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Taurine Inhibition of Metal-Stimulated Catecholamine Oxidation

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Taurine is an abundant amino acid found in mammalian tissues and it has been suggested to have cytoprotective functions. The aim of the present study was to determine if taurine had the potential to reduce oxidative stress associated with metal-stimulated catecholamine oxidation. Taurine and structural analogs of taurine were tested for their ability to inhibit metal-stimulated quinone formation from dopamine or L-dopa. Oxidative damage to proteins and lipids were also assessed *in vitro* and the effects of taurine were determined. Taurine (20 mM) was found to decrease significantly ferric iron (50–500 μM)- and manganese (10 μM)-stimulated L-dopa or dopamine oxidation. Taurine had no effect on zinc-induced dopamine oxidation and slightly potentiated copper- and $NaIO_4$-stimulated quinone formation. Ferric iron-stimulated lipid peroxidation was not affected by taurine (1–20 mM). Protein carbonyl formation induced by ferric iron (500 μM) and L-dopa (500 μM) was significantly reduced by 10 mM taurine. The cytotoxicity of L-dopa (250 μM) and ferric chloride (75 μM) to LLC-PK_1 cells was attenuated by 10 mM taurine or hypotaurine. Homotaurine alone stimulated L-dopa oxidation and potentiated the cytotoxic effects of ferric iron. Homotaurine was found to be cytotoxic when combined with L-dopa or L-dopa/iron. In contrast, hypotaurine inhibited quinone formation and protected LLC-PK_1 cells. These studies suggest that taurine may exhibit cytoprotective effects against the oxidation products of catecholamines by acting as a scavenger for free radicals and cytotoxic quinones.

Keywords: Antioxidants, Catecholamines, L-Dopa, Dopamine, Iron, Metals, Manganese, Taurine

INTRODUCTION

Taurine (2-aminoethane sulfonic acid) is a sulfur-containing amino acid that is present in millimolar concentrations in most mammalian tissues (Jacobsen and Smith, 1968). Taurine has been shown to be involved in a number of biological functions which include: osmoregulation, membrane stabilization, neuromodulation, cytoprotection, bile salt conjugation, and calcium modulation (Huxtable, 1992; Wright *et al.*, 1986). A role for taurine in cellular antioxidant defense mechanisms has been observed in a number of tissues (Timbrell *et al.*, 1995). Although taurine appears to have little intrinsic reactivity with oxidizing species (Aruoma *et al.*, 1988; Shi *et al.*, 1997), its high intracellular concentration may still allow it to directly scavenge free radicals. Taurine has clearly been shown to scavenge hypochlorous

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acid and form taurochloramines (Marquez and Dunford, 1994; Weiss *et al.*, 1982). Furthermore, taurine may bind reactive quinones involved in redox cycling and indirectly inhibit free radical formation (Tse *et al.*, 1976). The biosynthesis of taurine generates intermediates (hypotaurine and cysteamine) that also have free radical scavenging properties (Aruoma *et al.*, 1988; Huxtable, 1992). Glutathione synthesis is also indirectly linked to taurine synthesis since cysteine serves as a common precursor for both biosynthetic pathways (Beetsch and Olson, 1998). Thus, taurine directly and indirectly appears to be involved in cellular regulation of oxidative stress. The cytoprotective actions of taurine have been well documented (Redmond *et al.*, 1998; Timbrell *et al.*, 1995), but the mechanisms for these actions have not been clearly elucidated in all cases. The high taurine concentrations in tissues may confer some level of protection from oxidative stress.

Taurine is present in many tissues that contain high concentrations of catecholamines (Dawson and Wallace, 1992; Jacobsen and Smith, 1968). Catecholamines are known to be cytotoxic and increase oxidative stress (Basma *et al.*, 1995; Han *et al.*, 1996; Lai and Yu, 1997; Offen *et al.*, 1996; Stokes *et al.*, 1999). Catecholamines are known to undergo oxidation reactions to generate free radicals (H_2O_2, hydroxy, superoxide) and cytotoxic quinones (Basma *et al.*, 1995; Bindoli *et al.*, 1992; Graham *et al.*, 1978; Lai and Yu, 1997; Offen *et al.*, 1996; Pardo *et al.*, 1995). The oxidation of catecholamines is catalyzed by metals such as iron, copper and manganese (Archibald and Tyree, 1987; Donaldson *et al.*, 1980; Miller *et al.*, 1990; Shen and Dryhurst, 1998; Tanaka *et al.*, 1991). Metal-stimulated oxidation of proteins may also generate reactive catecholamines such as L-3,4-dihydroxyphenylalanine (L-dopa) via hydroxylation of tyrosine residues (Gieseg *et al.*, 1993; Morin *et al.*, 1998). Protein bound L-dopa may subsequently mediate other forms of oxidative damage (Morin *et al.*, 1998). Metal-stimulated catecholamine oxidation is capable of producing oxidative damage to DNA (Levay *et al.*, 1997; Spencer *et al.*, 1994). Metal-stimulated oxidation of catecholamines is thought to play a role in neurodegenerative diseases (Graham, 1984; Spencer *et al.*, 1994; Youdim *et al.*, 1993). Manganese neurotoxicity is also thought to be associated with catalytic mechanisms involving catecholamine oxidation in dopamine rich areas of the brain (Archibald and Tyree, 1987; Donaldson *et al.*, 1980; Graham, 1984; Shen and Dryhurst, 1998). Copper can stimulate catecholamine and protein oxidation as well as other forms of toxicity (Heikkila and Cabbat, 1978; Levay *et al.*, 1997). At present, it is unclear whether taurine under *in vivo* conditions serves as a scavenger for reactive quinones and free radicals derived from catecholamine oxidation.

Taurine content declines in peripheral tissues and the brain of aged rodents (Dawson and Wallace, 1992; Dawson *et al.*, 1999a; Strolin Benedetti *et al.*, 1990; 1991). We have recently found that there is a significant correlation between dopamine and taurine decline in the striatum of aged rats (Dawson *et al.*, 1999b). Other investigators have also reported that striatal taurine content decreases in aged rats (Strolin Benedetti *et al.*, 1990; 1991). The age-related decline in tissue taurine content could increase the susceptibility of critical tissue targets to free radical-mediated damage. The goal of the present series of experiments was to determine the effects of taurine to inhibit catecholamine oxidation induced by various metals. The effects of taurine to inhibit oxidative damage to protein *in vitro* and to attenuate cell death associated with iron-stimulated catecholamine oxidation were also examined.

MATERIALS AND METHODS

Measurement of Catecholamine Oxidation

The rate of catecholamine oxidation was monitored by measuring aminochrome formation

spectrophotometrically (Donaldson *et al.*, 1980; Heikkila and Cabbat, 1978). Aminochromes (quinoidal oxidation products) were detected at 480–490 nm using a Beckman DU 7000 diode array spectrophotometer. Experiments were conducted in phosphate buffered saline (PBS, pH = 7.4) at ambient temperatures (21–24°C) except for some experiments conducted at 37°C. The standard oxidation reactions were conducted in an assay volume of 3 ml and the rate of quinone formation was determined by measuring absorbance changes every 20 s for a total of 5 min. The rate of quinone formation (Δabsorbance/min) was linear with time and reaction rates were calculated by linear regression. Manganese and tyrosinase-induced oxidation of catecholamines occurred at very rapid rates and under these conditions reactions had to be performed in within 2 min to obtain linear reaction rates and absorbance changes were recorded every 5 s. In some cases absorbance was also determined at the end of the experiment to assess total aminochrome formation. Catecholamine oxidation rates were measured in the presence and absence of iron, manganese, copper and zinc. Catecholamine oxidation was also stimulated enzymatically with tyrosinase (Sigma, St. Louis, ≈2000 units/mg) or with the oxidizing agent, sodium periodate. The effects of taurine, taurine analogs and other amino acids were assessed for their ability to inhibit catecholamine oxidation induced by iron. All solutions (metals, amino acids and catecholamines) were made up fresh immediately prior to spectrophotometric assays. Specific experimental conditions and reagent concentrations are given in figure captions and in table legends.

L-Dopa Analysis by HPLC

Experiments were also conducted to determine directly if taurine inhibited the oxidation of L-dopa into quinones. The oxidation of L-dopa (100 μM) in the presence of ferric chloride (100 μM) was carried out in PBS in a 1 ml assay volume. The procedure was similar to that described above except the incubations were carried out for 2 h at 37°C in order to allow sufficient time for a significant loss of catecholamine. The reaction was stopped by the addition of 2 ml of ice cold 0.2 M perchloric acid containing 1 mM of Na_2EDTA and 1 mM glutathione to prevent further oxidation. The experimental conditions were as follows: (1) L-dopa only, (2) L-dopa + taurine (10 mM), (3) L-dopa + $FeCl_3$ and (4) L-dopa + taurine (10 mM) + $FeCl_3$ and (5) a control incubation of the L-dopa in PBS carried out at 4°C. L-Dopa was measured after at a 1 : 10 dilution in 0.05 M perchloric acid using a previously described HPLC assay (Kontur *et al.*, 1984; Wallace and Dawson, 1990). The samples gave a single chromatographic peak with no interfering peaks.

Protein Carbonyl Measurements

Oxidative damage to proteins was measured by determining carbonyl content in oxidatively modified proteins by the method of Levine *et al.* (1990). Carbonyl content was assessed by reaction with 2,4-dinitrophenylhydrazine and detected spectrophotometrically at 370 nm. Bovine γ-globulin (BGG, 1 mg/ml) was oxidatively damaged using either hydrogen peroxide (0.15%, 44 mM) or ferric chloride (250 μM) and L-dopa (250 μM). The assays were conducted in a total volume of 1 ml and oxidation reactions were carried out for either 30 or 60 min in a shaking water bath at 37°C. The oxidation reaction was stopped by precipitating the protein with 10% trichloroacetic acid (TCA). The protein pellet was washed with TCA-containing desferrioxamine (50 μM). Protein carbonyl content was then determined. The effects of 10–20 mM taurine to prevent oxidative damage were assessed and glutathione (10 mM) was used as a positive control in one experiment.

Lipid Peroxidation

Lipid peroxidation was estimated using the thiobarbituric acid (TBAR) test. TBAR were assayed

by a modification of the method described by Wills (1987). Malonaldehyde (TBAR) formation was quantitated using 1,1,3,3-tetraethoxypropane as a standard. The absorbancy of TBAR was read at 530 nm in a Beckman spectrophotometer. The ability of taurine (1–20 mM) to directly inhibit ferric chloride-stimulated lipid peroxidation was examined in adult Sprague–Dawley cerebral cortex. Rat cortex was homogenized in ice-cold PBS (pH = 7.0) and incubated (200 μg protein/tube) in the presence and absence of either ferric chloride (200 μM) and/or taurine (1–20 mM) for 15 min at 37°C in a shaking water bath. The reaction was stopped by the addition of 1 ml of a solution containing 0.8 M HCl, 12.5% TCA and 0.1 mM desferrioxamine. Aliquots from the samples were then assayed for TBAR formation in duplicate.

Renal Cell Culture and the Effects of Iron-Stimulated Catecholamine Oxidation on Cell Viability

LLC-PK_1 cells are a procine-derived renal epithelial cell line with characteristics of proximal tubular cells. LLC-PK_1 cells have a high capacity for dopamine synthesis and contain dopamine transporters and receptors (Dawson *et al.*, 1994). LLC-PK_1 cells were grown and maintained as previously described (Dawson *et al.*, 1994). Briefly, the cells (ATCC, passage 206–208) were grown in Delbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, streptomycin (100 μg/ml), penicillin (100 U/ml) and $NaHCO_3$ (44 mM). The cells were plated in 96-well culture dishes and incubated overnight prior to experimentation. The cells were treated with sterile saline containing 2.5 mM $CaCl_2$, 75 μM ferric chloride and 250 μM L-dopa for 60 min and then the cells were washed and fresh culture media (DMEM) was added. Calcium was added to maintain the attachment of cells to the culture plates during the incubation period. The ability of taurine and taurine analogs to attenuate cytotoxicity was evaluated. Taurine and taurine analogs were added simultaneously with iron/L-dopa treatment and the effects of a 60-min pre-treatment were also tested. The pre-treatment consisted of 60-min incubation in DMEM containing 10 mM taurine or taurine analogs and controls received fresh DMEM. The media was removed and the cells rinsed with sterile saline prior to treatment with iron/L-dopa treatment. Taurine and taurine analogs were also present during the cytotoxic exposure. The cells were allowed 24 h to recover and cytotoxicity (viability) was determined using an assay based on tetrazolium salt (MTT) reduction (Mosman, 1983). In addition to the MTT assay, total protein and DNA were measured to confirm the MTT results in pilot studies and all measures were closely correlated for LLC-PK_1 cells.

Statistical Analysis

In general, the data were subjected to analysis of variance (ANOVA) followed by planned comparisons using either Bonferroni's multiple comparison test for limited comparisons or the Newman–Keuls test when all groups were compared. When appropriate, nonparametric statistics (Kruskal–Wallis/Dunn's Multiple Comparison test) were used. In cases involving comparisons of only two means, a *t*-test was used. The statistical analyses were performed using GraphPad Prism Version 2.0 software (San Diego, CA). Data from metal-stimulated catecholamine oxidation studies were based on separate experiments repeated usually at least 5–6 times (n = 5–6). The data analysis was performed on the raw data and for graphic representation was sometimes presented as a percent of control. All data are expressed as mean ± standard error of the mean (SE).

RESULTS

Taurine caused a concentration-related decrease in quinone formation caused by ferric chloride (500 μM)-stimulated oxidation of L-dopa

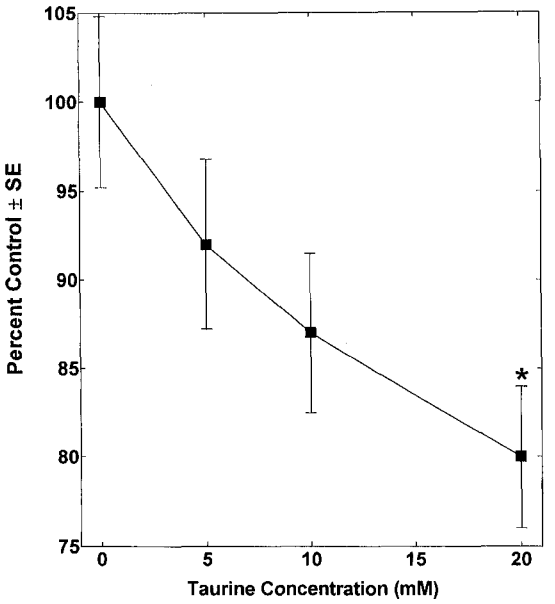


FIGURE 1 The effects of increasing concentrations of taurine to inhibit ferric chloride (500 μM)-stimulated L-dopa (500 μM) oxidation.

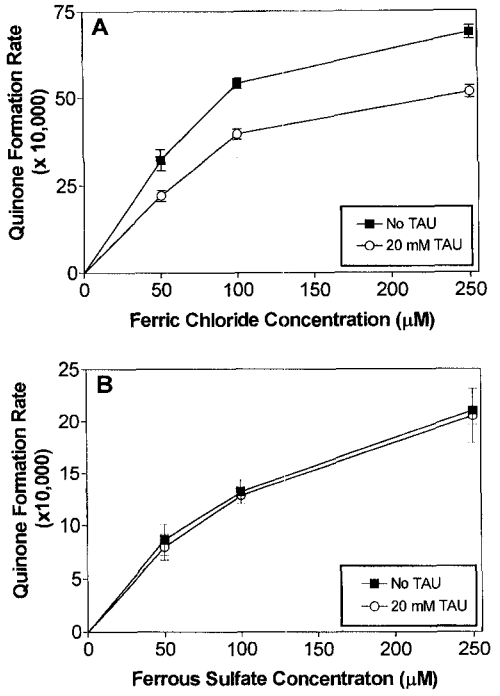
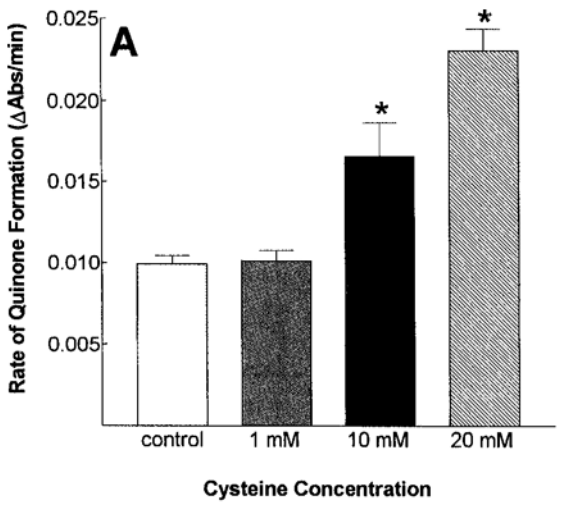


FIGURE 2 A. Taurine (20 mM) caused a significant ($p < 0.05$) reduction in Fe^{3+}-catalyzed oxidation of 500 μM Dopa at all three iron concentrations tested. B. Taurine (20 mM) did not show significant protection of Fe^{2+}-catalyzed oxidation of 500 μM L-dopa. Reactions were run for 5 min at room temperature (24°C) and were scanned at $\lambda = 490$ nm. Quinone formation rates represent the change in absorbance/min.

(500 μM) (Fig. 1). Ferric chloride-stimulated L-dopa oxidation in a concentration-dependent manner and 20 mM taurine significantly ($p < 0.01$) inhibited quinone formation at all ferric chloride concentrations tested (Fig. 2A). Ferrous sulfate-stimulated L-dopa oxidation, but to a lesser extent than ferric chloride (Fig. 2B). Addition of ascorbic acid (0.1 mM) to ferrous sulfate increased L-dopa oxidation slightly, but not to the level seen with ferric chloride (data not shown). Taurine (20 mM) did not inhibit ferrous sulfate-stimulated L-dopa oxidation (Fig. 2B). Hypotaurine, cystine, glutathione, and ascorbic acid inhibited iron-stimulated L-dopa oxidation (Table I). β-Alanine had no effect on L-dopa oxidation, but the taurine precursors, methionine and cysteine-stimulated L-dopa oxidation (Table I). Cysteine was tested at 1, 10 and 20 mM and both 10 and 20 mM produced a significant potentiation of L-dopa oxidation (Fig. 3A). Lysine, which has been reported to scavenge quinones (Kalyanaraman *et al.*, 1987), had only a modest effect on iron-stimulated quinone formation (Table I). Taurine analogs were also tested for their ability to inhibit dopamine (500 μM) oxidation stimulated by ferric chloride (100 μM). Taurine (sulfonic acid group) and hypotaurine (sulfinic acid group) inhibited quinone formation whereas β-alanine (carboxyl group) had no effect and isethionic acid (no amino group)-stimulated quinone formation (Fig. 4). Interestingly, extention of the carbon chain by one carbon (homotaurine) resulted in prominent potentiation of quinone formation in the presence of ferric iron and could significantly stimulate spontaneous (no added iron present) dopamine oxidation (Fig. 3B).

A number of experiments were conducted to examine the effects of buffer type (phosphate, Tris and borate) and reaction temperature (23°C



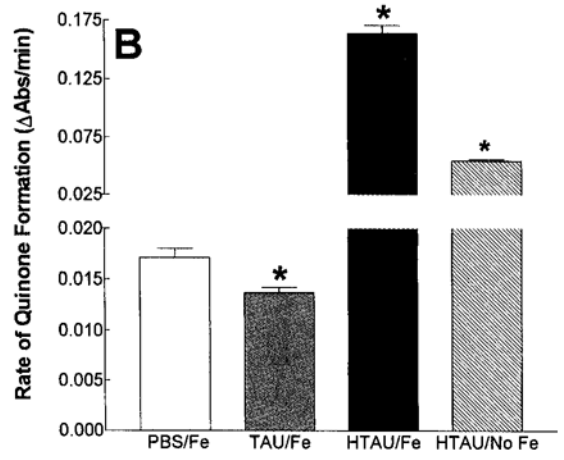


FIGURE 3 A. The effects of increasing concentrations of cysteine to potentiate ferric chloride (500 μM)-stimulated L-dopa (500 μM) oxidation. Cysteine at 10 and 20 mM significantly ($*p < 0.05$) increased quinone formation. B. Effects of homotaurine (HTAU, 20 mM) to significantly ($*p < 0.05$) stimulate dopamine (500 μM) oxidation in the presence and absence of ferric chloride (100 μM). Taurine (20 mM) was ran as control and inhibited ($*p < 0.05$) quinone formation.

TABLE I Effect of taurine and related amino acids on ferric chloride-stimulated L-dopa oxidation

Test compound	Number of replications	% Control
Taurine (20 mM)	5	75 ± 3^a
Taurine (20 mM)[b]	12	83 ± 3^a
Hypotaurine (20 mM)	14	87 ± 3^a
β-Alanine (20 mM)	15	96 ± 6^{ns}
Cystine (20 mM)	5	2 ± 2^a
Glutathione (20 mM)[b]	10	4 ± 3^a
Cysteine (20 mM)	9	222 ± 18^a
Methionine (20 mM)	9	121 ± 5^a
Lysine (20 mM)[b]	12	89 ± 4^{ns}
Ascorbic acid (0.5 mM)	5	77 ± 3^a

[a] $p < 0.05$, [ns] not significant. Ferric chloride (500 μM)-stimulated L-dopa (500 μM) oxidation. [b] Ferric chloride was 250 μM for these experiments. Control values (100%) represent the oxidation of L-dopa in the absence of taurine or test compounds.

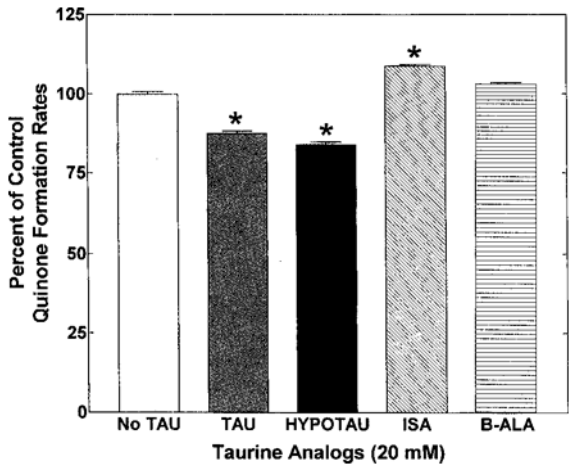


FIGURE 4 The effects of structural analogs of taurine to alter quinone formation rates. Taurine and analogs were all tested at 20 mM for their effects to alter quinone formation induced by 100 μM ferric chloride and 500 μM dopamine in PBS. Taurine and hypotaurine (HYPOTAU) inhibited and isethionic acid (ISA)-stimulated ($*p < 0.05$) dopamine oxidation.

versus 37°C). Taurine (20 mM) was able to significantly ($p < 0.05$) inhibit L-dopa oxidation stimulated by ferric iron (250–500 μM) in phosphate-buffered saline (pH = 7.4), 100 mM phosphate buffer (pH = 7.0), 100 mM borate buffer (pH = 10) and 50 mM Tris buffer (pH = 7.8) (data not shown). The reaction rates were temperature dependent and taurine produced better inhibition of quinone formation at 37°C than 23°C (Fig. 5), but this may simply be related to the higher reaction rates.

Taurine was able to significantly inhibit manganese-stimulated dopamine oxidation at low concentrations (50 μM) in PBS, but not at higher concentrations (Fig. 6). Tauriné was effective at inhibiting dopamine oxidation stimulated by

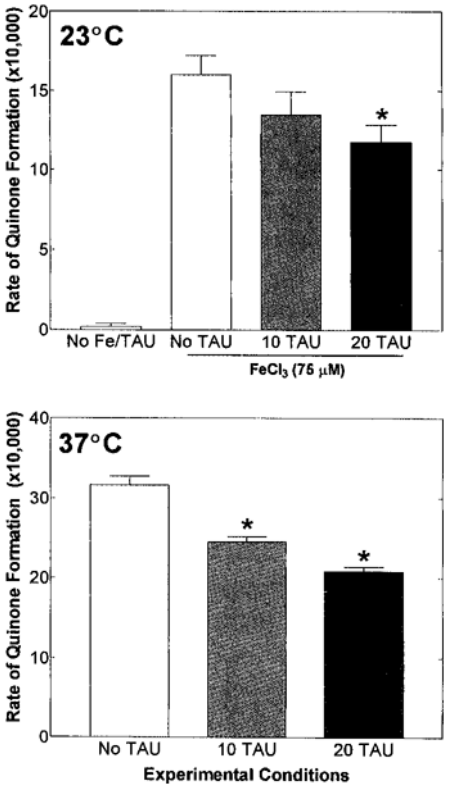


FIGURE 5 The effects of temperature on quinone formation rates (change in absorbance/min) were tested in the presence and absence of taurine (10 or 20 mM). L-dopa (250 μM) oxidation was stimulated by 75 μM ferric chloride and as expected, the rates of quinone formation increased as a function of elevated temperature. Taurine significantly ($*p < 0.05$) inhibited quinone formation. Note the low rate of spontaneous L-dopa oxidation in the absence of added iron and therefore this control was omitted from subsequent figures.

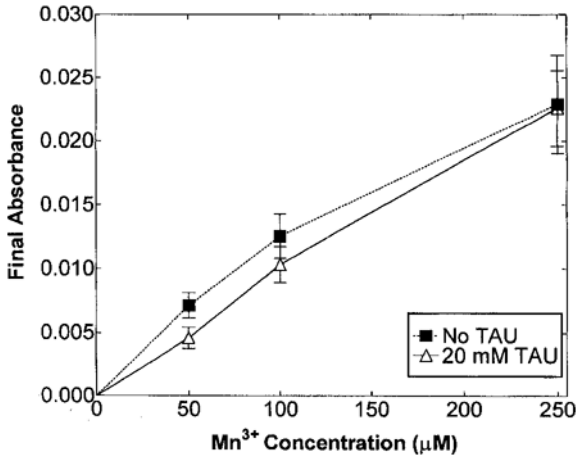


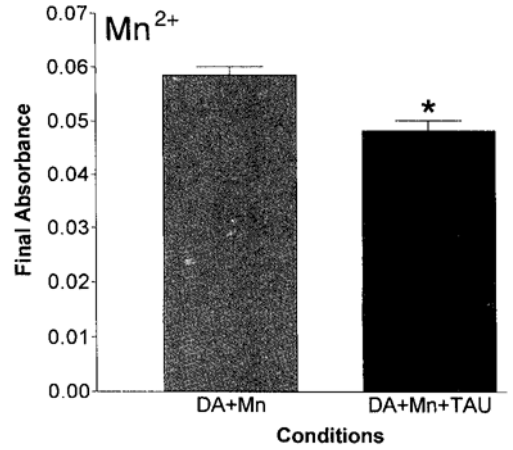
FIGURE 6 Dopamine (500 μM) oxidation induced by increasing concentrations of MnF_3. All experiments were carried out for 2 min at 37°C in PBS (pH = 7.4) and 20 mM taurine was tested for its ability to decrease quinone formation.

either Mn^{2+} or Mn^{3+} in Tris buffer (Fig. 7). Taurine had no effect on zinc-stimulated dopamine oxidation (Table II). Taurine significantly ($p < 0.05$) potentiated copper (Cu^{1+} and Cu^{2+})-stimulated dopamine oxidation at 50 and 100 μM (Table II).

Sodium nitroprusside (SNP) decomposes into nitric oxide (NO), cyanide and ferrous iron. NO has been shown to oxidize catecholamines and produce quinone derivatives (Yoshie and Ohshima, 1997). The stimulation of L-dopa (500 μM) oxidation with sodium nitroprusside (SNP, 500 μM) was significantly ($p < 0.01$) inhibited (64 ± 5%, $n = 6$) by taurine (20 mM). The reaction with SNP was carried out at room temperature in PBS (pH = 7.4) for 5 min and produced oxidation rates that were only about 10% of an equimolar concentration of ferric chloride.

The ability of taurine to inhibit quinone formation by enzymatic and nonenzymatic mechanisms not involving metals was explored. The oxidizing agent sodium periodate produced high rates of quinone formation that were not attenuated by taurine and in fact were significantly ($p < 0.05$) potentiated (Fig. 8A). Tyrosinase (Tase) can catalyze quinone formation and this enzymatic mechanism of dopamine oxidation was not affected by taurine (Fig. 8B). We also examined the spontaneous oxidation of the dopamine analog 6-OH-dopamine that is unstable and has a high rate of spontaneous oxidation in aqueous buffers at physiological pH. Taurine did not significantly inhibit the spontaneous oxidation of 6-OH-dopamine (data not shown).

Experiments were conducted to determine if taurine inhibited the formation of quinones by decreasing catecholamine oxidation directly.



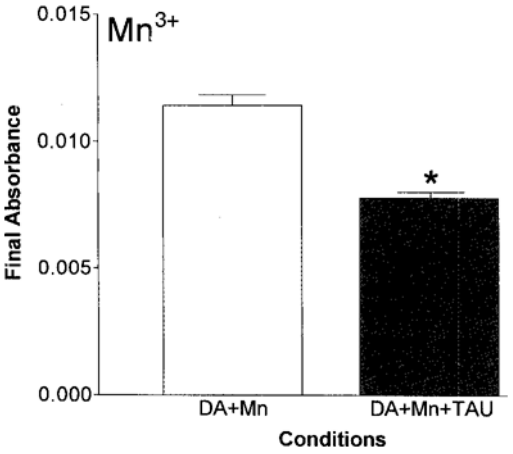
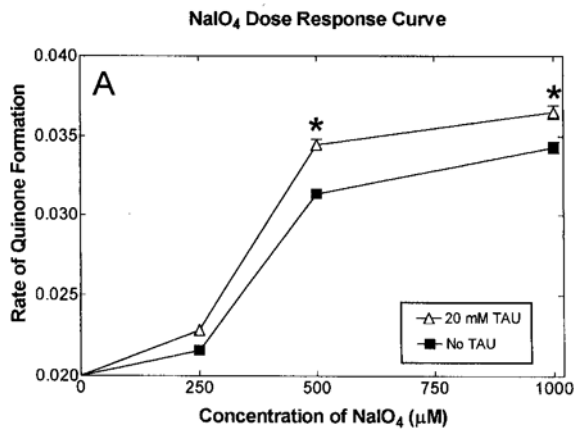


FIGURE 7 The ability of taurine (20 mM) to reduce 50 μM Mn^{2+} ($MnCl_2$)-catalyzed dopamine (DA, 500 μM) oxidation in 50 mM Tris buffer (pH 7.8). Reactions were run for a total of 5 min, at 37°C and scanned at $\lambda = 480$ nm. Mn^{+3} oxidation of dopamine (500 μM) was repeated using conditions identical to those in Fig. 6. Taurine showed significant protection ($^*p < 0.01$) from both Mn^{2+} and Mn^{3+}-catalyzed dopamine oxidation, but did not show a significant reduction in spontaneous oxidation of dopamine (data not shown).



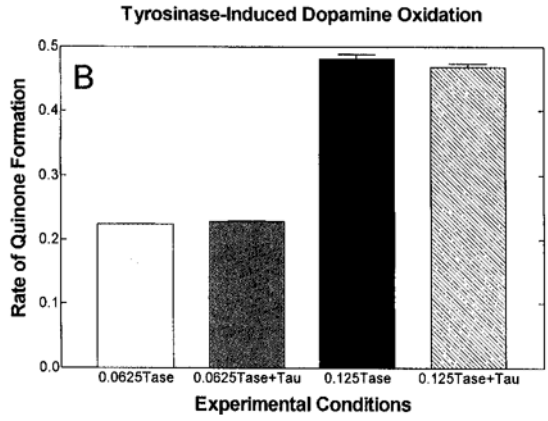


FIGURE 8 A. The ability of 20 mM taurine to alter the oxidation of 500 μM dopamine by sodium periodate ($NaIO_4$). The oxidation of dopamine by higher concentrations of $NaIO_4$ were significantly ($^*p < 0.05$) potentiated by taurine. The reactions were run for a total of 5 min at 25°C and scanned at $\lambda = 479$ nm. B. The lack of effect of 20 mM taurine (Tau) on the oxidation of 500 μM dopamine catalyzed by 0.0625 mg/ml and 0.125 mg/ml of the enzyme tyrosinase (Tase). Reactions were carried out for a total of 2 min at 25°C in phosphate buffer at pH 6.8. Rates of quinone formation represent the change in absorbance/min ± SE.

TABLE II Effects of taurine on dopamine oxidation rates in the presence of zinc or copper

Metal	10 μM	10 μM + TAU	50 μM	50 μM + TAU	100 μM	100 μM + TAU
Cu^{1+}	99 ± 1	114 ± 2	232 ± 6	259 ± 5*	336 ± 7	368 ± 9*
Cu^{2+}	107 ± 3	124 ± 2	258 ± 3	291 ± 4*	341 ± 5	377 ± 6*
Zn^{2+}	26 ± 1	22 ± 2	38 ± 2	38 ± 1	51 ± 1	51 ± 1

Samples ($n = 6$) were read ($\lambda = 480$ nm) for 5 min at 37°C in 50 mM Tris buffer (pH = 7.8). Dopamine (500 μM) oxidation was measured in the presence or absence of taurine (TAU, 20 mM). Taurine significantly ($^*p < 0.05$) potentiated copper (50 and 100 μM)-stimulated dopamine oxidation. Oxidation rates represent the change in absorbance/min × 10,000 ± SE.

TABLE III Effects of taurine on quinone formation and L-dopa oxidation

Group	Quinone formation	L-Dopa
A. L-Dopa 4°C Incubation	ND	110 ± 0.7
B. L-Dopa 37°C Incubation	0.002 ± 0.0007	103 ± 1.1^c
C. TAU (10 mM)	0.003 ± 0.0009	104 ± 0.4
D. $FeCl_3$ (100 μM)	0.017 ± 0.0011^a	90 ± 0.3^a
E. $FeCl_3$ + TAU (10 mM)	0.013 ± 0.0011^b	92 ± 0.8
F. $FeCl_3$ + TAU (20 mM)	0.012 ± 0.0011^b	94 ± 0.8^b

Quinone formation was determined by blanking with the 4°C samples to subtract spontaneous oxidation and reading the final absorbance at 490 nm. L-Dopa was assayed by HPLC and the values are expressed as nmol/tube ± SE. $n = 6$ per condition. $^a p < 0.05$ versus groups B and C. $^b p < 0.05$ versus group D. $^c p < 0.05$ versus group A.

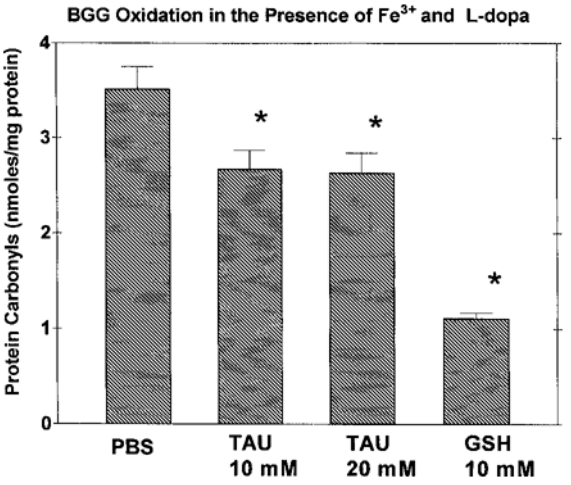


FIGURE 9 The oxidative damage to BGG (1 mg/ml) induced by L-dopa (500 μM) and ferric chloride (500 μM). Taurine (10 and 20 mM) and 10 mM glutathione (GSH) both significantly (*$p < 0.05$) decreased protein carbonyl content of oxidatively damaged BGG ($n = 6$ experiments and each condition was performed in triplicate in each experiment).

The results are presented in Table III. As in previous experiments, iron-stimulated quinone formation was inhibited by 20 mM taurine about 30% whereas L-dopa loss was reduced only about 4% (Table III). This reduction in the loss of L-dopa was statistically significant (Table III), but did not appear to account for the total reduction in quinone formation. The experiment was repeated under identical experimental conditions with dopamine and iron-stimulated dopamine oxidation was reduced by 10 mM taurine (92 ± 1.0 nmol/tube) compared to ferric chloride in the absence of taurine (88 ± 0.9 nmol/tube). Thus, taurine produced a consistent inhibition of iron-stimulated catecholamine oxidation, but of a smaller magnitude than the reduction in quinone formation.

Taurine protected BGG from the oxidative damage caused by either 30 or 60 min exposures to ferric chloride (250 μM) and L-dopa (250 μM). Taurine (20 mM) significantly ($p < 0.05$) inhibited ($47 \pm 3\%$, $n = 6$) protein carbonyl formation after 30 min of exposure, but 10 mM taurine only reduced the damage by 6%. Taurine (10 or 20 mM) and glutathione (10 mM) significantly ($p < 0.05$) decreased the carbonyl content of BBG treated with L-dopa and iron for 60 min (Fig. 9). Taurine did not inhibit protein carbonyl formation induced by H_2O_2, which caused more than two-fold greater damage than that produced by iron and L-dopa (data not shown).

Taurine (20 mM) did not inhibit basal or ferric chloride (200 μM)-stimulated TBAR formation in rat cerebral cortex homogenates (Fig. 10A). The effects of taurine on iron-stimulated lipid peroxidation were examined across a range of concentrations (1–20 mM) and again failed to inhibit iron-stimulated lipid peroxidation (Fig. 10B). It therefore appears unlikely that taurine's action to inhibit iron-stimulated quinone formation were due to simple chelation of iron.

Treatment of LLC-PK_1 cells with L-dopa (250 μM) and ferric chloride (75 μM) reduced cell viability about 40% (*$p < 0.05$) and both taurine and hypotaurine (10 mM) significantly (*$p < 0.05$) decreased cytotoxicity as indexed by the MTT assay (Fig. 11). Pre-treatment with taurine did not significantly enhance taurine's cytoprotective effects. Ferric chloride-stimulated L-dopa oxidation was determined under identical conditions as the cell culture experiments were conducted and taurine significantly ($p < 0.001$) decreased

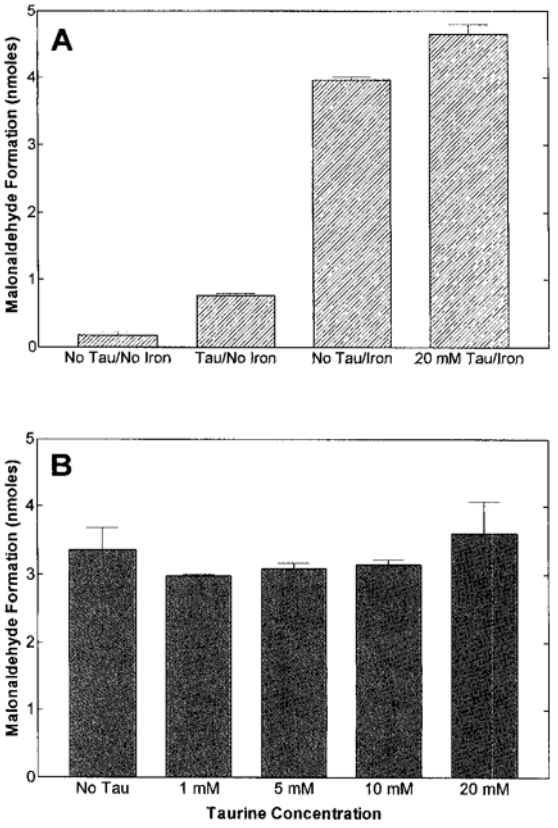


FIGURE 10 Ferric chloride (200 μM)-stimulated lipid peroxidation in crude homogenates of rat cerebral cortex (200 μg/protein/tube, $n = 4$). Taurine failed to alter iron-stimulated malonaldehyde formation.

quinone formation by 23% (10 mM) and 34% (20 mM) (Fig. 5). Taurine and hypotaurine blocked iron/L-dopa cytotoxicity, while homotaurine and 2-aminomethane sulfonic acid (data not shown) enhanced cytotoxicity (Fig. 11). Follow-up studies showed that 2-aminomethane sulfonic acid was cytotoxic when applied to LLC-PK_1 cells in the absence of iron/L-dopa (unpublished findings). Homotaurine alone had no effect on LLC-PK_1 cells, but it was cytotoxic if applied with L-dopa even without added ferric chloride (data not shown). These findings are consistent with the pro-oxidant effects of homotaurine seen in the quinone formation assays (Fig. 3B).

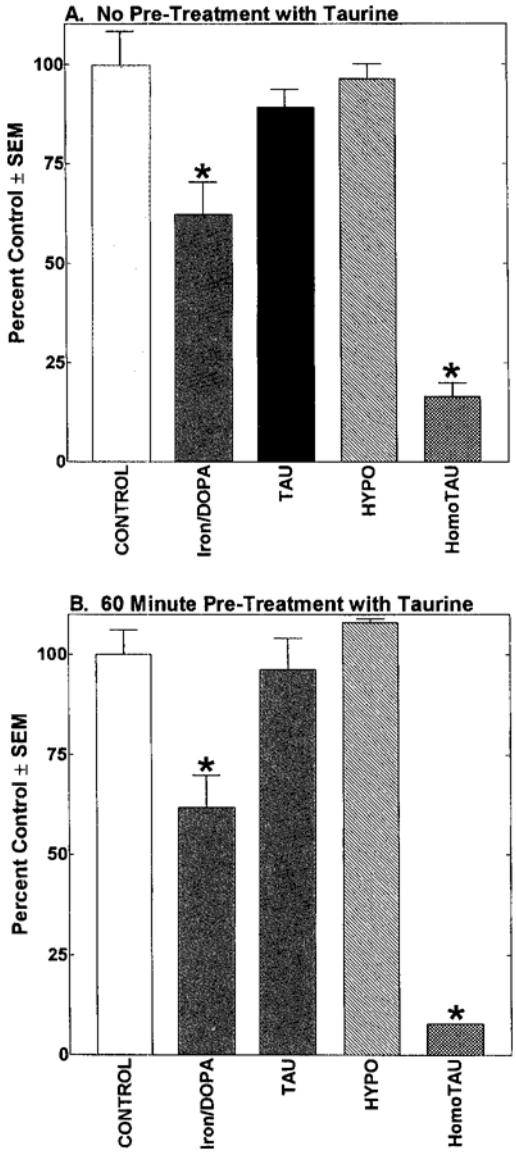


FIGURE 11 The cytoprotective effects of taurine in LLC-PK_1 cells treated with ferric chloride (75 μM) and L-dopa (250 μM). Iron/L-dopa treatment caused a significant ($^*p < 0.05$) decrease in cell viability that was blocked by 10 mM taurine or hypotaurine (HYPO). Homotaurine (HomoTAU) significantly ($^*p < 0.05$) potentiated cell death. A. LLC-PK_1 cells only treated with taurine or taurine analogs during the treatment with iron/L-dopa. B. LLC-PK_1 were treated with taurine or taurine analogs in culture media for 60 min prior to incubation with iron/L-dopa. Taurine and taurine analogs were also present during the iron/L-dopa treatment similar to conditions in A. These data are based on the average of 3–5 experiments using 8 wells per experimental condition in each experiment.

DISCUSSION

Taurine was clearly shown to inhibit L-dopa oxidation that was catalyzed by ferric chloride. Furthermore, this inhibition of L-dopa oxidation resulted in decreased oxidative damage to protein (BGG) and a reduction in cytotoxicity in cultured renal epithelial cells. Taurine did not attenuate L-dopa oxidation stimulated by the ferrous form of iron. The ferric form of iron is preferentially bound by the o-hydroquinone moiety of catecholamines which leads to the reduction of Fe^{3+} to Fe^{2+} with concomitant oxidation of the catecholamine (Miller *et al.*, 1990). The lower oxidation rates of L-dopa seen with the ferrous form of iron may preclude the detection of the antioxidant properties of taurine using the quinone formation assay. Taurine does not appear to inhibit the oxidation of catecholamines due to metal chelation since the stability constant for metal complexation by taurine is negligible (Wright *et al.*, 1986). Aruoma *et al.* (1988) also reported that taurine did not appear to chelate iron in a manner that affected hydroxyl radical formation from H_2O_2. Taurine was also effective across a range of ferric chloride concentrations and did not appear less effective at higher iron concentrations (Fig. 2). Taurine also failed to have any effect on iron-stimulated lipid peroxidation. We cannot, however, completely rule out a mechanism involving metal chelation or complexation.

We found that taurine inhibits iron-stimulated L-dopa oxidation in both borate buffer (pH = 10) and phosphate buffers (pH 7–7.4), but was slightly less effective in 50 mM Tris buffer (pH = 7) perhaps due to the chelating and free-radical scavenging properties of Tris buffers (Miller *et al.*, 1990). Taurine was effective in inhibiting manganese-induced dopamine oxidation in Tris buffer when the pH was higher (pH = 7.8), perhaps due to the higher oxidation rates seen at more basic pHs (Donaldson *et al.*, 1980). The high intracellular concentration and water solubility of taurine may function to protect vital cytosolic proteins and DNA (Messina and Dawson, in press) from oxidative damage arising from metal-stimulated catecholamine oxidation.

A number of studies have shown that catecholamines (dopamine and L-dopa) can produce oxidative stress and cytotoxicity. This cytotoxicity appears related to metal-catalyzed oxidation reactions which can be prevented by antioxidants (Basma *et al.*, 1995; Lai and Yu, 1997; Offen *et al.*, 1996; Pardo *et al.*, 1995; Shen and Dryhurst, 1998; Tanaka *et al.*, 1991). As previously suggested by Huxtable (1992), the synthesis of taurine may have a general cytoprotective effect by converting reactive cysteine and methionine to the antioxidant hypotaurine and subsequently to taurine. Cysteine alone or conjugated to reactive quinones can produce oxidative damage depending on cellular conditions. Furthermore, detoxification of free radicals and reactive intermediates by cysteine or glutathione may result in cytotoxic thiol derivatives (Monks *et al.*, 1992). Shen and Dryhurst (1998) have shown that both iron- and manganese-catalyzed oxidation of dopamine occurs in the presence of L-cysteine. Iron and cysteine can generate hydroxyl and cysteinyl radicals and dopamine-o-quinones react with cysteine to form 5-S-cysteinyldopamine (Shen and Dryhurst, 1998). Detoxification by taurine may be a biologically "safer" route that generates less reactive detoxification products. Taurine may serve as a nucleophile in addition reactions with reactive quinones (Tse *et al.*, 1976) and potentially stabilize quinones and prevent redox cycling which could serve as a source for the further generation of free radicals. The reaction rate for taurine is slow relative to the rate for intracyclization of the o-quinone that yields aminochromes (Tse *et al.*, 1976). In contrast, the sulfhydryl function of cysteine and glutathione undergoes fast rates of addition to o-quinones (Tse *et al.*, 1976). Our data showed that glutathione and cystine (not cysteine) almost completely blocked detectable quinone formation whereas, cysteine, homotaurine, and methionine increased quinone formation. Taurine was unable to decrease quinone formation caused by enzymatic mechanisms

or strong oxidants where rates of quinone formation were very high. Thus, taurine did not appear to simply scavenge quinones in a nonspecific manner. Taurine appeared to inhibit a specific mechanism of quinone formation associated with ferric iron or manganese. This is further suggested by the fact that hypotaurine was equipotent with taurine in inhibiting quinone formation although hypotaurine is a much better scavenger of hydroxyl radical (Aruoma *et al.*, 1988; Shi *et al.*, 1997). None-the-less, the high concentration of taurine in many tissues (5–60 mM) could over time significantly reduce the number of reactive quinones generated *in vivo* by ferric iron.

The methodology employed to measure quinone formation in these studies does not address the exact reaction mechanism responsible for the decline in quinone formation. Therefore, an apparent decline in the rate of quinone formation could result from an independent scavenging reaction by taurine that shifts the spectrophotometric characteristics of a putative quinone conjugate. Thus, the real rate of quinone formation could be unaffected, but the total quinones detected declined due to a conjugation reaction. As mentioned above, there did not appear to be a generalized conjugation of quinones by taurine since the inhibitory effects of taurine on quinone formation were restricted to ferric iron and manganese. Further studies will be required to fully elucidate the mechanism for taurine-induced reductions in measurable quinones derived from metal-stimulated catecholamine oxidation. Taurine had a modest effect to directly prevent the oxidation of either dopamine or L-dopa as determined by HPLC analysis. This could reduce redox cycling of quinones formed from metal-stimulated catecholamine oxidation reactions. The magnitude of the direct antioxidant effect (< 5% reduction) of taurine was smaller than the 20–30% reductions seen in quinone formation. This further strengthens the suggestion that taurine is scavenging quinones. Currently, we are developing methods to detect and quantitate putative tauro-quinone conjugates. Whatever the exact reaction mechanisms, taurine could reduce protein carbonyl formation and the cytotoxicity associated with iron-stimulated dopamine oxidation products.

The failure of taurine to inhibit zinc-stimulated catecholamine oxidation may relate to its lower intrinsic catalytic activity for oxidation reactions (Donaldson *et al.*, 1980). Zinc has also been shown to act synergistically with taurine to prevent various forms of cytotoxicity (Gottschall-Pass *et al.*, 1997). It is unclear why taurine enhanced copper-stimulated dopamine oxidation. A recent abstract (O'Brien *et al.*, 1999) suggests that taurine may form a stable complex with copper. It is unknown if this complex merely enhances copper solubility or if it also enhances catalytic activity for oxidation reactions. Taurine has been shown to attenuate copper toxicity in rats (Hwang *et al.*, 1998). Further experiments are necessary to understand how taurine interacts with these metals and their valence states.

The cell culture experiments showed that ferric iron and L-dopa were toxic to LLC-PK_1 cells. Previous studies have shown that treatment of LLC-PK_1 cells with L-dopa alone can induce oxidative stress and up-regulate glutathione production (Han *et al.*, 1996). Taurine and hypotaurine were shown to be equally effective in producing a cytoprotective effect in LLC-PK_1 cells consistent with their ability to inhibit quinone formation (Table I). This is in contrast to the fact that hypotaurine is a much better scavenger of hydroxyl radical than is taurine (Aruoma *et al.*, 1988; Shi *et al.*, 1997). Homotaurine accelerated quinone formation and enhanced L-dopa toxicity in the presence or absence of added iron. The mechanism for the pro-oxidant effects of homotaurine at present is unclear, but is consistent with what was seen with methionine and cysteine. Thiols are known to form quinone-thioethers that are reactive and potent nephrotoxicants and neurotoxins (Monks *et al.*, 1992). The experiments with no pre-treatment of the cells with taurine showed that simultaneous exposure of the cells to reactive quinones and taurine was

as effective as preloading the cells with taurine for an hour. Thus, much of the cytotoxicity may have been prevented at an extracellular site of action via inhibition of quinone formation.

Recently it has been shown that proteins that undergo attack by the hydroxyl radical can give rise to protein bound L-dopa which can cause damage to DNA (Gieseg *et al.*, 1993; Morin *et al.*, 1998). Iron or copper can also catalyze the oxidation of L-dopa and promote damage to DNA (Levay *et al.*, 1997; Spencer *et al.*, 1994). Our lab has conducted preliminary studies that have shown taurine at concentrations as low as 1 mM can inhibit oxidative damage to DNA caused by iron-stimulated L-dopa oxidation in the presence of added H_2O_2 (Messina and Dawson, 1998; in press). Taurine treatment resulted in significant reductions in hydroxylated bases found in calf thymus DNA after incubation with ferric iron, H_2O_2 and L-dopa (Messina and Dawson, in press). Thus, taurine can directly reduce quinone formation from catecholamines (L-dopa and dopamine) and reduce secondary markers (protein carbonyls and oxidized DNA) of oxidative damage. Iron can also mediate the oxidation of L-dopa to the excitotoxin, 2,4,5-trihydroxyphenylalanine (Newcomer *et al.*, 1995). Thus, taurine-mediated inhibition of iron-stimulated L-dopa oxidation would also be predicted to reduce the endogenous production of this neurotoxic agent.

Iron has been implicated in the pathogenesis of Parkinson's disease (Jellinger, 1999) and it has been proposed that metal–catecholamine interactions are responsible for the neurotoxicity (Graham, 1984; Shen and Dryhurst, 1998). Quinones formed from dopamine oxidation form adducts with thiols such as cysteine and significant increases in cysteinyl adducts of L-dopa and dopamine have been found in the substantia nigra of Parkinson's patients (Spencer *et al.*, 1998). The selective increase in iron in the substantia nigra appears related to neuromelanin (Jellinger, 1999) which forms via the polymerization of quinones. Iron is elevated in the substantia nigra with Parkinson's disease and appears to induce oxidative stress via a number of mechanisms (Jellinger, 1999). Treatment of Parkinson's patients with L-dopa could theoretically produce cytotoxicity since the oxidation of L-dopa can produce both free radicals (Basma *et al.*, 1995) and cytotoxic quinones (Pardo *et al.*, 1995). Reduced antioxidant defense mechanisms also appear to contribute to the neuronal loss in Parkinson's disease and taurine may play a role in protecting dopaminergic neurons from oxidative stress associated with elevated iron levels.

Aging has been shown to reduce striatal taurine concentrations and taurine content in other tissues that contain catecholamines (Dawson and Wallace, 1992; Dawson *et al.*, 1999a,b; Strolin Benedetti *et al.*, 1991). Furthermore, systemic administration of L-dopa to rodents produces reductions in the tissue content of taurine (Diederich *et al.*, 1997; Tyce and Owen, 1973) suggesting that taurine may be used *in vivo* to scavenge reactive quinones derived from L-dopa. It could be speculated that taurine may serve as an endogenous antioxidant and due to its high tissue concentrations may protect tissues from damage due to reactive quinones and oxygen radicals produced by catecholamine oxidation. Age-related reductions in taurine content could result in an increased susceptibility to oxidative damage and we have found that dietary taurine supplementation can reduce markers of oxidative damage to tissue proteins in aged rats (Dawson *et al.*, 1998). The dietary requirements for taurine may need to be reassessed in conditions associated with aging, metal toxicity or increased oxidative stress.

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