Biochemical Pharmacology, Vol. 55, pp. 2001–2006, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00075-6

Antioxidant Activity of Adrenergic Agents Derived from Catechol

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ABSTRACT. The ability of adrenergic catechol derivatives, including dobutamine, dopamine, and isoproterenol, to inhibit lipid peroxidation was examined. All the catechol derivatives we tested strongly inhibited lipid peroxidation. Dobutamine was a more powerful inhibitor of iron-catalyzed lipid peroxidation than the other agents, suggesting that part of the antioxidant activity of dobutamine is due to chelating iron. In addition, the catechol derivatives scavenged not only diphenylpicrylhydrazyl (DPPH) free radicals, but also 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cations and 2,2′-azobis-(2-amidinopropane)-dihydrochloride (AAPH) peroxyl radicals, indicating that the antioxidative activities of these agents are evidently due to scavenging free radicals. However, the rate constant of these catechol derivatives in scavenging hydroxyl radicals was $< 10^{10}$ M^{-1} sec^{-1}, suggesting that they may not protect against biological damage induced by hydroxyl radicals. BIOCHEM PHARMACOL **55**;12:2001–2006, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. antioxidant activity; dobutamine; dopamine; isoproterenol; lipid peroxidation; adrenergic drugs

Catechol derivatives, such as dopa and dopamine, produce reactive oxygens that cause tissue damage [1–7]. Dopa and dopamine in the presence of iron induce lipid peroxidation of rat liver microsomes [1, 2]. In addition, dopa, dopamine, and 3-*O*-methyldopa cause extensive oxidative DNA damage in the presence of copper traces and hydrogen peroxide [3]. Furthermore, Allen *et al.* [4] reported that catechol derivatives interact with iron and xanthine oxidase to produce highly reactive ($HO^{\cdot}$).† 6-Hydroxydopamine, which is a catechol derivative, is recognized as a neurotoxin because it causes degeneration of nerve terminals [5, 6]. These deleterious effects of catechol derivatives are associated with Parkinson's disease [7].

However, Liu and Mori [8] have demonstrated that monoamines, including catechol derivatives and serotonin, inhibit lipid peroxidation of brain homogenate, mitochondria, and microsomes catalyzed by iron, and they proposed that the monoamines act as excellent scavengers of free radicals to provide an antioxidative defense in the brain. Li *et al.* [9] also observed that dopa strongly suppresses lipid peroxidation of rat brain homogenate. Polyphenolic compounds, such as flavonoids [10], have a free radical scavenging activity that inhibits lipid peroxidation. We [11] reported that phenolic hydroxyl groups of the monoamines donate a proton to inhibit lipid peroxidation. However, the ability of catechol derivatives to inhibit lipid peroxidation varies with the substrate of lipid peroxidation used and depends on the mechanisms by which peroxidation is induced.

On the other hand, many catechol derivatives have been widely used as adrenergic drugs. However, their antioxidant activities have not been investigated. To understand the properties of catechol derivatives drugs, in this study we tried to evaluate these drugs as antioxidants, and found that dobutamine was a very strong inhibitor of lipid peroxidation.

MATERIALS AND METHODS

Chemicals

Dobutamine, dopamine, isoproterenol, metmyoglobin (from equine heart; Mb), and ABTS were obtained from the Sigma Chemical Co., and AAPH, ascorbic acid, and rose bengal were obtained from Wako Pure Chemical Industries Co. Ltd. Xantine oxidase (buttermilk) was purchased from Biozyme Laboratories Ltd.; TBA from the Merck Japan Co.; and *R*-phycoerythrin from Molecular Probes Inc. Other reagents were analytical-grade products from commercial suppliers.

Lipid Peroxidation

Rat liver microsomes were prepared by the method of Pederson and Aust [12]. Inhibition of lipid peroxidation was assayed as one measure of antioxidant activity. Peroxidation was induced by incubating microsomes with different substances. The formation of TBARS was determined as reported by Buege and Aust [13] with minor modifica-

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† *Abbreviations:* AAPH, 2,2′-azobis-(2-amidinopropane)-dihydrochloride; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); $ABTS^{\cdot +}$, ABTS radical cation; DOR, deoxyribose; DPPH, diphenyl-*p*-picrylhydrazyl; $HO^{\cdot}$, hydroxyl radicals; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; and TCA, trichloroacetic acid.

Received 9 May 1997; accepted 14 January 1998.

tions. The microsomal reaction system consisted of 0.1 mM of NADPH, 0.1 mM of ADP-Fe^{3+}, and microsomes (0.1 mg of protein/mL). The ascorbate-ADP-Fe^{3+} system consisted of 0.5 mM of ascorbate, 0.1 mM of ADP-Fe^{3+}, and microsomes (0.1 mg protein/mL), and the xanthine oxidase system contained 0.1 mM of hypoxanthine, 0.025 U of xanthine oxidase, microsomes (0.1 mg of protein/mL), and 0.1 mM of ADP-Fe^{3+}. The peroxidation reaction was performed in 10 mM of HEPES buffer containing 0.15 M of NaCl at pH 7.4. The AAPH system consisted of 10 mM of AAPH and microsomes (1.0 mg protein/mL) in 10 mM of HEPES buffer (pH 7.4) containing 0.15 M of NaCl. In the UV light system, quartz cells containing microsomes (0.1 mg of protein/mL) were placed on a Chromato-view transilluminator (Ultraviolet Product Inc.) for 1 h at room temperature. In the rose bengal system, test tubes containing microsomes (0.1 mg of protein/mL) and rose bengal (0.1 mM) were irradiated for 2 hr with a fluorescent lamp (40 W) at a distance of 20 cm at room temperature. Catechol-derived agents were added to the reaction mixtures before the start of the reaction. The peroxidation reaction was stopped by adding 1.0 mL of 30% TCA. The precipitate from microsomes was discarded after centrifuging for 10 min at 1500 *g*. The TBARS formation was assayed by measuring the absorbance at 535 nm after heating for 30 min at 100°, and the absorbance was expressed as nmol TBARS/mg protein using $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ [14]. Protein was measured by the bicinchoninic acid assay, using bovine serum albumin as a standard [15].

Scavenging Free Radicals

The ability of catechol derivatives to scavenge DPPH was measured by the method of Mellors and Tappel [16]. Radical cations [17] of ABTS ($ABTS^{\cdot +}$) were formed by interacting 150 μM of ABTS with 2.5 μM of activated metmyoglobin in 10 mM of phosphate buffer containing 0.15 M of NaCl at pH 7.4. Metmyoglobin (2.5 μM) was activated by adding 75 μM of H_2O_2. The ability of catechol derivatives to scavenge $ABTS^{\cdot +}$ was measured by the decrease in absorbance at 734 nm.

Fluorescence of R-Phycoerythrin

The loss of fluorescence of *R*-phycoerythrin was measured at 540-nm excitation and 575-nm emission [18]. The reaction mixture contained 17 nM of *R*-phycoerythrin, 40 mM of AAPH, and 20 μM of catechol derivatives in 75 mM of phosphate buffer (pH 7.0).

Deoxyribose Degradation

DOR is attacked by $HO^{\cdot}$ to form a product that reacts upon heating with TBA at low pH to form a chromogen [19]. The reaction mixture consisted of 1.0 mM of ascorbic acid, 0.1 mM of EDTA-Fe^{3+}, and 2.8 mM of DOR in 10 mM of phosphate buffer (pH 7.4) containing 0.15 M of NaCl. Catechol derivatives were added to the reaction mixture before the start of the reaction. The reaction was stopped by adding 30 μL of 30% TCA, and then 1.0 mL of 0.6% TBA was added to the reaction mixture. After heating for 8 min at 100°, the TBARS were determined by measuring the absorbance at 532 nm. The rate constant of catechol derivatives was calculated by the method of Halliwell *et al.* [20]. The DOR degradation in the presence of various concentrations of catechol derivatives was measured. The reaction rate of DOR is given by:

$$HO^{\cdot} = K_{DOR}[HO^{\cdot}][DOR]$$

The reaction rate of catechol derivative (CAT) is given by:

$$HO^{\cdot} = K_{CAT}[HO^{\cdot}][CAT]$$

Absorbance (A) obtained at the end of the experiment, taken as a measure of the rate of reaction, is given by:

$$A = K_{DOR}[HO^{\cdot}][DOR]$$

Absorbance (A_0), as a measure of the rate obtained in the absence of the catechol derivative, is given by:

$$A_0 = K_{DOR}[HO^{\cdot}][DOR] + K_{CAT}[HO^{\cdot}][CAT]$$

These equations can be combined to give:

$$1/A = 1/A_0(1 + K_{CAT}[CAT]/K_{DOR}[DOR])$$

where A is the absorbance in the presence of catechol derivatives at concentration [CAT] and A_0 is the absorbance in the absence of catechol derivatives. A plot of $1/A$ against [CAT] gave a straight line of slope $K_{CAT}/K_{DOR}[DOR]A_0$ with an intercept on the y-axis of $1/A_0$, and the rate constant for the reaction of catechol derivative with $HO^{\cdot}$ can be obtained from the slope of the line. The value of K_{DOR} used in this study was $3.1 \times 10^9\ M^{-1}\ sec^{-1}$ as reported by Halliwell *et al.* [20].

RESULTS

Inhibition of Lipid Peroxidation by Catechol-derived Agents

Figure 1 shows the structures of catechol derivatives and adrenergic agents used in this study. The inhibition of lipid peroxidation by adrenergic agents derived from catechol, including dobutamine, dopamine, and isoproterenol, was examined. Figure 2 shows that the catechol derivatives strongly inhibited the ADP–Fe^{3+}- and the NADPH-dependent microsomal lipid peroxidation measured by TBARS formation. The IC_{50} values of dobutamine, isoproterenol, and dopamine were approximately 4, 50, and 60 μM, respectively. Dobutamine inhibited the microsomal lipid peroxidation more strongly than dopamine and isoproterenol. The adrenergic drugs metaproterenol and terbutaline,

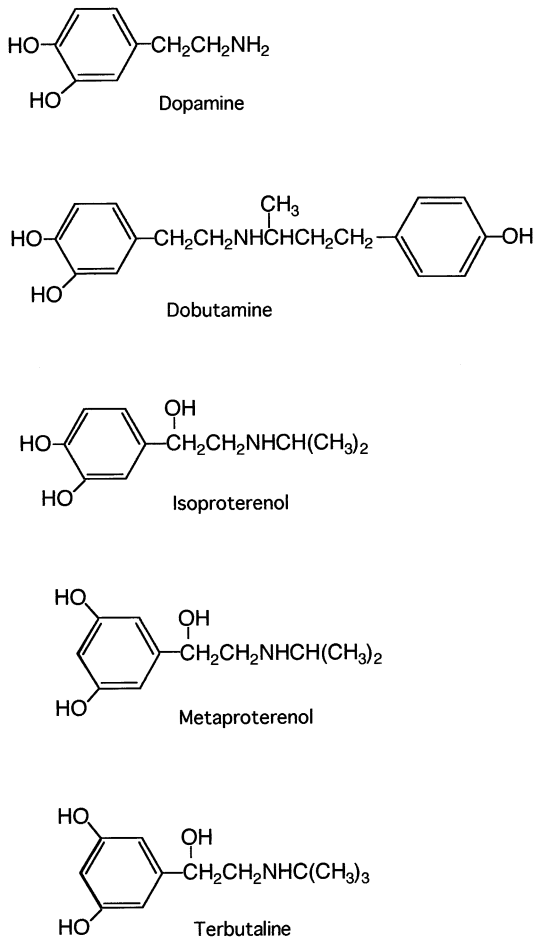

FIG. 1. Structure of adrenergic agents.

which have two hydroxyl groups at the meta position (Fig. 1), showed no activity in inhibiting lipid peroxidation (data not shown).

The ability of an antioxidant to inhibit lipid peroxidation varies with the mechanism by which peroxidation is induced. Therefore, the activity of the catechol derivatives to inhibit lipid peroxidation was examined further by using various reaction systems. In the reaction system of ascorbate–Fe^{3+} and xanthine-xanthine oxidase in the presence of ADP–Fe^{3+}, iron catalyzes the peroxidation reaction. Dobutamine, dopamine, and isoproterenol strongly inhibited the iron-catalyzed lipid peroxidations. The IC_{50} value of dobutamine was low, at about one order of magnitude over that of dopamine and isoproterenol (Table 1). Metaproterenol and terbutaline had no effect on the iron-catalyzed peroxidations (data not shown).

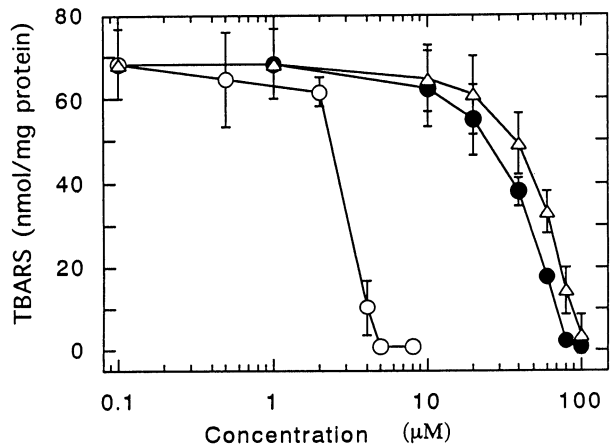

FIG. 2. Inhibition of microsomal lipid peroxidation by catechol derivatives. After incubation for 5 min, TBARS were measured as described in Materials and Methods. Each value represents the mean ± SD of five experiments. Key: (○) dobutamine; (●) dopamine; and (△) isoproterenol.

TABLE 1. Inhibition of various lipid peroxidations by catechol derivatives

Systems	Dobutamine	Dopamine	Isoproterenol
		IC_{50} (μM)	
Microsome	3.8 ± 1.0	45.8 ± 3.2	63.4 ± 6.8
Ascorbate–ADP–Fe^{3+}	2.4 ± 0.7	52.4 ± 9.3	137.8 ± 42.1
Xanthine oxidase	1.4 ± 0.4	30.8 ± 2.1	61.0 ± 3.4

After incubation for 5 min in the microsome system and for 30 min in the xanthine oxidase and ascorbate systems, the amount of TBARS formed was measured as described in Materials and Methods. Each value represents the mean ± SD of five experiments.

Polyphenols often chelate iron to inhibit iron-catalyzed lipid peroxidation [1]. However, some workers have reported that the dopa– or dopamine–iron complex causes lipid peroxidation [2, 9]. We confirmed that dopa–Fe^{3+} weakly induces microsomal lipid peroxidation, that dopa–ADP–Fe^{3+} does not, and that dobutamine– and isoproterenol–ADP–Fe^{3+} does not induce membrane lipid peroxidation.

We examined whether catechol derivatives inhibited the lipid peroxidations induced by AAPH, UV light, or rose bengal. In the presence of oxygen, AAPH decomposes to generate stable peroxyl radicals, which initiate lipid peroxidation [21]. Table 2 summaries the results. Dobutamine, dopamine, and isoproterenol inhibited AAPH-induced lipid peroxidation, and the order of inhibition was: dobutamine > dopamine > isoproterenol. Of interest, however, is the finding that dobutamine, compared with dopamine and isoproterenol, was not an effective inhibitor of AAPH-induced lipid peroxidation. The catechol derivatives also inhibited the photodynamic lipid peroxidation induced by

TABLE 2. Inhibitory effect of catechol derivatives on lipid peroxidation induced by AAPH or photodynamically

Systems	Dobutamine	Dopamine	Isoproterenol
		IC_{50} (μM)	
AAPH	1.1 ± 0.1	3.9 ± 1.1	5.9 ± 1.4
UV	1.0 ± 0.1	1.4 ± 0.6	3.3 ± 1.3
Rose bengal	5.5 ± 3.4	7.7 ± 3.3	77.2 ± 6.3

The drugs of catechol derivatives were added before the start of the peroxidant reaction. After incubation for 1 hr in the AAPH system or illumination for 1 hr under the UV system or 2 hr in the rose bengal system, the amount of TBARS formed was determined as described in Materials and Methods. Each value represents the mean ± SD of five experiments.

TABLE 3. Scavenging of DPPH and ABTS $\cdot^{+}$ radical cations by catechol derivatives

Additions	DPPHM $-\Delta A_{517\ nm/min}$	ABTS $\cdot^{+}$ $-A_{734\ nm/5\ min}$
Dobutamine	0.250	0.842
Dopamine	0.240	0.575
Isoproterenol	0.210	0.547
Metaproterenol	0.000	0.557
Terbutaline	0.000	0.599

Adrenergic agents (10 μM) were added to ethanolic DPPH (100 μM) solution. The decrease in DPPH absorbance at 517 nm after 1 min was measured. ABTS $\cdot^{+}$ were produced as described in Materials and Methods, and then the adrenergic agents were added to the reaction mixture. After 5 min, the decrease in ABTS $\cdot^{+}$ at 734 nm was measured. Each value represents the mean of three experiments. The variation was less than 10%

rose bengal and UV light. A little difference was observed between these agents in inhibiting the photodynamic lipid peroxidations. Only isoproterenol had little effect on the inhibition of lipid peroxidation induced by rose bengal under visible light; metaproterenol and terbutaline had no effect on the lipid peroxidations induced by AAPH, UV light, and rose bengal (data not shown).

Ability to Scavenge Free Radicals

We examined the ability of catechol derivatives to scavenge free radicals, and Table 3 summarizes the results. Dobutamine, dopamine, and isoproterenol decolorized the solution of stable DPPH. The ABTS $\cdot^{+}$ were produced by reacting metmyoglobin with H_2O_2 [17], and the scavenging capacity of the catechol derivatives was also tested. The catechol derivatives decolorized the solution of ABTS $\cdot^{+}$. These results indicate that dobutamine, dopamine, and isoproterenol scavenge DPPH and ABTS $\cdot^{+}$. Dobutamine was a more powerful scavenger of ABTS $\cdot^{+}$ than dopamine and isoproterenol. Metaproterenol and terbutaline did not react with DPPH, but scavenged ABTS $\cdot^{+}$.

Glazer [18] assessed the ability of various compounds to react with peroxyl radicals by monitoring the degradation of *R*-phycoerythrin. Figure 3 shows that the fluorescent degradation of *R*-phycoerythrin induced by AAPH was inhibited by the adrenergic agents. Catechol derivatives evidently scavenged, and metaproterenol also reacted with, the peroxyl radicals. Terbutaline caused little inhibition of the fluorescent degradation of *R*-phycoerythrin. These results indicate that catechol derivatives and metaproterenol scavenge AAPH peroxyl radicals.

Ability to Scavenge Hyroxyl Radicals

Among oxygen radicals, HO$^{\cdot}$ is extremely reactive to biological components and can be detected by its ability to degrade deoxyribose into fragments that generate pink chromogen upon heating with TBA at a low pH [19]. All adrenergic agents tested competitively inhibited deoxyribose degradation, suggesting that they are scavengers of

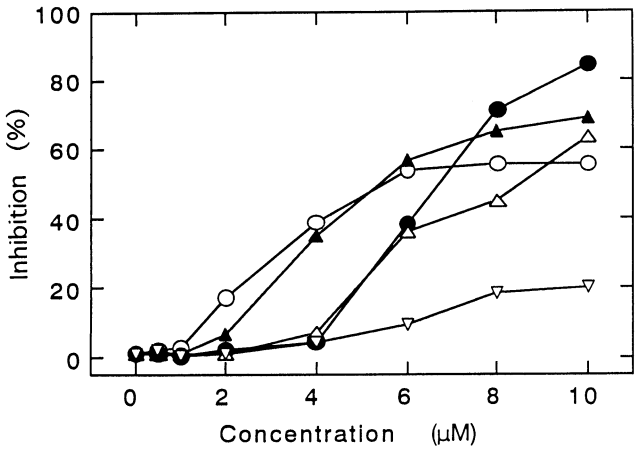

FIG. 3. Inhibition of fluorescent degradation of *R*-phycoerythrin by adrenergic agents. Fluorescent degradation of *R*-phycoerythrin caused by AAPH was measured and described in Materials and Methods. *R*-Phycoerythrin was almost degraded by incubation with 40 mM AAPH for 30 min. The fluorescence emission value of *R*-phycoerythrin was about 0.40 before the reaction with AAPH. Each point represents the mean of three experiments. Key: (○) dobutamine; (●) dopamine; (△) isoproterenol; (▲) metaproterenol; and (▽) terbutaline.

HO$^{\cdot}$. However, the rate constant of the catechol derivatives scavenging HO$^{\cdot}$ was $<10^{10}\ M^{-1}\ sec^{-1}$, indicating that dobutamine, dopamine, and isoproterenol were poor scavengers of HO$^{\cdot}$ (Table 4). Metaproterenol scavenged HO$^{\cdot}$ to the same extent as dobutamine, and terbutaline reacted more rapidly with HO$^{\cdot}$ than the catechol derivatives.

DISCUSSION

This study demonstrated that catechol derivatives, including dobutamine, dopamine, and isoproterenol, are efficient inhibitors of lipid peroxidation. A high inhibiting efficiency of dobutamine compared with isoproterenol and dopamine was found in iron-catalyzed lipid peroxidations. In contrast, only a little difference was observed between dobutamine, dopamine, and isoproterenol in the efficiency to inhibit lipid peroxidation induced photodynamically or by AAPH. No iron was involved in these reaction systems, suggesting that a part of the capacity of dobutamine to

TABLE 4. Rate constant of adrenergic agents to hydroxyl radicals

Additions	Rate constant for reaction with HO$^{\cdot}$ ($10^{10}\ M^{-1}\ sec^{-1}$)
Dobutamine	0.93 ± 0.20
Dopamine	0.35 ± 0.04
Isoproterenol	0.33 ± 0.08
Metaproterenol	0.91 ± 0.12
Terbutaline	1.30 ± 0.25

DOR degradation was measured by the generation of pink chromogen by heating with TBA at low pH. The rate constant was calculated as described in Materials and Methods. Each value represents the mean ± SD of five experiments.

inhibit lipid peroxidation was due to the ability to chelate iron involved in the peroxidation reaction. Lipid peroxidation has been suggested to be initiated by an iron–oxygen complex, such as Fe^{2+}–O_2–Fe^{3+} [22]. Dobutamine may act as an inhibitor of iron–oxygen complex generation.

The DPPH radical assay measures the most common natural antioxidants [23], especially phenols, such as vitamin E and hydroquinone [24]. Dobutamine, dopamine, and isoproterenol rapidly decreased DPPH and the $ABTS^{\cdot +}$ radical cations. In addition, fluorescent degradation of *R*-phycoerythrin by AAPH was inhibited. Evidently, these agents scavenge free radicals to inhibit lipid peroxidation. The high efficiency of catechol derivatives to scavenge free radicals may be due to the presence of hydrogen-donating hydroxyl groups in their molecules. However, adrenergic drugs, including metaproterenol and terbutaline, which have two phenolic hydroxyl groups at the meta position, caused little inhibition of lipid peroxidation. Although the data are not shown, we tested the scavenging abilities of catechol, hydroquinone, and resorcin on DPPH. The order of scavenging ability was catechol $\gg$ hydroquinone. Resorcin did not decolorize DPPH solution, indicating that it did not scavenge DPPH. These findings suggest that efficient inhibition of lipid peroxidation requires hydroxyl groups at the ortho position. Metaproterenol inhibited the fluorescent degradation of *R*-phycoerythrin, indicating that it scavenges AAPH peroxyl radicals. However, metaproterenol did not show inhibition of lipid peroxidations. Presumably, metaproterenol has a different reactivity between lipid peroxyl and AAPH peroxyl radicals.

Although catechol derivatives reacted with $HO^{\cdot}$, the rate constant was $<10^{10}\ M^{-1}\ sec^{-1}$. This value seems to be too low to protect most biological molecules from $HO^{\cdot}$. At least catechol derivatives did not effectively scavenge $HO^{\cdot}$. Some workers reported that catechol derivatives promote the induction of biological damage, such as DNA strand breaks and lipid peroxidation [25]. Li and Trush [26] suggested that the phenolic hydroxyl groups contribute to DNA damage induced by reactive oxygens generated by Cu^{2+} in the presence of H_2O_2. We confirmed that dobutamine and dopamine induce deoxyribose degradation in the presence of Cu^{2+} (5 μM) and that DNA strand breaks were also caused by dopamine in the presence of Cu^{2+} and H_2O_2 (data not shown). However, lipid peroxidation by Cu^{2+}–H_2O_2 was not inhibited by these catechol derivatives. Presumably, the DNA damage may be caused by $HO^{\cdot}$ generated site-specifically via oxidation of dopamine by a copper–redox cycle.

Barclay *et al.* [27] suggested that a hydrophilic chain-breaking antioxidant may have a therapeutic effect in the treatment of disorders, such as inflammation, that are suspected to involve lipid peroxidation. All catechol derivatives used in this study are hydrophilic agents. In many lipid peroxidations, iron mediates the peroxidation reaction. The data of this study suggest the possibility that among the catechol derivatives dobutamine may be used as an antioxidant drug for ischemia and reperfusion cellular injury.

References

1. Yagi K, Ishida N, Kodama S and Ohishi N, Induction of lipid peroxidation in rat liver microsomes by catecholamine–iron complex. *J Clin Biochem Nutr* **9:** 179–184, 1990.
2. Sotomatsu A, Nakano M and Hirai S, Phospholipid peroxidation induced by catechol–Fe^{3+} (Cu^{2+}) complex: A possible mechanism of nigrostriatal cell damage. *Arch Biochem Biophys* **283:** 334–341, 1990.
3. Spencer JPE, Jenner A, Aruoma Ol, Evans PJ, Kaur H, Dexter DT, Jenner P, Lees AJ, Marsden DC and Halliwell B, Intense oxidative DNA damage promoted by L-DOPA and its metabolites. Implications for neurodegenerative disease. *FEBS Lett* **353:** 246–250, 1994.
4. Allen DR, Wallis GL and McCay PB, Catechol adrenergic agents enhance hydroxyl radical generation in xanthine oxidase systems containing ferritin: Implications for ischemia/reperfusion. *Arch Biochem Biophys* **315:** 235–243, 1994.
5. Cohen G, Heikkila RE, Allis B, Cabbat F, Dembiec D, MacNamee D, Mytillneou C and Winston B, Destruction of sympathetic nerve terminals by 6-hydroxydopamine: Protection by 1-phenyl-3-(2-thiazolyl)-2-thiourea, diethyldithiocarbamate, methimazole, cysteamine, ethanol and *n*-butanol. *J Pharmacol Exp Ther* **199:** 336–352, 1976.
6. Sullivan SG, Sudarsky L and Stern A, Potentiating effect of catalase on inhibition by superoxide dismutase of 6-hydroxydopamine autoxidation. In: *Chemical and Biochemical Aspects of Superoxide and Superoxide Dismutase* (Eds. Bannister JV and Hill HAO), pp. 364–371. Elsevier, North-Holland, 1980.
7. Bindoli A, Rigobello MP and Deeble DJ, Biochemical and toxicological properties of the oxidation products of catechol amine. *Free Radic Biol Med* **13:** 391–405, 1992.
8. Liu J and Mori A, Monoamine metabolism provides an antioxidant defense in the brain against oxidant- and free radical-induced damage. *Arch Biochem Biophys* **302:** 118–127, 1993.
9. Li CL, Werner P and Cohen G, Lipid peroxidation in brain: Interaction of L-dopa/dopamine with ascorbate iron. Neurodegeneration **4:** 147–153, 1995.
10. Rice-Evans CA, Miller NJ and Paganga G, Structure–antioxidant activity relationship of flavonoids and phenolic acids. *Free Radic Biol Med* **20:** 933–956, 1996.
11. Miura T, Muraoka S and Ogiso T, Lipid peroxidation inhibited by monoamines. *Res Commun Mol Pathol Pharmacol* **93:** 57–67, 1996.
12. Pederson TC and Aust SD, Aminopyrine demethylase: Kinetic evidence for multiple microsomal activities. *Biochem Biophys Res Commun* **19:** 2221–2230, 1970.
13. Buege JA and Aust SD, Microsomal lipid peroxidation. *Methods Enzymol* **52:** 302–310, 1978.
14. Gonzalez Flecha BS, Repetto M, Evelson P and Boveris A, Inhibition of microsomal lipid peroxidation by α-tocopherol and α-tocopherol acetate. *Xenobiotica* **21:** 1013–1022, 1991.
15. Redinaugh MG and Turley RB, Adaptation of the bicinchoninic acid protein assay for use with microtiter plates and sucrose gradient fraction. *Anal Biochem* **153:** 267–271, 1986.
16. Mellors A and Tappel AL, The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. *J Biol Chem* **241:** 4353–4356, 1966.
17. Rice-Evans C and Miller NJ, Total antioxidant status in plasma and body fluids. *Methods Enzymol* **234:** 279–293, 1994.
18. Glazer AN, Phycoerythrin fluorescence-based assay for reactive oxygen species. *Methods Enzymol* **186:** 161–168, 1990.
19. Halliwell B and Gutteridge JMC, Formation of a thiobarbi-

turic-acid-relative substance from deoxyribose in the presence of iron salts. The role of superoxide and hydroxyl radicals. *FEBS Lett* **128:** 347–352, 1981.
20. Halliwell B, Gutteridge JMC and Auroma OL, The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* **165:** 215–219, 1987.
21. Miki M, Tamai H, Mino M, Yamamoto Y and Niki E, Free-radical chain oxidation of rat red blood cells by molecular oxygen and its inhibition by α-tocoperol. *Arch Biochem Biophys* **258:** 373–380, 1987.
22. Minotti G and Aust SD, The role of iron in oxygen radical mediated lipid peroxidation. *Chem Biol Interact* **71:** 1–19, 1989.
23. Blois MS, Antioxidant determinations by the use of a stable free radical. *Nature* **181:** 1199–1200, 1958.
24. Morel I, Lescoat G, Cillard P and Cillard J, Role of flavonoids and iron chelation in antioxidant action. *Methods Enzymol* **234:** 437–443, 1994.
25. Li Y and Trush MA, Oxidation of hydroquinone by copper: Chemical mechanism and biological effects. *Arch Biochem Biophys* **300:** 346–355, 1993.
26. Li Y and Trush MA, Reactive oxygen-dependent DNA damage resulting from the oxidation of phenolic compounds by a copper-redox cycle mechanism. *Cancer Res* **54:** 1895–1898, 1994.
27. Barclay LRC, Locke SJ, MacNeil JM, VanKessel J, Burton GW and Ingold KU, Autoxidation of micelles and model membranes. Quantitative kinetic measurements can be made by using either water-soluble or lipid-soluble initiators with water-soluble or lipid-soluble chain-breaking antioxidants. *J Am Chem Soc* **106:** 2479–2481, 1984.