Amino Acids (2002) 22: 351–368

Amino
Acids

Effects of sulfur containing amino acids on iron and nitric oxide stimulated catecholamine oxidation

M. Biasetti and **R. Dawson Jr.**

Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida, U.S.A.

Accepted October 10, 2001

Summary. Taurine is a free amino acid found in high concentrations in tissues containing catecholamines. The ability of taurine and its metabolic precursors to inhibit or stimulate catecholamine oxidation and subsequent quinone formation was examined. Ferric chloride was used as the catalyzing agent to stimulate L-dopa or norepinephrine oxidation and NO donors were also examined for their actions to stimulate quinone formation. Taurine attenuated iron-stimulated quinone formation from catecholamines suggesting that it may function as an endogenous antioxidant. Several other sulfur-containing amino acids (homocysteic acid, cysteine sulfinic acid and SAM) were found to inhibit catecholamine oxidation. Among other amino acids tested, homocysteine had biphasic effects; attenuating L-dopa oxidation catalyzed by ferric chloride and potentiating norepinephrine's oxidation catalyzed by both ferric chloride and sodium nitroprusside (SNP). Homotaurine and homocysteine (1 or 10 mM) greatly stimulated SNP-induced norepinephrine oxidation. Homotaurine potentiated quinone formation in the presence of ferric iron and this effect was attenuated by desferroxamine. In order to exclude a possible NO/iron interaction in SNP's oxidizing action, SIN-1 chloride, a specific NO-donor, was tested as an oxidizing agent. The failure of desferroxamine or taurine to attenuate SIN-1 oxidation of norepinephrine suggests that peroxynitrite-mediated oxidation was likely the dominant mechanism. Our results show that endogenous sulfur containing amino acids, like taurine, could serve a protective role to reduce cellular damage associated with both NO and metal-stimulated catecholamine oxidation.

Keywords: Amino acids – Taurine – Catecholamines – Ferric chloride – Nitric oxide – Homocysteine – Homotaurine

Introduction

Catecholamines are abundant in many tissues and are important in mediating neurotransmission and hormonal responses. Catecholamines also undergo

spontaneous oxidation and metals can greatly stimulate their oxidation (Miller et al., 1990). The oxidation of catecholamines can generate oxygen radicals and cytotoxic quinones (Bindoli et al., 1992; Monks et al., 1992). These oxidation products have been implicated in the pathophysiology of Parkinson's disease, congestive heart failure and certain types of cancer (Graham et al., 1978; Graham 1984; Monks et al., 1992; Terland et al., 1997; Cavalieri and Rogan, 2001). Previously, our laboratory had demonstrated that the amino acid taurine could decrease catecholamine oxidation as indexed by reduced quinone formation. Taurine can also reduce markers of oxidative damage such as protein carbonyls, DNA base hydroxylation and cell death caused by catecholamine oxidation products (Dawson et al., 2000a); Messina and Dawson, 2000). These previous studies focused primarily on dopamine and its precursor L-dopa and their neurotoxic potential. Free radicals and cytotoxic quinones may play a role in hypertension, atherogenesis and heart disease (Touyz, 2000; Terland et al., 1997). Both norepinephrine and dopamine are present in cardiovascular tissues and their oxidation could contribute to oxidative damage to vessel walls, the heart and renal tissue. Therefore, one of the major aims of the present study was to further study taurine and other sulfur containing amino acids and their ability to alter catecholamine oxidation rates.

The biosynthetic pathway for taurine determines the fate of many important sulfur containing amino acids and glutathione (Huxtable, 1992). Taurine can be synthesized from methionine via the transsulfuration pathway or more directly from cysteine. Catecholamines can be inactivated by the enzyme catechol-O-methyltranferase (COMT) which uses the sulfur containing amino acid, S-adenosylmethionine (SAM) as a methyl donor. Dopamine and dopamine-derived quinones are also conjugated with sulfate and cysteine respectively (Spencer et al., 1998). Cysteine conjugates of dopamine are very reactive and neurotoxic (Shen and Dryhurst, 1998). Another sulfur containing amino acid, homocysteine, has received notable attention as a risk factor for cardiovascular disease and it induces oxidative stress (Brattstrom and Wilcken, 2000; Nonaka et al., 2001). Thus, it is important to evaluate how taurine as well as its metabolic precusors may influence the oxidation state of catecholamines given their role in catecholamine metabolism.

Nitric oxide (NO) interacts with cysteine and nitrothiols likely mediate the vasodilatory actions of NO. NO has been shown to react directly with norepinephrine to form 6-nitronorepinephine which is postulated to have biologic activity (Shintaini et al., 1996; Daveu et al., 1997; Palumbo et al., 2001). Peroxynitrate, which can nitrate tyrosine residues, cannot convert catecholamines to their 6-nitro-derivatives and only produces oxidized derivatives (Daveu et al., 1997). Yoshie and Ohshima (1997) suggest that NO may oxidize catecholamines and form quinones which redox cycle forming superoxide radicals and promoting peroxynitite formation possibly leading to neuronal damage in Parkinson's disease and aging. The free radicals formed during catecholamine oxidation (superoxide radical and hydroxyl radical) could help accelerate the process of peroxynitrite formation from NO. Two characterized interactions between taurine and NO are the modulation of

taurine efflux by NO (Oja and Saransaari, 2000) and the inhibition of NO formation and iNOS expression by the chlorinated derivative of taurine, taurochloramine (Park et al., 1997). There has been little direct evidence for NO or $ONOO^-$ mediating catecholamine oxidation reactions and even less information concerning modulation by sulfur containing amino acids. Therefore, we also evaluated the stimulation of quinone formation by known NO donors and whether taurine and its analogs could attenuate NO's oxidizing actions.

Material and methods

L-Dopa oxidation stimulated by ferric chloride

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 490 nm using a Beckman DU 7,000 diode array spectrophotometer. Experiments were conducted in phosphate buffered saline (PBS, pH = 7.4) at ambient temperature (26–28°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 30 s for a total of 5 minutes. Reactions with high oxidation rates that yielded non-linear rates over that time span were ran for two minutes to obtain linear rates. Catecholamine oxidation rates were measured in the presence of ferric chloride (50 μM–250 μM). The effects of taurine, taurine analogs and other amino acids (1 mM–20 mM) were assessed for their ability to inhibit or potentiate catecholamine oxidation induced by ferric chloride. All solutions (ferric chloride, amino acids and L-dopa) were made up fresh immediately prior to spectrophotometric assays. Specific experimental conditions and reagent concentrations are given in figure captions.

Norepinephrine oxidation stimulated by ferric chloride

By reacting 500 μM norepinephrine and 250 μM ferric chloride for five minutes and then running a wavelength scan, we determined that the quinone product of norepinephrine had a maximal absorption at 510 nm, therefore, the rate of norepinephrine oxidation was measured spectrophotometrically using this wavelength. These experiments were conducted similar to those for L-dopa, but the ferric chloride was only tested at a final concentration of 250 μM. Taurine, taurine analogs and other amino acids were tested at 1 mM and 10 mM concentrations to evaluate their ability to inhibit or potentiate oxidation.

L-Dopa oxidation stimulated by sodium nitroprusside

These experiments were conducted as previously described but in order to understand the exact role nitric oxide may play in catecholamine oxidation, sodium nitroprusside (SNP) was used as the catalyzing agent at a 1 mM final concentration. By reacting 500 μM L-dopa and 1 mM sodium nitroprusside for five minutes and then running a wavelength scan, we determined that the quinones formed had a maximal absorption at 520 nm, therefore, the rate of catecholamine oxidation was measured spectrophotometrically using this wavelength.

Norepinephrine oxidation stimulated by sodium nitroprusside

After determining the optimal wavelength, the rate of norepinephrine oxidation induced by sodium nitroprusside was measured spectrophotometrically at 520 nm. These experiments were conducted as previously described above. Specific experimental conditions and reagent concentrations are given in figure captions.

Norepinephrine oxidation stimulated by SIN-1 chloride

3-Morpholinosydnonimine (SIN-1 chloride, TOCRIS, Ballwin, Mo), a NO donor, was tested at a final concentration of 0.1 mM obtained from a 3 mM stock solution made in PBS. The final concentration was chosen on the basis of the percentage of NO contained in 1 mM SNP. The SIN-1 stocks were made immediately prior to use. The oxidation rates were measured spectophotometrically at 520 nm. To rule out any possible interference, wavelength scans for both norepinephrine and SIN-1 were ran showing that NE and SIN-1 had a maximal absorption spectrum at 280 nm and 290 nm respectively. These experiments were conducted as described above.

Statistical analysis

Data from the oxidation study were based on separate experiments repeated at least 6 times ($n = 6$). The data analysis was performed on the raw data. Rate constants (Δabsorbance/minute) were used in data analysis since they were less variable than absolute final absorbance data. The statistical analyses were performed using GraphPad Prism version 2.0 software (San Diego, CA). All data are expressed as mean $\pm$ standard error of the mean (SEM). 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. Nonparametric analyses were used when appropriate. An asterisk (*) marks statistically significant data ($p < 0.05$). For graphic representation, data were sometimes presented as percent of control.

Results

L-Dopa oxidation stimulated by ferric chloride

Ferric chloride stimulated L-dopa oxidation in a concentration-dependent manner as previously seen (Dawson et al., 2000a). The addition of 10 mM taurine significantly ($p < 0.001$) reduced ferric chloride stimulated quinone formation overall, however, this reduction was not statistically significant at any single iron concentration tested (Fig. 1A). The temperature at which ferric chloride was tested seemed to play an important role on taurine's ability to significantly inhibit oxidation. Taurine's antioxidant effect was in fact more evident at higher oxidation rates obtained by increasing the incubation temperature up to 40°C (Fig. 1B). By testing taurine at a higher concentration (20 mM) and at ambient temperature (26°C) we did see a significant ($p < 0.05$) 40% decrease in the oxidation rates of L-dopa (500 μM) stimulated by 250 μM ferric chloride (data not shown). Thus, both taurine concentration and rate of oxidation are important variables that influence taurine's ability to inhibit quinone formation.

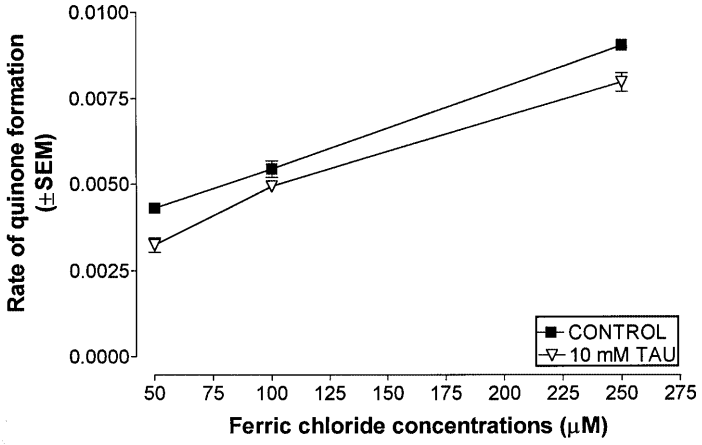

Fig. 1A. Effects of iron concentration on quinone formation. Ferric chloride stimulated quinone formation in a concentration dependent manner and 10 mM taurine attenuated oxidation. The two-way ANOVA showed that ferric iron significantly ($p < 0.001$) stimulated L-dopa oxidation and that 10 mM TAU had an overall significant effect in attenuating oxidation ($p < 0.001$) however this effect was not significant at any single ferric chloride concentration. Temperature = 26°C

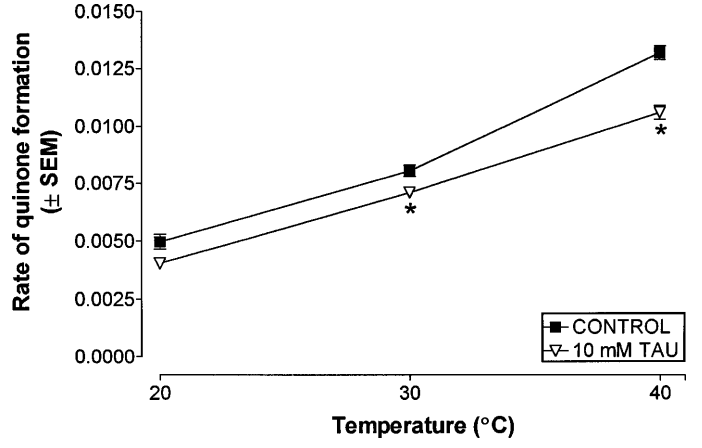

Fig. 1B. Effects of temperature on quinone formation. Increasing temperature stimulated ($p < 0.05$) quinone formation and 10 mM taurine significantly ($p < 0.05$) reduced oxidation in a rate dependent manner

To evaluate how taurine's antioxidant activity was related to its structure and metabolic precursors, analogs and precursors were tested at ambient temperature using 250 μM ferric chloride to stimulate oxidation rates. Although the concentrations of sulfur amino acid precursors employed exceeded their physiological concentrations, it allowed for structural comparison to effective concentrations of taurine. Cysteine (CYS) was tested at 10 mM and it produced a significant potentiation of L-dopa oxidation (Fig. 2A). As opposed to CYS, 10 mM homocysteine (HCYS) significantly reduced

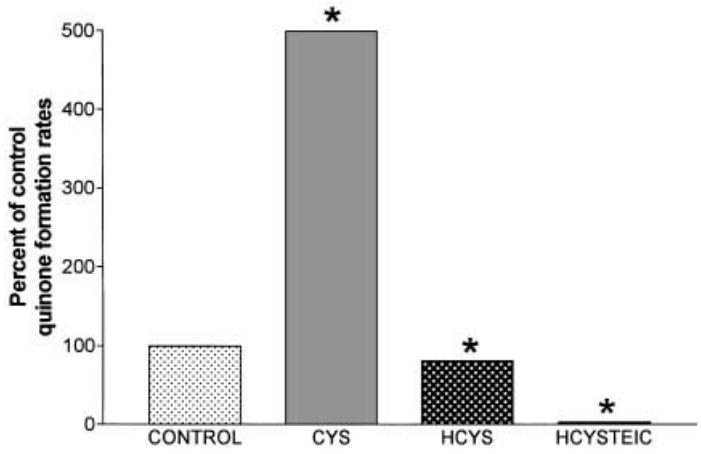

Fig. 2A. Effects of 10 mM cysteine and analogs on L-dopa oxidation. Cysteine (**CYS**, 10 mM) significantly potentiated L-dopa (500 μM) oxidation stimulated by 250 μM ferric chloride while 10 mM homocysteine (*HCYST*) and 10 mM homocysteic acid (Hcysteic acid) acted as antioxidants. Temperature = 27°C

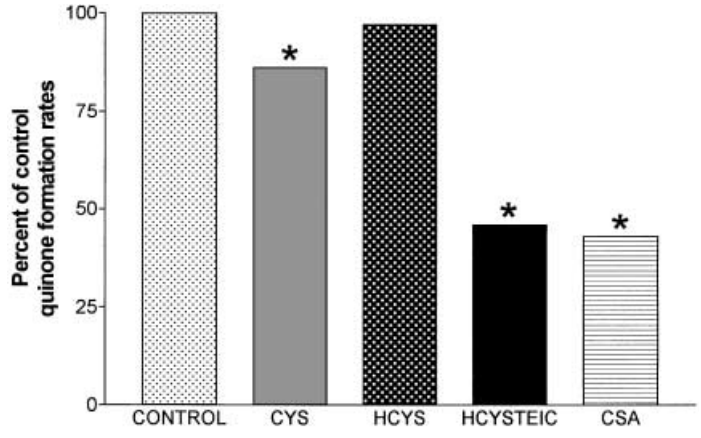

Fig. 2B. Effects of 1 mM cysteine and related analogs on L-dopa oxidation. Cysteine (*CYS*), homocysteic acid (Hcyst acid) and cysteine sulfinic acid (CysSulf) significantly (*$p < 0.05$) inhibited L-dopa (500 μM) oxidation induced by 250 μM ferric chloride. Temperature = 27°C

ferric chloride catalyzed oxidation of L-dopa (Fig. 2A). In the presence of homocysteic acid (10 mM) ferric chloride stimulated quinone formation was almost completely suppressed (Fig. 2A). These same amino acids were all tested at a concentration of 1 mM. The results obtained with 1 mM were similar to those obtained at 10 mM, with the exception of cysteine and homocysteine. Cysteine at 1 mM slightly reduced oxidation, whereas homocysteine had no effect (Fig. 2B). Cysteine sulfinic acid (CSA) was also tested at 1 mM and it significantly reduced oxidation similar to homocysteic acid (HCYSTEIC) (Fig. 2B). Thus, modification of chain length seems to play an important role modulating quinone formation.

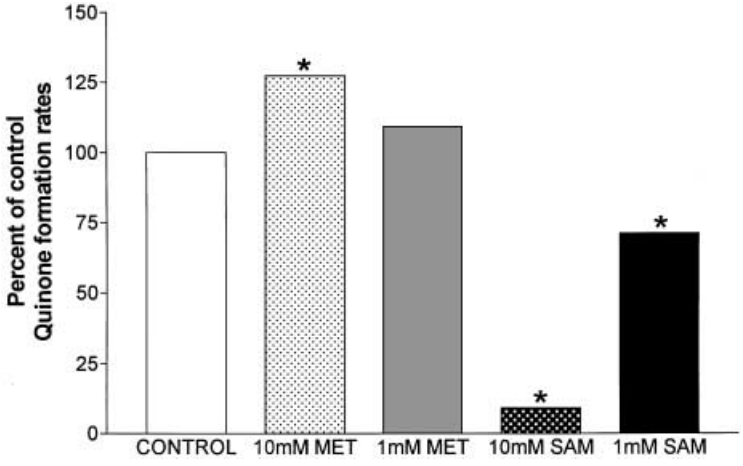

Fig. 3. Effects of methionine and S-adenosyl-methionine on L-dopa oxidation. Methionine (*MET*, 10 mM) (*p $<$ 0.05) and S-adenosyl methionine (*SAM*, 10 mM, 1 mM) (*p $<$ 0.05) respectively potentiated and inhibited L-dopa (500 μM) oxidation stimulated by 250 μM ferric chloride. Temperature = 28°C

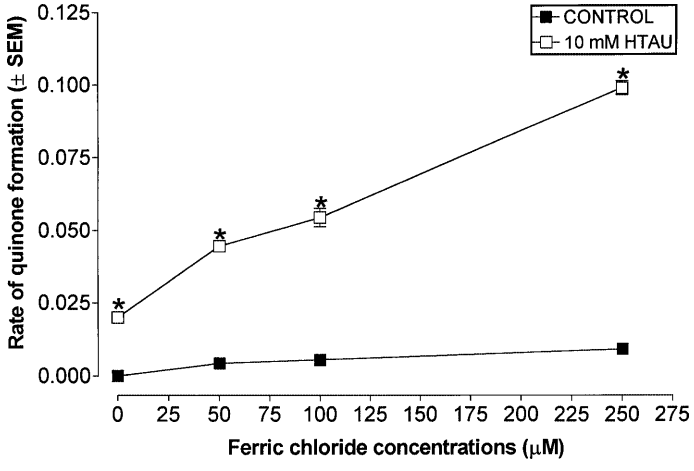

Fig. 4. Homotaurine's effects on L-dopa oxidation by ferric chloride. Homotaurine (10 mM) significantly (*p $<$ 0.05) potentiated oxidation of L-dopa in a concentration dependent manner. Temperature = 26°C

Methionine (10 mM) significantly potentiated oxidation while both 1 mM and 10 mM S-adenosyl-methionine (SAM) significantly inhibited oxidation, thus highlighting the importance of the S-adenosyl group in SAM's antioxidant activity (Fig. 3). Hypotaurine was also tested at 1 mM and 10 mM concentrations and though it reduced oxidation at its higher concentration this reduction was not statistically significant (data not shown).

Homotaurine (10 mM) significantly potentiated the oxidation of L-dopa (500 μM) both in the absence and presence of ferric chloride (50 μM, 100 μM or 250 μM) (Fig. 4). In order to understand the mechanism involved in

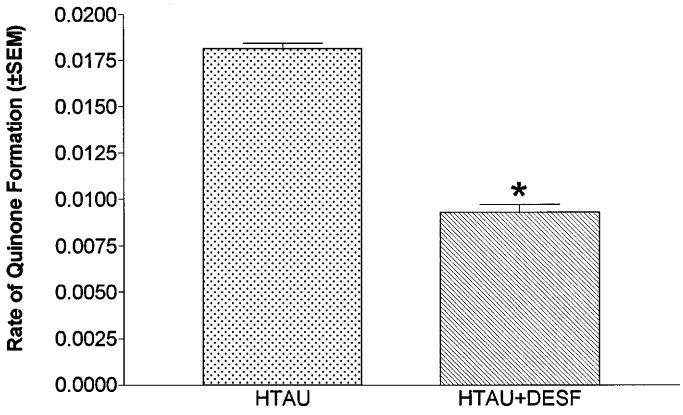

Fig. 5A. The effects of iron chelation by 1 mM desferroxamine on homotaurine-induced oxidation. Desferroxamine (*DESF*) significantly (*$p < 0.05$) inhibited L-dopa (500 μM) oxidation stimulated by 10 mM homotaurine (*HTAU*)

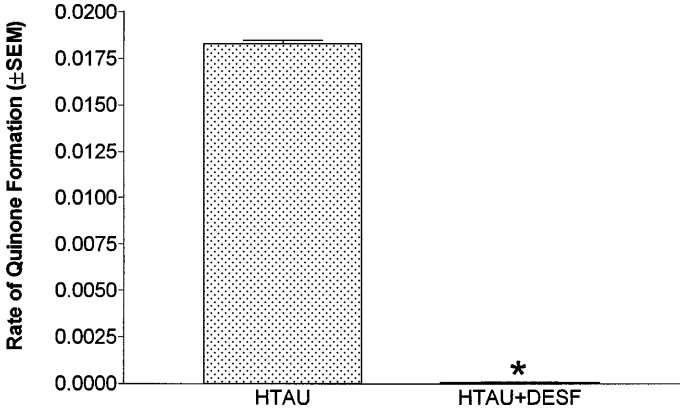

Fig. 5B. Effects of 5 mM desferroxamine effects on homotaurine-induced oxidation. Desferroxamine (*DESF*, 5 mM) significantly (*$p < 0.05$) inhibited L-dopa (500 μM) oxidation stimulated by 10 mM homotaurine (*HTAU*). Ferric chloride was not added in these experiments and the purpose of desferroxamine was to chelate trace levels of iron present in the buffer solutions. Temperature = 25.5°C for both experiments

homotaurine's oxidative effect, an iron-chelator (desferroxamine) was added to the L-dopa, which was then reacted with the 10 mM homotaurine in the absence of any added iron. The desferroxamine had a significant effect at both 1 mM and 5 mM concentrations to suppress quinone formation (Fig. 5A, 5B). The fact that it almost completely suppressed oxidation at its higher concentration suggests that iron interacts with homotaurine to stimulate quinone formation (Fig. 5B). All of the amino acids mentioned above were tested in the absence of ferric chloride and none, with the exception of 10 mM homotaurine, potentiated catecholamine oxidation. Thus, homotaurine can interact with the trace levels of metals in the buffer system and stimulate L-dopa oxidation.

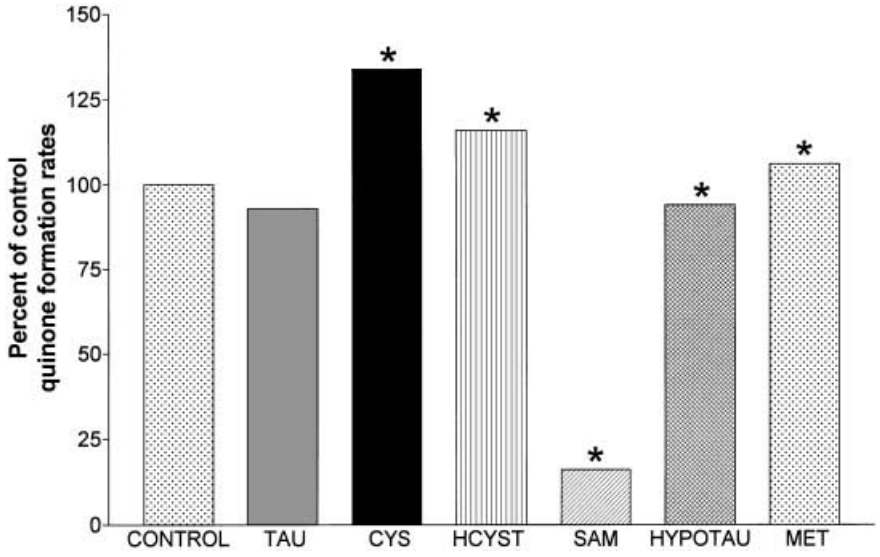

Fig. 6. Effects of sulfur containing amino acids (10 mM) on norepinephrine oxidation. Cysteine (*CYS*), homocysteine (*HCYST*) and methionine (*MET*) significantly (*$p < 0.05$) potentiated norepinephrine (500 μM) oxidation induced by 250 μM ferric chloride. SAM (10 mM) and hypotaurine (*HYPOTAU*) significantly (*$p < 0.05$) inhibited aminochrome formation. Standard errors were less than 2% for all groups. Temperature = 26°C

L-Dopa oxidation stimulated by sodium nitroprusside

SNP was a less potent oxidizing agent than ferric chloride. The oxidation rate of the control group (phosphate buffer pH = 7.4 + 500 μM L-dopa + 1 mM SNP) did not exceed 0.001 Δabs/min and at this low rate the only amino acid tested that had a significant ($p < 0.05$) effect to reduce oxidation by 20% was 10 mM hypotaurine (data not shown).

Norepinephrine oxidation stimulated by ferric chloride

The results obtained for norepinephrine were similar to what was seen for L-dopa. Taurine (10 mM) did not significantly reduce norepinephrine oxidation while 10 mM hypotaurine and 10 mM SAM had a significant antioxidant effect (Fig. 6). Cysteine (10 mM), homocysteine (10 mM) and methionine (10 mM) significantly potentiated oxidation (Fig. 6). These same amino acids were tested at 1 mM and had no significant effect on iron stimulated norepinephrine oxidation (data not shown). Homotaurine did not significantly potentiate norepinephrine's oxidation in the absence of ferric chloride but it did have a significant effect when 250 μM ferric chloride was added to either 20 mM or 10 mM HTAU (Table 1). As seen for the L-dopa, ferric chloride stimulated norepinephrine's oxidation in a concentration dependent manner and homotaurine (20 mM) significantly potentiatiated oxidation (Fig. 7).

Table 1. Homotaurine's (HTAU) effects on norepinephrine oxidation

Groups	Percent of control
Norepinephrine + $FeCl_3$	100 ± 2
HTAU (20 mM) − No added $FeCl_3$	64 ± 1[+]
HTAU (20 mM) + $FeCl_3$	726 ± 5*
HTAU (10 mM) + $FeCl_3$	569 ± 22*
HTAU (1 mM) + $FeCl_3$	132 ± 2

Data are expressed as mean ± SEM (n = 6 per group). HTAU (homotaurine) significantly (*$p < 0.01$) potentiated quinone formation from norepinephrine (500 μM) in the presence of ferric chloride (250 μM). Control rates (Norepinephrine + $FeCl_3$) represent quinone formation stimulated by 250 μM $FeCl_3$ in the absence of HTAU. [+]Note that normal norepinephrine oxidation rates in the absence of added iron are negligible, so HTAU did stimulate norepinephrine oxidation significantly ($p < 0.05$) above that of spontaneous (no added iron) rates.

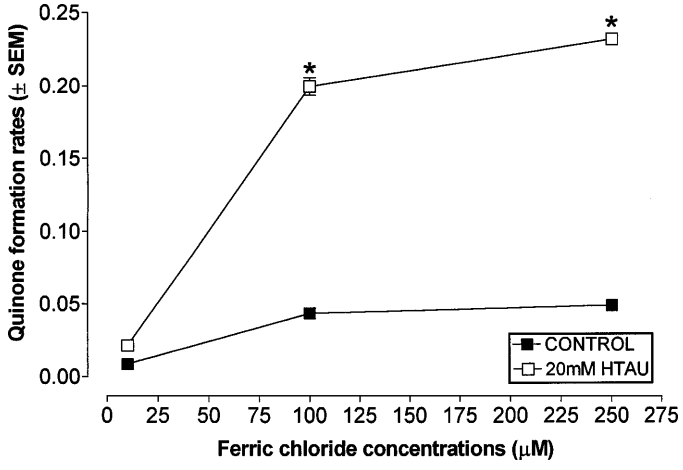

Fig. 7. Homotaurine's effects on norepinephrine's oxidation. Homotaurine (20 mM) significantly (*$p < 0.05$) potentiated oxidation in a concentration dependent manner. Increasing iron concentration had a significant ($p < 0.05$) effect to potentiate quinone formation. Temperature = 26°C

Norepinephrine oxidation stimulated by sodium nitroprusside and SIN-1 chloride

Both taurine (10 mM) and SAM (10 mM) acted as antioxidants by inhibiting the oxidation of norepinephrine (Table 2). Cysteine, homocysteine and homotaurine at both 1 mM and 10 mM concentrations all potentiated quinone formation induced by sodium nitroprusside (Table 2). Methionine at 10 mM also increased the oxidation of norepinephrine in the presence of SNP.

Table 2. Sulfur containing amino acids and their effects on norepinephrine oxidation induced by 1 mM SNP

Amino acids	Percent of control ± SEM
Control	100 ± 2
Tau − 10 mM	36 ± 4*
HTAU − 10 mM	1,744 ± 35*
HTAU − 1 mM	204 ± 8*
CYS − 10 mM	457 ± 8*
CYS − 1 mM	428 ± 6*
HCYS − 10 mM	698 ± 9*
HCYS − 1 mM	213 ± 4*
MET − 10 mM	204 ± 36*
SAM − 10 mM	0*

The control rate represents quinone formation from norepinephrine (500 μM) stimulated by 1 mM sodium nitroprusside (SNP) in the absence of any amino acid analog. Taurine, MET and SAM at 1 mM had no statistically significant effects on quinone formation (data not shown). (*$p < 0.01$ versus control rates).

In order to understand iron's role in sodium nitroprusside's mechanism of action, desferroxamine was added to the norepinephrine, which was then reacted with the SNP. Since 5 mM desferroxamine almost completely suppressed the aminochrome formation rate, a NO/iron interaction was likely (Fig. 8A, 8B). To confirm this interaction, a NO donor not containing iron, 3-morpholinosydnonimine, (SIN-1 chloride) was tested at 0.1 mM. This concentration was chosen on the basis of the percentage of NO contained in 1 mM SNP. Norepinephrine's oxidation was significantly ($p < 0.05$) potentiated by 0.1 mM SIN-1 (~70% increase) and the addition of 1 mM desferroxamine had no significant effect (data not shown). SIN-1 was tested at a higher concentration and 1 mM SIN-1 also significantly potentiated norepinephrine oxidation and this effect was also not attenuated by the addition of 1 mM desferroxamine (Fig. 9). These results clearly suggest that there is a NO/iron interaction responsible for sodium nitroprusside's ability to oxidize catecholamines.

Taurine's ability to block nitric oxide's catalyzing action on catecholamine oxidation was also tested. The addition of 1 mM (data not shown) or 10 mM taurine to 500 μM norepinephrine reacted with 1 mM SIN-1 had no significant effect (Fig. 9). Thus, the failure of both taurine and desferroxamine to attenuate the catalyzing action of SIN-1 suggests that peroxynitrite ($ONOO^-$) – mediated oxidation was likely the dominant mechanism.

Discussion

The results of these studies suggest several potential interactions between sulfur containing amino acids and commonly used drugs with oxidative potential, namely L-dopa and sodium nitroprusside (SNP). L-Dopa has been re-

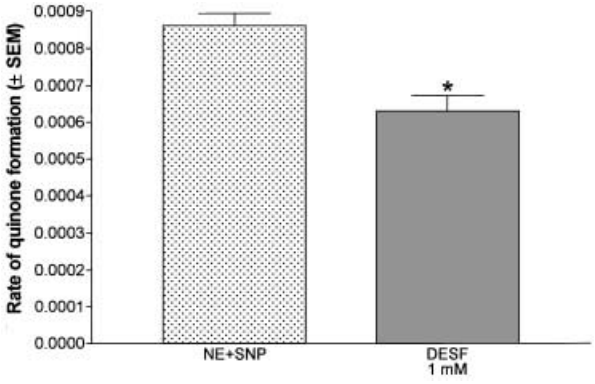

Fig. 8A. Desferroxamine (*DESF*) (1 mM) effects on norepinephrine oxidation by sodium nitroprusside. Desferroxamine (1 mM) significantly (*$p < 0.05$) reduced norepinephrine (500 μM) oxidation stimulated by 1 mM sodium nitroprusside

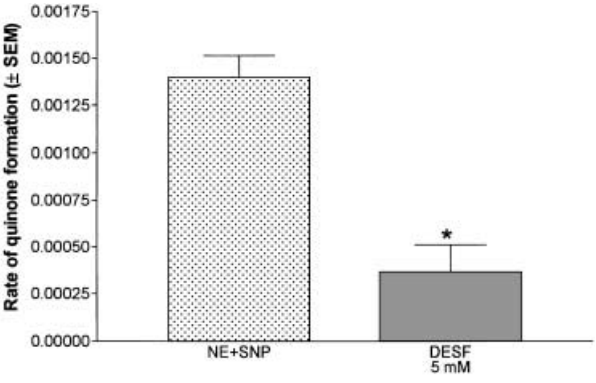

Fig. 8B. Desferroxamine (5 mM) induced reduction of norepinephrine oxidation. Desferroxamine (5 mM) significantly (*$p < 0.05$) reduced norepinephrine (500 μM) oxidation stimulated by 1 mM sodium nitroprusside

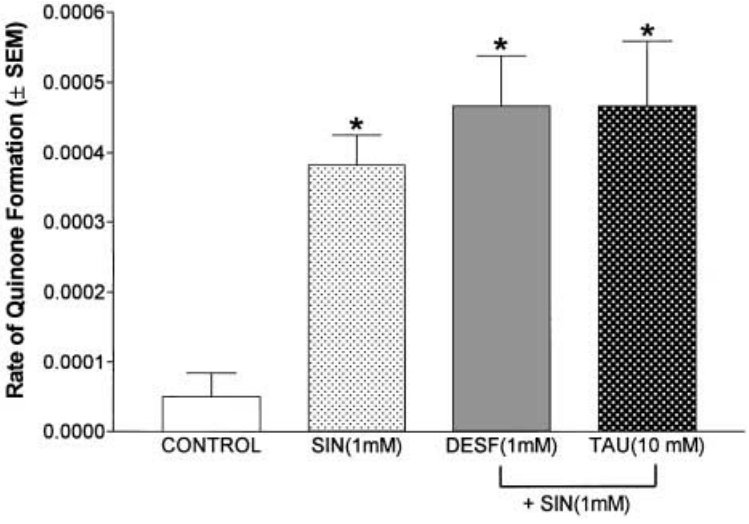

Fig. 9. Norepinephrine oxidation by SIN-1. SIN-1 (1 mM) significantly (*$p < 0.05$) potentiated norepinephrine (500 μM) oxidation. The addition of 1 mM desferroxamine or 10 mM taurine had no significant effect on SIN-1-induced quinone formation

ported to be neurotoxic (Basma et al., 1995; Pardo et al., 1995; Melamed et al., 1998; Jenner and Mitchell, 1998), but little is known about the various potential interactions between catecholamines and sulfur containing amino acids. It has been shown that chronic systemic L-dopa administration to rodents depletes both SAM and taurine pools (Daly et al., 1997; Diederich et al., 1997; Tyce and Owen, 1973) suggesting that these amino acids might be involved in scavenging reactive quinones derived from L-dopa *in vivo*. Our data establishes that both SAM and taurine reduces detectable quinones, but could be explained by an increase in quinone sulfur amino acid conjugates. This could potentially result in increased L-dopa available to undergo metal-stimulated oxidation reactions and less taurine and SAM available for scavenging these reactive quinones. Likewise, SNP or NO, in the presence of ferric iron, would be predicted to stimulate norepinephrine oxidation in the presence of homocysteine. The elevation of homocysteine in the plasma as a risk factor in cardiovascular disease could in part be due to the facilitation of vascular damage by catecholamine oxidation products. Diversion of available methionine and cysteine for homocysteine production could also lead to lower levels of taurine and glutathione for their respective cytoprotective functions.

Taurine may function as an endogenous antioxidant due to its high intracellular concentration in many tissues (5–60 mM) by protecting against oxidative damage caused by reactive quinones and oxygen radicals produced by catecholamine oxidation. Among taurine precursors, hypotaurine (sulfinic acid group) had some activity in inhibiting quinone formation but SAM, homocysteic acid and CSA were much better scavengers. The fact that hypotaurine has been shown to be a much better direct acting antioxidant than taurine (Aruoma et al., 1988; Mehta and Dawson, 2000) emphasizes the importance of the amino functional group in quinone scavenging reactions. The potential endogenous efficacy of these sulfur amino acids has to be put in the context that the *in vivo* levels of these taurine precusors are usually maintained at submillimolar levels.

Homocysteine and particularly homocysteic acid also attenuated iron-stimulated L-dopa oxidation while cysteine and methionine both potentiated it. Shen et al. (1998) demonstrated that ferric iron catalyzes the auto-oxidation of the neurotrasmitter dopamine in the presence of L-cysteine. Cysteine reacts with ferric iron generating the hydroxyl and cysteinyl radicals that respectively oxidize DA and form cysteinyl conjugates of DA. Cysteine can also undergo autoxidation in the presence of metals such as copper, generating hydrogen peroxide and cytotoxicity (Nath and Salahdeen, 1993). As suggested by Huxtable (1992), the synthesis of taurine may have a general cytoprotective effect by converting reactive cysteine and methionine to the antioxidants (SAM, CSA, homocysteic acid, hypotaurine) and subsequently to taurine.

As previously reported by our laboratory (Dawson et al., 2000a) the extention of the carbon chain by one carbon (homotaurine) resulted in prominent potentiation of quinone formation both in the presence and absence of added ferric chloride. In this study we focused on understanding homotaurine's catalytic action. To rule out possible interactions between

homotaurine and iron, an iron chelator (desferroxamine) was tested for its ability to block the actions of homotaurine. Heikkila et al. (1981) demonstrated that this iron-chelating agent inhibited both baseline as well as iron-stimulated oxidation of dopamine and norepinephrine. The addition of 5 mM desferroxamine almost completely suppressed norepinephrine oxidation suggesting that there is an interaction between homotaurine and iron in stimulating oxidation. A previous study reported little metal chelating potential for taurine (Wright et al., 1986), although a more recent study suggests taurine can bind copper(II)-dipeptide complexes at physiological pHs via its amino group (O'Brien et al., 1999). Our results suggest homotaurine may bind iron in a catalytically active state, whereas hypotaurine and taurine may bind iron and render it less catalytically active.

The interactions between catecholamines and nitric oxide were also examined. The NO donor, sodium nitroprusside (SNP), was used as the catalyzing agent. Since SNP had a weak effect on L-dopa we concentrated on nitric oxide's effects on norepinephrine oxidation. There is little reported data on how NO chemically interacts with L-dopa, although Daveu et al. (1997) have reported the reactivities of dopamine, norepinephrine and epinephrine, with nitric oxide and peroxynitrite. The aromatic ring of both dopamine and norepinephrine is nitrated at position 6C when these two catecholamines are reacted with NO in an oxygen containing buffer (pH 7.4). This specific nitration is due to the transient formation of N_2O_3 during the auto-oxidation of NO. It appears that peroxynitrite can oxidize catechols to o-quinones (Kerry and Rice-Evans, 1998; Daveu et al., 1997). The reaction of catecholamines with peroxynitrite results in the formation of oxidized derivatives but not 6-nitroderivatives. Our results would support this reaction mechanism and the generation of potentially cytotoxic quinones and free radicals. By directly measuring the rate of aminochrome formation spectrophotometrically, we determined that norepinephrine was oxidized in the presence of SNP, however, we could not rule out a possible NO/iron interaction since SNP releases both NO and iron. For this reason we tested 3-morpholinosydnonimine (SIN-1 chloride) which is exclusively a NO-donor, but easily forms peroxynitrite in the presence of superoxide radical. Our data shows that SIN-1 does potentiate norepinephrine's oxidation and the addition of either desferroxamine or taurine has no significant effect. In contrast, taurine could attenuate SNP's oxidation of norepinephrine but not the effects of SIN-1. This would suggest that taurine was more effective in disrupting metal-dependent catecholamine oxidation reactions.

Both homotaurine and homocysteine greatly potentiated norepinephrine oxidation. Previously we had found that 10 mM homotaurine could double peroxynitrate formation from SIN-1 (Mehta and Dawson, 2000). The failure of taurine and desferroxamine to attenuate SIN-1 action suggests that peroxynitrite-mediated oxidation was likely the dominant mechanism (Duveu et al., 1997). This can also be supported by our recent finding that it requires taurine concentrations above 50 mM to inhibit peroxynitrite formation derived from 0.1 mM SIN-1 (Mehta and Dawson, 2000). In fact, taurine slightly promoted peroxynitrate formation from SNP, so a quinone scavenging

mechanism for taurine would appear more likely than a reduction in peroxynitrate generation. It has not been determined whether taurine might scavenge catecholamine derived quinones *in vivo* and reduce super oxide availability for interaction with NO to cause peroxynitrite formation. Taurine has been shown to reduce oxidative stress in cultured vascular smooth muscle and block the decrease in gene expression and secretion of extracellular superoxide dismutase induced by exposure to homocyteine (Nonaka et al., 2001). Taurine is known to decrease NO formation and iNOS mRNA levels in cultured cells (Park et al., 1997). Likewise, nitrocatecholamines have recently been shown to inhibit all isoforms of NOS (Palumbo et al., 2001). Taurine is also the major endogenous scavenger of hypochlorous acid (HOCl) and recent studies have reported that HOCl reacts with nitrite to form nitrating reactive oxygen species (Marcinkiewicz et al., 2000; Lakshmi et al., 2000). Taurine by scavenging HOCl would be predicted to inhibit these types of nitrating reactions *in vivo*. Thus, it is possible *in vivo* that taurine could potentially reduce peroxynitrite formation via several different indirect mechanisms outlined above.

Although taurine and its analogs had similar effects on L-dopa and norepinephrine oxidation stimulated by either ferric chloride or SNP, it is important to note that homocysteine had biphasic effects. Interestingly, 10 mM homocysteine attenuated L-dopa oxidation catalyzed by ferric chloride while it potentiated norepinephrine's oxidation induced by ferric chloride or SNP. Rigobello et al. (2001) showed that adrenaline (epinephrine) could be oxidized by S-nitrosoglutathione in the presence of copper. In this case ferrous iron was ineffective, so further studies with copper or other metals may be important to predict potential relevant *in vivo* tissue-specific types of NO-mediated catecholamine oxidation reactions. NO in the presence of ferric iron and homocysteine could potentially stimulate norepinephrine oxidation in cardiovascular tissues and cause vascular damage. This is a very important finding with regard to cardiovascular disease and should be further investigated. This is the first study to examine homocysteine in the context of facilitating oxidative damage by metal-stimulated or NO-induced catecholamine oxidation. Homocysteine has been shown to potentiate copper-mediated neurotoxicity but did not reduce Fe(III) to Fe(II) (White et al., 2001). Only very high levels (10 mM) of homocysteine promoted iron-stimulated catecholamine oxidation, but 1 mM could double SNP-mediated norepinephrine oxidation. More studies examining the role of copper- and NO-homocysteine interactions would be insightful.

The associated depletion in taurine and SAM caused by L-dopa treatment (Daly et al., 1997; Diederich et al., 1997; Tyce and Owen, 1973) could render tissues more susceptible to oxidative injury since our data now implicates both taurine and SAM in scavenging these reactive quinones. Furthermore, homocysteine's role as a risk factor in cardiovascular disease should be examined in more detail as it relates to circulating catecholamines and metal catalyzed oxidative injury to vascular tissues. Our results show that both iron and NO interact with homocysteine to potentiate norepinephrine oxidation. During ischemia, infection or other conditions where more free

iron or NO is released, catecholamines such as dopamine or norepinephrine may be oxidized in sufficient amounts to damage vessel walls. Finally, homotaurine's pro-oxidant effect should be further studied with regard to the use of acamprosate (n-acetylhomotaurine) as a novel treatment for alcoholism. Wu et al. (2001) have shown acamprosate to be neurotoxic to cultured neurons and that it antagonizes the effects of endogenous taurine. Likewise, we have shown high concentrations of homotaurine to be toxic to renal cells (Dawson et al., 2000a). It is unclear how acamprosate interacts with metals or whether it can facilitate catecholamine oxidation *in vivo* since acetylation of the amino group may prevent metal binding activity. None-the-less, homotaurine has been reported to be an impurity in acamprosate (Fabre et al., 1999).

In summary, taurine at concentrations normally present intracellularly appears to reduce iron and SNP-induced quinone formation. Taurine has been shown to have cardioprotective and renoprotective effects in hypertension (Dawson et al., 2000b), but the exact mechanisms involved have never been fully explained. One additional mechanism can now be speculated to be a reduction in oxidative damage in cardiovascular tissues caused by catecholamine oxidation products.

Acknowledgements

The studies were funded in part by grants from the College of Pharmacy and Department of Pharmacodynamics at the University of Florida.

The authors wish to note that Nappi and Vass (J Biol Chem 276: 11214-11222, 2001) published a study after our work was accepted for publication that shows clear evidence of nitric oxide mediated oxidation of both L-Dopa and dopamine.

References

Auroma OI, Halliwell B, Hoey BM, Butler J (1988) The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 256: 251–255

Basma AN, Morris EJ, Nicklas WJ, Geller HM (1995) L-Dopa cytotoxicity to PC12 cells in culture is via its autoxidation. J Neurochem 64: 825–832

Bindoli A, Rigobello MP, Deeble DJ (1992) Biochemical and toxicological properties of the oxidation products of catecholamines. Free Rad Biol Med 13: 319–415

Brattstrom L, Wilcken DE (2000) Homocysteine and cardiovascular disease: cause or effect? Am J Clin Nutr 72: 315–323

Cavalieri E, Rogan E (2001) The key role of endogenous catechol estrogen-3, 4-quinones in tumor initiation. In: Creveling CR (ed) Role of quinone species in cellular toxicity. FP Grahma Publishing Co., Johnson City, TN, pp 247–260

Daly D, Miller JW, Nadeau MR, Selhub J (1997) The effect of L-dopa administration and folate deficiency on plasma homocysteine concentrations in rats. J Nutr Biochem 8: 634–640

Daveu C, Servy C, Dndane M, Marine P, Ducrocq M (1997) Oxidation and nitration of catecholamines by nitrogen oxides derived from nitric oxide. Nitric Oxide: Biol Chem 1: 234–243

Dawson R, Baker D, Eppler B, Tang E, Shih D, Hern H, Hu M (2000a) Taurine inhibition of metal stimulated catecholamine oxidation. Neurotox Res 2: 1–15

Dawson R, Liu S, Jung B, Messina S, Eppler B (2000b) Effects of high salt diets and taurine on the development of hypertension in the stroke-prone spontaneously hypertensive rats (SPSHR). Amino Acids 19: 643–665

de la Breteche' ML, Servy C, Lenfant M, Ducrocq C (1994) Nitration of catecholamines with nitrogen oxide in mild conditions. Tetrahedron Lett 35: 7231–7232

Diederich C, Milakofsky L, Hare TA, Hofford JM, Dadmarz M, Vogel WH (1997) Effects of L-Dopa/carbidopa administration on the levels of L-Dopa, other amino acids and related compounds in plasma, brain, and heart of the rat. Pharmacology 55: 109–116

Donaldson J, LaBella FS, Gesser D (1980) Enhanced autoxidation of dopamine as a possible basis of manganese neurotoxicity. Neurotoxicology 2: 53–64

Fabre H, Perrin C, Bosc N (1999) Determination of homotaurine as impurity in calcium acamprosate by capillary zone electrophoresis. J Chromatogr A 853/1–2: 421–430

Graham DG (1984) Catecholamine toxicity: a proposal for the molecular pathogenisis for manganese nuerotoxicity and Parkinson's disease. Neurotoxicology 5: 83–95

Graham DG, Tiffany SM, Bell WR, Gutknecht WF (1978) Autooxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds towards C1300 neuroblastoma cells in vitro. Mol Pharmacol 14: 644–653

Heikkila RE, Cabbat FS (1981) Inhibition of iron stimulated catecholamine degradation by the iron chelators detapac and desferal. Biochem Pharmacol 30: 2945–2947

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Jenner PG, Mitchell FB (1998) Levodopa neurotoxicity; experimental studies versus clinical relevance. Neurology 50: S39–S43

Kerry N, Rice-Evans C (1998) Peroxynitrite oxidises catechols to *o*-quinones. FEBS Lett 437: 167–171

Lakshmi VM, Hsu FF, Davis BB, Zenser TV (2000) Nitrating reactive nitric oxide oxygen species transform acetaminophen to 3-nitroacetaminophen. Chem Res Toxicol 13: 891–899

Marcinkiewicz J, Chain B, Nowak B, Grabowska A, Bryniarski K, Baran J (2000) Antimicrobial and cytotoxic activity of hypochlorous acid: interactions with taurine and nitrite. Inflamm Res 49: 280–289

Mehta TR, Dawson R (2001) Taurine is a weak scavenger of peroxynitrite and does not attenuate sodium nitroprusside toxicity to cells in culture. Amino Acids 20: 419–433

Melamed E, Offen D, Shirvan A, Djaldetti R, Barzilai A, Ziv I (1998) Levodopa toxicity and apoptosis. Ann Neurol 44: S149–S154

Messina S, Dawson R (2000) Attenuation of oxidative damage to DNA by taurine and taurine analogs. In: Della Corte L et al (eds) Taurine 4. Taurine and excitable tissues. Plenum Press, New York, pp 355–367

Miller DM, Buettner GR, Aust SD (1990) Transition metals as catalysts of "autooxidation" reactions. Free Rad Biol Med 8: 95–108

Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG (1992) Quinone chemistry and toxicity. Toxicol Appl Pharmacol 112: 2–16

Nath KA, Salahudeen AK (1993) Autoxidation of cysteine generates hydrogen peroxide: cytotoxicity and attenuation by pyruvate. Am J Physiol 264: F306–F314

Nonaka H, Tsujino T, Watari Y, Emoto N, Yokoyama M (2001) Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocysteine – amelioration of homocysteine-induced endoplasmic reticulum stress by taurine. Circulation 104: 1165–1170

O'Brien EC, Farkas E, Rockenbauer A, Nolan KB (1999) Metal compexes of taurine. The first reported equilibrium studies for complex formation by taurine at physiological pH; the copper (II)-glycyl-glycinate-taurine and the copper (II)-glycylaspartate-taurine systems. J Inorg Biochem 77: 135–139

Oja SS, Saransaari P (2000) Modulation of taurine release by glutamate receptors and nitric oxide. Prog Neurobiol 62: 407–425

Palumbo A, Astarita G, D'Ischia M (2001) Inhibition of neuronal nitric oxide synthase by 6-nitrocatecholamines under oxidative stress. Biochem J 356: 105–110

Pardo B, Mena MA, Casarejos MJ, Paino CL, De Yebenes JG (1995) Toxic effects of L-dopa on mesencephalic cell cultures: protection with antioxidants. Brain Res 682: 133–143

Park E, Schuller-Levis G, Jia J, Quinn MR (1997) Preactivation exposure of RAW 264.7 cells to taurine chloramine attenuates subsequent production of nitric oxide and expression of iNOS mRNA. J Leukoc Biol 61: 161–166

Rigobello MP, Scutari G, Boscolo R, Bindoli A (2001) Oxidation of adrenaline and its derivatives by S-nitrosoglutathione. Nitric Oxide 5: 39–46

Shen XM, Dryhurst G (1998) Iron and manganese catalyzed autooxidation of dopamine in the presence of L-cysteine: possible insights into iron and manganese-mediated dopaminergic neurotoxicity. Chem Res Toxicol 11: 824–837

Shintani F, Kinoshita T, Kanba S, Ishikawa T, Suzuki E, Sasakawa N, Kato R, Asai M, Nakaki T (1996) Bioactive 6-nitronorepinephrine identified in mammalian brain. J Biol Chem 271: 13561–13565

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998) Conjugates of catecholamine with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. J Neurochem 71: 2112–2122

Terland O, Flatmark T, Tangeras A, Gronberg M (1997) Dopamine oxidation generates an oxidative stress mediated by dopamine semiquinone and unrelated to reactive oxygen species. J Mol Cell Cardiol 29: 1731–1738

Touyz RM (2000) Oxidative stress and vascular damage in hypertension. Curr Hypertens Rep 2: 98–105

Tyce GM, Owen CA (1973) The effect of L-3, 4-dihydroxyphenylalanine administration on glucose metabolism in brain. J Neurochem 20: 1563–1573

White AR, Huang X, Jobling MF, Barrow CJ, Beyreuther K, Masters CL, Bush AK, Cappai R (2001) Homocysteine potentiates copper- and amyloid beta peptide-mediated toxicity in primary neuronal cultures: possible risk factors in the Alzheimer's-type neurodegenerative pathways. J Neurochem 76: 1509–1520

Wright CE, Talla HH, Lin YY (1986) Taurine: biological update. Ann Rev Biochem 55: 427–453

Wu JY, Jin H, Schloss JV, Faiman MD, Ningaraj NS, Foos J, Chen W (2001) Neurotoxic effect of acamprosate, n-acetyl-homotaurine, in cultured neurons. J Biomed Sci 8/1: 96–103

Yoshie Y, Ohshima H (1997) Synergistic induction of DNA strand breakage caused by nitric oxide together with catecholamine: implications for neurodegenerative disease. Chem Res Toxicol 10: 1015–1022

Authors' address: Dr. M. Biasetti, Department of Pharmacodynamics, JHMHC Box 100487, College of Pharmacy, University of Florida, Gainesville, FL 32608 U.S.A.

Received August 20, 2001