Oxidative stress and dysregulation of the taurine transporter in high-glucose-exposed human Schwann cells: implications for pathogenesis of diabetic neuropathy

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Askwith T, Zeng W, Eggo MC, Stevens MJ. Oxidative stress and dysregulation of the taurine transporter in high-glucose-exposed human Schwann cells: implications for pathogenesis of diabetic neuropathy. Am J Physiol Endocrinol Metab 297: E620–E628, 2009. First published July 14, 2009; doi:10.1152/ajpendo.00287.2009.—In human Schwann cells, the role of taurine in regulating glucose-induced changes in antioxidant defense systems has been examined. Treatment with high glucose for 7 days induced reactive oxygen species, increased 4-hydroxynoneal adducts (20 ± 5%, \( P < 0.05 \)) and poly-(ADP-ribosyl)ated proteins (40 ± 13%, \( P < 0.05 \)). Increases in these markers of oxidative stress were reversed by simultaneous incubation in 0.25 mM taurine. Both high glucose and taurine independently increased superoxide dismutase and catalase activity and decreased glutathione levels, but their effects were not additive. Glucose reduced taurine transporter (TauT) mRNA and protein in a dose-dependent manner with maximal decreases of 66 ± 6 and 63 ± 12%, respectively (\( P < 0.05 \) both). The \( V_{\text{max}} \) for taurine uptake was decreased in 30 mM glucose from 61 ± 5 to 42 ± 3 pmol·min\(^{-1} \)·mg protein\(^{-1} \) (\( P < 0.001 \)). Glucose-induced TauT downregulation could be reversed by inhibition of aldose reductase, a pathway that depletes NADPH and increases osmotic stress and protein glycation. TauT protein was increased more than threefold, and the \( V_{\text{max}} \) for taurine uptake doubled (\( P < 0.05 \) both) by prooxidants. TauT downregulation was reversed both by treatment with the antioxidant \( \alpha \)-lipoic acid, which increased TauT mRNA by 60% and \( V_{\text{max}} \) by 50% (\( P < 0.05 \) both), and by the aldose reductase inhibitor sorbinil, which increased TauT mRNA 380% and \( V_{\text{max}} \) by 98% (\( P < 0.01 \) both). These data highlight the potential therapeutic benefits of taurine supplementation in diabetic complications and provide mechanisms whereby taurine restoration could be achieved in order to prevent or reverse diabetic complications.

The relationship of hyperglycemia to the microvascular complications of diabetes is well established (13a, 76a, 76b), but the mechanisms contributing to the development and progression of complications are not well understood. Recently, increased oxidative/nitrosative stress has been implicated as a key pathogenic pathway (9, 18, 62, 78). Increased oxidative stress has been identified in the nerve (4, 24, 68), eye (54), vasculature (31), kidney (3, 32), and heart (29) in diabetic rodent models and in diabetic patients (23, 25, 43, 44). Antioxidant approaches have been shown to prevent or reverse complications in experimental diabetes (50). Glucose-driven oxidative stress occurs due to an imbalance in the production of reactive oxygen species (ROS) in concert with impaired antioxidant defense. Excess ROS production can have many downstream pathogenic effects within the cell, including protein nitrosylation, formation of poly(ADP-ribosyl)ated (PAR) protein polymers, and apoptosis (22, 35, 49, 51, 56, 63).

Flux through the enzyme aldose reductase (AR) pathway at key sites for diabetes complications has been viewed as a critical link between hyperglycemia, oxidative stress, and the cell dysfunction seen in diabetes (9, 42). This pathway increases intracellular fructose, sorbitol, and protein glycation while reducing the concentration of NADPH, an essential cofactor for regenerating reduced glutathione (GSH) (9, 46). Increased sorbitol production can contribute to osmotic imbalance, resulting in a compensatory reduction of other osmolytes, such as myo-inositol and taurine (42, 65, 74). Deficiency of these “compatible osmolytes” may result in their becoming rate limiting for normal metabolic functions (64, 66). Taurine is a sulfur-containing free amino acid that can function as an osmolyte, calcium modulator (36), and neurotransmitter (8). Taurine also exhibits antioxidant properties in some tissues (52), but the precise mode of its antioxidant actions remains unclear (61). Intracellular taurine depletion may result in wide-ranging metabolic perturbations, including impaired cellular response to oxidative/nitrosative stress with resultant cytotoxicity. Indeed hyperglycemia-induced taurine depletion has also been demonstrated in the nerve (67), lens (38), and mesangial cells (76) of diabetic rodents. Taurine replacement has been shown to attenuate oxidative stress in these tissues (50, 61, 73).

Intracellular taurine concentration is maintained by the Na\(^{+}\)Cl\(^{-}\)-dependent taurine transporter (TauT), which, in Madin-Darby canine kidney (MDCK) cells and retinal pigment epithelial cells (RPE) (26), is known to be regulated by glucose, oxidative stress, and changes in osmolarity. Disruption of taurine transport has been identified as the important pathway leading to its intracellular depletion (42, 65). In animal models of diabetic neuropathy, taurine supplementation has been shown to reduce lipid peroxidation, attenuate functional deficits, and ameliorate thermal and mechanical hyperalgesia (34, 36); however, the mechanisms involved as well as their cellular location remain unclear.

Schwann cells (SC) play an important role in maintaining peripheral nerve function, with actions including the synthesis and maintenance of nerve myelin (75). In diabetes, nerve demyelination and the resultant impairment of nerve conduction velocity are thought to reflect SC dysfunction (21). SCs are highly vulnerable to hyperglycemic toxicity (37, 69). They have been identified as the principal focus of oxidative/nitrosative stress in hyperglycemia and PAR in diabetic models (19, 78) by a mechanism mediated by AR, which is highly expressed. Disruption of taurine uptake in hyperglycemia could play an important role in SC dysfunction implicated in diabetic neuropathy.

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In this communication, we report the response of human SC antioxidant defense systems to high glucose exposure and the ability of taurine to attenuate oxidative stress in these cells and explore the potential mechanisms involved. We demonstrate paradoxical relationships between increased ROS production with upregulation of the antioxidant defense systems and decreased taurine transport with a potent effect of taurine to reduce ROS and its downstream consequences.

**METHODS**

**Cell culture.** The primary HSC strain (ScienCell, Carlsbad, CA) was cultured at 37°C in a 5% CO₂, humidified atmosphere in Dulbecco’s modified Eagles’ medium (DMEM, Lonza) supplemented with 10% FBS (fetal bovine serum), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine. Twenty-four hours prior to treatment medium was changed and replaced with DMEM supplemented with 5% fetal bovine calf serum (BCS) and containing high (10 and 30 mM) or normal (5 mM) glucose and other experimental conditions and passaged as required. Passages 6–9 were used in all experiments. For measurements with α-lipoic acid (ALA) and sorbitol, reagent stocks were dissolved in DMSO, and treatments were compared with control to which vehicle was added. ALA is a potent free radical scavenger that has been shown to have therapeutic benefits in both animal and clinical models of diabetic neuropathy; hence, in this study the effects of taurine have been compared with those of ALA. ALA can produce H₂O₂ (77); hence, this reagent was freshly made (100 μM) and treated with 100 U/ml catalase (CAT) at 37°C for 30 min to prevent the effect of H₂O₂, which acts as a source of oxidative stress. For measurements with taurine supplementation, 250 μM was used. Intracellular taurine content is typically in the millimolar range, 10–50 mM (1, 13a, 76a, 76b); hence, supplementation with 250 μM would not be expected to increase intracellular taurine content to supraphysiologic levels.

**Western blotting.** HSC were grown for 7 days in experimental conditions. At 7 days, cells were washed in Hank’s balanced salt solution (HBSS) and lysed in 2% SDS containing 1% protease inhibitor cocktail (Sigma). Protein concentration was measured using the bicinchoninic acid method. Sample (40 μg) was separated electrophoretically on a 10% SDS-polyacrylamide gel and electrotransferred onto a polyvinylidene difluoride (PVDF) membrane. The blots were blocked with 3% milk and incubated with primary antibodies [rabbit TauT (1:1,500, Millipore), 4-hydroxynoneal addsucts (4-HNE; 1:1,500, Merck), and mouse PAR antibody (1:1,500; Millipore)] prepared in 3% milk containing 0.05% Tween 20 + 0.05% sodium azide overnight at 4°C. Blots were probed with secondary antiseras conjugated to horseradish peroxidase (anti-rabbit IgG 1:4,000; GE Healthcare) and anti-mouse IgG (1:2,000; Sigma), and visualization was performed with ECL detection reagents (Roche). To confirm equal loading, membranes were stripped and reprobed with rabbit anti-actin (1:200; Sigma). Total content of 4-HNE, PAR proteins, and the 67-kDa TauT band were quantified by densitometry using a Syngene InGenius system and analyzed using Gene Tools software.

**Assay of ROS generation.** For measurement of intracellular ROS generation, HSC were grown for 7 days in treatment conditions. On day 5, cells were plated onto poly-L-lysine-coated slides. After culture for 2 more days, cells were washed twice in Dulbecco’s PBS and incubated in HBSS containing 10 μM OxyBurst green 2’7’dichlorodihydrofluorescein diacetate (H₂DCFDA) reagent for 1 h at 37°C. Cells were mounted on microscope slides and imaged on a fluorescence microscope at λ = 518 nm.

**Quantitative RT-PCR.** Quantitative RT-PCR (qRT-PCR) on HSC RNA was conducted using the Invitrogen SuperScript III Platinum One-Step qRT-PCR Kit according to the manufacturer’s instructions. Fam-labeled lux primer specific for the TauT boundary between exons 1 and 2 (5’-TCTGGGAGATCATCATTAGG-3’ and 3’-CGGCATTAG-CA).
CAACGGAGG-/H11032 and certified Joe-labeled actin primer (Invitrogen) as an internal control were used. Results were analyzed using SDS software using the ΔΔCt method and results expressed as relative quantity (RQ).

Taurine uptake. For measurement of intracellular taurine uptake, HSC were grown for 7 days in treatment conditions. On day 5, cells were plated onto 24-well plates and cultured for a further 24 h to reach 80% confluence. Cells were washed twice in serum-free DMEM prewarmed at 37°C. Uptake was initiated by adding warmed serum-free medium containing 6.9 nM [3H]taurine, and increasing concentrations of unlabeled taurine (1–50 μM) were added. Uptake was measured over 1 h where it was shown to be linear. All subsequent experiments were undertaken for 30 min at 37°C.

At the end of the incubation, the medium was aspirated and cells were washed twice in ice-cold HBSS. Cells were lysed by addition of 200 μl of 2% SDS and added to 2 ml of scintillation cocktail for liquid scintillation counting. Data are expressed as uptake in picomoles of taurine per milligram of protein per minute.

The influence of Na+ on uptake was investigated by performing the uptake in buffer containing 20 mM HEPES, 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and 5 mM glucose and comparing uptake in the same buffer, where choline chloride replaced NaCl for Na+-independent uptake. Between 1 and 50 μM taurine, the Na+-free uptake was negligible and hence was not routinely subtracted from measurement of Na+-dependent uptake.

Reduced glutathione, superoxide dismutase and catalase activity. Reduced glutathione (GSH), superoxide dismutase (SOD) activity, and CAT activities were measured by commercial kits (Bioassay Systems and Cayman Chemicals, Hayward CA and Ann Arbor, MI).

RESULTS AND DISCUSSION

Effect of hyperglycemia on TauT expression and taurine transport. Studies in RPE cells have demonstrated that TauT is down regulated by high glucose concentrations (42, 65). The effect of high glucose on the expression and activity of the TauT in HSC were explored. Changes in TauT gene expression were measured in HSC cells by qRT-PCR after exposure to 5, 7.5, 10, 15, 22, and 30 mM glucose (Fig. 1A) for 7 days. The abundance of TauT mRNA declined in a dose-dependent fashion with glucose treatment. Expression was reduced by 14% in 10 mM glucose with maximal effects (66 ± 6%, P < 0.05) in 30 mM glucose. No decrease was found when the osmotic control, 25 mM raffinose, was used in place of glucose.

In concert, the abundance of TauT protein was also decreased, as shown by Western blotting in Fig. 1B. Levels were reduced by 63 ± 12% (P < 0.05) in glucose concentrations above 15 mM. Again this effect was not reproduced by 25 mM raffinose.

Measurements of taurine uptake (Fig. 1C) demonstrated that 30 mM glucose reduced TauT Vmax by 41% from 61 ± 5 to 42 ± 3 pmol·min⁻¹·mg protein⁻¹ (P < 0.001) while increasing the Km (Michaelis-Menten constant) by 50% from 10 ± 2 to 16 ± 2 μM (P = 0.16). Again this reduction was not reproduced by 25 mM raffinose.
Effect of AR inhibition on TauT gene expression and taurine transport. In common with other osmoregulatory genes such as AR, TauT is transcriptionally upregulated by TonE (tonicity response element), contained in the 5′ region of the gene, in response to hypertonic stress (17, 26). Increases in intracellular sorbitol through greater AR flux may contribute to the transcriptional downregulation of TauT expression (42).

Inhibition of AR by cotreatment for 7 days with 10 μM sorbinil reversed the hyperglycemia-induced reduction of TauT gene expression, increasing gene expression to levels 2.9- and 3.8-fold above 5 mM glucose in 10 and 30 mM glucose, respectively (both P < 0.05; Fig. 2A). Kinetic studies confirmed that the AR inhibitor (ARI) restored TauT V_max, increasing to 98% above 5 mM from 54 ± 4 to 107 ± 14 pmol·min⁻¹·mg protein⁻¹, but with no change in K_m compared with untreated 30 mM glucose (Fig. 2B).

These data further support the compatible osmolyte hypothesis, which suggests that organic osmolytes such as sorbitol, taurine, and myo-inositol respond coordinately to changes in external osmolarity (10, 42), demonstrating a link between AR expression and TauT expression. Indeed, in SC, glucose flux through AR is seen as an important link between hyperglycemia and oxidative stress in diabetic neuropathy. Hence, AR inhibition depletes intracellular sorbitol, resulting in a compensatory increase in TauT expression. Genetic studies have indicated that patients with high AR expression are predisposed to complications including neuropathy, whereas patients with a low AR expression have a lower susceptibility to neuropathy (13, 19). The importance of SC AR expression in diabetic neuropathy has been confirmed in diabetic mice overexpressing AR in SC, which developed nerve GSH depletion and exacerbated nerve conduction velocity deficits (79), whereas AR knockout mice are protected from GSH depletion and experimental neuropathy (27).

Effect of oxidative stress on TauT expression and taurine transport. The TauT promoter also contains an antioxidant response element (ARE), which is activated in response to prooxidants (42). AREs are known to be activated by proxi-
nant stress, and genes known to contain ARE are involved in protecting the cell from oxidative damage. They include glutathione S-transferases (20) and γ-glutamylcysteine transferase catalytic and regulatory subunits (41).

The effects of prooxidants in HSC TauT protein expression were examined and are shown in Fig. 3. In 5 mM glucose, 6-h incubation with 100 μM H2O2 and 50 μM primaquine (PQ) increased TauT protein abundance two- and threefold, respectively (both P < 0.05). Cotreatment for 6 h with 30 mM glucose, however, completely prevented this prooxidant-induced increase.

Functional increases in protein were confirmed by measurement of taurine transport, which showed that 50 μM primaquine increased Vmax by 61% from 48 ± 4 to 78 ± 7 pmol⋅min⁻¹⋅mg protein⁻¹ (P < 0.005), a response that was almost completely abolished by 30 mM glucose (57 ± 4 pmol⋅min⁻¹⋅mg protein⁻¹). These data demonstrate the paradox whereby oxidative stress increases TauT expression, whereas high glucose (which increases oxidative and nitrosative stress by a wide range of pathways) downregulates TauT expression and taurine transport. These data also demonstrate that a component of glucose-induced stress is able to completely ablate the response of TauT to prooxidants.

**Effect of antioxidants on TauT gene expression and taurine transport.** The effects of the antioxidant ALA were explored and are shown in Fig. 4. In 5 mM glucose, ALA reduced TauT mRNA by 23 ± 15%, although this did not reach significance (P = 0.1). In 30 mM glucose, ALA completely prevented the glucose-mediated reduction of TauT gene expression, with TauT mRNA increased by 60 ± 10% over levels measured in 5 mM glucose (P < 0.05). ALA also reduced TauT expression at 5 mM glucose by 23 ± 15%, although this did not reach significance (P = 0.1). Similarly, kinetic studies indicated that ALA restored the 30 mM glucose-mediated reduction of TauT Vmax, resulting in a 51% increase above 5 mM glucose from 54 ± 4 to 82 ± 7 pmol⋅min⁻¹⋅mg protein⁻¹ (P < 0.05) and no significant change in Km vs. untreated 30 mM glucose.

These data demonstrate restoration of TauT expression by addition of an antioxidant. ALA would not be expected to alter sorbitol content; hence, it appears that antioxidants neutralize a component of glucose toxicity, such as glycation or nitrosylation, which impairs the TauT response to oxidative stress. This

![Fig. 6](image-url) Effect of high glucose and taurine on poly(ADP-ribosyl)ated (PAR) protein abundance. Equal protein loading was confirmed by β-actin. Quantitation was performed by densitometry on total bands, and data are expressed as means ± SE of 5 separate experiments. *P < 0.05 vs. 5 mM glucose, †P < 0.05 vs. respective glucose.
parallels studies in streptozotocin-diabetic rats in which ALA restored nerve taurine content without effecting sorbitol content (68).

**Effect of high glucose and taurine treatment on oxidative stress in HSC.** The changes in oxidative stress in HSC after chronic (7 day) exposure to 5, 10, and 30 mM glucose using H$_2$DCFDA reagent to assay ROS generation were explored (Fig. 5A). Fluorescence intensity was increased in 10 and 30 mM glucose compared with 5 mM glucose. This experiment was repeated three times with consistent increases. The increase in ROS induced by high glucose was abrogated by concurrent treatment with 250 µM taurine.

To confirm this finding, Western blotting against stable 4-HNE adducts, a product of lipid peroxidation, were performed on cells treated with high glucose for 7 days, shown in Fig. 5B. 4-HNE abundance was increased by 30 ± 4 and 20 ± 5% (both *P* < 0.05) on exposure to 10 and 30 mM glucose, respectively. Coincubation with 250 µM taurine reversed the glucose-induced increase of 4-HNE with a 9% reduction in 4-HNE abundance below untreated 5 mM glucose at both 10 and 30 mM (both *P* < 0.05 vs. respective glucose). At 5 mM there was a similar reduction in 4-HNE abundance, 9 ± 3% reduction (*P* = 0.2).

The evidence for the presence of increased oxidative/nitrosative stress in peripheral nerve from diabetic rodents is substantial (46, 47). However, the cellular localization of key sites for the generation of free radicals and their ultimate downstream cellular targets remain less well characterized. In experimental diabetic neuropathy, increased ROS abundance and nitrotyrosine immunoreactivity have been observed in the epineurial blood vessels (47), which may be etiologically important in the development of nerve perfusion deficits. Oxidative stress, PARP activation (12), and apoptosis in dorsal root ganglion neurons have also been implicated in the development of functional and structural deficits (59). The effect of taurine on oxidative stress markers has previously been explored in animal models of diabetes, where taurine has been found to reduce lipid peroxidation in the plasma (33), heart, muscle, liver, kidney (73), and sciatic nerve (50) and decrease superoxide formation in β-islet cells from rats infused with high glucose (72). Here, for the first time, we have demonstrated the ability of taurine to reduce glucose-induced ROS generation and lipid peroxidation in SC.

**Effect of high glucose on abundance poly(ADP-ribosyl)ated proteins.** One of the downstream effects of hyperglycemia-induced oxidative stress is DNA single-strand break activation of PARP. Activated PARP cleaves nicotinamide adenine dinucleotide (NAD$^+$), forming nicotinamide and ADP-ribose residues, the latter of which attaches to nuclear proteins forming poly(ADP-ribosyl)ated protein polymers. Accumulation of PARP results in inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which has been proposed to result in further diversion of glucose flux towards pathways involved in diabetic complications such as the polyol pathway (9). This relationship, however, remains controversial (45). High-glucose exposure has consistently been demonstrated to activate PARP, and in turn, PARP activation has been shown to increase lipid peroxidation (80). In animal models, PARP inhibition has been shown to prevent or reverse the development of experimental diabetic peripheral neuropathy and also decrease oxidative/nitrosative stress (16, 55).

As a measure of oxidative-stress-induced DNA damage, Western blotting of PAR proteins in cells incubated in high glucose for 7 days was performed, and the data are shown in Fig. 6. PAR protein abundance was increased dose dependently by 26 ± 7% in 10 mM and by 40 ± 13% in 30 mM glucose (*P* < 0.05), respectively. Coincubation with 250 µM taurine reduced the PAR to below control, with reductions at 5, 10, and 30 mM of 14 ± 2, 12 ± 19, and 3 ± 14%, respectively (*P* < 0.05 all vs. respective glucose).

Here, we have demonstrated for the first time that taurine can completely prevent the accumulation of PAR proteins. The effect of taurine on PAR protein abundance and lipid peroxidation is consistent with that observed for ARIs (14, 15, 53) and direct PAR inhibition (48). The mechanism of this effect is unknown; however, a mechanism involving the attenuation of oxidative or nitrosative stress is likely to be involved.

**Effect of high glucose on antioxidant defense systems.** Although the evidence for glucose-induced oxidative stress is well established, the response of the antioxidant defense system in HSC to high glucose is unclear and inconsistent. In a recent review, Maritim et al. (39) concluded that in models of diabetes GSH is decreased along with the activity of glutathione reductase, the activity of CAT is generally increased, and the response of SOD varies greatly with little correlation between age, model, or duration or type of diabetes studied. Miinea et al. (40) showed that, in immortalized rat SC exposed to high glucose, increased oxidative stress was associated with decreased SOD activity, GSH abundance, and glutathione reductase, whereas the activity of CAT remained unchanged. Those authors implicate increased GSH glycation as a factor leading to reduced SOD activity (1). As the response of the antioxidant defense system in nonimmortalized human SC has not been explored, we measured the activity of SOD, CAT, and concentration of GSH in cells incubated in 5, 10, and 30 mM glucose for 7 days (table 1).

The activity of SOD was increased by 70 ± 18% in 30 mM glucose (*P* < 0.05 vs. untreated). CAT activity was increased threefold at 10 and 30 mM glucose (both *P* < 0.05), clearly demonstrating mobilization of the antioxidant defense system in HSC. The abundance of GSH was reduced by 16 ± 6% at 10 mM and by 28 ± 4% at 30 mM glucose (*P* < 0.05). This may reflect NADPH depletion, a consequence of increased flux.

### Table 1. Effects of high glucose co-treated with either 250 µM taurine or 100 µM ALA on SOD and CAT activity and GSH concentration

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Treatment</th>
<th>SOD Activity, %control</th>
<th>CAT Activity, mmol·min$^{-1}$·mg$^{-1}$</th>
<th>GSH, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM</td>
<td>Untreated</td>
<td>100±11</td>
<td>139±48</td>
<td>36±1.6</td>
</tr>
<tr>
<td></td>
<td>Taurine</td>
<td>150±21</td>
<td>519±72</td>
<td>28±1.4</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>179±18</td>
<td>444±92</td>
<td>44±3.7</td>
</tr>
<tr>
<td>10 mM</td>
<td>Untreated</td>
<td>141±24</td>
<td>500±32</td>
<td>31±2.0</td>
</tr>
<tr>
<td></td>
<td>Taurine</td>
<td>142±10</td>
<td>493±78</td>
<td>29±1.8</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>168±16</td>
<td>606±87</td>
<td>32±1.5</td>
</tr>
<tr>
<td>30 mM</td>
<td>Untreated</td>
<td>170±18</td>
<td>349±69</td>
<td>26±1.5</td>
</tr>
<tr>
<td></td>
<td>Taurine</td>
<td>167±15</td>
<td>504±80</td>
<td>29±1.8</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>145±16</td>
<td>616±109</td>
<td>33±3.0</td>
</tr>
</tbody>
</table>

Data expressed as means ± SE. SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; ALA, α-lipoic acid. *P* < 0.05 vs. untreated 5 mM glucose.
through AR (9, 46). Whether the differences between this study and that of Miinea et al. are due to species differences known to occur in diabetes (11, 28, 39, 60) or due to the immortalization of the cells is yet to be determined.

Taurine is known to have a potent ability to reduce ROS and lipid peroxidation; however, the precise mechanism(s) of its antioxidant effect remains uncertain. Aruoma et al. (2) established that taurine is incapable of directly scavenging superoxide anion, hydroxyl radical, and hydrogen peroxide, although it is known to scavenge hypochlorite produced by morphonuclear leukocytes and eosinophils (61). Although of relevance in whole animal models, in isolated cell culture this mechanism does not account for the antioxidant effect observed, and many authors cite indirect mechanisms of ROS scavenging by taurine, e.g., by increasing the activities of other members of the antioxidant defense system (61).

We explored whether the effects of taurine on oxidative stress were due to an indirect action by altering the antioxidant defense system. The effect of taurine on SOD/CAT and GSH were measured alone and in combination with glucose and compared with actions of the antioxidant ALA. Taurine treatment (250 μM) resulted in a threefold increase in CAT activity (P < 0.05) and a 50 ± 21% increase in SOD activity (P = 0.144). ALA also increased SOD activity at 5 mM glucose by 79 ± 18% (P < 0.05) and CAT activity threefold (P < 0.05). The response of GSH to ALA, however, differed from that of taurine, as ALA increased GSH concentration at 5 mM glucose by 22 ± 10% (P < 0.05), whereas taurine decreased GSH concentration by 23 ± 4% (P < 0.05). The mechanism of this effect is unclear; however, since taurine and GSH are both synthesized from cysteine, depletion of GSH may reflect reduced synthesis due to decreased cysteine uptake (58) or structural differences between the antioxidants or varying targets of antioxidant activity.

In high glucose, taurine treatment had no additive effect on SOD or CAT activity and did not restore GSH content, whereas ALA restored GSH content. In experimental diabetic neuropathy, taurine treatment was found to reduce lipid peroxidation and attenuate deficits of nerve conduction, dorsal root ganglion neuron calcium signaling, endoneurial blood flow, and hyperalgesia (36). However, in this model, in composite nerve, no changes in SOD activity (73) or GSH abundance (50) were observed with taurine treatment, with taurine increasing oxidative stress and PAR abundance and SOD and CAT activity, whereas ALA did not restore GSH content, and did not increase antioxidant activity. The prooxidant effect of taurine depletion brought about by increased glycated hemoglobin (50) were observed with taurine treatment, with taurine increased oxidative stress and PAR abundance and SOD and CAT activity while disrupting the GSH system and taurine increased oxidative stress and PAR abundance and SOD and CAT activity (25).

Conclusion

In HSC, a key site for diabetic complications, glucose increased oxidative stress and PAR abundance and SOD and CAT activity while disrupting the GSH system and taurine transport. Taurine supplementation restored ROS, 4-HNE, and PAR back to levels found in normal glucose. This action was achieved without changes in classic antioxidant defense systems demonstrating direct antioxidant taurine actions. TauT expression and kinetics were restored by ARI as well as ALA, implicating an increase in intracellular sorbitol as well as nitrosylation or glycation in glucose-induced TauT downregulation. Together, these data suggest taurine supplementation as well as strategies directed toward prevention of taurine deple tion as potential therapies to help prevent or reverse diabetic neuropathy.

The antioxidant actions of taurine in high glucose occur with no change in the antioxidant defense; this is indicative of a direct antioxidant action such as being able to scavenge mitochondrial superoxide (57), which is heavily implicated in the pathogenesis of diabetic complications (9). Another possibility, based around work from Suzuki et al. (70, 71) and Kirino et al. (30), who found that taurine forms conjugates with uridines of mammalian mitochondrial tRNA and that in certain mitochondrial diseases these taurine modifications were lacking. This raises the possibility that taurine may be important in synthesis of mitochondrial proteins, such as those in the electron transport chain. Deficiency in synthesis of these proteins may lead to diversion of electrons from respiratory transport to oxygen, increasing formation of superoxide anion, and resulting in back-up glycolytic metabolites, increasing flux through pathways associated with diabetic complications such as the polyol pathway (61). This suggests that, rather than a direct antioxidant effect of taurine, taurine supplementation reverses the prooxidant effect of taurine depletion brought about by TauT downregulation.

GRANTS

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