Electron Paramagnetic Resonance Study on Free Radical Scavenging and/or Generating Activity of Dopamine-4-*O*-sulfate

Kazuyoshi KIRIMA,*[a] Koichiro TSUCHIYA,[a] Masanori YOSHIZUMI,[a] Taisuke KAMEDA,[a] Hitoshi HOUCHI,[b] Mami AZUMA,[b] and Toshiaki TAMAKI[a]

Department of Pharmacology[a] and Department of Pharmacy,[b] The University of Tokushima School of Medicine, Tokushima 770–8503, Japan. Received November 24, 2000; accepted February 16, 2001

The free radical scavenging and/or generating activity of dopamine-4-*O*-sulfate was examined and compared with that of dopamine. In humans, dopamine mostly exists in two isomeric forms of sulfate ester conjugates as metabolites; *i.e.*, dopamine-3-*O*-sulfate and dopamine-4-*O*-sulfate in the circulation. Dopamine is generally believed to be oxidized by molecular oxygen or another reactive oxygen species under physiological conditions, to form oxidized dopamine derivatives that are cytotoxic. However, it is not known whether dopamine conjugates are generated on interaction with reactive oxygen species or not. In the present study, we measured the susceptibility to oxidization of dopamine-4-*O*-sulfate by using electron paramagnetic resonance (EPR) spectroscopy and optical absorption spectrometry. Dopamine was easily oxidized and dopamine-derived radicals appeared, whereas dopamine-4-*O*-sulfate was not oxidized under physiological conditions. Furthermore, dopamine-4-*O*-sulfate did not react with a strong oxidizing agent, sodium periodate. These results suggest that dopamine-4-*O*-sulfate has resistance against autoxidation, and seems to be a stable metabolite of dopamine.

Key words dopamine-4-*O*-sulfate; dopamine; electron paramagnetic resonance; free radical; oxidation

With the increasing understanding of the cytotoxicity of free radicals and other reactive oxygen species as a major cause of human disease and aging, the roles of endogenous antioxidants have drawn attentions. Uric acid,[1] vitamin E,[2] vitamin C,[3] ubiquinone,[4] SH-compounds,[5] bilirubin,[6] and pyruvate[7] are recognized as endogenous non-enzymatic antioxidants, while catalase, superoxide dismutase and glutathione peroxidase are known to be enzymatic antioxidants.

Dopamine is the immediate metabolic precursor of norepinephrine and epinephrine. Dopamine interacts with dopamine receptors, then exerts its pharmacological effects.

In human and experimental animals, dopamine is present almost entirely as a sulfate conjugated form in the circulation,[8,9] and very little of the free form is presented in the plasma.[10] The physiological roles of sulfate conjugated dopamine are still unclear. Pharmacologically, conjugated dopamine has convulsive effects,[11] inhibits aldosterone secretion from cultured bovine adrenal cells,[12] and induces a constriction of some vascular beds.[13] One study, however, found that sulfate conjugated dopamine has no physiological activity.[14]

From the viewpoint of free radical chemistry, dopamine can serve electrons *via* its oxidation pathway (Chart 1). This enables dopamine to act as both an antioxidant and a prooxidant. It acts as an antioxidant by scavenging free radicals.[15—17] Recently, it was found that dopamine spontaneously react with molecular oxygen to produce reactive oxygen species and semiquinone dopamine radical,[18,19] which are toxic to catecholamine neurons.[20—23]

However, there is no evidence that sulfate conjugated dopamine acts as an antioxidant or a prooxidant. Therefore, in the present report, we compared dopamine and dopamine-4-*O*-sulfate for susceptibility to enzymatic and non-enzymatic oxidization *in vitro* using alkaline solution, horseradish peroxidase, UV irradiation, hydroxyl radical, superoxide anion radical, and sodium periodate.

Experimental

Chemicals Dopamine, hypoxanthine, xanthine oxidase (from butter milk), and peroxidase (from horseradish) were purchased from Sigma Chemical Co., (MO, U.S.A.), hydrogen peroxide from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and sodium metaperiodate from Acros Organics (NJ, U.S.A.). Dopamine-4-*O*-sulfate was kindly donated by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Other reagents were of the highest quality available from Wako Pure Chemical Industries Ltd.

Free Radical Analysis Using EPR Spectroscopy The free radical metabolites of dopamine and dopamine-4-*O*-sulfate were examined with an EPR spectrometer (JES-TE 300, JEOL Co., Ltd., Tokyo, Japan) equipped with a cavity and an aqueous quartz flat cell (JEOL Co., Ltd., Tokyo, Japan) at X-band (9.5 GHz). EPR data were analyzed by a computer (HP Apollo 9000 Series 400) with the software, ESPRIT 432 (JEOL Co., Ltd., Tokyo, Japan). Typical operating conditions of the EPR spectrometer were: power, 5 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.1 G; time constant, 0.03 s; sweep time, 1 min; temperature, 20 °C. Hyperfine coupling constants and spectral simulations were obtained with a computer program, Winsim.[24] Because of the short lives of radical species, a fast-flow method[25] was adopted for both Fenton[26] and horseradish peroxidase system.

UV Irradiation Experiment[27] Samples were irradiated with UV during passage through the quartz flat cell attached to the EPR cavity. The degassed (N_2 purge, 15 min) reaction mixture was slowly flowed through the cell (flow rate *ca.* 1 ml/min) to minimize the depletion of starting materials and buildup of light-absorbing materials. The irradiation source was 300-watt xenon arc light (Ushio, Tokyo, Japan).

Visible-UV Absorption Spectra Visible and UV absorption spectra were measured using a model 330 spectrophotometer (Hitachi Co., Tokyo, Japan). The spectrometer was operated between 250 nm and 600 nm at 25 °C. In the reference cell, 100 mM phosphate buffer (pH 7.4) was placed. Sample solutions consisted of 100 mM phosphate buffer (pH 7.4), 0.1 mM dopamine or dopamine-4-*O*-sulfate, and 0.2 mM sodium periodate.

Results

Effects of Alkaline on the Formation of Dopamine-Derived Radicals When dopamine (0.1 M) was mixed with a 1 M sodium hydroxide solution under aerobic conditions (pH 12—13), two kinds of EPR signals were detected with time (Figs. 1A, D), *i.e.*, primary and secondary dopamine radical.[19,28] The hyperfine coupling constants for the primary EPR signal (Fig. 1A: a_2^H=0.27 G, a_6^H=3.73 G, a_5^H=0.90 G, a_β^H=3.12 G) (Table 1) were very similar to those of reported

* To whom correspondence should be addressed. e-mail: kirima@basic.med.tokushima-u.ac.jp

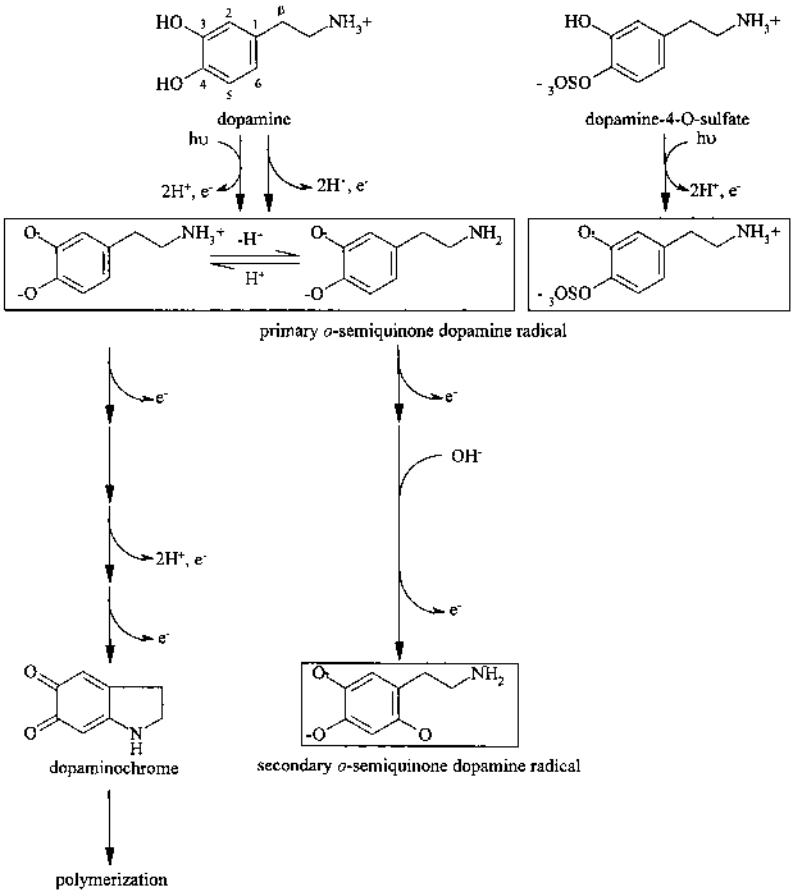

Chart 1. Proposed Oxidation Pathway of Dopamine and Dopamine-4-*O*-sulfate

for the autoxidation of dopamine at high pH.[19] Therefore, this radical was identified as a primary *o*-semiquinone dopamine radical.[19,28]

After 10 min incubation, the secondary *o*-semiquinone radical (Fig. 1D) appeared with the decrease of primary radical (Fig. 1C). The hyperfine splitting constants of this radical (a_2^H=0.56 G, a_5^H=0.84 G, a_β^H=3.31 G) (Table 1) corresponded to those of 6-O^- substituted *o*-semiquinone dopamine radical[29] obtained on nucleophilic attack of OH^- of the 6th position of dopamine *o*-quinone at high pH.[19]

On the other hand, dopamine-4-*O*-sulfate gave no EPR signal under these experimental conditions (Figs. 1G—I).

Effects of UV Irradiation on the Formation of Dopamine-Derived Radicals When dopamine was UV irradiated under anaerobic conditions, an apparent EPR signal was observed (Fig. 2A). The EPR parameters of this spectrum were a_2^H=0.43 G, a_6^H=3.57 G, a_5^H=0.94 G, and a_β^H=2.96 G (Table 1), identical to those of primary *o*-semiquinone dopamine radical.[19,28] No EPR signal was observed in the absence of dopamine (Fig. 2C). Dopamine-4-*O*-sulfate showed a weak EPR signal on UV irradiation under the same conditions (Fig. 2D).

Effects of the Horseradish Peroxidase-Hydrogen Peroxide System on the Formation of Dopamine-Derived Radicals When dopamine was mixed with horseradish peroxidase (40 nM) and hydrogen peroxide (200 μM) as an enzymatic oxidant, apparent EPR parameters (a_2^H=0.45 G, a_6^H=3.61 G, a_5^H=0.94 G, a_β^H=2.98 G) (Table 1) were observed in the complete system (Fig. 3A). And these parameters were

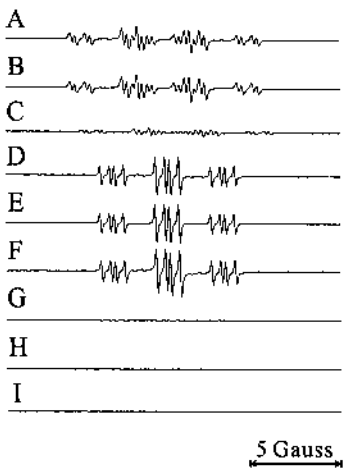

Fig. 1. Changes of EPR Spectra of Dopamine (A, C, D and F) and Dopamine-4-*O*-sulfate (G—I) in 1 M Sodium Hydroxide

A: 0.1 M dopamine in 1 M sodium hydroxide solution for 0.5 min incubation. B: computer simulation of spectrum A with proton hyperfine splitting constants of 0.27, 3.73, 0.90, and 3.12 G. C: same as A but for 5 min incubation. D: same as A but for 10 min incubation. E: computer simulation of spectrum D with proton hyperfine splitting constants of 0.56, 0.84, and 3.31 G. F: same as A but for 30 min incubation. G: 0.1 M dopamine-4-*O*-sulfate in 1 M sodium hydroxide solution for 0.5 min incubation. H: same as G but for 10 min incubation. I: same as G but for 30 min incubation. All reactions were carried out in air-saturated solution. Operating conditions of EPR were: power, 5 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.1 G; time constants, 0.03 s; sweep time, 1 min; temperature, 20 °C; receiver gain, 100.

Table 1. Electron Paramagnetic Resonance Parameters for Dopamine Derivatives

Parent compounds	Radical compounds	Hyperfine splitting constants				References
		a_2^H	a_5^H	a_6^H	a_β^H	
Dopamine	*o*-Semiquinone	0.27	0.89	3.73	3.13	Alkaline condition[19]
		0.28	0.91	3.70	3.11	pH 11[28]
		0.27	0.90	3.73	3.12	This work (Fig. 1)
		0.46	0.94	3.58	2.98	pH 7[28]
		0.43	0.94	3.57	2.96	This work (Fig. 2)
		0.45	0.94	3.61	2.98	This work (Fig. 3)
		0.47	0.92	3.57	3.01	This work (Fig. 4)
		0.45	0.93	3.56	2.98	This work (Fig. 5)
	6-OH-*o*-semiquinone	0.56	0.86	—	3.31	Alkaline condition[19]
		0.58	0.87	—	3.24	pH 11[28]
		0.56	0.84	—	3.31	This work (Fig. 1)
Dopamine-4-*O*-sulfate		0.43	0.94	3.57	2.96	This work (Fig. 2)

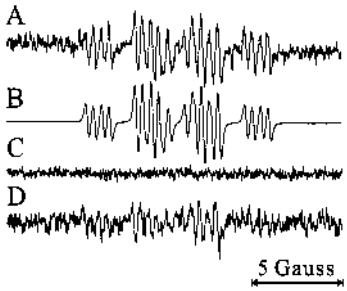

Fig. 2. EPR Spectra for Dopamine and Dopamine-4-*O*-sulfate Following UV Irradiation

A: radicals from photooxidation of 5 mM dopamine in 0.1 M phosphate buffer (pH 7.4). B: computer simulation of spectrum A with proton hyperfine splitting constants of 0.43, 3.57, 0.94, and 2.96 G. C: same as A but without dopamine. D: radicals from photooxidation of 5 mM dopamine-4-*O*-sulfate in 0.1 M phosphate buffer (pH 7.4). Operating conditions for EPR were: power, 5 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.2 G; time constants, 0.03 s (A, D), 0.1 s (C); sweep time, 15 min (A, D), 4 min (C); temperature, 20 °C; receiver gain, 10000 (A, D), 2000 (C). All spectra were measured under UV irradiation (300 watt).

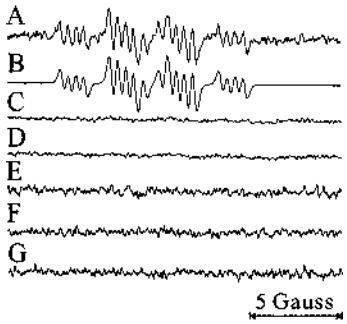

Fig. 3. EPR Spectra of the Reaction of Dopamine or Dopamine-4-*O*-sulfate with the Horseradish Peroxidase/Hydrogen Peroxide System

A: radicals from 5 mM dopamine in 0.1 M phosphate buffer (pH 7.4) with horseradish peroxidase/hydrogen peroxide. B: computer simulation of spectrum A with proton hyperfine splitting constants of 0.45, 3.61, 0.94, and 2.98 G. C: same as A but without hydrogen peroxide. D: same as A but without horseradish peroxidase. E: 5 mM dopamine-4-*O*-sulfate in 0.1 M phosphate buffer (pH 7.4) with horseradish peroxidase/hydrogen peroxide. F: same as E but without hydrogen peroxide. G: same as E but without horseradish peroxidase. Concentrations of horseradish peroxidase and hydrogen peroxide were 40 nM and 200 μM, respectively. Horseradish peroxidase and hydrogen peroxide were separately introduced into a flat cell by a modified fast-flow method. Operating conditions for EPR were: power, 20 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.2 G (A, B, E—G), 0.5 G (C, D); time constants, 0.1 s; sweep time, 1 min; temperature, 20 °C; receiver gain, 1250 (A, E—G), 2000 (C, D).

the same as those of the primary *o*-semiquinone dopamine radical.[19,28] No EPR signal appeared in the absence of hydrogen peroxide (Fig. 3C) or horseradish peroxidase (Fig. 3D) from the complete system. Dopamine-4-*O*-sulfate showed no EPR signal under these experimental conditions (Figs. 3E—G).

Effects of the Hypoxanthine–Xanthine Oxidase System on the Formation of Dopamine-Derived Radicals When dopamine was mixed with hypoxanthine and xanthine oxidase as a superoxide anion radical generating system, an apparent EPR signal (a_2^H=0.47 G, a_6^H=3.57 G, a_5^H=0.92 G, a_β^H=3.01 G) was observed (Fig. 4B). This radical was consistent with the primary *o*-semiquinone dopamine radical.[19,28] However, dopamine-4-*O*-sulfate showed no EPR signal under the experimental conditions (Fig. 4D).

Effects of Hydroxyl Radical on the Formation of Dopamine-Derived Radicals Because of the short lives of the hydroxyl radicals generated by the Fenton system,[26] we adopted a fast-flow method. As shown in Fig. 5A, a radical from dopamine (a_2^H=0.45 G, a_6^H=3.56 G, a_5^H=0.93 G, a_β^H=2.98 G) (Table 1) was detected after dopamine was mixed with hydrogen peroxide and ferrous. This radical was similar to the primary *o*-semiquinone dopamine radical.[19,28] In the absence of ferrous, no EPR signal was observed (Fig.

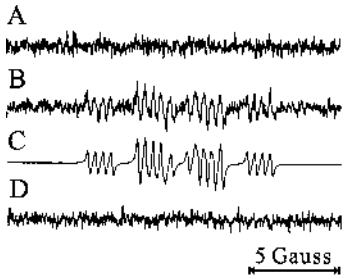

Fig. 4. EPR Spectra of the Reaction of Dopamine or Dopamine-4-*O*-sulfate with the Superoxide Anion Radical Generating System

A: control reaction mixture containing hypoxanthine (0.67 mM) and xanthine oxidase (0.09 units/ml) in 0.1 M phosphate buffer (pH 7.4). B: same as A but with 5 mM dopamine. C: computer simulation of spectrum B with proton hyperfine splitting constants of 0.47, 3.57, 0.92, and 3.01 G. D: same as A but with 5 mM dopamine-4-*O*-sulfate. The reaction was started by the addition of xanthine oxidase into a reaction mixture containing the sample and hypoxanthine. All EPR spectra were measured 1 min after the reaction had started. Operating conditions for EPR were: power, 5 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.1 G; time constants, 0.03 s; sweep time, 1 min; temperature, 20 °C; receiver gain, 1600.

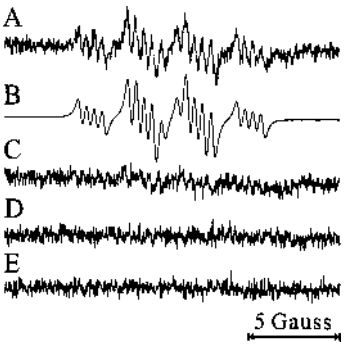

Fig. 5. EPR Spectra of the Reaction of Dopamine or Dopamine-4-*O*-sulfate with the Hydroxyl Radical Generating System

A: radicals from 5 mM dopamine in 0.1 M HEPES buffer (pH 7.4) obtained with the Fenton system. B: computer simulation of spectrum A with proton hyperfine splitting constants of 0.45, 3.56, 0.93, and 2.98 G. C: same as A but without ferrous. D: 5 mM dopamine-4-*O*-sulfate in 0.1 M HEPES buffer (pH 7.4) with the Fenton system. E: same as D but without ferrous. The combined solution of hydrogen peroxide and sample was introduced into the flat cell, then mixed with ferrous solution at a flow rate of 1 ml/min. Final concentrations of hydrogen peroxide, sample and ferrous ion were 10 mM, 5 mM and 1 mM, respectively. Operating conditions for EPR were: power, 20 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.2 G; time constants, 0.3 s; sweep time, 15 min; temperature, 20 °C: receiver gain, 5000.

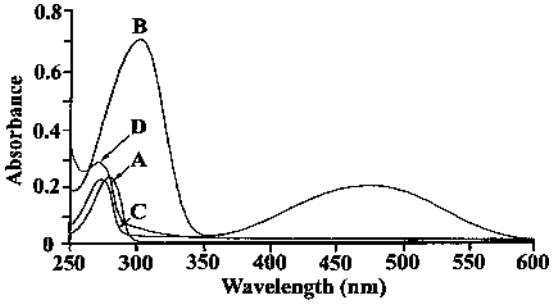

Fig. 6. Absorption Spectra of Dopamine or Dopamine-4-*O*-sulfate in the Presence of Sodium Periodate

A: 0.1 mM dopamine in 0.1 M phosphate buffer (pH 7.4). B: same as A but with 0.1 mM sodium periodate. C: 0.1 mM dopamine-4-*O*-sulfate in 0.1 M phosphate buffer (pH 7.4). D: same as C but with 0.1 mM sodium periodate. All spectra were measured 0.5 min after the mixing with $NaIO_4$ under aerobic conditions.

5C). Dopamine-4-*O*-sulfate shows no EPR signal under these experimental conditions (Figs. 5D, E).

Visible-UV Absorption Spectra of the Mixture of Dopamine or Dopamine-4-*O*-sulfate and Sodium Periodate We adopted a sodium periodate as an oxidant to compare the susceptibility for chemical oxidation between dopamine and dopamine-4-*O*-sulfate. Visible-UV absorption spectra were measured for the mixture of sodium periodate and dopamine or dopamine-4-*O*-sulfate aerobically. When dopamine was mixed with an equivalent amount of sodium periodate, two characteristic absorption peaks at 302 nm and 479 nm appeared (Fig. 6B). These peaks corresponded to the formation of dopaminochrome.[30] However, under the same experimental conditions, no absorption changes were observed with dopamine-4-*O*-sulfate even in the presence of sodium periodate (Fig. 6D). The increase of the absorption shoulder at around 250 nm in Fig. 6 was responsible for the incorporated sodium periodate.

Discussion

The pharmacological properties of dopamine vary with its concentration. At low concentrations, dopamine interacts with the D1-dopaminergic receptor which leads to vasodilation. At somewhat high concentrations, it cooperates with the β1-adrenergic receptor then exerts a positive inotropic effect on the myocardium. At high concentrations, dopamine acts on the α1-adrenergic receptor which is responsible for vasoconstriction.

In the circulation, 99% of dopamine was transformed into a sulfoconjugated form[31] by phenosulfotransferase which is localized in the gut,[32] liver,[33] kidney,[34] brain,[35] and platelets.[36] And dopamine sulfate is partially hydrolyzed by human arylsulfatase A, B and C *in vitro*, then converted to dopamine.[14] From the physiological study of free and sulfated dopamine during exercise, Yoshizumi *et al.* found a positive relationship between free dopamine and the conjugated form in plasma,[37] which suggests that dopamine-4-*O*-sulfate, not dopamine-3-*O*-sulfate,[38] acts as a reservoir of dopamine.

Dopamine is known to be an antioxidant and to protect neurocytes from oxidative stress[15—17] by scavenging reactive oxygen species. Meanwhile, dopamine also acts as a prooxidant through a reaction with molecular oxygen that produces reactive oxygen species.[18,19]

During the autoxidation of dopamine, *o*-semiquinone dopamine radicals,[19,28] –OH substituted *o*-semiquinone dopamine radicals[19,28,29] and 5,6-dihydroxyindole radicals[19] are produced. Photooxidation of dopamine also produced *o*-semiquinone dopamine radical.[28] These radical species are responsible for melamine formation by cyclization,[19] irreversible protein binding by nucleophilic addition[39] and cytotoxicity of catecholamine neurons,[20—23] which may relate to the progression of Alzheimer disease[40] and Parkinson disease.[41] Plasma soluble melanin formed from dopamine is toxic to human CD4+ lymphoblastic cells.[42] However, it is not known whether sulfoconjugated dopamine has the same redox properties as dopamine or not. Therefore, in the present study, we compared the antioxidant and prooxidant activities of dopamine-4-*O*-sulfate with those of dopamine.[43]

In the present study, dopamine was subjected to spontaneous oxidation at alkaline pH, reacted with reactive oxygen species such as superoxide anion radical and hydroxyl radical, then converted to radical species (Figs. 1, 4—5). Furthermore, dopamine was subtracted one electron by the horseradish peroxidase/hydrogen peroxide system then formed *o*-semiquinone dopamine radical (Fig. 3) as previously reported.[18]

Dopamine-4-*O*-sulfate, by contrast, showed no such formation of radicals in the presence of reactive oxygen species (Figs. 4, 5) or in the horseradish peroxidase-hydrogen peroxide system (Fig. 3) under physiological conditions *in vitro* (pH 7.4). In addition, dopamine-4-*O*-sulfate showed no change in absorption even on addition of $NaIO_4$, whereas dopamine did (Fig. 6).[30] Furthermore, the HPLC technique had applied to check the stability of dopamine-4-*O*-sulfate under these oxidative conditions mentioned above. No additional peaks were observed and the peak area of dopamine-4-*O*-sulfate was unchanged at any experimental conditions (date not shown). These results indicated that 1) dopamine-4-*O*-sulfate does not act as either an antioxidant or a prooxidant under our experimental conditions, 2) dopamine-4-*O*-sulfate itself cannot participate in the reaction to form melanin through the formation of *o*-semiquinone or *o*-

quinone compounds.

Generally, intrinsic catecholes are degraded to semiquinone radicals *via* both enzymatic and non-enzymatic pathways *in vivo*. And the generation of *o*-quinone through *o*-semiquinone radical causes the unfavorable effects of catechol derivatives, such as hepatic injury,[44] cardiotoxicity,[45] and hemolysis.[46] The fact that dopamine-4-*O*-sulfate is stable for the oxidization under physiological conditions may support the existence of a reservoir[47] of dopamine or a stable detoxicated product of dopamine.

References

1) Ames B. N., Cathcart R., Schwiers E., Hochstein P., *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6858—6862 (1981).
2) Burton G. W., Ingold K. U., *Ann. N. Y. Acad. Sci.*, **570**, 7—22 (1989).
3) Niki E., *World Rev. Nutr. Diet.*, **64**, 1—30 (1991).
4) Kawasaki T., *J. Nutr. Sci. Vitaminol.* (Tokyo), Spec, 552—555 (1992).
5) Terao K., Niki E., *J. Free Radic. Biol. Med.*, **2**, 193—201 (1986).
6) Stocker R., Yamamoto Y., McDonagh A. F., Glazer A. N., Ames B. N., *Science*, **235**, 1043—1046 (1987).
7) Andrae U., Singh J., Ziegler-Skylakakis K., *Toxicol. Lett.*, **28**, 93—98 (1985).
8) Johnson G. A., Baker C. A., Smith R. T., *Life Sci.*, **26**, 1591—1598 (1980).
9) Yoneda S., Alexander N., Vlachakis N. D., *Life Sci.*, **33**, 935—942 (1983).
10) Hashizume K., Yamatodani A., Yamamoto T., Wada H., Ogihara T., *Biochem. Pharmacol.*, **38**, 1891—1895 (1989).
11) Buu N. T., Duhaime J., Kuchel O., Genest J., *Life Sci.*, **29**, 2311—2316 (1981).
12) Racz K., Buu N. T., Kuchel O., De Lean A., *Am. J. Physiol.*, **247**, E431—E435 (1984).
13) Takeo S., Tanonaka K., Anan M., Hiraga S., *Arch. Int. Pharmacodyn. Ther.*, **289**, 60—71 (1987).
14) Demassieux S., Bordeleau L., Gravel D., Carrière S., *Life Sci.*, **40**, 183—191 (1987).
15) Liu J., Mori A., *Arch. Biochem. Biophys.*, **302**, 118—127 (1993).
16) Zareba M., Bober A., Korytowski W., Zecca L., Sarna T., *Biochim. Biophys. Acta*, **1271**, 343—348 (1995).
17) Korytowski W., Sarna T., Zar ba M., *Arch. Biochem. Biophys.*, **319**, 142—148 (1995).
18) Kalyanaraman B., Felix C. C., Sealy R. C., *Environ. Health Perspect.*, **64**, 185—198 (1985).
19) Sealy R. C., Puzyna W., Kalyanaraman B., Felix C. C., *Biochim. Biophys. Acta*, **800**, 269—276 (1984).
20) Cohen G., Heikkila R. E., *J. Biol. Chem.*, **249**, 2447—2452 (1974).
21) Jones D. C., Gunasekar P. G., Borowitz J. L., Isom G. E., *J Neurochem.*, **74**, 2296—2304 (2000).
22) Sachs C., Jonsson G., *Biochem. Pharmacol.*, **24**, 1—8 (1975).
23) Rotman A., *Life Sci.*, **21**, 891—900 (1977).
24) Duling D. R., *J. Magn. Reson. B*, **104**, 105—110 (1994).
25) Mason R. P., *Methods Enzymol.*, **105**, 416—422 (1984).
26) Sutton H. C., Winterbourn C. C., *Free Radic. Biol. Med.*, **6**, 53—60 (1989).
27) Kalyanaraman B., Felix C. C., Sealy R. C., *Photochem. Photobiol.*, **36**, 5—12 (1982).
28) Felix C. C., Sealy R. C., *Photochem. Photobiol.*, **34**, 423—429 (1981).
29) Adams R. N., Murrill E., McCreery R., Blank L., Karolczak M., *Eur. J. Pharmacol.*, **17**, 287—292 (1972).
30) Graham D. G., *Mol. Pharmacol.*, **14**, 633—643 (1978).
31) Dunne J. W., Davidson L., Vandongen R., Beilin L. J., Rogers P., *Life Sci.*, **33**, 1511—1517 (1983).
32) Conolly M. E., Davies D. S., Dollery C. T., Morgan C. D., Paterson J. W., Sandler M., *Br. J. Pharmacol.*, **46**, 458—472 (1972).
33) Häggendal J., *Acta Physiol. Scand.*, **59**, 255—260 (1963).
34) Rennick B., Quebbemann A., *Am. J. Physiol.*, **218**, 1307—1312 (1970).
35) Renskers K. J., Feor K. D., Roth J. A., *J. Neurochem.*, **34**, 1362—1368 (1980).
36) Hart R. F., Renskers K. J., Nelson E. B., Roth J. A., *Life Sci.*, **24**, 125—130 (1979).
37) Yoshizumi M., Nakaya Y., Hibino T., Nomura M., Minakuchi K., Kitagawa T., Katoh I., Ohuchi T., Oka M., *Life Sci.*, **51**, 227—234 (1992).
38) Strobel G., Werle E., Weicker H., *Biochem. Int.*, **20**, 343—351 (1990).
39) Scheulen M., Wollenberg P., Bolt H. M., Kappus H., Remmer H., *Biochem. Biophys. Res. Commun.*, **66**, 1396—1400 (1975).
40) Hegedus Z. L., *Toxicology*, **145**, 85—101 (2000).
41) Le W. D., Rowe D. B., Jankovic J., Xie W., Appel S. H., *Arch. Neurol.*, **56**, 194—200 (1999).
42) Montefiori D. C., Modliszewski A., Shaff D. I., Zhou J., *Biochem. Biophys. Res. Commun.*, **168**, 200—205 (1990).
43) Ackerman D. M., Hieble J. P., Sarau H. M., Jain T. C., *Arch. Int. Pharmacodyn. Ther.*, **267**, 241—248 (1984).
44) Elkington S. G., Schreiber W. M., Conn H. O., *Circulation*, **40**, 589—595 (1969).
45) Yates J. C., Dhalla N. S., *J. Mol. Cell Cardiol.*, **7**, 807—816 (1975).
46) Gottlieb A. J., Wurzel H. A., *Blood*, **43**, 85—97 (1974).
47) Buu N. T., Nair G., Kuchel O., Genest J., *J. Lab. Clin. Med.*, **98**, 527—535 (1981).