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COMMENTARY

# Modulation of 6-Hydroxydopamine Oxidation by Various Proteins

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**ABSTRACT.** The spontaneous autoxidation of the neurotoxin 6-hydroxydopamine proceeds by a free radical chain reaction involving the superoxide anion radical and produces the corresponding chromogen 6-hydroxydopamine quinone and hydrogen peroxide. The rate of this reaction is increased in the presence of ceruloplasmin and peroxidase, and reduced by superoxide dismutase, catalase, and DT-diaphorase. We report some explanations of why these proteins may increase or reduce the rate of autoxidation of 6-hydroxydopamine. BIOCHEM PHARMACOL 53;8:1065–1068, 1997. © 1997 Elsevier Science Inc.

**KEY WORDS.** 6-hydroxydopamine; superoxide dismutase; catalase; DT-diaphorase; ceruloplasmin; peroxidase

Animal models that reproduce symptoms of human pathological conditions have played an important role in the elucidation of physiopathological mechanisms as well as in the development of potential therapies. The use of neurotoxins to induce lesions in specific brain structures has led to the creation of experimental models of human neurological conditions and contributed greatly to recent progress in physiopathological aspects of neurology.

The neurotoxins 2,4,5-trihydroxyphenylalanine (6-hydroxydopa) and 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine) are intermediates in the formation of dopa and of dopamine and melanins, respectively [1]. 6-Hydroxydopamine accumulates in, and causes degeneration of, catecholamine-containing neurons when injected directly into or adjacent to cell clusters or nuclei of the brain, such as the noradrenergic locus ceruleus or the dopaminergic substantia nigra [2]. Injection of 6-hydroxydopamine into the ventricular systems of the brain damages catecholamine nerve terminals, but not the cell bodies. The amino acid analog 6-hydroxydopa penetrates freely into the brain, and its neurotoxicity is thought to be mediated by transformation to 6-hydroxydopamine [2].

The selective toxicity of 6-hydroxydopamine is attributable to its affinity for catecholamine reuptake systems, which result in its accumulation to critical levels in target neurons [3, 4]. Sachs and Jonsson [4] have estimated that the intraneuronal concentration of 6-hydroxydopamine required for neurotoxicity is as high as 20–50 mM. The ability to generate active oxygen species and 6-hydroxydopamine quinones underlies the toxic action of this agent [5–8]. Thus, 6-hydroxydopamine undergoes rapid autoxidation, reacting with molecular $O_2$ to yield a quinoidal product and several forms of reduced oxygen, including superoxide anion radical ($O_2^{-\cdot}$), hydroxyl radicals ($OH^{\cdot}$), and $H_2O_2$ [6]. These reactive species are thought to modify cellular components, thereby rendering them nonfunctional [9, 10]. Given that similar reactions of active oxygen species with brain components may be important in various aspects of aging, as well as in diseases such as Parkinson's disease, multiple sclerosis, senile dementia, and cancer, the autoxidation of 6-hydroxydopamine has been investigated extensively. It has been postulated that either $H_2O_2$ or 6-hydroxydopamine quinone may be directly responsible for the 6-hydroxydopamine-induced degeneration of adrenergic nerve terminals, although $O_2^{-\cdot}$, $OH^{\cdot}$, and 6-hydroxydopamine semiquinone may also contribute to the initiation of neuronal damage [11]. However, neither the mechanism of the autoxidation of 6-hydroxydopamine nor that of its cytotoxicity has been demonstrated definitively.

Neither $O_2$ [12] nor $H_2O_2$ [13] directly oxidizes 6-hydroxydopamine in the absence of some other reactant capable of acting as a co-oxidant. The relative contributions of $O_2$ and $H_2O_2$ [13] are affected by the relative amounts of $O_2$ and 6-hydroxydopamine present. Changes in the availability of propagating intermediates, substrates, and products are reflected in changes in the net redox state during the progress of the autoxidation of 6-hydroxydopamine. From these considerations, it is clear that the reaction of 6-hydroxydopamine with $O_2$ allows assessment of contributions of a variety of reactive species to the mechanisms by which $O_2$ is activated [14, 15]. Such an assessment can be undertaken by determining the inhibitory actions of selective scavengers or metal-chelating agents individually or in

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combination. A comprehensive study is needed, because combinations of scavengers have sometimes produced unexpected results, such as synergistic [12] or antagonistic [16] scavenger effects.

The following is a review of the proteins that may play an important role in regulating the extent of oxidation of 6-hydroxydopamine and thereby exacerbate or limit the resulting neuronal damage.

## OXIDATION OF 6-HYDROXYDOPAMINE

6-Hydroxydopamine undergoes spontaneous autoxidation in the presence of $O_2$ at physiological pH, yielding the corresponding quinoidal product, $H_2O_2$, $O_2^{-\cdot}$, and $OH^{\cdot}$ [12]:

$$6\text{-}QH_2 + O_2 \rightarrow 6\text{-}QH^{\cdot} + O_2^{-\cdot} + H^+ \quad (1)$$

$$6\text{-}QH_2 + O_2^{-\cdot} + H^+ \rightarrow 6\text{-}QH^{\cdot} + H_2O_2 \quad (2)$$

$$6\text{-}QH^{\cdot} + O_2 \rightarrow 6\text{-}Q + O_2^{-\cdot} + H^+ \quad (3)$$

$$6\text{-}QH^{\cdot} + O_2^{-\cdot} + H^+ \rightarrow 6\text{-}Q + H_2O_2 \quad (4)$$

where $6\text{-}QH_2$ is defined as 6-hydroxydopamine; $6\text{-}QH^{\cdot}$, as 6-hydroxydopamine semiquinone; and 6-Q, as 6-hydroxydopamine quinone.

Hydrogen peroxide generated from reaction (2) is an effective oxidant for 6-hydroxydopamine [17], reacting as follows:

$$6\text{-}QH_2 + H_2O_2 \rightarrow 6\text{-}Q + 2H_2O \quad (5)$$

The mechanism of autoxidation of 6-hydroxydopamine (initiation, propagation, and termination), the effects of metal-chelating agents, and the formation of $O_2^{-\cdot}$ and $OH^{\cdot}$ have been described previously [18].

## INHIBITION OF 6-HYDROXYDOPAMINE OXIDATION

### *Superoxide Dismutase*

Superoxide dismutase (EC 1.15.1.1), a ubiquitous enzyme that protects cells and tissues from injury induced by $O_2^{-\cdot}$, catalyzes the dismutation of $O_2^{-\cdot}$ to $H_2O_2$ and $O_2$. In this reaction, one molecule of $O_2^{-\cdot}$ is oxidized to $O_2$, while a second is reduced to $H_2O_2$. Superoxide dismutase activity was first described by McCord and Fridovich [19] with a copper–zinc protein from bovine erythrocytes, the X-ray structure of which was subsequently determined [20].

The inhibitory effect of superoxide dismutase on the spontaneous autoxidation of 6-hydroxydopamine was demonstrated by Heikkila and Cabbat [21]. Superoxide dismutase (100 μg/mL) reduced the rate of spontaneous quinone formation from 0.25 mM 6-hydroxydopamine by 93%, and the initial rate of $O_2$ consumption by 6-hydroxydopamine was also reduced in the presence of superoxide dismutase. These researchers concluded that $O_2^{-\cdot}$ is formed during the spontaneous autoxidation of 6-hydroxydopamine and that it catalyzes this reaction. Local concentrations of superoxide dismutase may be important in determining the susceptibility of neurons to 6-hydroxydopamine. Heikkila and Cabbat [21] developed a sensitive assay for superoxide dismutase based on the autoxidation of 6-hydroxydopamine; the amounts of superoxide dismutase activity in rat brain, liver, and spinal cord detected by this assay were similar to those determined by other assays.

### *Catalase*

Catalase (EC 1.11.1.6) contains ferriprotoporphyrin IX as a prosthetic group that directly contributes to substrate activation. The enzyme mediates the oxidation of various substances by $H_2O_2$, and, in particular, catalyzes the oxidation of $H_2O_2$ itself and that of ethanol.

Catalase inhibits the spontaneous autoxidation of 6-hydroxydopamine [22]; at a concentration of 15 μg/mL, it reduces the rate of the spontaneous quinone formation from 0.25 mM 6-hydroxydopamine by 50%. The most important role of catalase in protection against 6-hydroxydopamine toxicity may be to prevent $OH^{\cdot}$ formation by inhibiting the Fenton reaction between iron and $H_2O_2$.

### *DT-Diaphorase*

DT-Diaphorase [NAD(P)H-quinone oxidoreductase, EC 1.6.99.2] is a flavoprotein that catalyzes the oxidation of NADH and NADPH at equal rates by two-electron reduction of quinonic compounds to hydroquinones. The presence of DT-diaphorase in rat brain was first demonstrated by Thomas and Pearse [23], and it has been isolated subsequently from many species of animals, plants, bacteria, and fungi [24].

DT-Diaphorase has been proposed to play a role in the detoxification of quinones [24], and the enzyme purified from soybean (*Glycine max*) was shown recently by Rescigno *et al.* [25] to inhibit the autoxidation of 6-hydroxydopamine. In the presence of NADH, DT-diaphorase (0.4 μg/mL) inhibited the autoxidation of 6-hydroxydopamine (50 μM) by 37%; after consumption of the NADH, autoxidation of 6-hydroxydopamine resumed at the same rate as that observed in the absence of enzyme [25]. Rescigno *et al.* [25] concluded that DT-diaphorase may play an important role in neuroprotection by maintaining low concentrations of 6-hydroxydopamine and other toxic quinones (!).

In general, reducing agents, such as ascorbate or NAD(P)H and DT-diaphorase, may amplify cellular toxicity by reducing 6-hydroxydopamine. The reduced forms then recycle through oxidative reactions with molecular oxygen to increase the production of free radical intermediates. As shown in reactions 6 and 7, a paradoxical situation arises in which DT-diaphorase amplifies oxidative damage:

$$6\text{-}QH_2 + O_2 \rightarrow 6\text{-}Q + [O_2^{-\cdot}, OH^{\cdot}, H_2O_2] \quad (6)$$

$$6\text{-}Q + NAD(P)H \xrightarrow{\text{DT-diaphorase}} 6\text{-}QH_2 + NAD(P)^+ \quad (7)$$

## ENHANCEMENT OF 6-HYDROXYDOPAMINE OXIDATION

### *Ceruloplasmin*

Ceruloplasmin, a blue copper oxidase, catalyzes the four-electron reduction of $O_2$ by substrates without releasing $O_2^{-\cdot}$ or $H_2O_2$ as intermediates. Copper is bound to the enzyme with different coordinations that characterize three classes of sites: (i) type 1, or "blue," copper, which is responsible for an intense absorption band around 600 nm and is paramagnetic, with a coupling constant in the EPR spectrum that is unusually narrow, parallel, and hyperfine; (ii) type 2 copper, which does not contribute appreciably to the visible spectrum and whose EPR spectrum is typical of more regularly coordinated copper complexes; and (iii) type 3 copper, which is EPR silent and thought to consist of a pair of antiferromagnetically coupled Cu(II) ions [26, 27]. It has been proposed that the physiological function of ceruloplasmin includes mediating the oxidation of ferrous iron and its incorporation into apotransferrin [28].

Medda *et al.* [29] demonstrated that ceruloplasmin catalyzes the oxidation of 6-hydroxydopamine to the corresponding quinone. The rate of the spontaneous quinone formation from 0.4 mM 6-hydroxydopamine was increased by 100% in the presence of 0.11 μM ceruloplasmin. The kinetic parameters and pH dependence of 6-hydroxydopamine oxidation catalyzed by ceruloplasmin were similar to those observed with classical substrates of this enzyme. Ceruloplasmin-mediated oxidation of 6-hydroxydopamine, unlike spontaneous oxidation, did not result in appreciable production of $H_2O_2$. 6-Hydroxydopamine was the first natural substrate whose oxidation was shown to be catalyzed by ceruloplasmin, suggesting that this enzyme may protect neurons from 6-hydroxydopamine-induced damage by preventing the accumulation of toxic by-products.

### *Peroxidase*

Peroxidase (hydrogen donor oxidoreductase, EC 1.11.1.7), which catalyzes the oxidation of various substrates by $H_2O_2$ or substituted peroxides, is widely distributed and has been isolated from many higher plants [30]. Although the wide distribution of this enzyme suggests that it may perform an important function, its role is not clear because of the large number of reactions that it catalyzes and the existence of many isozymes [31].

Padiglia *et al.* [32] showed that horseradish peroxidase as well as other peroxidases facilitate the spontaneous oxidation of 6-hydroxydopamine to 6-hydroxydopamine quinone, and they suggested that this latter compound may function as a hydrogen donor for the enzyme. The $H_2O_2$ formed during the oxidation reaction is utilized by peroxidase for the oxidation of other 6-hydroxydopamine molecules. The rate of spontaneous quinone formation from 0.4 mM 6-hydroxydopamine was increased by 50% in the presence of horseradish peroxidase (95 ng/mL) [32]. Thus, peroxidase may serve to prevent the accumulation of $H_2O_2$ during the oxidation of 6-hydroxydopamine.

Catalase inhibits the spontaneous autoxidation of 6-hydroxydopamine, removing hydrogen peroxide [reaction (5)], while peroxidase increases this oxidation, accelerating reaction (5), using 6-hydroxydopamine as electron donor.

## CONCLUDING REMARKS

In light of our findings and in agreement with previous reports, we have emphasized that the neurotoxicity of 6-hydroxydopamine results from its spontaneous oxidation by molecular oxygen. The reactions of free radicals on biomolecules and tissues are important in physiology and pathology. The autoxidation of 6-hydroxydopamine is intimately involved with its destructive properties, and local concentrations of antioxidant proteins (i.e. superoxide dismutase, catalase, peroxidase, ceruloplasmin, DT-diaphorase) may regulate the degree of susceptibility of various tissues to this compound.

6-Hydroxydopamine cannot be used for the enzymatic determination of these proteins in crude extracts, since different amounts of superoxide dismutase, catalase, peroxidase, ceruloplasmin, and DT-diaphorase may interfere with each of these assays.

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