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Oxidation of DOPAC by nitric oxide: effect of superoxide dismutase

João Laranjinha* and Enrique Cadenas†

**Faculty of Pharmacy and Center for Neurosciences, University of Coimbra, Portugal*

†*Department of Molecular Pharmacology & Toxicology, School of Pharmacy, University of Southern California, Los Angeles, California, USA*

Abstract

This study aimed to characterize the redox interaction between 3,4-dihydroxyphenylacetic acid (DOPAC) and nitric oxide (·NO), and to assess the reductive and oxidative decay pathways of the DOPAC semiquinone originating from this interaction. The reaction between DOPAC and ·NO led to the formation of the DOPAC semiquinone radical, detected by electron paramagnetic resonance (EPR) and stabilized by Mg^{2+}, and the nitrosyl anion detected as nitrosylmyoglobin. The EPR signal corresponding to the DOPAC semiquinone was modulated as follows: (i) it was suppressed by glutathione and ascorbic acid with the formation of new EPR spectra corresponding to the glutathionyl and ascorbyl radical, respectively; (ii) it was enhanced by Cu,Zn-superoxide dismutase; the enzyme also accelerated the decay of the semiquinone species to DOPAC quinone. These results are interpreted as a one-electron oxidation of DOPAC by ·NO; the reductive decay of the semiquinone back to DOPAC was facilitated by reducing agents, such as glutathione and ascorbate, whereas the oxidative decay to DOPAC quinone was facilitated by superoxide dismutase. The latter effect is understood in terms of a reversible conversion of nitrosyl anion to ·NO by the enzyme. The biological relevance of these reactions is also discussed in terms of the reactivity of peroxynitrite towards DOPAC as a model with implications for aerobic conditions.

Keywords: DOPAC, dopamine, nitric oxide, Parkinson's disease, superoxide dismutase.

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Nitric oxide (·NO) is a diffusible free radical and membrane permeant neurotransmitter in brain (Moncada *et al*. 1991; Dawson and Dawson 1998). Physiologically ·NO is produced by a constitutive neuronal ·NO synthase (nNOS) following glutamate stimulation of NMDA receptors and the subsequent cellular influx of calcium. Excessive stimulation of NMDA receptors may increase ·NO concentration in brain, but expression of an inducible, Ca^{2+}-independent NOS isoform (iNOS) may also occur with the consequent increase in ·NO concentration (Dawson and Dawson 1998). ·NO reacts at near diffusion-limited rates with other free radicals and with transition metals in proteins such as guanylate cyclase and haemoglobin, but despite its free radical character ·NO is remarkably unreactive with most biological molecules at the low concentrations produced *in vivo* (Beckman 1996). However, excessive production of ·NO is neurotoxic and has been implicated in a variety of neurological disorders (Dawson and Dawson 1998), including Parkinson's disease, which is characterized by the loss of dopaminergic neurones in the nigrostriatal system.

Two salient features of Parkinson's disease may be relevant to the context of this study. First, the toxic effects of ·NO in Parkinson's disease are highlighted by recent findings showing that induction of iNOS in glial cells contributes to degeneration of dopamine-containing neurones in a mice model of Parkinson's disease (Liberatore *et al*. 1999), and that inhibition of nNOS prevents Parkinsonism in animal models (Hantraye *et al*. 1996; Przedborski *et al*.

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Address correspondence and reprint requests to João Laranjinha Faculty of Pharmacy and Center for Neurosciences, University of Coimbra, Couraça dos Apóstolos, 51, r/c, 3000 Coimbra, Portugal.
E-mail: laranjin@ci.uc.pt

Abbreviations used: DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DOPAC, 3,4-dihydroxyphenylacetic acid; DTPA, diethylenetriamine pentacetic acid; EPR, electron paramagnetic resonance; GSH, glutathione; GS·, glutathionyl radical, iNOS, NOS isoform; Mb–Fe^{III}, metmyoglobin; Mb–Fe^{III}–NO, nitrosylmyoglobin; nNOS, neuronal ·NO synthase; SOD, superoxide dismutase.

1996; Dehmer *et al*. 2000; Klivenyi *et al*. 2000). Second, in Parkinson's disease, the metabolism of dopamine is a well known source of oxidative stress leading to cell death; dopamine-induced neurotoxicity has been attributed to the generation of toxic quinones and semiquinones (Graham *et al*. 1978; Hastings *et al*. 1996), autoxidation with production of superoxide anion and hydrogen peroxide (Hoyt *et al*. 1997), consumption of antioxidants (Cohen *et al*. 1997), and inhibition of the mitochondrial respiratory chain (Ben-Shachar *et al*. 1995). It was also observed that dopamine causes apoptotic death in neurones by an early insult to mitochondria affecting components of the respiratory chain (Wallace *et al*. 1997; Berman and Hastings 1999). In brain, 3,4-dihydroxyphenylacetic acid (DOPAC) is a major metabolite of dopamine (Kopin 1985) resulting from two oxidative steps catalysed by monoamine oxidase and aldehyde dehydrogenase activities, both located at the mitochondrial level (Tank *et al*. 1981; Ambroziak and Pietruszko 1991; Keung and Vallee 1998).

This study aimed to characterize the redox interaction between ·NO and DOPAC, and to assess the reductive and oxidative decay pathways of the DOPAC semiquinone radical, an intermediate originating from the aforementioned interaction, and evaluating the reactivity of peroxynitrite towards DOPAC, a model with implications for aerobic experimental conditions.

Materials and methods

Chemicals and biochemicals

DOPAC, glutathione (GSH), ascorbic acid, and Cu,Zn-superoxide dismutase were purchased from Sigma Chemical Co. (St Louis, MO, USA). ·NO gas (Praxair, Danbury, CT, USA) was passed through 5 M NaOH (two passages) to remove contaminating nitrogen oxides in an apparatus using only stainless steel or glass tubing. Nitric oxide-saturated solutions were prepared by flowing ·NO gas (treated as described above) through deoxygenated ultra pure water (obtained from a Milli-Q system; Millipore Company, Bedford, MA, USA) for 30 min. Peroxynitrite was synthesized from sodium nitrite and hydrogen peroxide according to the procedure described by Beckman *et al*. (1994). All other chemicals were of analytical grade.

Nitric oxide measurements

The reaction between ·NO gas and DOPAC was performed in a vessel under controlled conditions such that head space was always 1/10th of total volume (to minimize and reproduce ·NO partition into the vessel atmosphere). This enabled us to perform reproducible experiments. Using gas-tight syringes, several volumes of ·NO gas were transferred to the reaction mixture, vortexed and the concentrations measured electrochemically using the ISO-NO Mark II nitric oxide meter and sensor from WPI (Sarasota, FL, USA). The ·NO electrode was calibrated by filling the reaction chamber with KI/H_2SO_4 (0.1 M), and titrating with stock solutions of KNO_2. Calibration was also performed using ·NO-saturated solutions (prepared as described previously; Koppenol 1998).

Spectroscopic measurements

Electron paramagnetic resonance (EPR) spectra were recorded on a Brucker ECS 106 spectrometer (Bruker Instruments Inc., Billerica, MA, USA) at room temperature (24°C) using the following instrument settings (unless otherwise stated): microwave frequency, 9.8 GHz; microwave power, 20 mW; sweep width, 30 G; modulation amplitude, 0.96 G; time constant, 0.65 s; sweep rate, 20 G/min. Reaction mixtures challenged with ·NO gas (treated as described above) or with ·NO-saturated solutions were immediately transferred (avoiding contamination with oxygen) to bottom-sealed Pasteur pipettes and the EPR spectra were recorded.

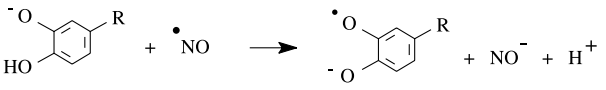
o-Semiquinone radicals were measured under conditions of spin-stabilization with 0.2 M Mg^{2+}. This approach, which significantly enhances radical concentration allowing the unambiguous identification of semiquinone species, has been widely applied to the study of catecholamine-derived radicals (Kalyanaraman *et al*. 1984; Kalyanaraman *et al*. 1987).

Absorption spectra were recorded on a Hitachi U3110 absorption spectrophotometer (Hitachi Instruments Inc., San Jose, CA, USA). Spectra of metmyoglobin were recorded in the 450–680 nm range. Metmyoglobin concentration was calculated from $\varepsilon_{630} = 3.42\ \text{mM}^{-1}\ \text{cm}^{-1}$ and nitrosylmyoglobin from $\varepsilon_{575} = 10.5\ \text{M}^{-1}\ \text{cm}^{-1}$ (Antonini and Brunori 1971). DOPAC quinone formation was followed at 415 nm.

Results

Oxidation of DOPAC by ·NO

The semiquinone form of DOPAC, stabilized by Mg^{2+}, was detected by EPR upon incubation of DOPAC with ·NO under anaerobic conditions. The EPR signal intensity increased linearly with increasing ·NO concentrations (Fig. 1) and decreased with time. In the presence of O_2, the semiquinone radical of DOPAC is also observed, but linearity between radical intensity and ·NO concentration is lost, suggesting that competing reactions are occurring (not shown). Under anaerobic conditions, and at variance with DOPAC, dopamine semiquinone radical was not detected upon incubation of dopamine with ·NO (not shown). The formation of the DOPAC semiquinone suggests a one-electron oxidation of DOPAC by ·NO (reaction 1):



(1)

with concomitant reduction of ·NO to nitrosyl anion (NO^-). The formation of the latter was confirmed by detection of nitrosylmyoglobin (reaction 2; $k_2 = 6.4 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$) (Goretski and Hollocher 1988):

$$\text{Mb}[\text{Fe}^{\text{III}}] + \text{NO}^- \rightarrow \text{Mb}[\text{Fe}^{\text{III}}] - \text{NO} \qquad (2)$$

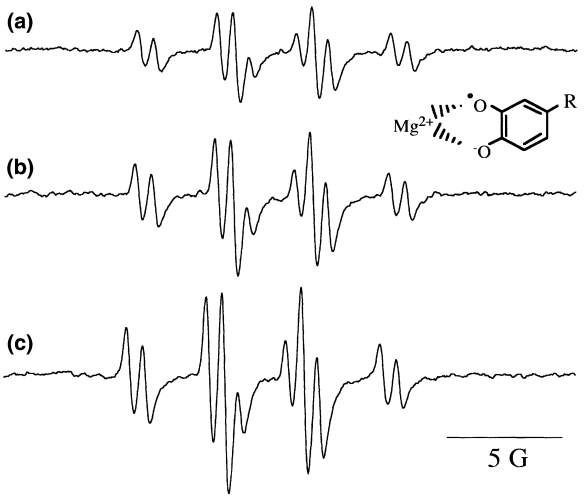


Fig. 1 Oxidation of DOPAC by nitric oxide: EPR spectra of DOPAC semiquinone. The reaction mixture consisted of 1 mM DOPAC in 0.1 M Tris/0.1 mM DTPA buffer, pH 7.4 (final volume 1 mL). The reaction mixture was purged with He. The reaction was initiated by addition of nitric oxide gas under controlled conditions to yield solutions containing 38 (a), 170 (b) and 335 μM (c) of nitric oxide. Spectra are representative of several independent experiments. Instrument settings and other assay conditions as described in the Materials and methods section.

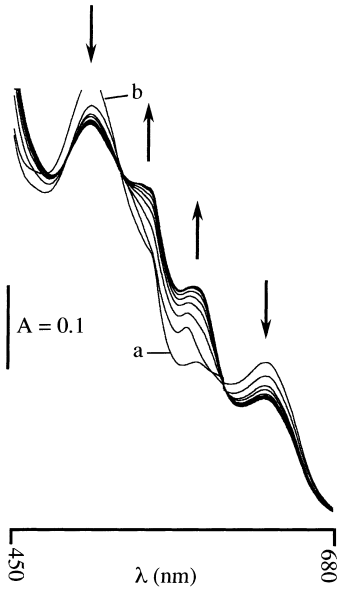


Fig. 2 Detection of nitrosyl anion by nitrosylmyoglobin formation. Metmyoglobin spectral changes following the addition of DOPAC and nitric oxide. The reaction mixture consisted of 50 μM metmyoglobin, 1 mM DTPA and 1 mM DOPAC in 50 mM phosphate buffer, pH 7.4, purged with He. The reaction was initiated by adding nitric oxide gas (30 μM final concentration). Scans were recorded with 5 min intervals. Upward and downward arrows indicate increase and decrease of absorbance, respectively. (a) absorption spectrum of metmyoglobin. (b) Absorption spectrum of nitrosylmyoglobin. Ascorbate added at the end of the reaction exerted no spectral modifications.

incubation of metmyoglobin with DOPAC/·NO resulted in a decrease in the 503 and 630 nm absorption of metmyoglobin, and an increase at 542 and 580 nm; isosbestic points were at 602, 531 and 481 nm. The absorption at 578–580 nm is characteristic of nitrosylmyoglobin (Antonini and Brunori 1971): under the experimental conditions of Fig. 2, approximately 90% of ·NO was recovered in the form of nitrosylmyoglobin.

Oxidation of DOPAC by peroxynitrite

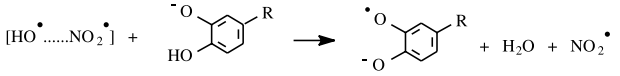
DOPAC semiquinone radical is rapidly formed upon incubation of DOPAC with peroxynitrite ($ONOO^-$), the product of the reaction between $O_2^{-}\cdot$ and ·NO (reaction 3; $k_3 = 1.9 \times 10^{10}$ M^{-1} s^{-1}; Huie and Padmaja 1993):

$$O_2^{-} + \cdot NO \rightarrow ONOO^- \qquad (3)$$

The EPR spectrum shown in Fig. 3 supports the notion of oxidation of DOPAC by an oxidant contained in the cage formed by $ONOO^-$ protonation to peroxynitrous acid (reaction 4) in a manner similar to that described for the oxidation of ubiquinol by peroxynitrite (Schopfer *et al.* 2000)

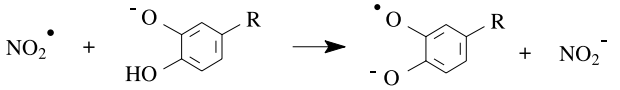
$$ONOO^- + H^+ \rightarrow ONOOH \Leftrightarrow HO^{\cdot} \ldots\ldots NO_2^{\cdot}]. \qquad (4)$$

It is likely that DOPAC semiquinone formation follows the mechanism outlined in reaction 5:



(5)

This does not rule out a second oxidation by nitrogen dioxide ($NO_2\cdot$) (reaction 6), a potent oxidant ($E^{0}_{,NO_2\cdot/NO_2^-} = 1.0$ V) also expected to be formed during the trimolecular reaction of ·NO with O_2 (a mechanism viable in aerobic conditions) (reaction 7):



(6)

$$2\,\cdot NO + O_2 \rightarrow\rightarrow 2NO_2^{\cdot} \qquad (7)$$

Upon incubation of dopamine with peroxynitrite, an EPR signal, ascribed to the semiquinone obtained after the first one-electron oxidation of dopamine, was also observed (Fig. 3b).

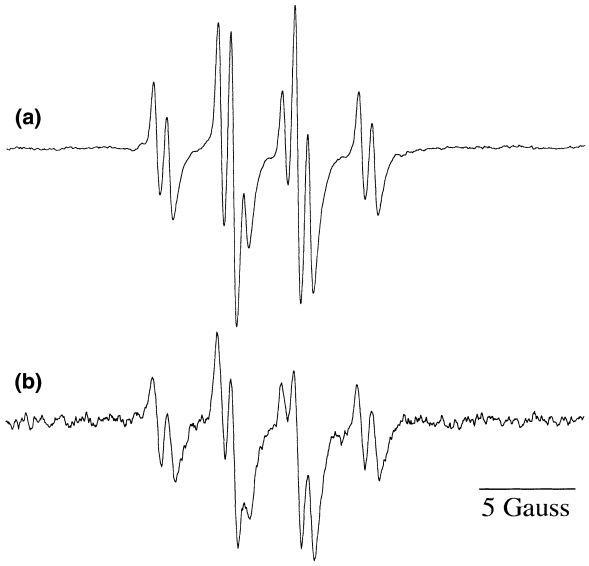


Fig. 3 Oxidation of DOPAC and dopamine by peroxynitrite: EPR spectra of DOPAC and dopamine semiquinones. The reaction mixture consisted of 1 mM DOPAC (a) or 1 mM dopamine (b) in 0.1 M Tris/0.1 mM DTPA buffer, pH 7.4 (final volume 1 mL). The reaction was initiated by a bolus addition of 400 μM peroxynitrite. Instrument settings and other conditions as described in the Materials and methods section. Spectra (a) and (b) are represented under different intensity scales.

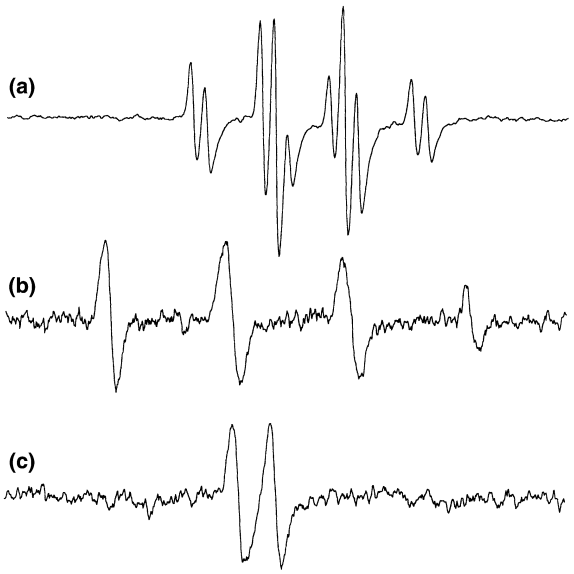
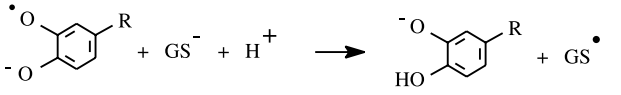


Fig. 4 Formation of glutathionyl and ascorbyl radicals during the interaction of DOPAC with ·NO. (a) EPR spectrum of DOPAC semiquinone. Assay conditions as in Fig. 1. (b) EPR spectrum of the glutathionyl radical-DMPO adduct. The reaction mixture consisted of 1 mM DOPAC, 50 μL nitric oxide gas, 200 mM DMPO in 110 mM NaCl/20 mM phosphate buffer, pH 7.4. 1 mM GSH was added to the buffer after DOPAC and nitric oxide. Sweep width: 80 G. (c) Spectrum of ascorbyl radical. Assay conditions as in (b) but sweep width 30 G and 1 mM ascorbate instead of GSH in the absence of the spin trap, DMPO. Also, spectrum (a) is represented in a different scale intensity relatively to (b) and (c).

Formation of glutathionyl and ascorbyl radicals during the oxidation of DOPAC by ·NO

The EPR signal ascribed to DOPAC semiquinone (Fig. 4a) and formed during the oxidation of DOPAC by ·NO in anaerobiosis was abolished by GSH: spin trapping EPR showed a new signal ascribed to the adduct of thiyl radical of GSH (glutathionyl radical) with DMPO ($a^N = 15.4$ G; $a^H = 16.2$ G; Fig. 4b). Likewise, the EPR signal of the DOPAC semiquinone was abolished by ascorbate and a new signal corresponding to the characteristic doublet of ascorbyl radical ($a^H = 1.8$ G) was observed (Fig. 4c).

These findings suggest that DOPAC semiquinone oxidizes glutathione and ascorbate with formation of glutathionyl and ascorbyl radicals, respectively (reaction 8).



(8)

Note that these experiments were carried out in anaerobiosis; hence, the redox transition DOPAC semiquinone → DOPAC (reductive decay) is expected to be facilitated by glutathione and ascorbate, and rules out the involvement of oxyradicals derived from DOPAC semiquinone autoxidation in the formation of glutahionyl and ascorbyl radicals.

Effect of superoxide dismutase on DOPAC oxidation by ·NO

In anaerobic conditions, Cu,Zn-superoxide dismutase elicited the following effects on the oxidation of DOPAC by ·NO.

Superoxide dismutase increased the EPR signal intensity of DOPAC semiquinone arising from the oxidation of DOPAC by ·NO

Figure 5 shows the decay of DOPAC semiquinone radical with time in the absence (Fig. 5a) and the presence of superoxide dismutase (Fig. 5b). The magnitude of the increase was dependent on superoxide dismutase concentration. Superoxide dismutase had no effect on DOPAC in the absence of ·NO. The effect of different concentrations of superoxide dismutase on the decay of the DOPAC semiquinone EPR signal is shown in Fig. 6.

The enhancement of the EPR signal decay intensity by superoxide dismutase, and its faster decay in the presence of the enzyme, may be understood in terms of the removal of the nitrosyl anion product (reaction 1) by Cu^{2+}-superoxide

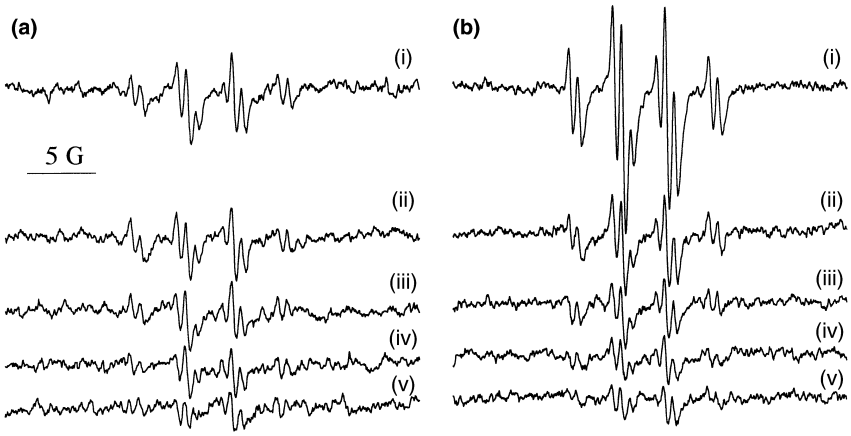


Fig. 5 Effect of superoxide dismutase on DOPAC semiquinone formation. The reaction mixture consisted of 1 mM DOPAC in 0.1 M Tris/0.1 mM DTPA buffer, pH 7.4. The reaction was initiated by addition of 10 μL nitric oxide gas. (a) Control. Spectra of DOPAC radical taken at (i) 0, (ii) 3, (iii) 6, (iv) 9, and (v) 15 min. (b) As in (a) but in the presence of 12.5 μM Cu,Zn-superoxide dismutase. Instrument settings and other conditions are described in the Materials and methods section.

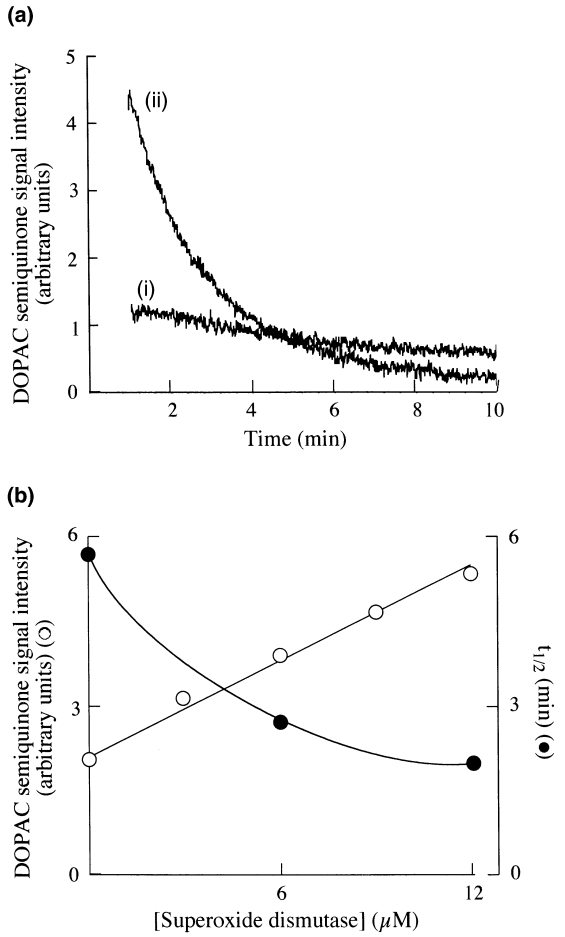


Fig. 6 Effect of superoxide dismutase on DOPAC semiquinone signal intensity and decay. (a) Time course of DOPAC semiquinone decay in the absence (i) and presence (ii) of 25 μM superoxide dismutase. Assay conditions as in Fig. 4. (b) Dependence of signal intensity and $t_{1/2}$ on superoxide dismutase concentration.

dismutase (reaction 9). This leads to two effects: first, it enhances the oxidation of DOPAC by ·NO, thereby increasing the signal intensity, and second, it accelerates the decay of the DOPAC semiquinone. The kinetic control of ·NO-mediated DOPAC oxidation by superoxide dismutase appears to be accomplished within a cycle involving the ·NO → NO^- transition during DOPAC oxidation (reaction 1) and the NO^- → ·NO transition coupled to the reduction of Cu^{2+} in superoxide dismutase (reaction 9), displacing reaction 1 to the right:

$$SOD-Cu^{2+} + NO^- \rightarrow SOD-Cu^{+} + \cdot NO \qquad (9)$$

The redox role of copper in superoxide dismutase in the enhancement of the DOPAC semiquinone EPR signal was substantiated by two approaches: first, cyanide inhibited the enhancing effect elicited by superoxide dismutase (Fig. 7c), and second, the effect of superoxide dismutase was mimicked by copper (Fig. 7d); as expected, the copper-enhancement of the DOPAC semiquinone signal was not sensitive to cyanide (Fig. 7e). Of note, copper (sulphate) used here is not a catalytic model for the general activity of SOD but a model for the activity of copper in SOD on the decay of the DOPAC radical.

Superoxide dismutase competed with myoglobin for NO^-

Figure 8 shows the absorption spectral changes occurring during the oxidation of DOPAC by ·NO and the ensuing nitrosylmyoglobin formation. A 50% inhibition of nitrosylmyoglobin formation was observed in the presence of superoxide dismutase. The effects of two superoxide dismutase concentrations on the time course of nitrosylmyoglobin formation are shown in Fig. 8(c). This inhibitory effect on nitrosylmyoglobin formation is a

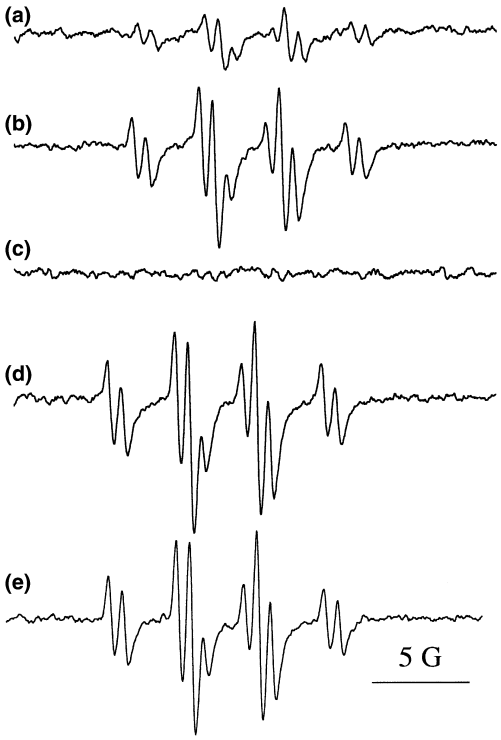
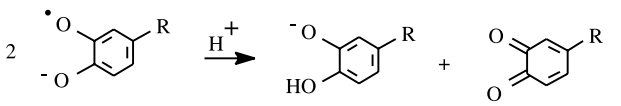


Fig. 7 Modulation of DOPAC semiquinone signal intensity: role of copper. The reaction mixture consisted of 1 mM DOPAC in 0.1 M Tris, pH 7.4. The reaction mixture was purged with He. The reaction was initiated by the addition of 10 μL nitric oxide gas. (a) No further additions. (b) *Plus* 12 μM superoxide dismutase. (c) *Plus* 12 μM superoxide dismutase and 100 μM cyanide. (d) *Plus* 12 μM $CuSO_4$. (e) *Plus* 12 μM $CuSO_4$ and 100 μM cyanide.

consequence of the competition between metmyoglobin (reaction 2) and superoxide dismutase (reaction 9) for NO^-.

Superoxide dismutase enhanced the formation of DOPAC quinone

Accumulation of DOPAC quinone in the presence of superoxide dismutase occurred at initial rates 2.5-fold faster than in its absence (Fig. 9). The formation of DOPAC quinone (in the absence and presence of superoxide dismutase) in anaerobic conditions is expected to occur by disproportionation (reaction 10):



(10)

Discussion and conclusions

This study – carried out under anaerobic conditions – provides evidence for the oxidation of DOPAC by ·NO and the modulation of this redox reaction by glutathione, ascorbate and Cu,Zn-superoxide dismutase.

Formation of DOPAC semiquinone

The primary reaction proceeds with production of DOPAC semiquinone and NO^-, their occurrence confirmed by EPR and absorption (nitrosylmyoglobin formation) spectroscopy, respectively (reactions 1 and 2). The formation of nitrosylmyoglobin cannot provide an indirect measure of the rate of DOPAC oxidation by ·NO, for it may impose a kinetic control on the primary reaction: although nitrosylmyoglobin formation proceeds at modest rates ($k_2 = 6.4 \times 10^5$ M^{-1} s^{-1}), the removal of NO^- by the haemoprotein may accelerate DOPAC oxidation. It is also expected that the monoanionic form of DOPAC (reaction 1) is participating in the electron transfer reaction with ·NO, for deprotonation is a requisite condition for electron transfer. DOPAC quinone formation may be expected to originate from disproportionation (reaction 10) as shown in pulse radiolysis studies for the case of melanine precursor, 3,4-dihydroxyphenylalanine (Chedekel *et al*. 1984; Thompson *et al*. 1985).

Reductive decay of DOPAC semiquinone

Based on the suppression of the DOPAC semiquinone signal and the appearance of a glutathionyl or ascorbyl radical signal in reaction mixtures containing glutathione and ascorbate, respectively, it may be surmised that these electron donors favoured the reduction of DOPAC semiquinone back to DOPAC (reaction 8). The reduction of DOPAC semiquinone by ascorbate is thermodynamically feasible [$E(A^{\cdot -}/A^-) = 0.282$ mV] (Koppenol and Butler 1985), whereas that by glutathione [$E(GS^{\cdot}/GS^-) = 0.85$–$1.3$ V] (Surdhar and Armstrong 1986) may be driven by either the decay pathways of the glutathionyl radical (e.g. its conjugation with thiolate: $GS^{\cdot} + GS^- \rightarrow GSSG^{\cdot -}$) or – as in the conditions of Fig. 4 – its reaction with the spin trap ($k_{DMPO + GS^{\cdot}} = 10^7$–$10^8$ M^{-1} s^{-1}) (Davies *et al*. 1987).

Acceleration of DOPAC semiquinone formation and oxidative decay

Superoxide dismutase accelerates DOPAC semiquinone formation as well as the decay of the semiquinone species: this effect may be interpreted on the basis of a redox cycle involving the reversible conversion of nitrosyl anion to ·NO by superoxide dismutase (Murphy and Sies 1991); ·NO thus formed, would potentiate DOPAC semiquinone formation and its faster decay. In the experimental conditions in this study – assays carried out in anaerobic conditions – the accumulation of DOPAC quinone (Fig. 9) by autoxidation may be ruled out, whereas disproportionation appears to be the major mechanism underlying the formation of the quinone (Thompson *et al*. 1985).

The effect of superoxide dismutase on the fate of DOPAC semiquinone and the primary reaction between DOPAC and

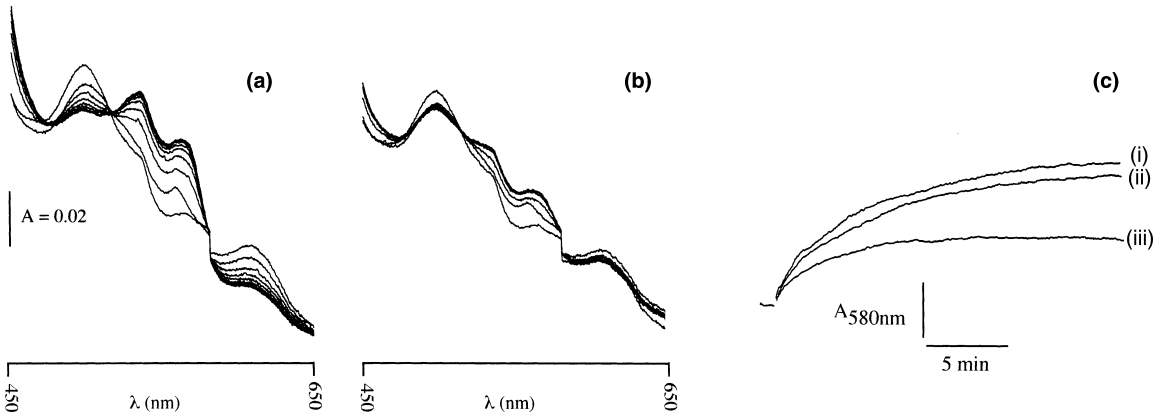


Fig. 8 Effect of superoxide dismutase on nitrosylmyoglobin formation. (a) Assay conditions: 0.5 mM DOPAC, 5 μM metmyoglobin, 1 mM DTPA in 50 mM potassium phosphate buffer, pH 7.4, and supplemented with 28 μM nitric oxide to initiate the reaction. (b) As in (a) in the presence of 1.7 μM superoxide dismutase. (c) Time course of nitrosylmyoglobin formation. Assay conditions as in (a): (i) no further additions; (ii) *plus* 4 μM superoxide dismutase; (iii) *plus* 16.6 μM superoxide dismutase.

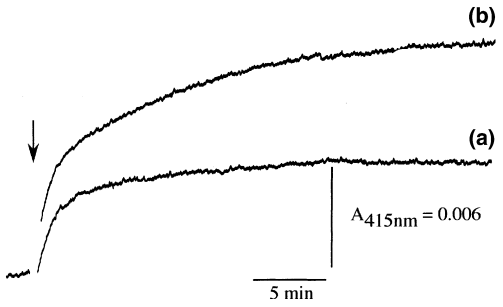


Fig. 9 Time course of DOPAC quinone formation. Assay conditions: 2 mM DOPAC and 0.2 M Mg^{2+} in He-purged 0.1 M Tris buffer, pH 7.4; the reaction was started with the addition of 28 μM nitric oxide (arrow). (a) control; (b) *plus* 4 μM superoxide dismutase. Other assay conditions as in the Materials and methods section.

·NO needs further analysis within the frame of its physiological implications: the effect of superoxide dismutase described here in connection with DOPAC metabolism is expected to shift the redox status of the cell towards a more oxidized state. This notion may be strengthened by the augmentation of DOPAC quinone levels, the reutilization of ·NO within a reductive pathway (see above and reaction 1) associated with an increase in DOPAC quinone levels, and channeling of the Cu^{+}/Cu^{2+} cycles in superoxide dismutase to nitrogen metabolism rather than $O_2^{\cdot-}$ reduction. Of course, these notions depend on the steady state levels of the individual reactants and the rate constants of the reactions involved.

Biological significance

The implications of this study for biological systems gain significance in view of the following.

(i) Glutamate potentiates simultaneously ·NO production and the toxic effects of dopamine towards neuronal cells (Garthwaite and Boulton 1995).

(ii) ·NO regulates dopamine release in the hippocampus of rats (Segieth *et al*. 2000; Wegener *et al*. 2000) through activation of the guanylate cyclase pathway (Peterson *et al*. 1995), and also affects dopamine reuptake (Cook *et al*. 1996).

(iii) ·NO activates tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of dopamine) in PC12 cells (Roskoski and Roskoski 1987).

(iv) The activity of the recently described nitric oxide synthase in mitochondria (mtNOS) (Ghafourifar and Richter 1997; Giulivi *et al*. 1998) provides a site-specific setting for the oxidation of DOPAC by ·NO: first, the steady-state level of ·NO in the mitochondrial matrix is expected to be high, partially as a result of the activity of mtNOS ($\approx 3 \times 10^{-8}$ M; Boveris *et al*. 2000); second, and at least temporarily, DOPAC synthesis is expected to occur in the mitochondrial matrix via the activity of the rather unspecific aldehyde dehydrogenase (Tank *et al*. 1981; Ambroziak and Pietruszko 1991; Keung and Vallee 1998); third, the production of $O_2^{\cdot-}$, by the mitochondrial respiratory chain [enhanced by ·NO inhibition of the bc_1 segment (Poderoso *et al*. 1996) in an antimycin-like fashion] contribute to peroxynitrite formation via the diffusion-controlled rate of the reaction between ·NO and $O_2^{\cdot-}$. Given this scenario, the oxidation of DOPAC by $ONOO^{-}$ (Fig. 3) may be an important source of DOPAC semiquinone and ensuing redox transitions of this species. Therefore, it may be surmised that, in perfect aerobic conditions, NO would oxidize DOPAC to *o*-semiquinone indirectly through peroxynitrite formation.

Thus, either dopamine or its metabolites may be potential targets for ·NO and derived oxidants, affecting the physio-

logical chemistry of both ·NO and catecholamines. In line with this hypothesis, it has been shown that ·NO promoted nitration of dopamine in aerobic solutions, and that the nitrated dopamine failed to increase 2′,5′-cyclic adenosine monophosphate (cAMP) formation in cultured striatal neurones (Daveu *et al*. 1997). The modulation of the DOPAC/·NO interaction by ascorbate and glutathione gains further significance when considering that these antioxidants are present in the mM range in the brain. Concerning the effects elicited by superoxide dismutase, it may be noted that its activity is increased in *substantia nigra* in Parkinson's disease (Saggu *et al*. 1989).

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