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Antioxidant Effects of Serotonin and L-DOPA on Oxidative Damages of Brain Synaptosomes

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Antioxidant effects of serotonin and L-DOPA on neuronal tissues were examined by studying the oxidative damages of brain synaptosomal components. The study further explored the mechanism by which they exert protective actions. Serotonin and L-DOPA (1 μM to 1 mM) significantly inhibited lipid peroxidation of brain tissues by either Fe^{2+} and ascorbate or t-butyl hydroperoxide in a dose dependent fashion. Protective effect of serotonin on the peroxidative actions of both systems was greater than that of L-DOPA. Protein oxidation of synaptosomes caused by Fe^{2+} and ascorbate was attenuated by serotonin and L-DOPA. Protein oxidation more sensitively responded to L-DOPA rather than serotonin. Serotonin and L-DOPA (100 μM) decreased effectively the oxidation of synaptosomal sulfhydryl groups caused by Fe^{2+} and ascorbate. The production of hydroxyl radical caused by either Fe^{3+}, EDTA, H_2O_2 and ascorbate or xanthine and xanthine oxidase was significantly decreased by serotonin and L-DOPA (1 mM). Equal concentrations of serotonin and L-DOPA restored synaptosomal Ca^{2+} uptake decreased by Fe^{2+} and ascorbate, which is responsible for SOD and catalase. Protective effects of serotonin and L-DOPA on brain synaptosomes may be attributed to their removing action on reactive oxidants, hydroxyl radicals and probably iron-oxygen complex, without chelating action on iron.

Key Words: Antioxidant action, Serotonin, L-DOPA, Synaptosomes

INTRODUCTION

The oxidative stress has been shown to cause necrosis and apoptosis. Reactive oxygen species appear to evoke necrotic cell death. Reaction of oxidants with lipids disrupts ion gradients of membrane and produces plasma membrane rupture (Kane et al, 1993). Inhibition of superoxide dismutase produces apoptosis in PC12 cells and in spinal cord neurons (Rothstein et al, 1994; Troy & Shelanski, 1994). Implication of oxidants in the neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis has been suggested (Simonian & Coyle, 1996). H_2O_2 is produced in the synthesis of dopamine by tyrosine hydroxylase (Olanow & Arendash, 1994). In addition, iron level is increased in the substantia nigra in Parkinson's disease (Dexter et al, 1991). The oxidative stress is increased by catalyzing the production of hydroxyl radicals from H_2O_2 by the Fentone reaction. Nerve cells exposed to β-amyloid peptides produce H_2O_2, and both vitamin E and catalase protect cells from its toxic action (Behl et al, 1992; Behl et al, 1994). Mutations in the cytosolic Cu/Zn superoxide dismutase gene are reported to be associated with familial amyotrophic lateral sclerosis (Rosen et al, 1993).

Serotonin and L-DOPA have been reported to exhibit protective effects on oxidative tissue damages. Serotonin depresses lipid peroxidation of microsomes by Fe^{3+}, ADP and NADPH system (Tse et al, 1991). N-Acetylserotonin decreases the peroxidation of linoleic acid induced by 2,2′-azobis (2-amidinopropane) (Longoni et al, 1997). Serotonin is reported to scavenge superoxide anion and hypochlorous acid (HOCl). However, serotonin exhibits pro-oxidant action in the ferric-bleomycin system and cause DNA damage (Chan & Tang, 1996). Thus, serotonin shows

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antioxidant and pro-oxidant effects. L-DOPA inhibits peroxidations of mitochondria (Narayanaswami & Sies, 1990) and ox-brain phospholipids (Spencer et al, 1996). L-DOPA is currently used in symptomatic treatment of Parkinson's disease. However, it induces apoptosis in cultured postmitotic chick sympathetic neurons (Ziv et al, 1997). L-DOPA autoxidizes and liberates reactive oxygen species, such as superoxide anion and hydroxyl radicals (Spencer et al, 1996). It may serve to exacerbate the neurodegenerative process.

Reactive oxygen species appear to be involved in pathogenesis of several neurodegenerative disorders. In this study, effects of serotonin and L-DOPA on neuronal tissues were examined on the oxidative damages of synaptosomal lipids and proteins of cerebral cortex by either Fe^{2+} and ascorbate or t-butyl hydroperoxide. Their effects were observed in altered synaptosomal Ca^{2+} uptake by oxidative damages. The present study suggests that serotonin and L-DOPA exert protective actions on neuronal cells against reactive oxidants.

METHODS

Materials

5-Hydroxytryptamine (serotonin), L-3,4-dihydroxyphenylalanine (L-DOPA), superoxide dismutase (from bovine blood, SOD), catalase (from bovine liver), dimethyl sulfoxide (DMSO), 1,4-diazabicyclo (2,2,2) octane (DABCO), ascorbic acid, NADPH, t-butyl hydroperoxide (t-BuOOH), xanthine, xanthine oxidase, 2-thiobarbituric acid (TBA), 2,4-dinitrophenylhydrazine (DNPH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), 2-αdeoxyribose, diethylenetriaminepentaacetic acid (DTPA), o-phenanthroline and arsenazo III were purchased from Sigma-Aldrich Inc. (St. Louis, MO, U.S.A.). Other chemicals were of analytical grade.

Preparation of cerebral synaptosomes

Synaptosomal particles were prepared from rat cerebral cortex according to the method of Hajós (1975). Male Sprague-Dawley rats weighing about 150~200 g were used. The animals were killed by decapitation. After removing blood clot, cerebral cortices were placed in 9 volume of cold 0.3 M sucrose and homogenized with a teflon glass homogenizer. Homogenizing solution was centrifuged at 1,500 g for 10 min. The supernatants were recentrifuged at 9,000 g for 20 min. The pellet was dispersed in 5 ml of 0.3 M sucrose. The suspension was layered over 20 ml of 0.8 M sucrose and centrifuged at 9,000 g for 25 min. A synaptosomal fraction was obtained at 0.8 M sucrose layer. The synaptosomal fraction was diluted with 6 volume of diluting solution (contains 145 mM NaCl, 5 mM KCl, 10 mM dextrose, 1.3 mM $MgCl_2$ and 20 mM Tris-maleate, pH 7.4) and was centrifuged at 15,000 g for 20 min. The pellet was resuspended in diluting solution, and protein concentration was determined using the Bradford method as described in the Bio-Rad protein assay kit.

Preparation of brain homogenates: Rat brain homogenate (10%) was prepared in 0.15 M KCl and was centrifuged at 800 g at 10 min. The supernatant was used in the experiments.

Measurement of lipid peroxidation

Lipid peroxidation of synaptosomes was estimated from measuring malondialdehyde concentration by thiobarbituric acid method. Brain synaptosomes (0.4 mg protein/ml) were contained in the reaction mixture consisting of 150 mM KCl, oxidants, and 50 mM NaH_2PO_4, pH 7.4. Reaction was initiated by addition of oxidant. After 30 min of incubation, the reaction was terminated by adding 1.0 ml of 1% TBA in 50 mM NaOH and 1.0 ml of 2.8% trichloroacetic acid (Gutteridge et al, 1982). The concentration of malondialdehyde was expressed as nmol/mg protein using the molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ (Buege & Aust, 1978).

Assay of carbonyl groups

The oxidation of synaptosomal proteins was measured by carbonyl assay using 2,4-dinitrophenylhydrazine (DNPH) (Levine et al, 1993). One ml of reaction mixtures, which contain 1 mg protein/ml of synaptosomes, was treated with 10 μM Fe^{2+} and 100 μM ascorbate for 30 min. A 4 ml of 10 mM DNPH in 2.5 M HCl was added to mixtures, and tubes were left for 1 h at room temperature in the dark. The mixtures were treated with 20% trichloroacetic acid and 10% of same reagent, sequentially. After centrifugation, pellets were washed three times with 4 ml

of ethanol : ethyl acetate mixture (1 : 1) solution. The final pellets were dissolved in 2 ml of 6 M guanidine HCl solution and were left for 15 min at 37°C with mixing. Absorbance of the supernatants was read at 370 nm, and protein carbonyls were calculated using the molar extinction coefficient of 2.2×10^4 $M^{-1}cm^{-1}$.

Measurement of sulfhydryl content

Synaptosomes (1.0 mg protein/ml) were incubated in 100 mM Tris-HCl, pH 7.4 buffer medium and were treated with 10 μM Fe^{2+} and 100 μM ascorbate for 30 min at 37°C. The assay mixture (1.0 ml) was mixed with the same volume of 2 M $HClO_4$, and the mixture was centrifuged at 12,000 g for 8 min. The supernatant (100 μl) was added to the assay mixture containing 0.6 mM DTNB, 200 μM NADPH and 0.1 M potassium phosphate, pH 7.0 to make a total volume of 980 μl. Twenty μl of GSH reductase (6 U/ml) was added to initiate the assay. The formation of 5-thio-2-nitrobenzoic acid was measured spectrophotometrically at 412 nm. The total amount GSH was determined from a standard curve obtained with known amounts of GSH standards (Hung & Lee, 1998).

Assay of thiobarbituric acid reactivity of 2-αdeoxyribose

Amount of hydroxyl radical generated was estimated from TBA reactivity of 2-αdeoxyribose (Halliwell & Gutteridge, 1981). The reaction mixtures contained 2 mM 2-αdeoxyribose, 50 μM $FeCl_3$, 50 μM EDTA, 500 μM H_2O_2, 100 μM ascorbate (or 0.2 mM xanthine and 42.1 mU/ml xanthine oxidase), 150 mM KCl, 50 mM NaH_2PO_4 buffer, pH 7.4 and other compounds in a final volume of 1.0 ml. After 30 min of incubation, the reaction was stopped by adding 1.0 ml of 1% TBA in 50 mM NaOH and 1.0 ml of 2.8% trichloroacetic acid. The fluorescence was measured at the wavelengths of excitation, 532 nm and emission, 553 nm.

Assay of ferrous ion binding

Binding effects of serotonin and L-DOPA on ferrous ion was assayed by using formation of ferrous ion-o-phenanthroline complex (Aust et al, 1993). Measurement of ferrous ion oxidation was done in 2 ml of reaction mixture containing serotonin (or L-DOPA) and 30 mM NaCl, pH 7.0 at 37°C. Reaction was started by adding 100 μM Fe^{2+} and then was stopped by mixed with 10 mM o-phenanthroline. Absorbance change was measured at 512 nm, and oxidation rate was calculated using molar extinction coefficient of 1.11×10^4 $M^{-1}cm^{-1}$.

Measurement of Ca^{2+} uptake by synaptosomes

The rate and extent of Ca^{2+} uptake by synaptosomes was assayed by monitoring the absorbance change of calcium sensitive dye, arsenazo III, at 675~685 nm with a dual wavelength-split beam spectrophotometer (Aminco Chance) (Åkerman & Heinonen, 1983). Experiment was performed in the assay medium consisting of synaptosomal particles (0.75 mg protein/ml), 100 μM arsenazo III, 130 mM KCl, 1.3 mM $MgCl_2$, 30 μM $CaCl_2$ and 20 mM Tris-maleate, pH 7.4 at 30°C. The Ca^{2+} uptake by synaptosomes was induced by addition of 1 mM ATP, and the uptake amount was estimated from measuring the change of absorbance in the same assay mixture without synaptosomes after addition of $CaCl_2$.

Statistical analysis

Data were expressed as means$\pm$S.D.. Statistical analysis was performed by using Student's t-test for paired data.

RESULTS

Protective actions of serotonin and L-DOPA on lipid peroxidation of synaptosomes

Iron catalyzes production of reactive oxygen intermediates and stimulates oxidative tissue damages. Effects of serotonin and L-DOPA on lipid peroxidation of cortex synaptosomes of the rat brain were studied. Fig. 1 shows that 10 μM Fe^{2+} and 100 μM ascorbate caused peroxidation of synaptosomal lipids, and at 30 min of incubation, 14.49 ± 0.57 nmol/MDA/mg protein (n=5) was produced. Serotonin and L-DOPA significantly inhibited lipid peroxidation of synaptosomes by Fe^{2+} and ascorbate in a dose dependent fashion. With incubation of serotonin and L-DOPA (100 μM), the peroxidation was decreased by 71.3% and 14.9%, respectively. Protec-

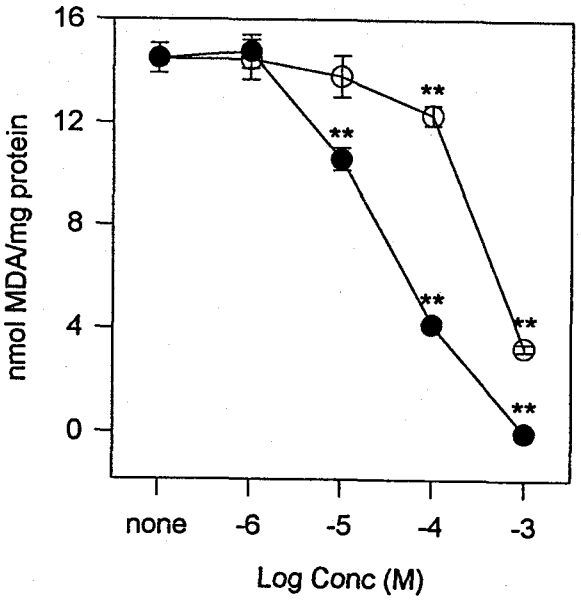

Fig. 1. Inhibition of Fe^{2+} and ascorbate-induced lipid peroxidation by serotonin and L-DOPA. Synaptosomes of brain cortex (0.4 mg protein/ml) were treated with 10 μM $FeSO_4$ and 100 μM ascorbate for 30 min. Values are means ± S.D., n=5. ●, serotonin and ○, L-DOPA. **p< 0.01 by Student's *t*-test.

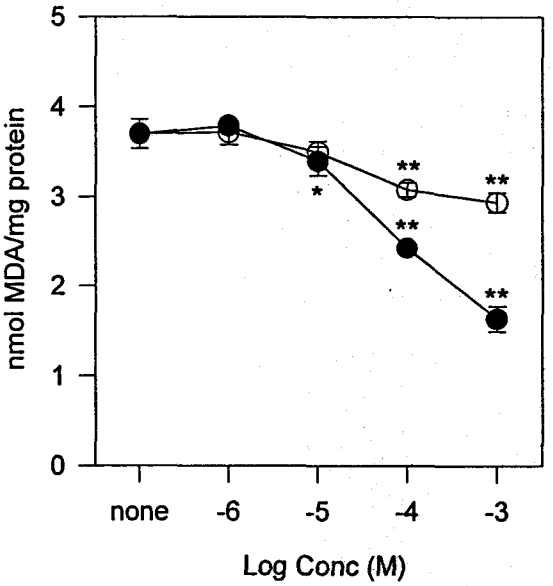

Fig. 3. Attenuation of t-butyl hydroperoxide-induced lipid peroxidation by serotonin and L-DOPA. Brain homogenates (0.4 mg protein/ml) were treated with 0.5 mM t-butyl hydroperoxide for 30 min. Values are means ± S.D., n=5. ●, serotonin and ○, L-DOPA. *p<0.05, **p <0.01 by Student's *t*-test.

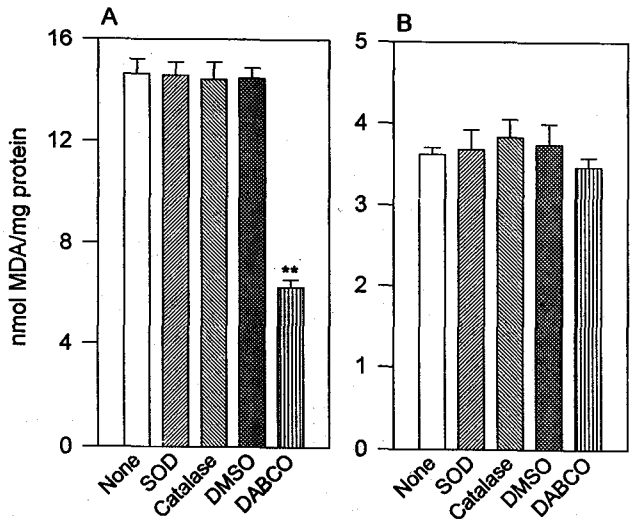

Fig. 2. Effects of oxidant scavengers on synaptosomal lipid peroxidation. Synaptosomes and brain homogenates (0.4 mg protein/ml) were treated with either 10 μM $FeSO_4$ and 100 μM ascorbate (A) or 0.5 mM t-butyl hydroperoxide (B) in the presence of scavengers. Values are means ± S.D., n=5. none, no addition; SOD, 30 μg/ml SOD; Catalase, 30 μg/ml catalase; DMSO, 10 mM DMSO; DABCO, 10 mM DABCO. **p<0.01 by Student's *t*-test.

tive effect of serotonin was greater than that of L-DOPA. Involvement of reactive oxygen species in lipid peroxidation caused by Fe^{2+} and ascorbate was observed. As shown in Fig. 2, Fe^{2+} and ascorbate-induced lipid peroxidation was not affected by 30 μg/ml SOD, a scavenger of superoxide anion, 30 μg/ml catalase, a scavenger of H_2O_2 and 10 mM DMSO, a scavenger of hydroxyl radical, whereas DABCO (10 mM), a scavenger of singlet oxygen, inhibited the peroxidation by 57.3%.

Antioxidant effects of serotonin and L-DOPA were also studied on lipid peroxidation of brain homogenates by t-butyl hydroperoxide (t-BuOOH). Incubation of brain homogenates with 0.5 mM t-BuOOH produced 3.70±0.16 nmol/mg protein of MDA (n=5) at 30 min. Dose-dependent inhibition was observed in the presence of serotonin and L-DOPA. t-BuOOH-induced lipid peroxidation of brain homogenates was decreased by 100 μM of serotonin and L-DOPA, and 34.3% and 16.8% of inhibitions were observed (Fig. 3). Similar to peroxidative action of Fe^{2+} and ascorbate, inhibitory action of serotonin was greater than that of L-DOPA. The effects of oxidant scavengers on lipid peroxidation were examined. All scavengers

Table 1. Effects of serotonin and L-DOPA on protein oxidation of synaptosomes

	Carbonyls
	nmol/mg protein
Fe^{2+} + ascorbate	10.28±0.43
+ Serotonin 100 μM	4.41±0.68**
+ L-DOPA 100 μM	1.41±0.06**
+ SOD 30 μg/ml	10.09±0.29
+ Catalase 30 μg/ml	9.19±0.37*
+ DMSO 10 mM	8.57±0.49**
+ DABCO 10 mM	5.65±0.43**

Synaptosomes of brain cortex (1 mg protein/ml) were treated with 10 μM $FeSO_4$ and 100 μM ascorbate for 30 min. Values are means±S.D., n=3. *p<0.05, **p<0.01 by Student's *t*-test.

Table 2. Inhibition of the oxidation of synaptosome sulfhydryl groups by serotonin and L-DOPA

	Sulfhydryl content
	(nmol/mg protein)
No addition	31.12±0.27
Fe^{2+} + ascorbate	15.69±0.77
+ Serotonin 100 μM	28.49±0.75
+ L-DOPA 100 μM	18.54±0.57
+ SOD 30 μg/ml	15.19±0.73
+ DMSO 10 mM	14.98±0.64
+ DABCO 10 mM	14.02±0.28

Synaptosomes of brain cortex (1 mg protein/ml) were treated with 10 μM $FeSO_4$ and 100 μM ascorbate for 30 min. Values are means±S.D., n=4.

used (SOD, catalase, DMSO and DABCO) failed to inhibit lipid peroxidation caused by t-BuOOH. Thus, the other oxidizing species may be involved in t-BuOOH-caused lipid peroxidation.

Inhibition of protein oxidation of synaptosomes by serotonin and L-DOPA

Protein oxidation can be assayed by measuring carbonyl levels. Synaptosomal proteins were oxidized by addition of 10 μM Fe^{2+} and 100 μM ascorbate.

Table 3. Scavenging actions of serotonin and L-DOPA on hydroxyl radical

	Deoxyribose degradation	
Compounds	Fe^{3+}, EDTA, H_2O_2 and ascorbate	X./X.O.
No addition	1.306±0.026	69.7±3.5
Serotonin 100 μM	0.810±0.016**	44.5±2.4**
L-DOPA 100 μM	–	36.1±1.7**
DMSO 10 mM	0.099±0.004**	61.6±2.1**
Sod. formate 10 mM	0.201±0.006**	63.8±0.8*

2-α Deoxyribose (2 mM) was treated either with 50 μM Fe^{3+}, 50 μM EDTA, 500 μM H_2O_2 and 100 μM ascorbate (expressed as Δ absorbance) or with 0.2 mM xanthine and 42.1 mU/ml xanthine oxidase (expressed as fluorescence unit). Values are means±S.D., n=5. *p<0.05, **p<0.01 by Student's *t*-test.

After 30 min of incubation, 10.28±0.43 nmol/mg protein of carbonyls was produced. Serotonin and L-DOPA (100 μM) decreased the formation of protein carbonyls significantly and attenuated it by 57.1% and 86.3%, respectively (Table 1). In contrast to lipid peroxidation, protein oxidation responded to L-DOPA more sensitively than to serotonin. Effects of oxidant scavengers on the protein oxidation were also different from lipid peroxidation. Stated concentrations of catalase, DMSO, and DABCO exhibited inhibitory effects on protein oxidation induced by Fe^{2+} and ascorbate, while the effect of SOD was not detected.

The sulfhydryl groups are essential for the maintenance of cellular functions. Oxidation of cellular sulfhydryl groups can produce alteration of cellular function. Incubation of synaptosomes with 10 μM Fe^{2+} and 100 μM ascorbate caused a significant. oxidation of sulfhydryl groups. Defensive actions of serotonin and L-DOPA on the sulfhydryl groups against the oxidants were studied. As can be seen in Table 2, serotonin and L-DOPA (100 μM) decreased the oxidation of sulfhydryl groups of synaptosomes by Fe^{2+} and ascorbate significantly. However, stated concentrations of the oxidant scavengers (SOD, DMSO and DABCO) did not affect the oxidation of sulfhydryl groups.

Scavenging actions of serotonin and L-DOPA on hydroxyl radical

Hydroxyl radical produced by either Fe^{3+}, EDTA, H_2O_2 and ascorbate or xanthine and xanthine oxidase were assayed by the method of 2-α deoxyribose degradation. The production of hydroxyl radical caused by 50 μM Fe^{3+}, 50 μM EDTA, 500 μM H_2O_2 and 100 μM ascorbate was significantly inhibited by 10 mM of DMSO and sodium formate. Meanwhile, the xanthine and xanthine oxidase (0.2 mM and 42.1 mU/ml, respectively)-induced production of hydroxyl radical did not respond to the scavengers that much. Table 3 shows that serotonin and L-DOPA (100 μM) significantly reduced the production of hydroxyl radical caused by either Fe^{3+}, EDTA, H_2O_2 and ascorbate or xanthine and xanthine oxidase. In the mixture containing Fe^{3+}, EDTA, H_2O_2 and ascorbate, the scavenging effect of L-DOPA on hydroxyl radical could not be observed because of red color development.

Effects of serotonin and L-DOPA on autoxidation of iron

Ferrous ion rapidly oxidized at 37°C of reaction mixtures, and within 1 min, it almost completely oxidized. Iron oxidation was assayed using iron chelator, o-phenanthroline. DTPA (1 mM), an iron chelator, interfered with oxidation of iron, and the oxidation very slowly proceeded. One hundred μM of serotonin weakly inhibited iron oxidation, but its effect finished within 1 min of postaddition (Fig. 4). The effect of 100 μM L-DOPA on iron oxidation was not detected.

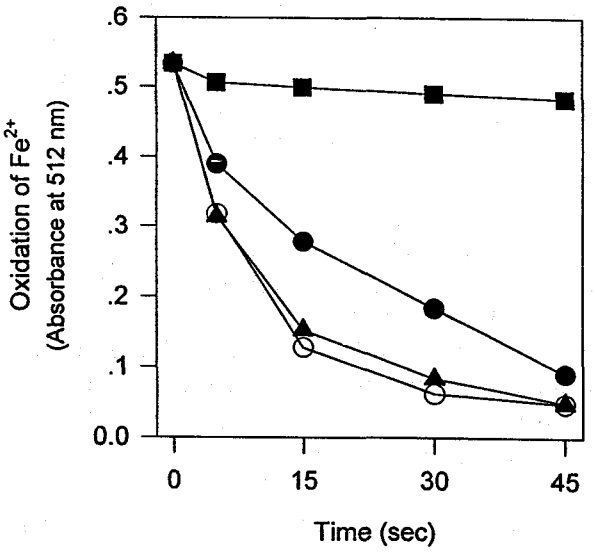

Fig. 4. Effects of serotonin and L-DOPA on iron oxidation. Ferrous ion (100 μM) was mixed with serotonin or L-DOPA, and at stated times, remaining reduced form was measured. Values are means of three experiments. ○, no addition; ●, 100 μM serotonin; ▲, 100 μM L-DOPA; ■, 1 mM DTPA.

Reverse of depressed synaptosomal Ca^{2+} uptake by serotonin and L-DOPA

Brain synaptosomes have the membrane potential and can accumulate Ca^{2+} when triggered by depolarizing agents, including K^+. In this experiment, the

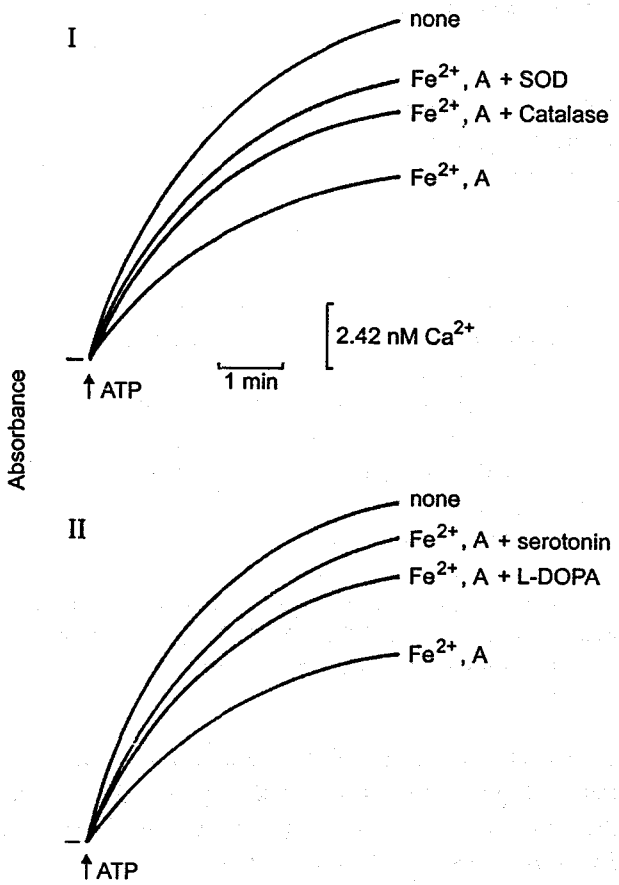

Fig. 5. Reverse of Fe^{2+} and ascorbate-induced depression of synaptosomal Ca^{2+} uptake by serotonin and L-DOPA. Brain synaptosomes were treated with 10 μM $FeSO_4$ and 100 μM ascorbate (A) in the presence of 10 μg/ml of SOD and catalase (I) and 100 μM of serotonin and L-DOPA (II). Ca^{2+} uptake was initiated by addition of 1 mM ATP.

Ca^{2+} uptake by synaptosomes was made by adding ATP, and it was gradually increased. The amount of Ca^{2+} uptake by synaptosomal fractions in K^{+}-rich medium was 16.1 nmol/mg protein at 4 min. As shown in Fig. 5, the ATP-induced Ca^{2+} uptake was decreased by pretreament with 10 μM Fe^{2+} and 100 μM ascorbate, which is markedly reversed by addition of serotonin and L-DOPA (100 μM). SOD and catalase (10 μg/ml) also showed reversal effects on the depressed Ca^{2+} uptake.

DISCUSSION

Free radicals are thought to be involved in the pathogenesis of adult-onset neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease. Iron promotes effectively tissue damages by oxidants (Halliwell & Gutteridge, 1989). Autoxidation of iron produces reactive oxygen metabolites, including hydroxyl radical and iron-oxygen complexes, and these metabolites appear to cause the damages of biological molecules. Fe^{2+} plus ascorbate and Fe^{3+}-ADP-NADPH effectively evoke lipid peroxidation of liver microsomes.

Degradation of hyaluronic acid by Fe^{2+}, H_2O_2 and ascorbate and oxidation of collagen by Fe^{2+} and H_2O_2 are effectively attenuated by addition of hydroxyl radical scavengers. However, the peroxidative action of Fe^{2+} plus ascorbate or t-BuOOH on brain tissue lipids was not affected by DMSO as well as SOD and catalase (Fig. 2). Hydroxyl radical may not play a central role in lipid peroxidation as described in previous report (Halliwell & Gutteridge, 1989). Instead of hydroxyl radical, iron-oxygen complexes appear to be involved in lipid peroxidation. The inhibitory effect of DABCO on lipid peroxidation caused by Fe^{2+} and ascorbate indicates that singlet oxygen is involved in damage of synaptosomal lipids, while its role in t-BuOOH-induced lipid peroxidation was not suggested.

t-BuOOH is a prooxidant, which enters cells and endogenously produce radicals, such as butoxyl radical (Cadenas & Sies, 1982). The t-BuOOH-induced lipid peroxidation of erythrocyte membrane ghosts is weakly inhibited by DABCO, while the effects of SOD, catalase, mannitol and DMSO do not occur (Sugiyama et al, 1996). However, protective effect of DABCO on erythrocyte membrane was not observed in the present study using brain tissues. Serotonin and L-DOPA exert protective effects on the oxidative damage of synaptosomes.

Since the lipid peroxidation caused by Fe^{2+} and ascorbate appears to be ascribed to iron-oxygen complexes and peroxyl radicals, serotonin and L-DOPA may have a defensive action on synaptosomal lipids against those oxidants. Inhibition of t-BuOOH-induced lipid peroxidation by serotonin and L-DOPA suggests that they exert antioxidative effects on the oxidative tissue damages, which are non-metal ion dependent.

The oxidative modification of cellular proteins evokes interaction of carbonyl groups with amino acid residues of proteins (Levine et al, 1993). The assay for the carbonyl content of proteins is used as the definitive method for the measurement of metal-catalyzed oxidation. In contrast to lipid peroxidation, designated scavengers of oxidants except SOD decreased the oxidation of synaptosomal proteins evoked by Fe^{2+} and ascorbate. Thus, it is indicated that reactive oxygen species, such as H_2O_2, hydroxyl radical and singlet oxygen, are participated in the iron-catalyzed oxidation of proteins. Metal ion-stimulated degradation of collagen is known to be well protected by four scavengers used in this study. Coincided with this finding, the protein oxidation of brain synaptosomes was significantly attenuated by hydroxyl radical scavenger. From the different responses of lipids and proteins to oxidants, the oxidative mechanism in proteins may be different from that in lipids, and various tissue components against the oxidants appear to show different responses. Remarkable inhibitions of lipid and protein oxidation by serotonin and L-DOPA suggest that they are effective and potent antioxidants.

Free sulfhydryl groups are highly reactive species and are essential for the maintenance of cellular functions. Sulfhydryl groups can provide protection against endogenous and exogenous oxidants. Sulfhydryl content correlates well with cell viability in vitro (Thomas et al, 1986). The oxidation of synaptosomal sulfhydryl groups caused by Fe^{2+} and ascorbate was not decreased by the addition of SOD and DMSO. Even DABCO did not protect the oxidation of sulfhydryl groups. Sulfhydryl groups react with the oxidants and then get oxidized. This oxidation is probably not arrested by antioxidants. However, serotonin and L-DOPA significantly inhibited the oxidation of synaptosomal sulfhydryl groups. They inhibit the oxidation of cell components, sul-

fhydryl groups, against oxidants and may provide maintenance of cell functions.

Since hydroxyl radical seems to be involved in the oxidation of synaptosomal proteins and to play a role in lipid peroxidation, scavenging actions of serotonin and L-DOPA on hydroxyl radical were examined. The production of hydroxyl radical was measured with degradation of 2-α deoxyribose by either Fe^{3+}, EDTA, H_2O_2 and ascorbate or xanthine and xanthine oxidase. Protective effect of hydroxyl radical scavengers on 2-α deoxyribose degradation confirms specificity of the detection method, as previous report (Halliwell & Gutteridge, 1981). Serotonin has been shown to promote hydroxyl radical production in Fe^{3+}-EDTA/H_2O_2-deoxyribose system (Marshall et al, 1996). The effect of L-DOPA on iron-dependent hydroxyl radical generation from H_2O_2 is thought to be complex, and the low concentration is known to stimulate hydroxyl radical production (Spencer et al, 1996). In contrast to these views, serotonin and L-DOPA (100 μM) showed significant scavenging actions on hydroxyl radical in the present experiment, and this effect will provide a defense on synaptosomes against the oxidants.

The antioxidant actions of serotonin and L-DOPA on oxidative damage of synaptosomes were studied with the respect to iron chelation. The autoxidation of ferrous ion at 37°C reaction mixtures, which was apparently arrested by addition of DTPA, was weakly inhibited by serotonin, while the effect of L-DOPA was not detected. Serotonin is reported to exert a reducing action on Fe^{3+} to Fe^{2+}. In the assay system using o-phenanthroline, serotonin (100 μM) showed a 0.012/30 sec of absorbance change and a 1.1 nM/30 sec of reduction rate on iron (data not shown). Thus, reducing action of serotonin is very weak, and the action may not influence oxidative tissue damages.

Neurotransmitters are stored in synaptic vesicles and are released by fusion of these vesicles to the plasma membrane, which are triggered by Ca^{2+} influx through Ca^{2+} channels. Hydrogen peroxide has been shown to enhance the elevated cytosolic Ca^{2+} concentration in synaptosomes elicited by high K^+ depolarization (Tretter et al, 1997). However, dopamine inhibits Ca^{2+} uptake induced by veratrine in cortical and striatal slices (Crowder & Bradford, 1987) and reduces inward Ca^{2+} current in retinal ganglion cells (Liu & Lasater, 1994). Similar to these data on dopamine effects, the Ca^{2+} uptake by brain synaptosomes was apparently decreased by pretreament of Fe^{2+} and ascorbate. The restoration of depressed Ca^{2+} uptake by SOD and catalase indicates the participation of reactive oxygen species in the depressant action of Fe^{2+} plus ascorbate on synaptosomes. Serotonin and L-DOPA appear to attenuate the oxidative stress-induced depression of synaptosomal Ca^{2+} uptake through scavenging or defensive actions on reactive oxidants. In conclusion, serotonin and L-DOPA may exert protective actions on neuronal cells against reactive oxidants.

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