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Taurine is a weak scavenger of peroxynitrite and does not attenuate sodium nitroprusside toxicity to cells in culture

T. R. Mehta and **R. Dawson, Jr.**

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

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Summary. Many studies have suggested an antioxidant role for taurine, but few studies have directly measured its free radical scavenging activity. The aim of the present study was to directly determine the action of taurine and taurine analogs to inhibit peroxynitrite-mediated oxidation of dihydrorhodamine 123 (DHR) to rhodamine. Taurine was also tested to determine if it could attenuate the toxicity of sodium nitroprusside (SNP) to neuronal cultures. Taurine at concentrations above 30 mM had a modest ability to inhibit peroxynitrite formation derived from SIN-1. Hypotaurine could inhibit peroxynitrite formation from both SIN-1 (↓75%) and SNP (↓50%) at 10 mM. Other taurine analogs (homotaurine, β-alanine & isethionic acid) slightly potentiated DHR oxidation by SIN-1. Short-term (1-hour) treatment of PC12 cultures with either SNP (1–2 mM) or taurine (20–40 mM) appeared to induce cellular proliferation. In contrast, 24-hour treatment with SNP (1 mM) induced cell death. Combination treatments with taurine and SNP appeared to interact in an additive fashion for both cell proliferation and neurotoxic actions. It appears unlikely that taurine is a major endogenous scavenger of peroxynitrite.

Keywords: Amino acids – Taurine – Peroxynitrite – Hypotaurine – Sodium nitroprusside – Neurotoxicity – Nitric oxide

Introduction

Taurine (2-aminoethanesulfonic acid) is an abundant amino acid found in millimolar concentrations in mammalian cells. The high intracellular concentrations of taurine are essential for its role as an organic osmolyte, but the mechanisms for its other putative cellular functions are not as well characterized (Huxtable, 1992). Taurine appears to act as an inhibitory neuromodulator due to its interactions at GABA, glycine and a putative taurine receptor (Huxtable, 1989; Flint et al., 1998). A substantial literature exists regarding taurine's role in the modulation of cellular calcium levels

(Huxtable, 1989, 1992). Taurine has also been shown in many *in vitro* and *in vivo* studies to have cytoprotective effects (Timbrell et al., 1995) and these actions are often attributed to an antioxidant mechanism (Storey et al., 1983; Pasantes-Morales et al., 1985; Gordon et al., 1986; Laidlaw et al., 1989; Milei et al., 1992; Nakamori et al., 1993; Raschke et al., 1995). Studies that have directly assessed the free radical scavenging potential of taurine have shown minimal direct chemical scavenging actions against many oxygen-derived radicals (Aruoma et al., 1988; Shi et al., 1997). In contrast, a recent study (Kilic et al., 1999) did show scavenging activity for millimolar concentrations of taurine against both superoxide and peroxide. Taurine has been well established as the major cellular scavenger for hypochlorous acid (HOCl, Weiss et al., 1982; Cantin, 1994; Marcinkiewicz, 1997). One factor that is often not adequately addressed in the assessment of the antioxidant role of taurine is its high intracellular concentrations in certain cell types. Thus the question remains, could even modest antioxidant activity relative to well-known antioxidants (glutathione, ascorbic acid or vitamin E etc.) still have biologic relevance in cells that contained high concentrations (10–70 mM) of taurine? Furthermore, no systematic studies have been published that clearly identifies the direct antioxidant potential of taurine against a wide spectrum of oxidants.

Taurine has recently been shown to protect hepatocytes against injury mediated by the nitric oxide (NO, Redmond et al., 1996). NO has been shown to have both cytotoxic and cytoprotective effects in many tissues and cell lines (Dalkara and Moskowitz, 1997). NO can also react with superoxide radical ($O_2\bullet^-$) to form peroxynitrite ($ONOO^-$) (Beckman and Koppenol, 1996). Neutrophils release both NO and $O_2\bullet^-$ during the respiratory burst and peroxynitrite can be formed at sites of inflammation. Peroxynitrite is a very reactive oxidant that has been implicated in many pathogenic mechanisms. Peroxynitrite is known to induce strand breaks in DNA and cause nitration of tyrosine residues in proteins (Briviba et al., 1999). A number of low molecular weight antioxidants such as reduced glutathione (GSH), cysteine and methionine are known to scavenge peroxynitrite. The aim of the present study was to examine taurine and taurine analogs for their ability to scavenge peroxynitrite. A second aim was to determine if taurine could protect cells from exogenous NO toxicity.

Materials and methods

Peroxynitrite scavenging assay

Materials

Dihydrorhodamine 123 (DHR), 3-morpholinosydnonime (SIN-1), diethylenetriaminepentaacetic acid (DETAPAC), bovine liver catalase, sodium nitroprusside (SNP), taurine [2-aminoethanesulfonic acid], hypotaurine [2-aminoethanesulfinic acid], homotaurine [3-aminopropanesulfonic acid], β-alanine, and isethionic acid [2-hydroxyethanesulfonic acid] were purchased from Sigma (St. Louis, MO).

Dihydrorhodamine 123 assays

Peroxynitrite formation can be determined by the oxidation of DHR to rhodamine, producing a characteristic yellow-green color (Kooy et al., 1994). The peroxynitrite-mediated oxidation of DHR was performed as described by Lomonosova et al. (1998). A 250μM solution of DHR was prepared in DMSO. The solution was purged with nitrogen gas and stored at $-20°$C. All other solutions were prepared in 0.1 M phosphate buffer (pH 7.4) just prior to usage. All reaction mixtures contained 50μM DHR 123, 0.1 mM diethylenetriaminepentaacetic acid (DETAPAC) and 130 U/ml bovine liver catalase. Catalase and DETAPAC were added to prevent non-specific oxidation of DHR 123 (Lomonsova et al., 1999). SIN-1 releases NO$\cdot$ and $O_2\cdot^-$, and peroxynitrite production was induced from 0.025, 0.05, and 0.10 mM SIN-1 for 180 minutes. Comparison was made between groups containing one of the three SIN-1 doses and 10 mM taurine. All conditions were tested and replicated at least three times (N = 3) in separate experiments. Samples were read spectrophotometrically at 500 nm at 30, 60, 90 and 180 minutes.

The initial study showed that 0.10 mM SIN-1 was found to produce an optimal level of peroxynitrite for measurement and was used in all subsequent experiments. In the first experiment, it was also determined that 90 minutes was an adequate time to measure peroxynitrite formation, and thus readings were taken for the rest of the experiments at 30, 60, and 90 minutes. The abilities of taurine (10, 20, 30, 50, and 60 mM), hypotaurine (10 mM), β-alanine (20, 50 mM), homotaurine (10, 20 mM), or isethionic acid (10, 20 mM) to prevent peroxynitrite formation were also tested.

The DHR assay was also repeated using sodium nitroprusside (SNP) as the NO-donor. First, 20 mM taurine was tested against peroxynitrite formation from 0.63, 1.25, 2.5, 5 and 10 mM SNP. Spectrophotometric readings were taken at 30, 60, 90, and 180 minutes and also at 24 hours. An optimal absorbance was produced by 1.25 mM SNP, and in the following experiments, 30 and 60 mM taurine, along with 10 mM hypotaurine, were tested for effects on peroxynitrite production from 1.25 mM SNP.

Cell culture experiments

Materials

PC12 cells were purchased from American Type Culture Collection (Manassas, VA). Fetal bovine serum, penicillin/streptomycin solution, horse serum, rat tail collagen, and RPMI-1640 media were purchased from GibcoBRL (Grand Island, NY) SK-N-SH cells were generously donated by Dr. Kelly Gridley. Sodium nitroprusside and 3-(4,5-dimethlythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO). Matrigel basement membrane was generously donated by Dr. Yun Ju He, as were some PC12 cells.

Cell culture conditions

PC12 cells were cultured in RPMI-1640 media supplemented with 10% horse serum, 5% fetal bovine serum, and a penicillin (50 units/ml)-streptomycin (25μg/ml) solution, as described previously by Greene and Tischler (1976). The cells were incubated at 37°C with 5% CO_2. For cell viability experiments with treatments added for one hour, 96 well plates were coated the day before cell plating with 100μl rat tail collagen (16.6 ml collagen/150 mL 0.02 N acetic acid). For experiments with treatments to be added for 24 hours, 50μl Matrigel basement membrane was added to wells of the 96 well plate. The cells were plated by adding 100μl of a cell suspension followed by a 100μl media in each of the 96 wells to achieve a density of 50,000–75,000 cells/well. Dr. Kelly Gridley provided

SK-N-SH cells in 24 well plates at a density of 500,000 cells/well. SK-N-SH cells were grown in RPMI-60 media as previously described (Gridely et al., 1998).

Cytotoxicity and cell viability

For cell viability experiments, the NO donor sodium nitroprusside (SNP) was used. For 96 well plates, culture media was removed and the cells were rinsed once with 100 μl phosphate buffered saline (PBS, pH = 7.4), while 24 well plates were rinsed with 200 μl PBS per well. SNP treatments were added for one-hour or 24 hours. SNP was dissolved in PBS for use in one-hour treatment experiments. SNP used for 24-hour treatments was dissolved in serum-free RPMI-1640 media. Treatments were added at a volume of 200 μl in 96-well plates, while a 1 mL volume was added to cells in 24-well plates. Adjustments were made in the taurine concentrations used in individual experiments (10–40 mM) in an attempt to minimize taurine-induced changes in cell viability so as to not complicate the interpretation of combination treatments. The use of concentrations above 40 mM were avoided since this concentration was shown in the first experiment to stimulate cell proliferation.

For 1-hour experiments, the treatments were removed, 200 μl media was then added to the cells, and the plates were incubated overnight. The next day, 50 μl 0.02 mg/ml MTT, dissolved in PBS was added to media in 96 well plates for 4 hours to determine cell viability. In the case of 24 well plates, 200 μl MTT was added to existing media. Viability was determined by the reduction of MTT to formazan (Van de Loosdrecht et al., 1994). After 4 hours incubation, media was aspirated from wells, and 200 μl acidic isopropanol (20 mL 2 N HCl in 500 mL isopropanol) was added to cells in 96 well plates, while 500 μl was added to cells in 24 well plates. The 96 well plates were read directly, whereas three 100 μl aliquots were taken from each well of the 24 well plates and put into three wells of a new 96 well plate and absorbance was measured. Absorbance, which decreases as cell death increases, was measured with a plate reader (SLT Lab Instruments, Austria) at 550 nm with 690 nm background.

Statistical analyses

A one-way ANOVA followed by Dunnett's multiple comparision tests was used for statistical analysis of data when comparing NO-donor treatments, taurine treatments, and combination treatments to control treatments. Data are expressed as mean ± SEM.

Results

Peroxynitrite formation from SIN-1

A concentration dependent increase in rhodamine absorbance due to SIN-1 was seen using the dihydrorhodamine 123 assay, indicating increased peroxynitrite formation (Fig. 1A). For all three SIN-1 concentrations, 10 mM taurine did not significantly decrease rhodamine absorbance (Fig. 1A). In the second experiment, the ability taurine (10 or 20 mM) and 10 mM hypotaurine (hypotau) to prevent peroxynitrite formation from 0.1 mM SIN-1 were tested (Fig. 1B). Only 10 mM hypotaurine significantly decreased absorbance and peroxynitrite formation ($p < 0.0001$), while 10 and 20 mM taurine had no significant effect on peroxynitrite formation. A third experiment tested 50 mM taurine, homotaurine (HTau), isethionic acid (Iseth) and β-alanine

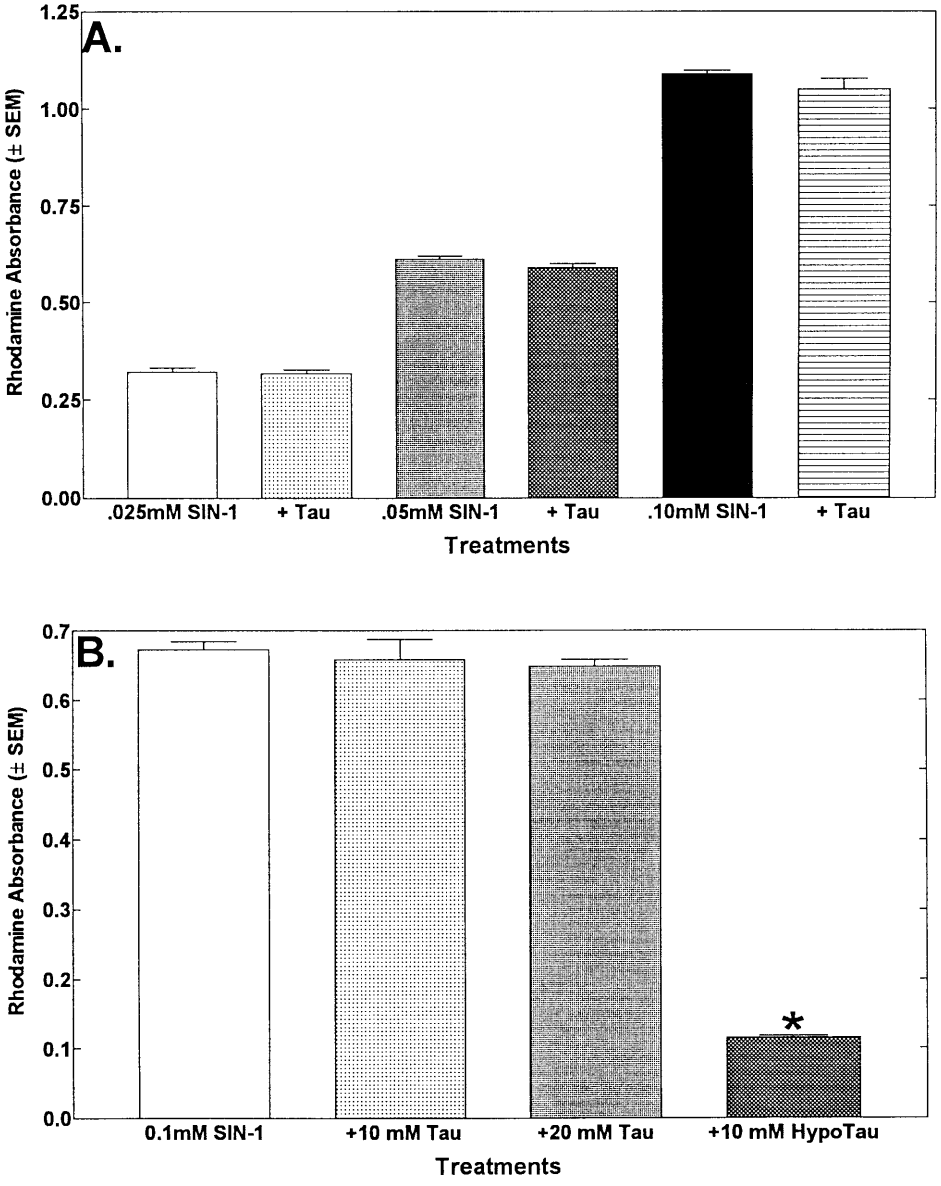

Fig. 1. A. The concentration dependent effects of SIN-1 to increase peroxynitrite formation as indexed by the oxidation of DHR to rhodamine. Peroxynitrate formation was measured for 180 minutes and DHR oxidation was significantly ($p < 0.001$) increased with increasing SIN-1 concentrations. Taurine (Tau, 10 mM) had no effect on DHR oxidation. (N = 3) **B.** The effects of taurine and hypotaurine (hypotau) on 0.1 mM SIN-1 induced peroxynitrite formation. Taurine (10 or 20 mM) had no effect on DHR oxidation, whereas hypotaurine (10 mM) significantly reduced DHR oxidation (*$p < 0.0001$). (N = 6, 90 minute incubation)

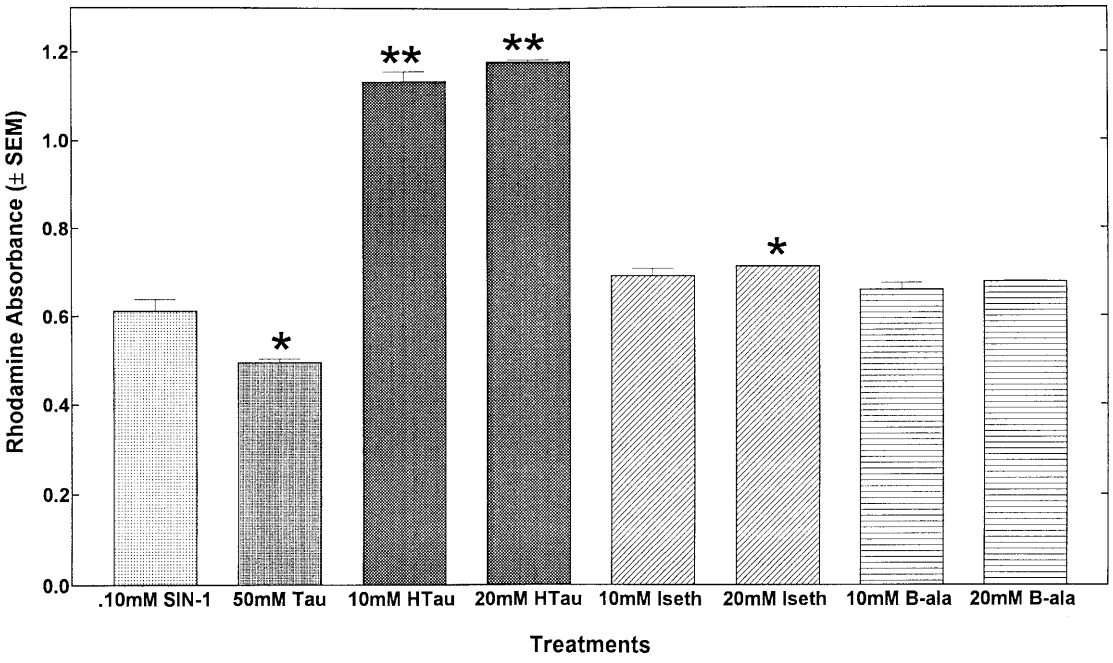

Fig. 2. The effects of taurine (50 mM) and taurine analogs to alter peroxynitrate formation from SIN-1. Taurine significantly (*$p < 0.05$) inhibited DHR oxidation, whereas homotaurine (Htau, **$p < 0.0001$) and isethionic acid (Iseth, *$p < 0.05$) potentiated DHR oxidation. β-Alanine (B-ala, 10–20 mM) had no effect on DHR oxidation. (N = 3)

(B-ala) (Fig. 2). Only 50 mM taurine significantly decreased rhodamine absorbance ($p < 0.05$). Rhodamine absorbance was significantly increased by 10 mM and 20 mM homotaurine ($p < 0.0002$ and $p < 0.0001$ respectively), and 20 mM isethionic acid ($p < 0.05$). In the final experiment, higher concentrations of taurine and β-alanine were tested (Fig. 3). Only 50 and 60 mM taurine were found to significantly decrease rhodamine absorbance ($p < 0.05$ and $p < 0.0001$ respectively). β-Alanine (20 and 50 mM) significantly increased rhodamine absorbance ($p < 0.0001$ and $p < 0.05$ respectively).

Peroxynitrite formation from SNP

A time course experiment was first performed with SNP that followed peroxynitrite formation for 24 hours since subsequent cell culture treatments would employ 24-hour treatments. For the first 90 minutes, absorbance resulting from peroxynitrite formation from SNP increased in a concentration-dependent manner (data not shown). However, by 180 minutes, 10 mM SNP showed a trend for less peroxynitrite formation than 2.5 or 5 mM SNP (Fig. 4). At 24 hours, both 5 and 10 mM SNP showed less absorbance than 1.25 and 2.5 mM SNP (Fig. 4). Taurine (20 mM) significantly

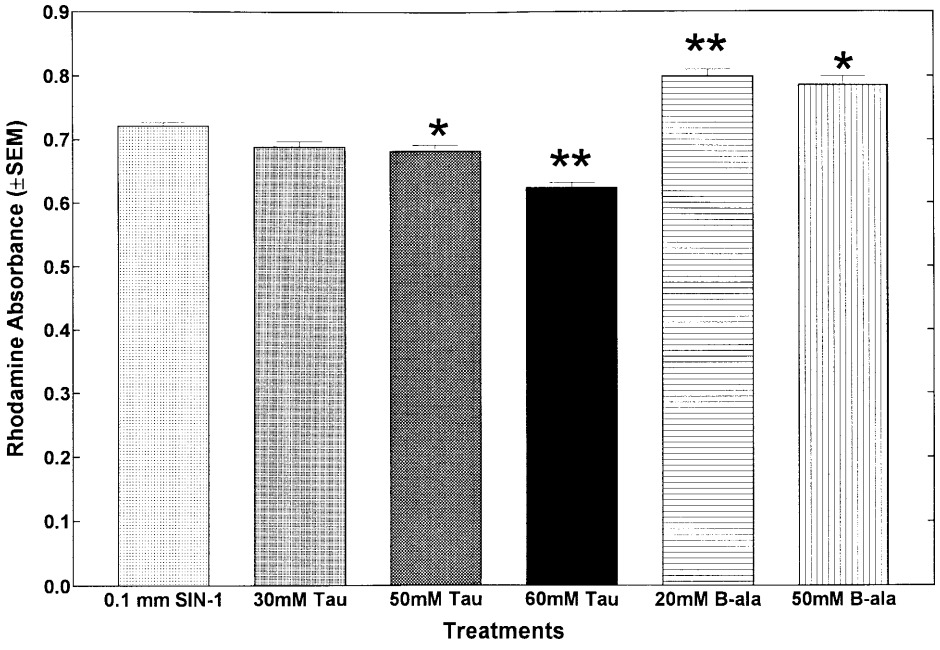

Fig. 3. The effects of high concentrations of taurine and β-alanine (*B-ala*) on 0.1 mM SIN-1-induced oxidation of DHR. Taurine (50–60 mM) significantly (*$p < 0.05$) decreased and β-alanine significantly (**$p < 0.001$) increased DHR oxidation. (N = 6)

increased peroxynitrite formation at SNP concentrations of 1.25 mM or higher ($p < 0.05$) (Fig. 4).

The effects of taurine and hypotaurine on peroxynitrite formation from 1.25 mM SNP were tested. Taurine (30 and 60 mM) significantly increased peroxynitrite formation for the initial 180 minutes ($p < 0.05$) and 10 mM hypotaurine significantly decreased rhodamine absorbance ($p < 0.001$) (Fig. 5). Increasing taurine from 30 to 60 mM had no effect to further enhance rhodamine absorbance and in fact it appeared that 20 mM taurine (Fig. 4) appeared somewhat more effective. Over the 24 hours only 10 mM hypotaurine significantly attenuated peroxynitrite formation ($p < 0.0001$) and taurine had no significant effect [data not shown].

A range (10–60 mM) of concentrations of β-alanine, homotaurine and taurine were tested for their effects to modify the pH of the 0.1 M phosphate buffer (pH = 7.40) system used in the DHR oxidation experiments. These experiments were performed to examine if the inhibitory effects of taurine on DHR oxidation were due to acidification of the reaction mixture. Both taurine and β-alanine had only slight effects to decrease the pH by 0.06 pH units at 60 mM. Homotaurine (60 mM) decreased the pH by 0.02 units. Since taurine decreased and β-alanine had the opposite effect on DHR oxidation and both had the same slight action on pH, it was concluded that the minor change in pH was not an important variable.

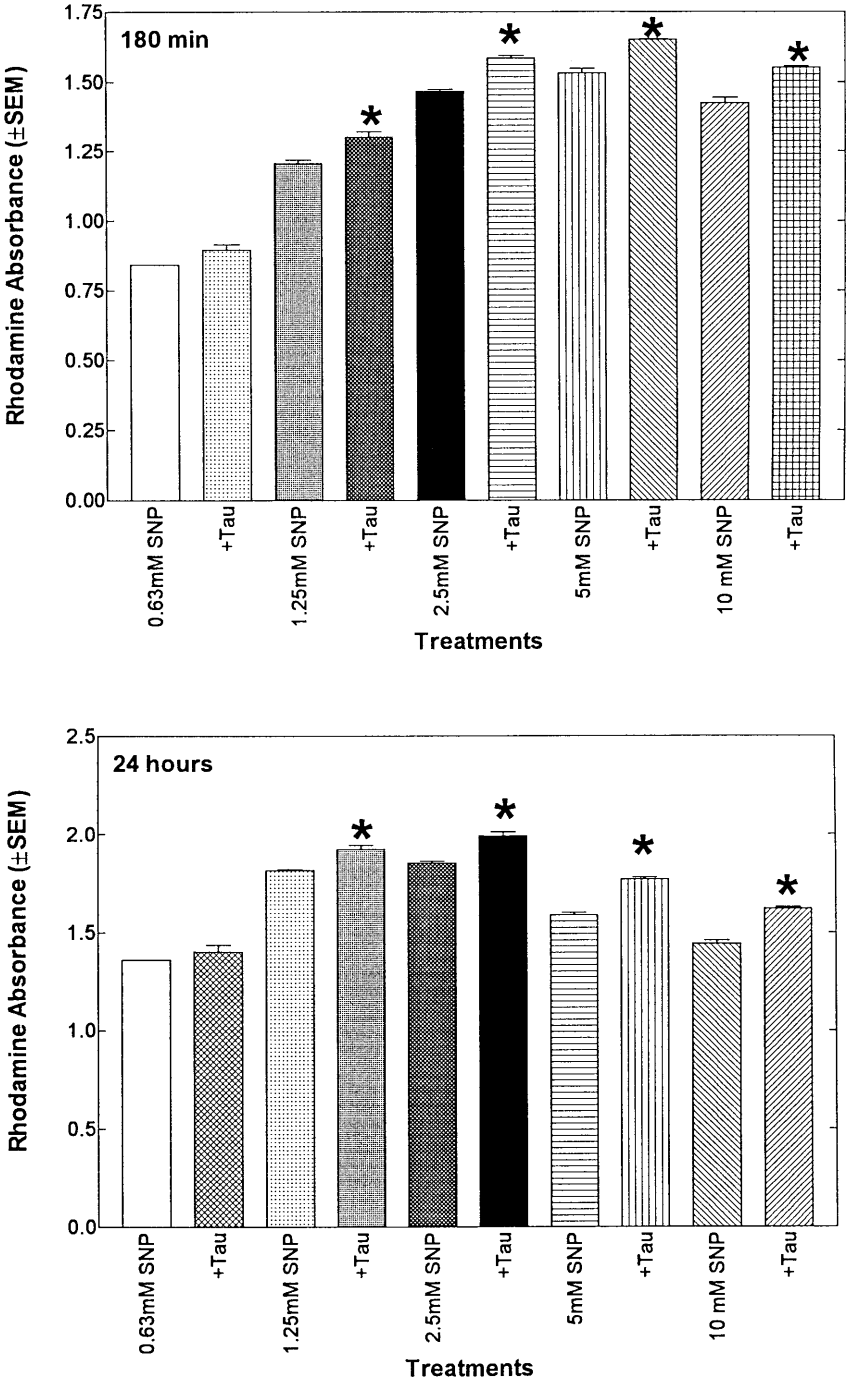

Fig. 4. SNP-induced oxidation of DHR to rhodamine after 180 min or 24 hour incubations. Increasing concentrations of SNP caused the oxidation of DHR and taurine (20 mM) had a significant (*$p < 0.05$) effect to potentiate peroxynitrite formation. (N = 3)

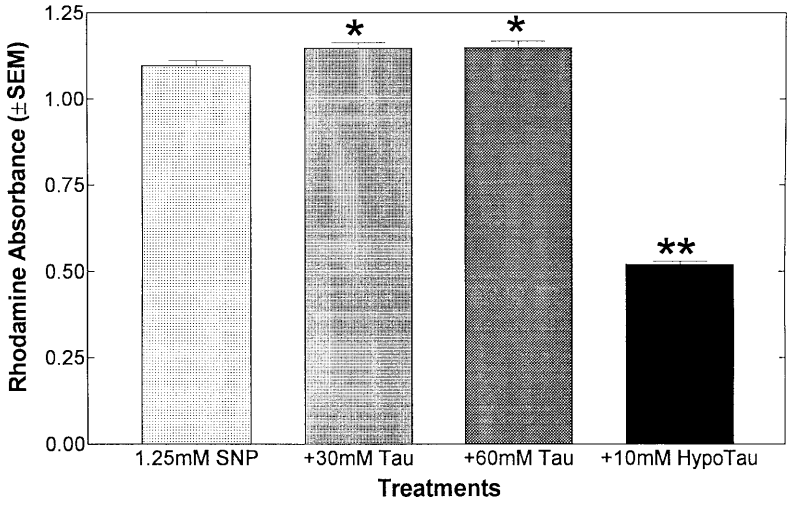

Fig. 5. The effects of high concentrations of taurine or 10 mM hypotaurine on SNP-induced oxidation of DHR after a 180 minute incubation. Taurine slightly (*$p < 0.05$) increased and hypotaurine decreased (**$p < 0.001$) peroxynitrite formation induced by SNP. (N = 6)

Table 1. Effects of SNP and taurine on cell viability after 1 or 24 hours of treatment

Groups	PBS	Taurine	SNP	SNP + Taurine
A. PC12-1h	100.0 ± 4.2	115.8 ± 4.0*	131.4 ± 4.5**	131.6 ± 4.3**
B. PC12-1h	100.0 ± 5.7	117.1 ± 5.5	144.2 ± 6.5**	168.5 ± 4.9**
C. PC12-24h	100.0 ± 5.0	81.9 ± 4.8*	67.6 ± 4.6**	54.2 ± 3.1**
D. SK-N-SH-24h	100.0 ± 4.3	98.4 ± 1.7	75.2 ± 2.8**	58.8 ± 1.8**

Data are expressed as a percent of the PBS control groups ± SEM. (N = 18–30 wells per treatment condition) Experiment A used 40 mM taurine and 1 mM SNP and the combination treatment employed 10 mM taurine and 0.25 mM SNP. Experiment B utilized 20 mM taurine and 2 mM SNP alone and in combination. Experiment C employed 30 mM taurine and 1 mM SNP for all treatment conditions. Experiment D used 20 mM taurine and 1 mM SNP alone and in combination. (*$p < 0.05$, **$p < 0.01$ versus PBS control group).

Cell culture experiments

Sodium nitroprusside (SNP) was used as the NO-donor to induce neurotoxicity in PC12 cells. Two experiments were performed with SNP added to cells for 1 hour. In the first experiment (Table 1), both 40 mM taurine ($p < 0.05$) and 1 mM SNP ($p < 0.01$) increased cell proliferation. When combined 10 mM taurine and 0.25 mM SNP ($p < 0.01$) also increased cell proliferation compared to the control group (PBS treatment) (Table 1). In the second experiment, the results basically followed the same pattern (Table 1). MTT absorbance and cell proliferation were increased by 20 mM taurine but the increase was nonsignificant (Table 1). Cell proliferation was enhanced by 2 mM SNP ($p < 0.01$), while the combination of taurine and SNP also

significantly increased cell proliferation compared to controls ($p < 0.01$) (Table 1). The effects of the combination treatment appeared additive.

PC12 cell death was significantly increased due to 24 hour treatment with 30 mM taurine ($p < 0.05$) and 1 mM SNP also decreased cell viability ($p < 0.01$) (Table 1). The combination of the two increased ($p < 0.01$) cell death even more than that caused by SNP alone, again appearing to be additive. A similar experiment was repeated in SK-NSH cells and provided comparable results. Cell viability was unaffected by 20 mM taurine, while 1 mM SNP significantly increased cell death ($p < 0.01$). The combination of taurine and SNP significantly decreased cell viability ($p < 0.01$).

Discussion

Overall, these studies demonstrated that taurine had low intrinsic antioxidant activity to prevent the oxidation of DHR by peroxynitrite. In contrast, hypotaurine was effective at scavenging peroxynitrite, an effect consistent with its sulfinic acid functional group and its ability to accept oxy radicals. Interestingly, other taurine analogs potentiated DHR oxidation and taurine had a similar action when SNP was the NO donor. At least two mechanisms could be involved in amino acids stimulating SNP-induced DHR oxidation, either an enhancement of superoxide or hydroxyl radical formation or stabilization of an intermediate of SNP (Aleryani et al., 1999). Our data really cannot distinguish among these possibilities and additional studies to explore the mechanism of this effect were beyond the scope of our original study design. Recently O'Brien et al. (1999) presented evidence that taurine could form complexes with copper, but these complexes were less stable than complexes with β-alanine. Metal complexes are know to act as catalyst in the generation of oxygen radicals and thus we could speculate that these amino acids may have interacted with trace metals in the reaction mixture despite the presence of a chelator (DETAPAC). Peroxynitrite formation from SNP is complex and it is likely that SNP or its decomposition products reacted in solution to generate small amounts of peroxynitrite and other oxygen radicals (Aleryani et al., 1999). This explained the need to use much higher concentrations of SNP to generate comparable amounts of peroxynitrite relative to SIN-1. Only strong oxidants such as peroxynitrite and HOCl are able to nonenzymatically oxidize DHR (Briviba et al., 1999). Taurine slightly reduced DHR oxidation at concentrations above 30 mM and the cytosolic concentration of taurine in human neutrophils has been estimated to be 50 mM (Green et al., 1991). In contrast, cytosolic concentrations of hypotaurine are less than 1 mM in neutrophils (Green et al., 1991). Thus, it appears unlikely that taurine or hypotaurine are major extracellular scavengers of peroxynitrite generated by neutrophils. Taurine concentrations are high enough intracellularly in neutrophils to modestly inhibit peroxynitrite formation. The possibility that taurine interacts endogenously with other antioxidants in synergistic ways to increase it efficacy cannot be discounted (Keys and Zimmerman, 1999).

The cell culture studies clearly suggest that even high extracellular concentrations of taurine could not reduce the cytotoxic effects of SNP. Redmond et al. (1996) demonstrated that 4 mM taurine could not directly reduce SNP-induced necrosis in cultured hepatocytes. These authors showed that exogenous taurine could reduce apoptosis and necrosis in hepatocytes by inhibiting NO and oxy radical formation, but the actions were attributed to a suppression of NO synthase (NOS) mRNA (Redmond et al., 1996). Previous studies have shown that taurine is chlorinated by HOCl to form taurine chloramine (Weiss et al., 1982; Thomas et al., 1983) and that taurine chloramine can reduce NO formation and inhibit the expression of iNOS mRNA (Marcinkiewicz et al., 1995; Park et al., 1995, 1997). Likewise, Boldyrev et al. (1999) recently demonstrated that 1 mM taurine could not reduce either free radical generation or cytotoxicity caused by SIN-1 treatment of cerebellar granule cells. Therefore, despite the high intracellular concentrations of taurine in neutrophils it is unlikely that taurine alone serves a major function to directly scavenge peroxynitrite or reduce its cytotoxicity. Hypotaurine can scavenge peroxynitrite, but its intracellular concentrations are low relative to its potential scavenging activity. We did not chose to test taurine at 50–60 mM in our cell culture model because our preliminary studies showed that high taurine concentrations (30–40 mM) appeared to stimulate cellular proliferation. This would have complicated the interpretation of potential protective effects of taurine against NO toxicity by a strictly free radical scavenging mechanism.

Short-term treatment (1-hour) with SNP did not produce cell death, but in fact appeared to serve as stimulus for cell proliferation. High extracellular taurine concentrations (20–40 mM) also slightly potentiated cellular proliferation in PC12 cells. Other investigators have also shown that taurine can enhance cellular proliferation in certain cell types (Chen et al., 1998), but have antiproliferative effects in others (Zhang et al., 1999). We have previously found that 1-hour taurine treatment (1–10 mM) had no significant effect on cellular proliferation in renal cells (LLC-PK1) or PC12 cells (unpublished observations). The high extracellular concentrations of taurine used in these studies could have a number of nonspecific actions (osmotic etc.) on cellular proliferation. Likewise, SNP has been shown to both increase (Ziche et al., 1997) and decrease (Garg et al., 1992) cellular proliferation depending on cell type and experimental conditions. It did appear that taurine and SNP acted in an additive fashion for both the cell proliferative and cytotoxic actions. One potential common mechanism to account for these interactions could be NO and taurine modulation of basic fibroblast growth factor (bFGF) calcium-dependent signaling mechanisms. Both NO and taurine have been shown to interact with signaling pathways regulated by bFGF resulting in cellular proliferation or cytoprotective mechanisms (Ziche et al., 1997; El Idrissi et al., 1998). At present, we cannot address the mechanism that accounts for interactions between taurine and SNP.

Homotaurine produced a marked increase in SIN-1 mediated DHR oxidation. We have previously found that homotaurine also promoted catecholamine oxidation to quinones (Dawson et al., 2000). Homotaurine

was also found to enhance the cytotoxicity of L-dopa to renal cells in culture (Dawson et al., in press). It is unclear how the extension of the carbon chain of taurine promotes oxidation of catecholamines and DHR. β-Alanine and isethionic acid both modestly stimulated DHR oxidation by SIN-1. Taurine slightly potentiated SNP-induced oxidation of DHR and appeared to weakly enhance the cellular toxicity of the 24-hour SNP treatment. We have previously found that taurine could slightly potentiate the oxidation of catecholamines by $NaIO_4$ or copper (Dawson et al., 2000). Peroxynitrite is know to be toxic to PC12 cells (Estévez et al., 1995), but SNP toxicity is complicated by the release of NO, cyanide and iron, all of which have adverse effects on cells. Thus, it is unclear if taurine's potentiation of SNP toxicity was due to enhanced peroxynitrite formation. Peroxynitrite can induce cell death by apoptotic mechanisms (Estévez et al., 1995), whereas taurine has been shown to either inhibit (Redmond, et al., 1998; Wu et al., 1999) or have no effect on apoptotic cell death (Hansson et al., 1996; Wedi et al., 1999).

Tang et al. (1996) reported a biphasic effect of taurine on the neurotoxicity of excitatory amino acids with low concentrations (0.1–10 mM) potentiating toxicity and high concentrations (>20 mM) protecting primary neuronal cultures. These authors hypothesized that low concentrations of taurine may activate Cl^- channels and potentiate toxin-mediated cell swelling, whereas high taurine concentrations may improve osmoregulation or protect neurons against calcium overload. Taurine has been shown to activate both GABA (Huxtable, 1989) and glycine (Hussy et al., 1997; Sebra et al., 1998) receptor-mediated chloride conductances. It is possible that even high concentrations of taurine could potentiate neuronal injury caused by SNP by augmenting chloride-mediated cell swelling. Sebra et al. (1998) suggested that taurine activation of glycine-gated chloride flux was associated with a blunting of intracellular calcium elevation due to endotoxin. It is difficult to speculate what role taurine-mediated chloride flux might have played in potentiating neuronal death caused by 24-hour SNP treatment. It is clear that even high concentrations of taurine failed to protect PC12 or SK-N-SH cells from SNP toxicity and in fact augment its toxicity.

In summary, taurine had weak effects to block peroxynitrite formation whereas hypotaurine had a significant inhibitory action to block DHR oxidation, albeit at 10 mM. It appears unlikely that taurine plays a major role as a direct scavenger of peroxynitrite. Taurine was found to act in an additive manner to potentiate the cell proliferative effect of acute (1-hour) SNP treatment. Taurine could also act in an additive manner to enhance the neurotoxic effect of 24-hour SNP treatment. Further studies are needed to examine potential common cellular signaling pathways where NO and taurine interact.

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Authors' address: Dr. Ralph Dawson, Jr., Department of Pharmacodynamics, College of Pharmacy, University of Florida, Box 100487, Gainesville, FL 32610, U.S.A.

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