radicals, shifts the balance in favour of aminochrome redox-cycling. Ultimately, the oxidative stress generated in this way during redox cycling of aminochrome may cause dopaminergic cell death in the substantia nigra. Redox-cycling of aminochrome may be prevented, however, by the concerted action of a number of phase II biotransformation enzymes. NQO and glutathione transferase catalyze the two-electron reduction of aminochrome or the reductive conjugation of aminochrome to reduced GSH, respectively, yielding products that are amenable to further detoxication. In contrast to its adverse effect on the stability of DA-semiquinone, by counteracting the autooxidation of DA-hydroquinone into DA-semiquinone, SOD promotes the detoxication of aminochrome via NQO. Abbreviations: GSH, glutathione; GST, glutathione transferase; NADPH cyt P450 red., NADPH-cytochrome P450 reductase; NQO, NAD(P)H:quinone oxidoreductase; SOD, superoxide dismutase; ST, sulfotransferase; and UGT, UDP glucuronosyltransferase.

results obtained by others using rat brain sections [43, 44]. Moreover, abundant expression of Mu class GSH transferases, including the Mu 2–2 form, also has been reported in the human substantia nigra [40]. At this point it is important to realize, however, that although the outcome of a small number of genetic-linkage studies suggests a possible connection with

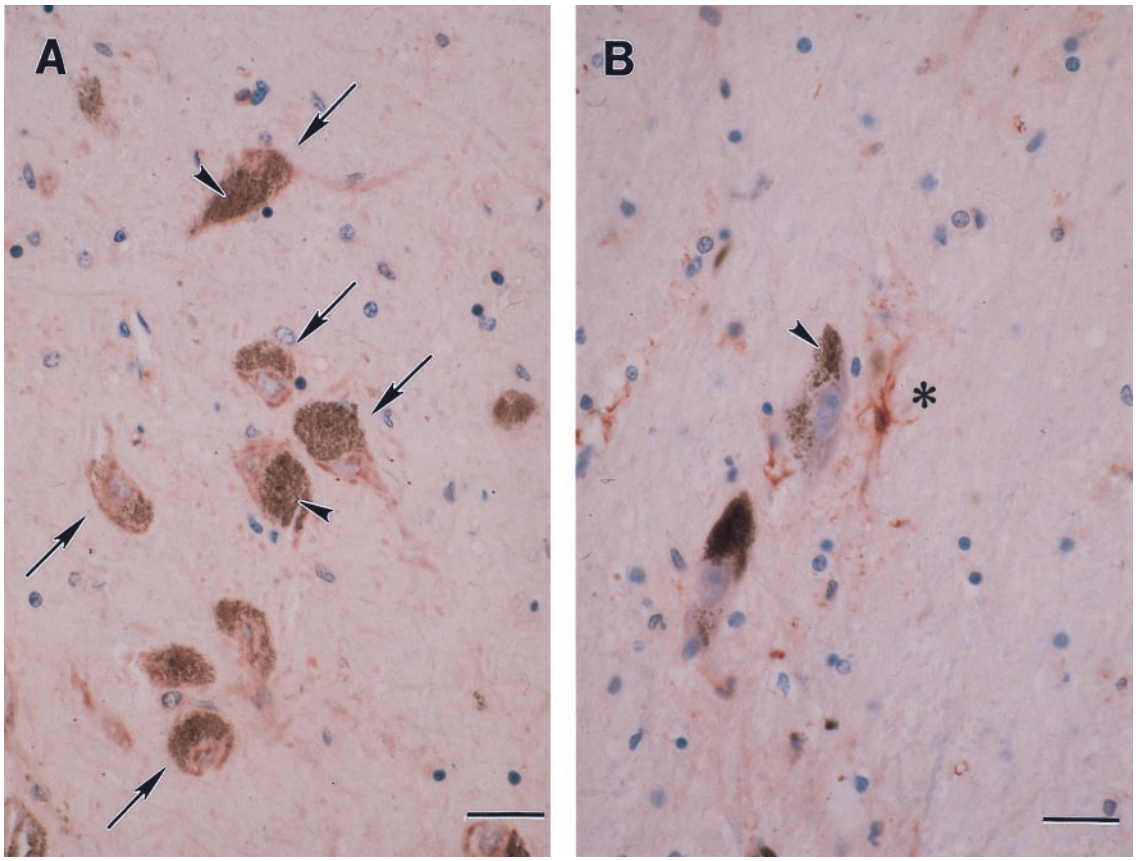

FIG. 3. Photomicrographs of paraffin sections of human substantia nigra showing NQO-immunoreactivity in neuromelanin containing neurons (panel A, arrows) and in glial cells (panel B, asterisk). Arrowheads indicate the granular neuromelanin pigment. Following antigen retrieval in 10 mM citric acid, immunoreactivity for NQO was demonstrated using a combination of two monoclonal antibodies raised against purified recombinant human NQO protein (courtesy of Dr. D. Siegel; University of Colorado Health Sciences Center, Denver, CO, U.S.A.; for details, see Siegel *et al.*, *Clin Cancer Res* 4: 2065–2070, 1998), biotinylated goat anti-mouse secondary antibodies, avidin–biotin–peroxidase complexes, and the red-colored AEC/H_2O_2 as chromogen. Sections were counterstained with hematoxylin. Scale bar = 30 μm.

PD [45, 46], to our knowledge, thus far no data have been produced showing impaired functioning of these phase II enzymes in the Parkinsonian brain [47].

PHASE II BIOTRANSFORMATION AS A TARGET FOR NEUROPROTECTION IN PD

Given also their expression in relevant brain structures, the pivotal role of certain phase II biotransformation enzymes in the inactivation of DA-derived cytotoxic quinones makes these antioxidant proteins attractive targets for the development of innovative neuroprotective agents to treat PD. In contrast to other fields of biomedicine, however [34, 48], until now the therapeutic potential of manipulation of phase II enzyme activity has not been explored actively in the neurosciences. For the purpose of neuroprotection in PD, drugs capable of stimulating the activity of both NQO and GSH transferase(s) would be expected to be of special therapeutic interest. Interestingly, efforts to establish pharmacological means of cancer chemoprevention have proven that, besides their capability to neutralize a broad spectrum of electrophilic substances, NQO and GSH transferase(s) share their inducibility by these toxicants and also by a large array of other, structurally diverse chemicals of both natural and synthetic origin [34, 49–51]. In fact, some of these compounds, including phenolic antioxidants (e.g. butylated hydroxyanisole, tea polyphenols, and phytoestrogens) [34, 50, 52], aromatic isothiocyanates (e.g. sulforaphane) [53], and nonsteroidal anti-inflammatory drugs (e.g. indomethacin and ibuprofen) [54], have been suggested to confer cellular protection via a coordinated up-regulation of the expression of phase II enzymes, in particular NQO and GSH transferase. This effect is most likely mediated by activation of a so-called antioxidant response element present in the promoter regions of the respective genes [50, 55]. Recently, redox-mediated stimulation of this antioxidant response element has been shown to be instrumental also in brain cells for the simultaneous induction of NQO and GSH transferase activity [56].

In the context of phase II enzyme induction, a particularly interesting and well studied class of compounds is the dithiolethiones, a cyclic, sulfur-containing group of agents,

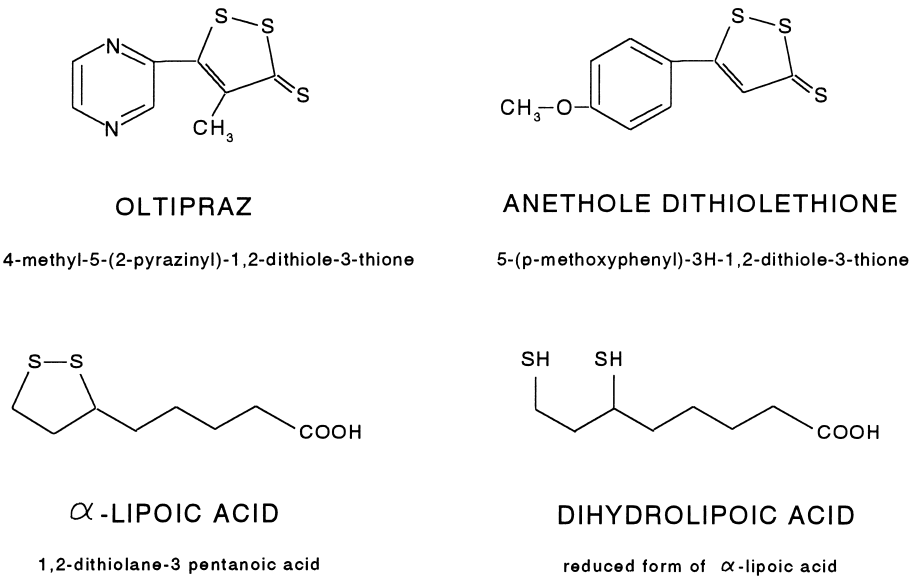

FIG. 4. Chemical structures of the dithiolethiones oltipraz and anethole dithiolethione and of the oxidized and reduced forms of α-lipoic acid.

originally described as constituents of cruciferous vegetables [57]. The dithiolethiones, of which oltipraz and anethole dithiolethione are currently available for use in humans (Fig. 4) [58, 59], not only increase the activity of phase II biotransformation enzymes in various cellular preparations *in vitro* [60–62], but are active also *in vivo* in animals and humans with only minor side-effects reported [57–59]. Concomitant with their stimulatory effect on phase II biotransformation, dithiolethiones are especially attractive in that they also boost general cellular antioxidant capacity by acting as regular oxidant scavengers [58, 63], by up-regulating the activity of ROS-scavenging enzymes such as SOD [64], by modulating redox-sensitive gene transcription (e.g. inhibition of peroxide-mediated activation of NF-κB) [65], by increasing the expression of metal-binding proteins such as ferritin [66], and by stimulation of those enzymes responsible for the maintenance of reduced GSH pools, in particular γ-glutamyl cysteine synthetase, glutathione disulfide-reductase, and glucose-6-phosphate dehydrogenase [57, 63, 67, 68]. Indeed, such dual characteristics have been shown recently to contribute considerably to the capacity of phase II enzyme inducers to protect against DA neurotoxicity, at least *in vitro* [69]. Interestingly, dithiolethiones show a distinct structural similarity with α-lipoic acid (Fig. 4), a natural thiol antioxidant that has attracted considerable clinical interest for its potential use as a neuroprotectant in the treatment of neurodegenerative disorders, including PD [70]. Besides similar structural features such as vicinal thiol groups, dithiolethiones and α-lipoic acid also appear to exhibit similar effects on the cellular response to oxidative stress [71, 72]. Thus, these compounds are anticipated to have a common intracellular mechanism of action. Upon entering the cell, the disulfide bond in α-lipoic acid is reduced to form dihydrolipoic acid (Fig. 4) [70, 71]. In contrast to α-lipoic acid itself, dihydrolipoate has been found recently to prevent the binding of quinones released during DA oxidation to the reduced sulphydryl groups present in cysteine or GSH [25]. This, albeit indirectly, suggests that intracellular reduction may be important also for dithiolethiones to be effective in protecting neurons against DA toxicity. However, although the highly lipophilic dithiolethiones are taken up intracellularly [73], their subsequent metabolic fate and the biological activity of the metabolites produced await further characterization.

CONCLUDING REMARKS

Based upon the evidence presented in the current commentary, we conclude that improved understanding of the pro- and antioxidant pathways operative in autooxidative DA breakdown may be instrumental to open up new avenues for the development of alternative and effective neuroprotectants for clinical use in PD. Although a wealth of data is available about the products formed in this process and the molecular mechanisms responsible for their neurotoxicity, less is known concerning the mechanisms involved in the detoxication of these products in the brain. Nevertheless, in our opinion there is now sufficient evidence to underscore an important role for phase II biotransformation enzymes, in particular NQO and GSH transferases, therein. Therefore, from the clinical point of view, it will be a great challenge to test whether drugs identified primarily by their capability to stimulate the activity of these phase II biotransformation enzymes in the brain will indeed confer neuroprotection in PD. As a matter of fact, given the availability of compounds such as the dithiolethiones, which in addition to phase II enzyme induction exhibit a broad spectrum antioxidant capacity, and considering the recent advances in brain imaging techniques, which allow monitoring of putative neuroprotective effects in (pre-)symptomatic PD patients [74], nothing seems to stand in the way of the initiation of pioneering studies investigating

this issue both in experimental PD (models) and in patients affected by the disease.

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