Iron-Mediated Generation of the Neurotoxin 6-Hydroxydopamine Quinone by Reaction of Fatty Acid Hydroperoxides with Dopamine: A Possible Contributory Mechanism for Neuronal Degeneration in Parkinson's Disease

Alessandro Pezzella,[†] Marco d'Ischia,[†] Alessandra Napolitano,[†] Giovanna Misuraca,[‡] and Giuseppe Prota*[,†]

Department of Organic and Biological Chemistry, University of Naples "Federico II", Via Mezzocannone 16, I-80134 Naples, Italy, and Department of Experimental Pharmacology, University of Naples "Federico II", Via D. Montesano 49, Naples, Italy

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Exposure of dopamine to an excess of linoleic acid 13-hydroperoxide (13-hydroperoxyoctadecadienoic acid) in the presence of ferrous ions in Tris buffer, pH 7.4, resulted in a relatively fast, oxygen-independent reaction exhibiting first-order kinetics with respect to both catecholamine and metal concentrations. Product analysis in the early stages revealed the presence of significant amounts of the quinone of the neurotoxin 6-hydroxydopamine, together with some aminochrome and ill-defined melanin-like material. Quinone formation required the presence of iron, either in the ferrous or ferric form, and was unaffected by peroxidase, catalase, and hydroxyl radical scavengers, e.g. mannitol, as well as biologically relevant antioxidants, like ascorbate and glutathione. Hydrogen peroxide proved as effective as linoleic acid hydroperoxide in inducing dopamine oxidation and conversion to 6-hydroxydopamine quinone. Metal chelators, including EDTA and bipyridyl, markedly suppressed quinone formation without, however, inhibiting dopamine oxidation. These and other results are consistent with a hydroxyl radical independent hydroxylation/oxidation mechanism basically different from the Fenton reaction, which involves direct interaction of the peroxide with a dopamine−Fe(III) chelate generated during the process.

Introduction

A persisting condition of oxidative stress, with more or less severe perturbation of the intracellular redox equilibria and permanent deterioration of critical cell structures, is definitely recognized as a core pathogenetic factor underlying selective degeneration or dysfunction of the dopaminergic neurons of the substantia nigra in Parkinson's disease.[1−3] Circumstantial evidence accumulated over the past decades suggests that, whatever the primary cause of injury, an intricate cascade of intra- and extraneutronal reactions is set in motion which, in the aftermath of the insult, may induce a chronic oxidative diversion of dopamine metabolism against a failing line of antioxidant defense.[1b] Although the consequences of this metabolic derangement have not been fully elucidated at the molecular level, credit has been given to the possibility that aberrant oxidation of dopamine could result in the generation of neurotoxic species impairing basic metabolic functions and leading eventually to neuron death.[2c] Arguments supporting this view derived from the discovery by Tranzer and Thoenen[4] in 1968 that an oxidized dopamine derivative, 6-hydroxydopamine (**1**) was able to cause selective destruction of peripheral catecholaminergic nerve endings, possibly via spontaneous oxidative conversion to yield potentially toxic quinone species. The consequent burst of interest in the neurotoxic properties of **1** spurred intense research efforts aimed at demonstrating endogenous generation of this neurotoxin as a potential contributory mechanism in the etiopathogenesis of Parkinson's disease and other severe mental disorders.[5] A hint came, *inter alia*, from a report claiming the formation of **1** in the caudate nucleus of the rat brain following administration of a large dose of methylamphetamine, which is known to destroy dopamine nerve terminals.[6] Analytical difficulties and certain pitfalls inherent in the methodologies for detection of **1** in vivo, however, hindered substantial progress in this area. Moreover, the actual ability of **1** or its quinone (**2**) to induce destruction of dopaminergic nerve terminals when generated in trace amounts was questioned.[5,7] Nonetheless, the 6-hydroxydopamine hypothesis of neurotoxicity in Parkinson's disease was never abandoned, being corroborated by model in vitro experiments demonstrating that oxidation of dopamine under conditions of physiological relevance may result in the formation of sufficient amounts of **1** to justify its implication in neurodegenerative diseases. Among the oxidizing systems that have been reported to produce some **1** from dopamine are the Fenton system,[8] the enzyme tyrosinase,[9] and Mn(II) in the presence of oxygen,[10] although the latter two have been disproved.[11]

More recently, in a reexamination of this issue, it was found that nucleophilic addition of hydrogen peroxide to dopamine quinone produced, e.g. by peroxidase/hydrogen peroxide oxidation, may lead to the formation of **1** in relatively good yields.[11] Since enhanced production of hydrogen peroxide, derived in part from upregulated monoamine oxidase activity, is well documented in Parkinson's disease,[1c,12] it seems conceivable that under appropriate conditions a mechanism similar to that observed in vitro becomes operative in vivo.

As an extension of that study, we have surveyed the reaction behavior of dopamine with a number of metabolic products of oxidative stress that are putatively formed and accumulated in the degenerating nigrostri-

* Address all correspondence to: Professor Giuseppe Prota, Department of Organic and Biological Chemistry, University of Naples "Federico II", Via Mezzocannone 16, I-80134 Naples, Italy. Phone: +39-81-7041249. Fax: +39-81-5521217. E-mail: prota@cds.unina.it.
[†] Department of Organic and Biological Chemistry.
[‡] Department of Experimental Pharmacology.
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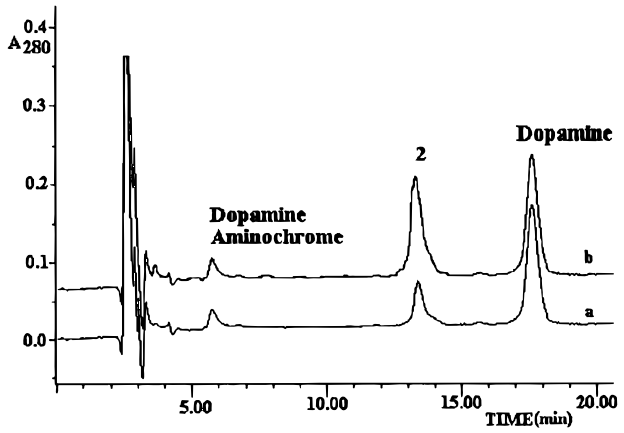


Figure 1. HPLC elution profile of the products formed by oxidation of dopamine (500 μM) with linoleic acid 13-hydroperoxide (5 mM) and ferrous ions (350 μM) in 0.1 M Tris buffer, pH 7.4, at 37 °C, at 20 min reaction time. Trace A, reaction mixture; trace B, the same as A, spiked with authentic **2**.

atal tract. In this paper we report that, in addition to hydrogen peroxide, hydroperoxides derived from oxidation of polyunsaturated fatty acids, e.g. linoleic acid, are also effective in inducing the conversion of the catecholamine to **2** in the presence of ferrous ions.

Results

Exposure of 500 μM dopamine to 5 mM linoleic acid 13-hydroperoxide (13-hydroperoxyoctadecadienoic acid) in 0.1 M Tris buffer, pH 7.4 at 37 °C, resulted in little or no detectable conversion of the catecholamine over periods of time of up to 1 h. However, when ferrous ions at 350 μM concentration were added to the incubation mixture, a relatively fast reaction occurred, indicated by the rapid disappearance of the substrate and the development of a reddish coloration denoting aminochrome formation. A similar effect was also observed with other lipid hydroperoxides, like those produced by enzymatic oxidation of arachidonic acid. HPLC analysis of the reaction mixture after about 20 min (Figure 1) showed expectedly the presence of dopamine aminochrome as well as of another significant component of the oxidation mixture whose chromatographic and spectrophotometric properties (λ_{max} = 495 nm) proved identical to those of 2-hydroxy-5-(2-aminoethyl)-1,4-benzoquinone (**2**), the quinone of **1**[11] (Scheme 1).

This structural assignment was confirmed by electrospray-mass spectrometric (ES/MS) analysis of the product isolated by ion-exchange chromatography and/or preparative HPLC, exhibiting a detectable pseudomolecular peak at *m*/*z* 167, as well as by $NaBH_4$ reduction, leading to a compound indistinguishable from authentic **1**. Attempts to identify other significant products of dopamine oxidation met with failure under a variety of conditions, nor was there evidence for other isomeric hydroxydopamines, e.g. 5-hydroxydopamine. Apparently, the mass balance was accounted for by chromatographically ill-defined, melanin-like materials. Figure 2 illustrates the time course of dopamine oxidation and product formation from 500 μM dopamine and 350 μM ferrous ions in the presence and in the absence of linoleic acid 13-hydroperoxide, as a control. In the former case, substrate consumption was fast and resulted in the pronounced formation of **2**, peaking at 5 min and decreasing below detection limits after ca. 60

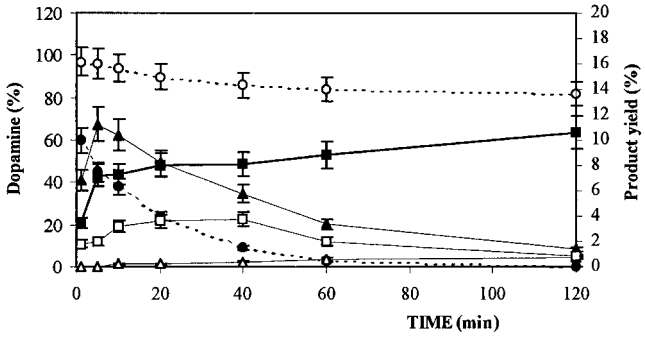
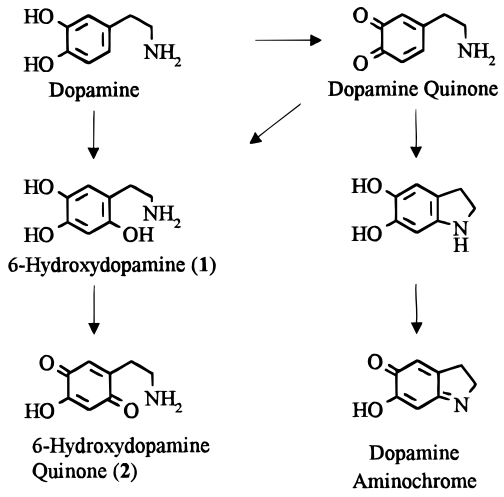


Figure 2. Time course of dopamine oxidation (dashed lines, left axis) and percent product yields (right axis) by oxidation of 500 μM dopamine with 350 μM ferrous ions in the presence and in the absence of 5 mM linoleic acid 13-hydroperoxide. Dopamine decay: (●) with hydroperoxide; (○) without hydroperoxide. Formation of **2**: (▲) with hydroperoxide; (△) without hydroperoxide. Aminochrome formation: (■) with hydroperoxide; (□) without hydroperoxide.

Scheme 1. Oxidation Chemistry of Dopamine Showing the Competition between Aminochrome and 6-Hydroxydopamine Formation



min, when most of the dopamine was consumed. No substantial change in the reaction kinetics and course was observed under an oxygen-free atmosphere or using Fe(III) ions in place of Fe(II). In the absence of hydroperoxide, dopamine concentration decreased at a much slower rate and reached a plateau at about 15% substrate consumption after 60 min, at which time only trace amounts of **2** could be detected.

Dopamine oxidation followed first-order kinetics for at least 50% of the hydroperoxide/Fe(II)-promoted reaction (Figure 3). The reaction showed also first-order dependence with respect to the metal concentration, as apparent from a logarithmic plot of the initial rates of dopamine consumption vs Fe(II) concentration, which gave a straight line with a slope of 1.05 (Figure 4).

No reliable information could be obtained about the kinetic dependence of the reaction course on hydroperoxide concentration because of difficulties in obtaining reproducible data under pseudo-first-order conditions.

Figure 5 shows the effect of Fe(II) concentration on the relative yield of formation of **2** vs aminochrome in the early phases of the hydroperoxide-promoted reaction.

Notably, a marked increase in the yield of **2** was observed with increasing iron concentration, the ratio

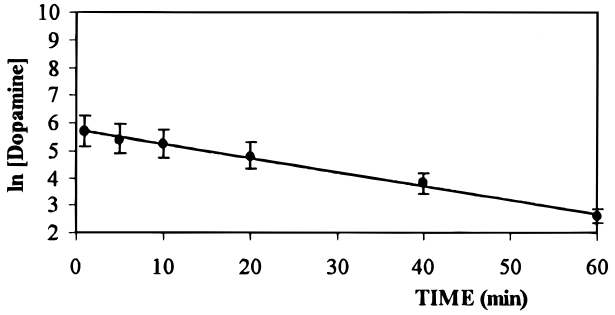


Figure 3. Plot of ln dopamine concentration against time, $r^2 = 0.89$. Slope = 1.1 ± 0.1. Reaction conditions are as in Figure 1.

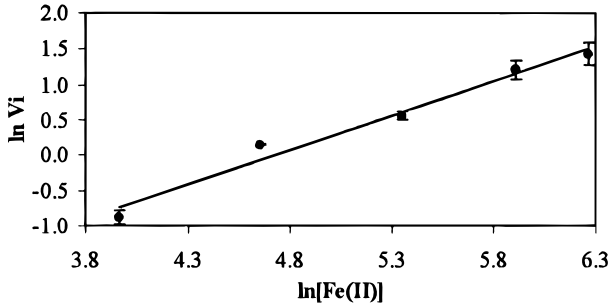


Figure 4. Logarithmic plot of initial rates of dopamine oxidation (μM s^{-1}) vs Fe^{2+} concentration, $r^2 = 0.98$. Dopamine was oxidized as detailed in Figure 1. Initial rates of dopamine oxidation were determined after 1 min. Slope = 1.05 ± 0.05.

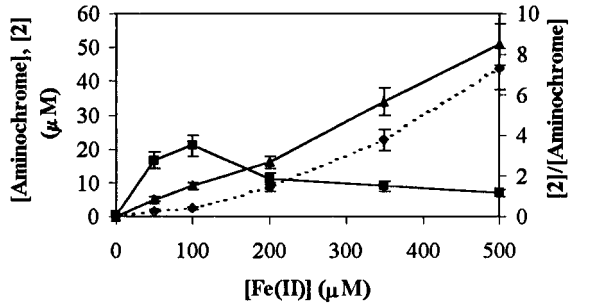


Figure 5. Plots of **2** (▲) and aminochrome (■) concentration (left axis) and **2**/aminochrome ratio (dashed line, right axis) against Fe^{2+} concentration.

becoming greater than 1 when Fe(II) concentration was about 0.7 times that of dopamine.

Table 1 shows the effects of various experimental parameters and additives on the extent of dopamine oxidation and product formation determined after 1 min.

Use of phosphate as a buffer caused a marked decrease in the rate of the hydroperoxide/Fe(II)-promoted reaction, compared to Tris and HEPES, with concomitant alteration of the product distribution toward the aminochrome. Positively or negatively charged surfactants like mirystyltrimethylammonium bromide and sodium dodecyl sulfate (SDS) exerted little or no influence on hydroperoxide/Fe(II)-promoted dopamine oxidation.

Hydrogen peroxide proved as effective as linoleic acid 13-hydroperoxide in inducing dopamine oxidation and formation of **2** in the presence of Fe(II) ions. In the absence of Fe(II), peroxidase was unable to utilize linoleic acid 13-hydroperoxide as a cosubstrate to oxidize dopamine, whereas in the presence of the metal it did not affect the reaction course. Sodium periodate, in the presence of Fe(II), induced the rapid oxidation of

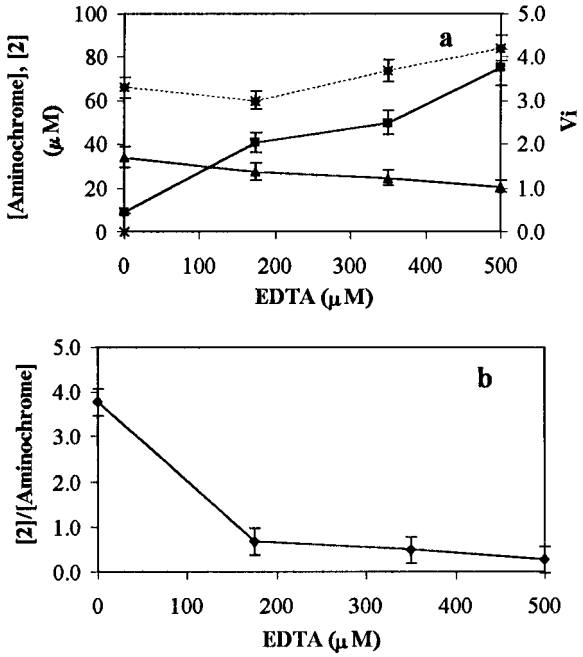


Figure 6. (a) Plot of **2** (▲) and aminochrome (■) concentration (left axis) and initial rate of dopamine oxidation (dashed line, right axis) vs EDTA concentration. (b) Plot of **2**/aminochrome ratio vs EDTA concentration. Dopamine oxidation was carried out in the presence of linoleic acid 13-hydroperoxide and Fe^{2+} as detailed in Figure 1.

dopamine without forming detectable **2**. No **2** was also formed when dopamine was oxidized with tyrosinase or periodate in the presence of hydroperoxide, but without added metal.

Of the various antioxidants and radical scavengers tested, mannitol and catalase were virtually inactive on the hydroperoxide/Fe(II) system, whereas ascorbic acid and glutathione, which are present in high concentrations in brain tissues,[3c] markedly inhibited accumulation of the aminochrome without, however, affecting formation of **2** and only slightly affecting dopamine consumption. No detectable reaction of glutathione with **2** could be observed in the relevant oxidation mixture. Particularly worthy of note was the effect of metal chelators, viz. bipyridyl and EDTA. While bipyridyl, unlike EDTA, dramatically accelerated the rate of dopamine oxidation by the hydroperoxide/Fe(II) system, both chelators virtually suppressed formation of **2**.

In further experiments the effect of EDTA on dopamine oxidation with 5 mM hydroperoxide and 350 μM ferrous ions in Tris buffer was investigated in detail. A plot of the initial rates of dopamine decay against EDTA concentration (Figure 6a) showed a slight increase in the oxidation rate with increasing chelator concentrations. Analysis of the **2**/aminochrome product ratio as a function of EDTA concentration (Figure 6b) provided confirmatory evidence for a concentration-dependent ability of the metal chelator to suppress formation of **2**.

Spectrophotometric investigation of the oxidation mixtures revealed in the early stages the presence of a well detectable blue chromophore with an absorption maximum at 575 nm, due probably to a dopamine−Fe(III) chelate.[13] This was confirmed by a comparison with the absorption features of dopamine solutions containing Fe(III) salts, as well as by the lack of significant shift of the dopamine absorption maximum in the presence of Fe(II) under rigorously an oxygen-

Table 1. Effect of Experimental Parameters and Additives on the Rate of Dopamine Oxidation and Product Yields[a]

peroxide	buffer (0.1 M)	additive (concn)	initial rate (μM s^{-1})[b]	yield of **2** (%)[c]	aminochrome yield (%)[c]
none	Tris	nihil			
13-HPODE[d]	Tris	*f*			0.1
13-HPODE	Tris	nihil	3.3	6.8	1.8
13-HPODE	HEPES	nihil	2.9	7.0	1.9
13-HPODE	phosphate	nihil	1.2	1.8	3.9
H_2O_2	Tris	nihil	3.0	7.2	2.7
13-HPODE	Tris	SDS (0.2 M)	3.2	7.0	1.5
13-HPODE	Tris	MTMAB[e] (0.2 M)	3.2	6.8	2.0
13-HPODE	Tris	mannitol (0.2 M)	3.1	6.6	2.0
13-HPODE	Tris	catalase (150 units/mL)	3.0	6.9	2.5
13-HPODE	Tris	tyrosinase (30 units/mL)[f]	1.7	1.6	4.3
13-HPODE	Tris	peroxidase (5 units/mL)	3.7	7.0	1.5
13-HPODE	Tris	peroxidase (5 units/mL)[f]	0.7	0.6	0.7
none	Tris	$NaIO_4$ (0.45 mM)	7.5	0.1	10
13-HPODE	Tris	$NaIO_4$ (0.45 mM)[f]	7.5	0.1	12
13-HPODE	Tris	EDTA (0.5 mM)	3.7	4.1	15
13-HPODE	Tris	bipyridyl (0.9 mM)	7.5	1.9	17
13-HPODE	Tris	glutathione (0.1 mM)	3.0	6.9	0.1
13-HPODE	Tris	ascorbate (0.1 mM)	2.3	5.2	0.1

[a] Reaction conditions as in Figure 1, unless otherwise stated. [b] Determined at 1 min reaction time. SD ≤ 10%. [c] Average of three determinations, SD ≤ 10%. [d] Linoleic acid 13-hydroperoxide. [e] Myristyltrimethylammonium bromide. [f] No iron added.

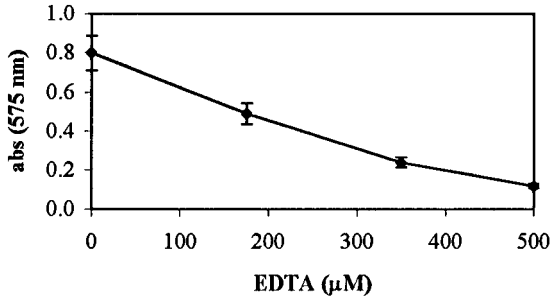


Figure 7. Plot of the absorbance at 575 nm of the dopamine−Fe(III) chelate vs EDTA concentration in 0.1 M Tris buffer, pH 7.4. Dopamine and Fe^{3+} concentration were 500 and 350 μM.

free atmosphere. Addition of increasing concentrations of EDTA to the dopamine−Fe(III) system caused a proportional decrease of the blue chromophore, due evidently to decomposition of the dopamine−Fe(III) chelate (Figure 7).

Discussion

Abnormal iron accumulation[3,14] and an up to 10-fold increase in the levels of lipid hydroperoxides,[15] along with elevated production of hydrogen peroxide,[1] figure prominently among those pathogenetic processes which are specifically associated with an oxidative stress condition in the parkinsonian substantia nigra. While these changes are indicative of toxic processes involving crucial membrane lipid components, whether and by what mechanism(s) they may directly affect the dopamine pool in degenerating nitrostriatal neurons remains an open issue. A novel hint is offered by the results of the present study, which provide a chemical basis to envisage a substantial conversion of dopamine to the proximate oxidation product of the neurotoxin 6-hydroxydopamine in hydroperoxide-rich environments, provided that iron is present in the reaction milieu.

From the chemical viewpoint, the reaction described in this paper presents a number of peculiar features which may be worthy of comment. Firstly, formation of **2** does not seem to proceed via nucleophilic addition of the hydroperoxide group onto dopamine quinone, since chemical oxidation of dopamine in the presence of linoleic acid 13-hydroperoxide, but in the absence of iron, i.e. under conditions where dopamine quinone is definitely formed, failed to afford any detectable **2**. Against a significant involvement of dopamine quinone is also the lack of inhibitory effects on the formation of **2** of glutathione and ascorbic acid, which are known to deplete transiently generated *o*-quinones via either nucleophilic addition[16] or reduction mechanisms.[17]

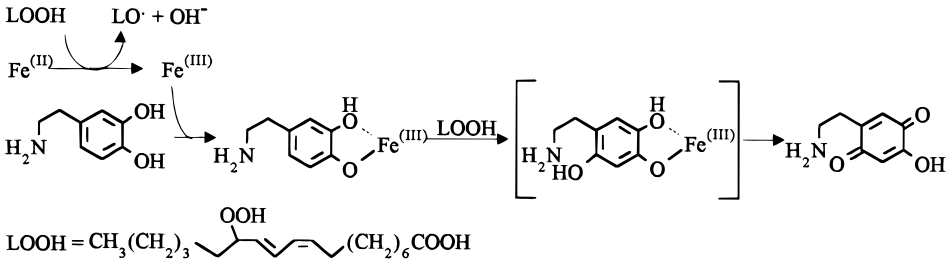
In fact, the observed inhibitory effect of metal chelators clearly indicates that formation of **2** strictly depends on the generation of a dopamine−Fe(III) complex and, by implication, that the latter is the species actually suffering hydroxylation/oxidation. In this frame, the competition between the 6-hydroxydopamine and the aminochrome routes would be governed by the partitioning of iron between the catecholamine chelate and other possible complexes within the reaction milieu.

Another issue concerns the mechanism of hydroxylation/oxidation of the dopamine−Fe(III) complex. The process evidently does not depend on oxygen, requires a hydroperoxide as co-substrate, and does not involve hydroxyl radicals as the attacking species, thus differing significantly from Fenton-type reactions. In accord with this interpretation are both the lack of effect of mannitol, a known OH radical scavenger and, by contrast, the inhibitory effect of EDTA, a commonly used iron chelator for Fenton reactions. Furthermore, the generation of hydroxyl radicals, which typically follows one-electron reduction of hydrogen peroxide by ferrous ions, is incompatible with the basic mode of decomposition of alkyl hydroperoxides, leading mainly to alkoxyl radicals and hydroxyl anions.[18]

On this basis, a mechanism could be envisaged in which iron chelation confers to dopamine a marked susceptibility to attack at the 6-position of the catechol ring by the hydroperoxide (Scheme 2). Although this reaction seemed to be regiospecific, because of the failure to detect other isomeric hydroxydopamines, the poor mass balance and the different stability of isomeric hydroxydopamines did not allow this point to be definitely assessed.

An interesting implication of our results relates to the observed inability of glutathione and ascorbic acid to prevent the iron/peroxide-dependent generation of **2**. On

Scheme 2. Schematic Outline of the Proposed Mechanism of Conversion of Dopamine to **2**, Highlighting Formation of the Iron−Dopamine Chelate as the Critical Step



a chemical basis, this suggests the possible occurrence within catecholaminergic neurons of neurotoxic processes which defy counteraction by the primary line of antioxidant defense in the brain. These and other related processes, revolving around the oxidative interaction of dopamine with iron,[19] may overall contribute to progressive impairment of crucial cellular structures and functions and, eventually, hasten the final demise of the neuron.

Apart from the possible involvement in neuronal degeneration, it is tempting to speculate here that the observed peroxide/iron-induced catecholamine oxidation may play a role in the biosynthesis of neuromelanin, the characteristic age pigment responsible for the dark coloration of the neuron perykaria in human substantia nigra.[20,21] In nigral neurons, a mechanism may be envisioned whereby basal generation of hydrogen peroxide and lipid peroxides in the presence of iron evokes a slow, yet constant oxidation of a minor part of the dopamine pool leading to the gradual intraneuronal deposition of melanin pigments.

Conclusions

The present study throws light on a hitherto overlooked hydroxyl radical independent mechanism of conversion of dopamine to the proximate oxidation product of the neurotoxin 6-hydroxydopamine. Although basically different from the hydroxylations induced by the peroxidase/hydrogen peroxide system and by the Fenton reaction, the proposed reaction process and the previous ones are not mutually exclusive and may all contribute to the catecholamine degenerative chemistry associated with dopaminergic neuron loss.

Of course, it is too early to propose any causal relationships between the observed chemistry and the process of neuronal degeneration. Considering that iron and peroxide concentrations exceed basal levels and increase dramatically at advanced stages of substantia nigra degeneration in Parkinson's disease, it is conceivable that formation of **2** becomes significant only after the primary toxic processes are underway, perhaps contributing to amplify those degenerative events that lead eventually to dopaminergic neuron death.

If supported in further studies, the mechanism described in the present paper may contribute to create an improved background in which to fit many of the still unconnected observations on the biochemical pathology of Parkinson's disease and may yield new clues for the development of innovative neuroprotective interventions based on the control of peroxide and iron metabolism.

Experimental Section

UV spectra were recorded on a Perkin-Elmer Lambda 7 spectrophotometer having the cell compartment controlled at 37 ± 0.1 °C with circulating water. HPLC analyses were performed with a Gilson instrument equipped with model 305 pumps and a model 317 UV detector. ES/MS spectra were determined with a VG BIO-Q triple quadrupole mass spectrometer.

Materials. Dopamine hydrochloride, sodium periodate, ferrous sulfate heptahydrate, ethylenediaminetetraacetic acid disodium salt (EDTA), myristyltrimethylammonium bromide, bipyridyl, and nonstabilized hydrogen peroxide (35% solution in water) were from Aldrich Chemie (Steinheim, Germany). 6-Hydroxydopamine hydrobromide, 5-hydroxydopamine hydrobromide, linoleic acid, arachidonic acid, ascorbic acid, reduced glutathione, soybean lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12, 110 000 units/mg) type IB, mushroom tyrosinase (*o*-diphenol:O_2 oxidoreductase, EC 1.14.18.1, 6300 units/mg), horseradish peroxidase (donor H_2O_2 oxidoreductase, EC 1.11.1.7, 200 purpurogallin units/mg, $E_{430}/E_{275} = 2.0$) type II, catalase from bovine liver (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6, 9740 units/mg) were purchased from Sigma Chemicals (St. Louis, MO). Dopamine aminochrome was prepared by aerial oxidation of 5,6-dihydroxyindoline hydrobromide[11] in sodium bicarbonate buffer, pH 8.0. 6-Hydroxydopamine quinone (**2**) was prepared by sodium periodate oxidation of 6-hydroxydopamine as reported previously.[11]

Analytical Conditions. HPLC was performed using a Spherisorb S5 ODS 2 column (1 mL/min) and a 10 × 250 mm Alltech Econosil C_{18} column (6 mL/min) for analytical and preparative runs, respectively. Detection was carried out at 280 nm. Mobile phase: 0.05 M phosphate buffer, pH 3.0, containing 10 mM sodium 1-octanesulfonate−acetonitrile, 93:7.

Synthesis of Linoleic Acid 13-Hydroperoxide. Linoleic acid 13-hydroperoxide was synthesized enzymatically by oxidation of linoleic acid with soybean lipoxygenase. To a solution of linoleic acid (1 mM) in 0.1 M borate buffer, pH 9.0, was added lipoxygenase (2000 units/mL) under vigorous stirring. The reaction was monitored spectrophotometrically at 234 nm (conjugated diene formation), and when the absorbance plateaued, it was stopped by acidification to pH 4.0 with 3 M HCl. The mixture was extracted three times with chloroform, and the organic layers were washed with saturated aqueous NaCl, dried over anhydrous sodium sulfate, and evaporated to dryness. The resultant hydroperoxide, obtained as a colorless oil, was dissolved in methanol and stored at −20 °C. Hydroperoxide concentration was checked prior to each experiment using a molar extinction coefficient of 27 000 M^{-1} cm^{-1} at 234 nm.[22]

Arachidonic acid 15-hydroperoxide was prepared by a similar procedure.

Linoleic Acid 13-Hydroperoxide-Induced Oxidation of Dopamine. Kinetic Experiments. A solution of dopamine (0.5 mM) in 0.1 M Tris buffer (unless otherwise stated), pH 7.4, was treated with appropriate volumes of a freshly prepared stock solution of ferrous sulfate in water, up to the desired concentration, and of linoleic acid 13-hydroperoxide (5 mM final concentration). When required, solutions of ferric salts or hydrogen peroxide were used in place of ferrous salts and linoleic acid 13-hydroperoxide, respectively. Additives, e.g. enzymes, surfactants, metal chelators, and antioxidants, were added to the desired concentration, as indicated in the Results section as well as in the legends to figures. The mixture was incubated under stirring at 37 °C in a thermostated water

bath. Aliquots of the reaction mixture were periodically withdrawn, acidified to pH 1.0 with 3 M HCl, and centrifuged at 7000 rpm for about 15 min, and the aqueous layer was injected into the HPLC. Identification and quantification of dopamine, aminochrome, and **2** were carried out by comparing retention times and integrated peak areas with external calibration curves for authentic samples. Product identification was also secured by coinjection of an aliquot of the reaction mixture with the appropriate volume of a stock solution of the relevant compound. All experiments were run at least in triplicate. Statistical parameters were determined by linear least-squares fitting.

Isolation and Characterization of 2 by Ferrous Ion Promoted Oxidation of Dopamine with Linoleic Acid 13-Hydroperoxide. Dopamine (10 mg, 0.5 mM) was oxidized with linoleic acid 13-hydroperoxide (5 mM) and ferrous sulfate (0.35 mM) in Tris buffer, pH 7.4, as described above. After 1 h, or when most of the dopamine was consumed, the reaction mixture was chromatographed on a column (15 × 1 cm) of Dowex 50W X4 (100−200 mesh). After being washed with 0.01 M HCl (50 mL), the column was eluted with 0.1 M and 0.5 M HCl (50 mL each, 2-mL fractions). The fractions eluted with 0.5 M HCl contained **2** as apparent from spectrophotometric analysis and comparison of the HPLC elutographic properties of the main product with those of authentic **2**. The appropriate fractions were concentrated at room temperature with a rotary evaporator, care being taken to avoid evaporation to dryness, and analyzed within 24 h by ES/MS.

An aliquot of the concentrated fractions containing **2** was neutralized with crystals of sodium phosphate, treated with an excess of sodium borohydride, and then analyzed by HPLC. Formation of **1** was confirmed by coinjection with an authentic sample.

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