The effects of nitric oxide (NO) on both tyrosinase/O2-and horseradish peroxidase/H2O2-mediated oxidations of dopamine and its o-dihydric phenol precursor L-dopa were compared with autooxidative processes and quantitatively assessed by oxidative and reductive electrochemical detection systems. In peroxidase/H2O2/NO-catalyzed reactions, significantly more substrate was oxidized than in the corresponding control incubations lacking NO. In tyrosinase/O2/NO-promoted reactions the total amounts of l-dopa and dopamine oxidized were significantly less than the amounts of the substrates oxidized by enzyme alone. These data indicate that the activity of the heme protein peroxidase was enhanced by NO, whereas tyrosinase, a copper-containing monoxigenase, was inhibited. The NO-mediated reduction of tyrosinase/O2 activity may be attributed to the formation of an inhibitory copper-nitrosyl complex. An oxidized nitrodopamine derivative, considered to be either the quinone or semiquinone of 6-hydroxynitrosodopamine, was generated in peroxidase/H2O2/NO-mediated reactions with dopamine along with two oxidized melanin precursors, dopamine quinone and dopaminechrome. No corresponding nitroso compound was formed in reactions involving l-dopa or in any of the tyrosinase-mediated reactions. The formation of such a noncycled nitrosodopamine represents an important alternative pathway in catecholamine metabolism, one that by-passes the formation of cytoprotective indole precursors of melanin. The results of this investigation suggest that cellular integrity and function can be adversely affected by NO-promoted oxidations of dopamine and other catechols, reactions that not only accelerate their conversion to reactive quinones but also form potentially cytotoxic noncycled nitroso derivatives. Reduced levels of dopamine in the brain through NO-enhanced oxidation of the catecholamine will almost certainly be manifested by diminished levels of the dopamine-derived brain pigment neuromelanin.

The selective degeneration of melanized nigrostriatal dopaminergic neurons and attendant functional impairments of associated neuronal pathways are primary neuropathological manifestations of Parkinson’s disease (PD).1 Neuronal degeneration in PD is accompanied by the progressive depletion of the neurotransmitter dopamine, as well as the pigment derived from its autoxidation, neuromelanin (1–3). Several mechanisms have been proposed to account for the selective vulnerability of the pigmented nigrostriatal dopaminergic neurons in PD, but the pathogenesis of this disease remains largely enigmatic. The neuropathology associated with PD has been attributed to oxidative stress resulting from the toxic effects of certain reactive intermediates of oxygen (ROI) (4), most notably the hydroxyl radical (·OH), a highly reactive molecule generated by the interaction of hydrogen peroxide (H2O2) with superoxide anion (O2·−), transition metals (Fe2+ or Cu2+), or nitric oxide (NO) (5, 6).

The preferential targeting and destruction of nigrostriatal dopaminergic neurons in PD implicates as cytotoxic molecules substances specifically generated as by-products of dopamine metabolism in the brain. Dopamine autoxidation produces dopamine quinone (DAQ), an electrophilic molecule that rapidly cyclizes to form dopaminechrome (DAC). Subsequent reactions generate inoioles that polymerize to form a brown-black pigment termed eumelanin. The autoxidation of l-dopa, the carboxylated precursor of dopamine, is similar and results in the formation of dopaquinone (DOQ) and dopachrome (DOC) en route to forming eumelanin (Fig. 1). If cysteine or glutathione are present during the oxidation of the dopamine or l-dopa, the quinones derived from these catechols react with the thiols to form compounds that ultimately polymerize to yield a reddish-yellow pigment termed pheomelanin. Neuromelanin appears to be a heteropolymer derived in part from dopamine and comprises pheomelanin and eumelanin (1, 7).

If the oxidation of brain dopamine represents a source of potentially neurotoxic molecules, endogenous or exogenous factors that influence the rate of oxidation of the brain dopamine, or that alter the biochemistry and reactivity of its metabolites, will likely play a critical role in neuronal homeostasis. Frequently associated with ROI-mediated cytotoxic processes are reactive intermediates of nitrogen (RNI) derived from nitric oxide (NO) through the action of nitric oxide synthase. Nitric oxide is produced in virtually all cell types, including neurons, endothelial cells, macrophages, and malignant melanocytes. Despite a widely acknowledged beneficial role of NO in numerous and diverse physiological processes, this diffusible free radical has been shown to react with O2 and ROI to spawn an array of reactive molecules that can aggressively target biomolecules. Included in the arsenal of potentially cytotoxic RNI are nitrogen dioxide (NO2), nitrous anhydride (N2O3), nitrite neochrome; DOC, dopachrome; DOQ, dopaquinone; RNI, reactive intermediates of nitrogen; NO, nitric oxide; GSH, reduced glutathione; DFE, diethylamine-NO-generating complex; HPLC-ED, high performance liquid chromatography with electrochemical detection; 6NDAQ, 6-nitroso dopamine quinone; 6NDASQ, nitrosodopamine semiquinone; 60HDA, trihydroxyphenyl quinone.

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‡ The abbreviations used are: PD, Parkinson’s disease; ROI, reactive intermediates of oxygen; DAQ, dopamine quinone; DAC, dopami-
Effects of NO on the Oxidations of L-dopa and Dopamine

11215

Experimental Procedures

Chemicals—The following chemicals were obtained from Sigma Chemical Co. (St. Louis, MO): l-dopa (1,3,4-dihydroxyphenylalanine), dopamine (3,4-dihydroxyphenethylamine) mushroom tyrosinase (E.C. 1.14.18.1, 3970 units/mg), horseradish peroxidase (E.C. 1.11.1.7, 175 purpuragolin units/mg), and reduced glutathione (GSH). Diethylamine-NO-generating complex (DEA) was obtained from Research Biomedicals International (Natick, MA). Chelex 100 was purchased from Bio-Rad Laboratories (Richmond, CA). Stock solutions of all components were prepared daily in ultrapure reagent-grade water obtained with a Milli-Q system (Millipore, Bedford, MA) and kept at 4 °C for a maximum period of 3 h and then discarded.

Reaction Mixtures and Enzyme Activity—Standard reaction mixtures for both enzymatic and nonenzymatic oxidations of L-dopa and dopamine were composed of 0.5 mM substrate in a total volume of 100 μl of bicarbonate buffer (100 mM NaCl/25 mM NaHCO3; pH 7.2). For enzyme-mediated oxidations, the reaction mixtures contained either 0.01 μg of tyrosinase (3970 units/mg) or 0.25 μg of peroxidase (175 purpuragolin units/mg) and 8 mM H2O2. Oxidations mediated by NO were initiated by the incorporation of 2.5–10 mM DEA into the reaction mixtures. Iron-mediated oxidations were initiated by incorporating into the reaction mixtures 0.3 mM FeCl3-EDTA complex. Following incubations at 22 °C for various specified intervals, 5-μl aliquots were removed from each reaction mixture and analyzed by high performance liquid chromatography with electrochemical detection (HPLC-ED). Any modifications in composition or concentrations of the standard reaction mixtures are specified where required. Comparative and quantitative measurements of enzyme-mediated oxidations were made using HPLC-ED to measure substrate oxidation in standard reaction mixtures with and without NO. Specific activity was expressed as picomoles of substrate depleted per minute/μg of protein under the standard conditions established for the study. Reduced glutathione (100 nmol) was added to reaction mixtures to note the effects of the reductant on the production of o-quinones.

Control incubations were conducted by excluding substrate, NO, enzyme, or iron, as was appropriate for each experiment. The possibility of OH production resulting from metal contaminants was excluded in experiments that compared rates of oxidation in test solutions with those observed in identical control mixtures that were either pretreated with the metal-chelating ion exchange resin, Chelex 100, or that contained the specific iron chelator desferrioxamine.

HPLC-ED Analyses of Catechol Oxidations—A sensitive and specific salicylate hydroxylation assay was used in conjunction with HPLC-ED to monitor the enzymatic and nonenzymatic oxidations of L-dopa and dopamine. The HPLC system consisted of a Bioanalytical Systems (West Lafayette, IN) LC-4B amperometric detector with a glassy carbon working electrode and an Ag/AgCl reference electrode. The working electrode was maintained at an oxidative potential of +650 mV to monitor the o-diphenols dopa and dopamine, and a reductive potential of −100 mV to detect their oxidized products, such as o-quinones, semiquinones, and eumelanochromes (e.g., dopachrome, dopamine). In both reductive and oxidative modes, instrument sensitivity was maintained at 50 nA. The solvent system was comprised of 25 mM citrate buffer (pH 3.0) containing 2.5% acetonitrile, 0.5 mM sodium octylsulfate, and 0.7 mM disodium EDTA. All separations were made with an Alltech Spherisorb ODS 5-μm reverse phase column using a flow rate of 1.0 ml/min. Known amounts of different catecholamines occasionally were incorporated into control reaction mixtures to serve as internal standards. The correlation coefficient of the calibration curves established for the standards typically was greater than 0.98.

Data Analyses—All experiments were replicated at least three times. Results are presented as the means ± S.D. (of four experiments) of the determinations specified. Differences between mean values were evaluated using the Student’s t test. The difference between two means was considered significant when p < 0.05.

RESULTS

Chromatographic Analyses—In initial experiments autoxidations and tyrosinase/O2-mediated oxidations were analyzed by electrochemical detection under reductive conditions (−100 mV) with l-dopa as the substrate and incubation periods ranging from 30 s to 42 min. In subsequent experiments, assay periods ranging from 2 to 12 min were used, under which conditions two oxidized metabolites of l-dopa were detected in tyrosinase/O2-mediated reactions, DOQ and DOC (Fig. 2). In reaction mixtures lacking enzyme, the initial product formed during the autoxidation of l-dopa was DOQ, which first appeared in 12-min incubations (Fig. 3). The autoxidation of dopa also generated DOC, but this metabolite was detected only in reaction mixtures incubated for periods longer than 20 min (not presented). NO-mediated oxidations of dopa also generated...
DOQ (Fig. 3). There was no evidence of DOC in any of the NO mixtures that were incubated for periods up to 42 min. However, both DOQ and DOC were generated by the iron-mediated oxidation of dopa (Fig. 4).

Two oxidized metabolites of dopamine, DAQ and DAC, were likewise generated during autoxidation and in reactions mediated by tyrosinase/O$_2$ (not presented) and iron (Fig. 4). The extent of production of the two quinoids of 1-dopa and dopamine in 2-min assays was dependent on substrate concentration (Fig. 5). The addition of GSH to the reaction mixtures diminished the levels of the two oxidized metabolites of dopamine, DAQ (Fig. 6) and DAC (not shown). In tyrosinase/O$_2$-mediated reactions the amount of DAQ was reduced by nearly 60% 6 min after the addition of 100 nmol of GSH and by 86% after 42-min incubation (Fig. 6). GSH had a similar reducing affect on the oxidized metabolites of dopa generated by autoxidation and tyrosinase/O$_2$- and iron-mediated oxidations (not presented). Interestingly, the production of the two oxidized metabolites of dopamine were completely inhibited when GSH was incorporated into reaction mixtures prior to the addition of enzyme. These observations are consistent with other experimental evidence showing that antioxidants such as GSH and ascorbate can effectively abrogate the NO-induced oxidation of dopamine (15) and that GSH and cysteine react readily with the $\alpha$-quinones of dopa and dopamine to form reduced glutathional and cysteinyl conjugates that can be detected with oxidative electrochemical methods (28).

Significantly ($p < 0.5$) more dopamine and 1-dopa were oxidized in reaction mixtures containing peroxidase/H$_2$O$_2$ and NO than were oxidized by peroxidase/H$_2$O$_2$ alone (Fig. 7). In 6-min incubations the percentages of 1-dopa oxidized separately by NO, peroxidase/H$_2$O$_2$, and peroxidase/H$_2$O$_2$/NO were 22.5, 32, and 77%, respectively. Completely different results were obtained with tyrosinase/O$_2$-mediated oxidations where signifi-
cantly ($p < 0.5$) less substrate was consumed in reaction mixtures containing NO (Fig. 7). A series of incubations was conducted to ascertain if the NO-mediated augmentation of peroxidase activity and the inhibition of tyrosinase activity were dependent on the concentration of NO. In 1-min incubations containing 2.5, 5, or 10 mM NO, the rates of oxidation of L-dopa by peroxidase/H$_2$O$_2$ were significantly elevated above controls (lacking NO; 0.15 nmol/min), averaging 0.19, 0.31, and 0.428 nmol/min, respectively (Fig. 8B). The augmented activity of peroxidase/H$_2$O$_2$ at each concentration tested was significantly ($p < 0.05$) higher than the additive effect of NO-mediated oxidation (Fig. 8A) plus the activity obtained by enzyme alone (Fig. 8B). With tyrosinase, the opposite was observed. At each concentration of NO tested (2.5, 5, or 10 mM), the activity of tyrosinase/O$_2$ was significantly lower than control incubations lacking NO (Fig. 8B).

The above experiments employed oxidative detection methods (HPLC-ED, +650 mV) to monitor catechol consumption. These tests were replicated with reductive detection methods (HPLC-ED, −100 mV) to document the quinoid metabolites generated during the oxidations of dopa and dopamine. Under these conditions the presence of elevated levels of quinones produced in incubations containing enzyme/NO mixtures would correlate with the amount of substrate oxidized. However, quinone production in both the tyrosinase/O$_2$/NO- and peroxidase/H$_2$O$_2$/NO-mediated reactions were significantly lower ($p < 0.05$) than in reaction control mixtures containing only enzyme (Fig. 9). Quinone levels generated in reaction mixtures containing substrate and NO, substrate alone (autoxidation), and substrate and H$_2$O$_2$ are provided for comparison (Fig. 9).

Under the specific reductive and oxidative conditions established for this investigation, no additional product was detected in any of the tyrosinase/O$_2$/NO-mediated reactions, or in the peroxidase/H$_2$O$_2$/NO-mediated oxidation of L-dopa. Of considerable interest, however, was the detection of an oxidized nitrosonodopamine molecule (P#1) generated during the peroxidase/H$_2$O$_2$/NO-mediated catalysis of dopamine (Fig. 10). The presence of this nitro derivative indicates that horseradish peroxidase/H$_2$O$_2$ can promote the nitrosation of dopamine. Under the conditions established for this investigation, the nucleophilic addition of NO or other RNI such as NO$_2$ to oxidized derivatives of dopamine would most likely generate a noncyclic molecule such as 6-nitrosodopamine quinone (6NDAQ), or nitrosodopamine semiquinone (6NDASQ) (Fig. 11). The possibility that the molecule identified only in the peroxidase/H$_2$O$_2$/NO-mediated catalysis of dopamine was derived by hydroxylation (i.e., a trihydroxyphenyl quinone, 6OHDA) instead of nitration was considered, but it was reasoned that such a
hydroxylation product also would have had to form in peroxidase/H₂O₂ reactions lacking NO. Therefore, the nitrosation of dopamine was considered to be the pathway most likely involved in the product (P#1) seen in Fig. 10.

DISCUSSION

This investigation documents the effects of NO on the autoxidation and tyrosinase/O₂- and peroxidase/H₂O₂-mediated oxidations of dopamine and its α-dihydric phenol precursor L-dopa. In the absence of NO, the quinoids of dopamine (DAQ, DAC) and L-dopa (DOQ, DOC) that formed in enzyme-mediated oxidations were reduced in a time-dependent manner by the addition of GSH. When GSH was incorporated into reaction mixtures prior to the addition of enzyme, no products were observed, confirming the oxidative nature of these molecules. In peroxidase/H₂O₂/NO-catalyzed reactions, the total amounts of dopa and dopamine oxidized were significantly higher than the additive amounts of the substrates oxidized by NO and enzyme separately. In tyrosinase/O₂/NO-catalyzed reactions, the total amount of α-diphenol substrate oxidized was less than the amount of substrate oxidized by enzyme alone. These data indicate that the activity of the heme protein peroxidase was enhanced by NO, whereas tyrosinase, a copper-containing monooxygenase, was inhibited.

**FIG. 7.** Representative chromatograms depicting for comparative purposes the diminished levels of L-dopa that were manifested when the catechol was oxidized by tyrosinase (TASE) or peroxidase (PASE). Chromatographic conditions were +650 mV, 50 nA, and a flow rate of 1 ml/min.

**FIG. 8.** A, the rate of oxidation of L-dopa in reaction mixtures containing varying concentrations (0 = buffer, 2.5, 5, or 10 mM) of NO. B, comparative analyses of the rates of dopa oxidation by tyrosinase/O₂ (TASE) and peroxidase/H₂O₂ (PASE) with and without NO. Only in the peroxidase/H₂O₂ + NO mixtures were the rates of L-dopa augmented. Data were obtained by HPLC-ED using the chromatographic conditions given in Fig. 7 to measure substrate consumption. Each 5-μl sample of reaction mixture analyzed by HPLC-ED initially contained 1 pmol of dopa, and either 0.025 μg of tyrosinase or 0.5 μg of peroxidase and 0.7 nmol of H₂O₂. S.D. are <1% of the mean values, which were derived from three replicate experiments.

**FIG. 9.** Data documenting lower levels of DAQ generated in reaction mixtures composed of both enzyme and NO than those formed in reaction mixtures containing enzyme alone. The amount of DAQ resulting from the autoxidation of dopamine, as well as from oxidations caused by NO and H₂O₂, are provided for comparison. Data were obtained by HPLC-ED using the chromatographic conditions given in Fig. 2 to measure the production of oxidized metabolites of dopamine. Each 5-μl sample of reaction mixture analyzed by HPLC-ED initially contained 1 pmol of dopamine, and either 0.025 μg of tyrosinase, or 0.5 μg of peroxidase and 0.7 nmol of H₂O₂. Reaction mixtures with NO contained 25 nmol of DEA. The amount of H₂O₂ in reaction mixtures lacking enzyme was 0.7 nmol. All data points represent the means of three replicate tests. S.D. are <4% of the mean value.
Because NO reacts at near diffusion-controlled rates with the iron center of heme-containing proteins (e.g. guanylyl cyclase, oxyhemoglobin, cytochrome p450) (29), this activity likely accounts for the enhanced peroxidase-mediated oxidation of L-dopa and dopamine we observed in this study with horseradish peroxidase. NO can bind reversibly with both ferric (Fe$^{3+}$) and ferrous (Fe$^{2+}$) states of iron (29, 30) and has been shown to form heme nitrosyl complexes with horseradish peroxidase (31). However, NO has been shown to have an inhibitory effect on such heme-containing enzymes as glutathione peroxidase (32, 33), glutathione reductase (34), lipoygenase (35), lactoperoxidase (36), and thyroid peroxidase (37). Studies with glutathione peroxidase suggest that NO and its derivatives directly inactivate the enzyme, resulting in an increase in intracellular peroxides that are responsible for cellular damage (33). Disparities among reports describing the ability of NO to augment or inhibit enzyme activity may result from differences in the concentration of NO, which appear to influence its association/dissociation rates with the iron redox center of some heme-containing enzymes (29, 30).

Whether NO reacts with metals at the active sites of enzymes depends on the affinity of NO for the metal and the relative concentrations of enzyme, O$_2$, and NO. When NO is present in much lower concentrations than O$_2$, the formation of nitrogen dioxide (NO$_2$) is initiated by the reversible reaction of NO with O$_2$ to form the nitrosyldioxyl radical (ONOO$^-$) (Reaction I). The subsequent rate-limiting step is the addition of a second nitric oxide to ONOO$^-$ forming a compound that rapidly decomposes by hemolytic cleavage of the O–O bond to form NO$_2$ (Reactions II–IV). Hydrogen bonding with water stabilizes ONOO$^-$ and makes it hydrophilic. The nitrosyl-dioxyl radical, which possesses about the same energy as is released by the hydrolysis of ATP under standard conditions (31), can dissociate NO from ferrous heme proteins, thereby inactivating the enzyme.

\[
\text{NO} + \text{O}_2 \rightleftharpoons \text{ONO}^- \\
\text{REACTION I}
\]

\[
\text{NO} + \text{ONO}^- \rightleftharpoons \text{ONO}_2^- \\
\text{REACTION II}
\]

\[
\text{ONO}_2^- \rightarrow 2\text{NO}_2 \\
\text{REACTION III}
\]

\[
(2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2 \text{ net}) \\
\text{REACTION IV}
\]

Thus, the reaction rate for NO binding to an enzyme must be faster than the formation of ONOO$^-$ by the reaction of NO with O$_2$. When nitric oxide synthase is activated, NO will first associate with the enzyme due to the faster reaction rate with the metal than with O$_2$. Because of the high O$_2$ concentrations relative to enzyme, NO will gradually be bound as ONOO$^-$, and

![Graphical Representation](image_url)
a gradual loss of enzyme activity will be manifested.

The NO-mediated inhibition of tyrosinase/O_2 activity reported in this investigation with the two phenolic precursors of melanin as substrates may be attributed to differences in the concentration of exogenous NO and/or to the formation of a copper-nitrosyl complex that may diminish, rather than enhance enzyme activity. The binding of NO to the binuclear copper center of tyrosinase has been shown to yield a dipolar-coupled pair in which both copper ions bind NO (38). The mechanism by which nitrosyl-copper complexes form at the active site of tyrosinase to diminish enzyme activity has not been established. Tyrosinase is unique in that it catalyzes two distinct reactions, an initial hydroxylation of monophenols to o-diphenols, and the ensuing oxidation of o-diphenols to o-quinones. Based on chemical and spectroscopic studies of its binuclear copper-active site, three forms of the enzyme have been characterized; oxy-tyrosinase, deoxy-tyrosinase, and met-tyrosinase (38, 39). The oxidation of o-diphenols to their corresponding o-quinones can be mediated either by the oxy-form of tyrosinase or the met-form. It is proposed that NO or its derivative, NO_2, may interact with the met-form of tyrosinase to yield transient noncatalytic forms of the enzyme.

Effects of NO on the Oxidations of L-Dopa and Dopamine

Fig. 12. A, tyrosinase is a dinuclear type-3 copper-containing metalloprotein that reversibly binds and activates dioxygen. Each copper center has three histidine (His) coordinates, and the active site is accessible to phenolic substrates. The oxy-form of tyrosinase reacts catalytically with both monophenols and diphenols, whereas the met-form reacts only with diphenols. Such catalytic interactions produce transient complexes that dissociate with the release of o-quinones. In its reduced or deoxy-form the enzyme reversibly binds O_2, generating the oxy-form of the enzyme. The deoxy-form is reverted back to the oxy-form by addition of O_2. The met-form of tyrosinase can be converted to the oxy-form by addition of H_2O_2 (40). The tyrosinase-mediated conversion of monophenols starts with their binding to the oxy-form of the enzyme, a reaction that produces the o-diphenol and the met-form of the enzyme. Thus, the oxidation of o-diphenols to their corresponding o-quinones can be mediated either by the oxy-form of tyrosinase or the met-form. B, it is proposed that NO or its derivative, NO_2, may interact with the met-form of tyrosinase to yield transient noncatalytic forms of the enzyme.

This investigation documents for the first time the production of an oxidized nitrodopamine derivative generated by the peroxidase/H_2O_2/NO-mediated catalysis of dopamine. The molecule, which is considered to be either a noncyclic quinone (6NDQA) or semiquinone (6NDASQ) of nitrodopamine (Fig. 11), was generated along with two oxidized melanin precursors, DAQ and DAC. Except for the production of melanin precursors, no nitroderivative was detected in the peroxidase/H_2O_2/NO-mediated catalysis of L-dopa or in any of the tyrosinase/O_2/
Effects of NO on the Oxidations of L-Dopa and Dopamine

NO-mediated reactions. These findings support recent studies that reported a reduced 6-nitroderivative to be the principal reaction product emanating from the activity of catecholamines with nitrogen oxides derived from NO (9, 10, 45). The oxidation of such a nitroderivative of dopamine by univalent or divalent electron transfer would generate 6NDAHQ and 6NDAQ, respectively (Fig. 11), and thus account for the nitroderivative molecule detected in this investigation.

It is not known why substrate nitration was not evident in peroxidase/H$_2$O$_2$/NO-promoted oxidations of l-dopa, when such a reaction was manifested with dopamine, the decarboxylated derivative of l-dopa. Because peroxidase can use both NO and H$_2$O$_2$ as substrates to catalyze the nitrosation of tyrosine residues (46), the rate of enzyme/H$_2$O$_2$ oxidation of L-dopa may exceed that of dopamine, limiting the extent of competitive involvement of NO in nitrating l-dopa. Also, it is possible that the electrochemical conditions established for this investigation were inappropriate for detecting nitroderivatives of l-dopa or that nitration of l-dopa was prevented by the side-chain CO$_2$H group of the catechol. The absence of substrate nitration in tyrosinase/O$_2$/NO-mediated reactions with either l-dopa or dopamine may document a significantly different method of converting o-diphenols to o-quinones than that employed by peroxidase/H$_2$O$_2$. This possibility highlights the importance of several recent studies documenting the presence of tyrosinase gene expression via the cGMP pathway (19, 52). In our previous studies (19, 52) and melanogenesis are enhanced by NO modulating tyrosinase activity and enzyme activation and augmentation.

Whether the effects of NO on the enzyme-mediated oxidations of dopamine and l-dopa are contributory but less than additive, as we found with tyrosinase/O$_2$, or synergistic, as with peroxidase/H$_2$O$_2$, the cells in which these reactions occur are likely to manifest significantly altered catechol dynamics. The formation of nonylized nitrosodopamine quinones or semiquinones represents an important alternative pathway in the metabolism of catecholamine, because it by-passes the formation of melanogenic precursors, such as DHI or DHICA, which are considered to be cytotoxic (56–58) (Fig. 1). The formation and possible role of neuromelanin in the etiology of Parkinson’s disease continue to be issues of considerable interest (56, 59), because, as a redox-active polymer, the pigment has the capacity to engage in electron transfer processes. Depending on the prevailing intra- and extracellular environments, melamins can react with different reducing or oxidizing species and, thus, be either cytotoxic or cytotoxic (24, 60). The capacity of neuromelanin to bind iron through –OH phenolic units (2) reduces the involvement of the metal in OH production by the Fenton reaction and contributes to the antioxidant functions of the pigment. The pro-oxidant or antioxidant functions of the pigment are dependent on its redox equilibrium, a feature readily altered by the presence of transition metal ions, changes in pH, and interactions with reactive intermediates of oxygen (ROI) and nitrogen (RNI). Thus, NO fluxes in the brain can have a significant effect on the function(s) of neuromelanin.

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