Taurine replacement attenuates hyperalgesia and abnormal calcium signaling in sensory neurons of STZ-D rats

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Taurine replacement attenuates hyperalgesia and abnormal calcium signaling in sensory neurons of STZ-D rats. Am J Physiol Endocrinol Metab 288:E29–E36, 2005; doi:10.1152/ajpendo.00168.2004.—The etiology of painful diabetic neuropathy is poorly understood, but may result from neuronal hyperexcitability secondary to alterations of Ca2+ signaling in sensory neurons. The naturally occurring amino acid taurine functions as an osmolyte, antioxidant, Ca2+ modulator, inhibitory neurotransmitter, and analgesic such that its depletion in diabetes may predispose one to neuronal hyperexcitability and pain. This study reports the effects of taurine replacement on hyperalgesia and sensory neuron Ca2+ homeostasis in streptozotocin-diabetic (STZ-D) rats. Nondiabetic and STZ-D rats were treated with a 2% taurine-supplemented diet for 6–12 wk. Thermal hyperalgesia and mechanical allodynia were determined by measuring hindpaw withdrawal latency to radiant heat and the withdrawal threshold to the von Frey anesthesiometer. Intracellular Ca2+ signaling was explored in neurons from L4–L6 dorsal root ganglia (DRG), using fura 2 fluorescence. Taurine replacement of diabetic rats attenuated deficits of nerve conduction and prevented reductions of mechanical and thermal withdrawal threshold and latency, respectively. In small DRG sensory neurons from diabetic rats, recovery of intracellular Ca2+ concentration ([Ca2+]i) in response to KCl was slowed and 73% corrected by taurine. The amplitudes of caffeine and ATP-induced [Ca2+]i transients were decreased by 47 and 27% (P < 0.05), respectively, in diabetic rat DRG sensory neurons and corrected by 74 and 93% (P < 0.05), respectively, by taurine replacement. These data indicate that taurine is important in the regulation of neuronal Ca2+ signaling and that taurine deficiency may predispose one to nerve hyperexcitability and pain, complicating diabetes.

Pain; diabetic neuropathy; allodynia

The rising prevalence of diabetes mellitus in the United States (3) is presenting new challenges for the management of chronic microvascular complications, including distal symmetrical sensorimotor polyneuropathy (DPN; see Refs. 16 and 41). In diabetes, damage to large-diameter myelinated sensory nerve fibers results in vibratory and position sensory deficits and eventually distal weakness, whereas defects of small thinly or unmyelinated sensory nerve fibers can result in neuropathic pain, dysesthesias, and/or paresthesias (53, 73). Symptoms such as tactile allodynia and hyperalgesia (53, 73) can significantly impair activity, metabolic control, and quality of life and lead to depression. Indeed, painful DPN can be refractory to treatment with conventional pharmacological therapy (7, 73), emphasizing the importance of the discovery and development of new therapeutic approaches that ideally can target both large-fiber and small-fiber deficits.

Increased oxidative and nitrosative stresses (4, 8, 18, 38, 42, 60) have emerged as leading candidates in the pathogenesis of DPN. A direct relationship has emerged between measures of oxidative stress and the development of nerve blood flow and nerve conduction deficits (4, 8, 38, 42, 60) as well as impaired neurotrophism (20, 43). Oxidative stress has also recently been invoked as a contributing factor to painful DPN (9, 72). However, attenuation of oxidative stress alone may be insufficient to completely alleviate neuropathic pain complicating diabetes, emphasizing the need to impact additional metabolic pathways implicated in pain pathogenesis.

In diabetes, alterations in neuronal Ca2+ signaling may contribute to the development of DPN and pain (24, 66). Diabetes increases the current amplitude of multiple voltage-dependent Ca2+ currents (23, 67), and Ca2+ influx activates nitric oxide (NO)/cGMP/protein kinase G pathways; blockade of this pathway decreases experimentally induced pain (40). In diabetic rats, protein kinase A, protein kinase C (PKC), and NO second messenger systems contribute to hyperalgesia, whereas N-methyl-D-aspartate receptor-mediated events are not thought to be involved (2). Additionally, persistent elevations of cytosolic Ca2+ have been implicated in neuronal degeneration (45) and apoptosis (21). Therefore, attenuation of oxidative stress and amelioration of abnormal Ca2+ signaling have emerged as important therapeutic targets in DPN.

Taurine (2-aminoethanesulfonic acid) functions as an important endogenous antioxidant (25, 43, 44), Ca2+ modulator (28, 35), neurotransmitter (14), and osmolyte (58, 59), such that its intracellular depletion could promote chronic cytotoxicity in diabetes. We have previously identified taurine depletion in the sciatic nerve of streptozotocin-diabetic (STZ-D) rodents and demonstrated that taurine replacement (using a 1–2% taurine-supplemented diet) attenuates nerve oxidative stress (43) and ameliorates neurovascular, metabolic, neurotrophic, and functional deficits (43, 49). We have proposed that the metabolic properties of taurine uniquely implicate its depletion as a critical factor in the pathogenesis of painful DPN (59). The studies reported herein aimed to explore this hypothesis by assessing the potential of taurine replacement to ameliorate...
hyperalgesia and abnormal Ca\textsuperscript{2+} signaling in sensory neurons of STZ-D rats.

MATERIALS AND METHODS

All experiments were performed in accordance with regulations specified by the National Institutes of Health Principles of Laboratory Animal Care, 1985 Revised Version, and the University of Michigan Protocol for Animal Studies.

Animal model. Barrier-sustained, caesarean-delivered male Wistar rats (200–300 g) were acclimatized for 1 wk before being fasted overnight and rendered diabetic by an intraperitoneal injection of STZ (45 mg/kg; Sigma-Aldrich, St. Louis, MO) in 0.2 ml of 10 mM citrate buffer, pH 5.5. Diabetes was defined as a nonfasting plasma glucose ≥200 mg/dl in tail vein blood (One Touch II; Lifescan, Milpitas, CA) 48 h after STZ injection. Animals were subsequently randomly assigned to the following four experimental groups: nondiabetic controls, nondiabetic animals treated with a 2% taurine-supplemented diet (Sigma), untreated diabetic controls, and diabetic animals treated with a 1% taurine-supplemented diet. The taurine dose was reduced to compensate for the twofold increase of food consumption in diabetic rats and to achieve comparable circulating taurine levels and normalization of taurine content in the sciatic nerve. This was done to avoid potentially detrimental excess tissue taurine (59) and was maintained for periods of 6 (experiment 1) or 12 (experiment 2) wk. Less than 1% of diabetic rats became nondiabetic and were not used for further experiments. In experiment 1, animals were killed by an overdose of urethane and, in experiment 2, animals were killed by CO\textsubscript{2} narcosis.

Measurements of nerve conduction velocity. Initially, in experiment 1, the effects of taurine therapy on diabetes-induced nerve conduction velocity (NCV) slowing were explored. Rats were anesthetized with a mixture of 40–90 mg/kg ketamine and 5–15 mg/kg xylazine injected intraperitoneally. For sciatic motor NCV (MNCV), the left sciatic nerve was stimulated proximally at the sciatic notch and distally at the ankle via bipolar electrodes with supramaximal stimuli (8 volts) at 20 Hz. The latencies of the compound muscle action potentials were recorded via bipolar electrodes from the first interosseous muscle of the hindpaw and measured from the stimulus artifact to the onset of the negative M-wave deflection. MNCV was calculated by subtracting the distal latency from the proximal latency, and the result was divided into the distance between the stimulating and recording electrode. Digital sensory NCV (SNCV) was recorded in the digital nerve to the second toe by stimulating with a square wave pulse of 0.05-ms duration, utilizing the smallest-intensity current that resulted in a maximal amplitude response. The sensory nerve action potential was recorded behind the medial malleolus. Sixteen responses were averaged to obtain the position of the negative peak. The maximal SNCV was calculated by measuring the latency to the onset/peak of the initial negative deflection and the distance between stimulating and recording electrodes. In all measurements, body temperature was monitored by a rectal probe and maintained at 37°C with a warming pad. Hindlimb skin temperature was also monitored by a thermistor and maintained between 36 and 38°C by radiant heat.

Measurement of mechanical sensitivity. Next, in experiment 1, in a parallel experiment, the effects of taurine therapy on mechanical and thermal sensitivity were explored in nondiabetic and diabetic rats. Measurements were made on alternate hindpaws separated in time by at least 1 h. Tactile allodynia was determined by quantifying the withdrawal threshold of the hindpaw in response to mechanical stimulation using a von Frey anesthesiometer (model 2290C; IITC Life Science, Woodland Hills, CA). The test was conducted between 9:00 and 11:30 AM each day. The rats were placed in individual Plexiglas boxes on a stainless steel mesh floor and were allowed to acclimate for at least 20 min. A 0.5-mm-diameter polypropylene rigid tip was used to apply a force to the plantar surface of the hindpaw. The latency of the withdrawal was recorded by the anesthesiometer. The anesthesiometer was calibrated before each recording. The test was repeated four to five times at ~5-min intervals on each animal, and the mean value was calculated.

Measurement of thermal sensitivity. To determine the sensitivity to noxious heat, rats were placed within a Plexiglas chamber on a transparent glass surface and allowed to acclimate for at least 20 min. A thermal stimulation meter (IITC Life Science Instruments) was used. The device was activated after placing the stimulator directly beneath the planter surface of the hindpaw. The paw withdrawal latency in response to the radiant heat was recorded. Individual measurements were repeated four to five times, and the mean value was calculated as the thermal threshold.

Isolation of dorsal root ganglion neurons. In experiment 2, nondiabetic and untreated and taurine-treated diabetic animals were maintained for 12 wk, at which time point animals were killed by CO\textsubscript{2} narcosis. Isolated, acutely dissociated dorsal root ganglia (DRG) neurons from lumbar 4–6 regions were aseptically prepared. DRG neurons were dissected, trimmed, minced, and then incubated at 37°C in DMEM (GIBCO-BRL, Grand Island, NY) containing 0.3% collagen type II (Sigma) for 50 min, followed by 0.1% bovine trypsin (Sigma) for 10 min. Ganglia were dissociated into single cells by trituration through a flame-constricted pipette. The small DRG neurons (<30 μm diameter) were then plated on poly-l-lysine-coated coverslips, which were incubated in room air supplemented with 7% CO\textsubscript{2} at 37°C for 1 h before recordings were made. Typically, four to six DRG neurons were isolated from each animal.

Measurement of intracellular free Ca\textsuperscript{2+} levels in DRG neurons. To measure intracellular Ca\textsuperscript{2+} transients, DRG neurons were incubated with physiological salt solution (PSS) containing 140 mM NaCl, 5 mM KHCO\textsubscript{3}, 1 mM MgCl\textsubscript{2}, 2.2 mM CaCl\textsubscript{2}, 10 mM glucose, and 10 mM HEPES, pH 7.2, with 10 μM fura 2-AM (Molecular Probes, Eugene, OR) for 20 min at room temperature and then washed with PSS to allow cytoplasmic deesterification of the Ca\textsuperscript{2+}-sensitive fluorescent dye. The dishes were then removed to a chamber system that allowed for perfusion with selected PSS solution. Fura 2 fluorescence was monitored using dual-wavelength microspectrofluorometry (SPEX Industries AR-CM system; Edison). The fluorescence ratio (340/380) was converted to an intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]i) by the equation of Grynkiewicz et al. (22).

The relationship of fura 2 fluorescence to [Ca\textsuperscript{2+}]i, was calibrated using an in vivo approach in which DRG neurons were dialedyzed with various solutions. The [Ca\textsuperscript{2+}]i, values were calculated from the equation [Ca\textsuperscript{2+}]i = K\textsubscript{dm}(R – R\textsubscript{min})(R\textsubscript{max} – R), where K\textsubscript{dm} is the fluorescence ratio recorded after 20 min of incubation of cells in Ca\textsuperscript{2+}-free (2 mM EGTA) PSS containing fura 2-AM and 4 μM ionomycin, and R\textsubscript{max} was determined the same way except with an external solution containing 10 mM Ca\textsuperscript{2+}-; the constant K\textsubscript{dm} was determined by back calculation, using fluorescence ratio values measured in a Ca\textsuperscript{2+}-EGTA PSS (with ionomycin) buffered to a 130 mM free Ca\textsuperscript{2+} concentration together with the determined R\textsubscript{min} and R\textsubscript{max} values. On average, both R\textsubscript{min} and R\textsubscript{max} used for intracellular Ca\textsuperscript{2+} calibration were measured from 30–40 small DRG cells. The value K\textsubscript{dm} was 3,335 nM, and R\textsubscript{min} and R\textsubscript{max} were 1.04 and 13.3, respectively.

Statistical analysis. Data are expressed as means ± SE. Differences among experimental groups were determined by ANOVA, and the significance of between-group differences was assessed by the Student-Newman-Keuls multiple-range test. Significance was defined as P ≤ 0.05. If the variances for the variables were found to differ significantly, a logarithmic transformation was performed that corrected the unequal variances. All analyses were then performed on the transformed data. When between-group variance differences could not be normalized by log transformation (data sets for body weights, plasma glucose, and some metabolic parameters), the data were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA, followed by the Bonferroni/Dunn test for multiple comparisons.
Table 1. Effect of taurine replacement and diabetes on body weight and plasma glucose values

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Plasma Blood Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>19</td>
<td>459±59</td>
<td>74±7</td>
</tr>
<tr>
<td>ND + T</td>
<td>11</td>
<td>495±64</td>
<td>77±15</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>346±51*</td>
<td>411±88*</td>
</tr>
<tr>
<td>D + T</td>
<td>21</td>
<td>376±71*</td>
<td>418±105*</td>
</tr>
</tbody>
</table>

Values are means ± 1 SD. ND, nondiabetic; ND + T, ND treated with taurine; D, untreated diabetic; D + T, diabetic treated with taurine. *P < 0.05 vs. ND.

RESULTS

Body weights and plasma glucose levels from experiment 1 are shown in Table 1. As expected, after 6 wk, body weights were lower in diabetic animals compared with nondiabetic controls. Blood glucose concentration was increased approximately sixfold in diabetic rats compared with control groups. Taurine treatment of nondiabetic or diabetic rats had no significant effects on either body weight or plasma glucose concentration.

Effects of diabetes and taurine replacement on MNCV and SNCV. The effects of diabetes and taurine replacement on MNCV and SNCV slowing are shown in Fig. 1, A and B. As expected (43, 49), sciatic MNCV was reduced by 24% (P < 0.001) after 6 wk of untreated diabetes (57 ± 1 and 44 ± 1 m/s in nondiabetic and diabetic rats, respectively). Taurine replacement partially corrected this deficit by 31% (P < 0.001; 48 ± 1 m/s; Fig. 1A). In contrast, digital SNCV was reduced by 16% (P < 0.001) in diabetic rats (38 ± 3 and 32 ± 0.5 m/s in nondiabetic and diabetic rats, respectively) and was 72% corrected (P < 0.01; 36 ± 1 m/s) by taurine replacement to levels that were not significantly different from controls (Fig. 1B). Taurine treatment was without effect in nondiabetic control animals (data not shown).

Effect of diabetes and taurine replacement on mechanical thresholds. The effects of diabetes and taurine replacement on mechanical and thermal thresholds are shown in Fig. 1, C and D (NCV measurements were also measured in these animals, and the results were consistent with those reported above). The paw withdrawal threshold to the von Frey anesthesiometer was reduced by 65% (P < 0.01) in untreated diabetic rats (24 ± 3 g) compared with the nondiabetic control animals (69 ± 4 g; Fig. 1C), consistent with diabetes-induced tactile allodynia. Taurine replacement resulted in a 90% (P < 0.01) correction of the threshold to levels that were indistinguishable from the nondiabetic control group (62 ± 10 g). Although the mechanical threshold of nondiabetic control animals treated with taurine was 19% greater than that of untreated nondiabetic control animals (82 ± 8 g), this difference did not achieve statistical significance (P = 0.1).

Effect of diabetes and taurine replacement on thermal sensitivity. The paw withdrawal latency in response to the radiant heat stimulus was reduced by 30% (P < 0.01) in untreated
diabetic rats (14 ± 1 s) compared with control animals (20 ± 1 s; Fig. 1D). Taurine replacement in diabetic animals resulted in a 95% (P < 0.01) correction of this deficit (19 ± 1 s). Taurine treatment was without effect in nondiabetic control rats (20 ± 1 s).

Effect of diabetes and taurine replacement on Ca\(^{2+}\) signaling in small DRG sensory neurons. Next, the effects of diabetes and taurine replacement on DRG neuron [Ca\(^{2+}\)], transients were explored after 12 wk of experimental diabetes. This prolonged duration of experimental diabetes was chosen, since, in addition to hyperalgesia, which is an acute but transient finding in STZ-diabetic rats, we wished to assess the effect of taurine replacement on persistent alterations of [Ca\(^{2+}\)], signaling that have been implicated not only in pain pathways but also in neuronal degeneration (45) and apoptosis (21, 52).

Parallel experiments to those described below were, however, also performed in animals with shorter durations of experimental diabetes, which confirmed that these abnormalities of Ca\(^{2+}\) signaling and sensitivity to taurine replacement were present simultaneously as diabetes-induced hyperalgesia (data not shown).

Like before, body weights were decreased and plasma glucose values increased sixfold in diabetic rats and were unaffected by taurine replacement (data not shown). Resting [Ca\(^{2+}\)], was determined in small DRG sensory neurons from the different experimental groups. No difference was found in resting [Ca\(^{2+}\)], between any of the groups (54 ± 8 nmol/l in nondiabetic controls, n = 32, 63 ± 11 nmol/l in untreated diabetic controls, n = 24, and 63 ± 8 nmol/l in diabetic animals treated with a 1% taurine-supplemented diet, n = 32).

Effect of KCl. Initially, a solution containing 50 mM KCl (in mM: 90 NaCl, 50 KCl, 5 KHCO\(_3\), 1 MgCl\(_2\), 10 glucose, 10 HEPES, and 2.2 CaCl\(_2\), pH 7.2) was applied for 15 s to depolarize the DRG neurons and open membrane voltage-gated Ca\(^{2+}\) channels. No significant differences in the amplitude of the depolarization-induced [Ca\(^{2+}\)], transients was observed in neurons from diabetic or taurine-treated animals (839 ± 55 nmol/l in nondiabetic controls, n = 32, 745 ± 86 nmol/l in untreated diabetic controls, n = 21, and 766 ± 51 nmol/l in diabetic animals treated with a 1% taurine-supplemented diet, n = 33). However, a consistent deceleration of recovery of [Ca\(^{2+}\)], in response to membrane depolarization was observed in DRG neurons from untreated diabetic animals (Fig. 2). In DRG neurons from nondiabetic control animals, [Ca\(^{2+}\)], recovered to the basal level with a time constant of 252 ± 27 s. In contrast, the restoration of [Ca\(^{2+}\)], in diabetic neurons was prolonged over twofold (541 ± 31 s, P < 0.01). Taurine replacement in diabetic rats corrected this deficit by 73% (331 ± 35 s, P < 0.01).

Effect of caffeine. To explore potential mechanisms contributing to delayed recovery of [Ca\(^{2+}\)], in small DRG sensory neurons from diabetic rats, endoplasmic reticulum Ca\(^{2+}\) storage was assessed in the different animal groups with the use of caffeine, which stimulates Ca\(^{2+}\)-gated Ca\(^{2+}\) release from the endoplasmic reticulum stores (64). In our present study, 100% of cells responded to caffeine.

The application of 20 mmol/l caffeine for 20 s resulted in transient elevations of [Ca\(^{2+}\)], in all experimental groups, a response that was unaffected by the use of Ca\(^{2+}\)-free extracellular solution (Fig. 3). The amplitudes of caffeine-induced [Ca\(^{2+}\)], transients were significantly (P < 0.001) smaller in diabetic rats (373 ± 48 nmol/l) compared with nondiabetic control animals (704 ± 71 nmol/l; Fig. 3). Taurine replacement in diabetic rats was found to correct this deficit by 91% (638 ± 50 nmol/l; P < 0.01).

Effect of ATP. Activation of purinoreceptors by ATP raises [Ca\(^{2+}\)], by a number of different mechanisms, including the stimulation of Ca\(^{2+}\) entry via plasmalemmal Ca\(^{2+}\) channels and P2X receptors (34) and inositol trisphosphate-induced Ca\(^{2+}\) release from endoplasmic reticulum. Therefore, the effects of diabetes and taurine replacement on ATP-sensitive [Ca\(^{2+}\)], transients were explored. Despite the use of 100 μM ATP, <50% cells were found to be responsive to stimulation in control animals, and 34 and 36% of cells had a response to ATP in diabetic animals and diabetic animals treated with a 1% taurine-supplemented diet, respectively. Neurons were considered to be responding when the amplitude of the [Ca\(^{2+}\)], increase evoked by ATP exceeded 50 nM. Representative examples of the ATP-induced [Ca\(^{2+}\)], response are shown in Fig. 4. The ATP-induced Δpeak [Ca\(^{2+}\)], was decreased by 65% (P < 0.05) in diabetic rats (144 ± 44 nmol/l) compared with nondiabetic controls (410 ± 69 nmol/l). Taurine replacement resulted in a 92% (P < 0.05) correction of this deficit (376 ± 83 nmol/l) to levels that were not significantly different from healthy control animals.

DISCUSSION

The etiology of painful DPN is poorly understood, but may result from neuronal hyperexcitability secondary to alterations of Ca\(^{2+}\) signaling in sensory neurons (24, 66). The naturally occurring amino acid taurine functions as an osmolyte, anti-
sensitization (11, 68) and hyperalgesia or allodynia (51). Damage to small myelinated afferent fibers has also been found in animal models and diabetic patients (30, 69). Reduced mechanical threshold of C-fiber nociceptors (61) and increased nociceptor spontaneous activity have been demonstrated in STZ-D rats (11, 61). Processing of nociceptive information may also be profoundly affected by diabetes. Structures of the descending antinociceptive pathway involved in pain processing include the hypothalamus, the nucleus accumbens, the mesencephalon, and the limbic systems (27). Taurine is a ubiquitous amino acid found at high concentrations with the peripheral nervous system (59), but also within the central nervous system, especially in the hypothalamus, a location implicated in its antihypertensive/antisympathetic actions (46). Therefore, deficits of taurine metabolism at multiple levels could have contributed to the development of DPN in this model. In this report, we chose to explore the effect of taurine replacement on Ca\textsuperscript{2+} transients in small sensory neurons, since abnormal Ca\textsuperscript{2+} signaling has been implicated in the development of altered nociception (66) and neurodegeneration (26, 45, 66).

Sensory neuropathy has been consistently associated with abnormal Ca\textsuperscript{2+} signaling in the DRG of diabetic rodents (24, 26, 66). Moreover, serum from diabetic patients produces elevations of neuronal Ca\textsuperscript{2+} influx and increased programmed cell death (48, 57). Diabetes increases the current amplitude of multiple voltage-dependent Ca\textsuperscript{2+} currents (23, 67). In this report, experimental diabetes resulted in wide-ranging abnormalities of Ca\textsuperscript{2+} signaling in small DRG neurons, which were manifest as prolongation of recovery of cytosolic Ca\textsuperscript{2+} after

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.pdf}
\caption{[Ca\textsuperscript{2+}], transients induced by the application of 20 mM caffeine recorded from small DRG sensory neurons at 12 wk from ND, D, and D + T rats; \(n = 4–6\) animals in each experimental group. Typically, each animal contributed 3–5 DRG sensory neurons to each measurement. A: representative examples of caffeine-induced [Ca\textsuperscript{2+}], transients from an ND rat, a D rat, and a D + T rat. Area under the curve (expressed as %ND control): ND 100 \pm 17\%, D 99 \pm 15\%, and D + T 110 \pm 12\%. B: average values for amplitude of caffeine-induced [Ca\textsuperscript{2+}], transients from ND (\(n = 17\)), D (\(n = 18\)), and D + T (\(n = 35\)). Data are group means \(\pm 1\) SE. Statistical analysis: ND vs. D and D vs. D + T, *\(P < 0.05\).}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{figure4.pdf}
\caption{[Ca\textsuperscript{2+}], transients induced by the application of 100 \(\mu\text{M}\) ATP for 20 s recorded from small DRG sensory neurons at 12 wk from ND, D, and D + T rats; \(n = 4–6\) animals in each experimental group. Typically, each animal contributed 3–5 DRG sensory neurons to each measurement. A: representative examples of ATP-induced [Ca\textsuperscript{2+}], transients from an ND rat, a D rat, and a D + T rat. Area under the curve (expressed as %ND control): ND 100 \pm 33\%, D 34 \pm 5\%, and D + T 67 \pm 24\%. B: average values for amplitude of ATP-induced [Ca\textsuperscript{2+}], transients from ND (\(n = 15\)), D (\(n = 13\)), and D + T (\(n = 15\)). Data are group means \(\pm 1\) SE. Statistical analysis: ND vs. D and D vs. D + T, *\(P < 0.05\).}
\end{figure}

... modulator, inhibitory neurotransmitter, and analgesic (25, 28, 35, 43, 44, 58, 59) such that its depletion in diabetes may predispose one to neuronal hyperexcitability and pain. Taurine replacement of STZ-D rats attenuated deficits of nerve conduction and prevented reductions of tactile and thermal withdrawal threshold and latency, respectively. In small DRG sensory neurons from diabetic rats, recovery of [Ca\textsuperscript{2+}], in response to KCl was slowed and partially corrected by taurine. The amplitudes of caffeine and ATP-induced [Ca\textsuperscript{2+}], transients were smaller in small DRG neurons from diabetic rats and partially or completely corrected by taurine replacement, respectively. Data indicate that taurine is important in the regulation of neuronal Ca\textsuperscript{2+} signaling and that taurine deficiency may predispose one to nerve hyperexcitability and pain, complicating diabetes.

Six weeks of diabetes was sufficient to result in MNCV and SNCV deficits, which were accompanied by the development of both thermal hyperalgesia and tactile allodynia. Taurine replacement attenuated MNCV slowing but completely prevented sensory deficits. STZ-D rats have been extensively utilized as a model for human painful DPN, since they exhibit decreased nociceptive thresholds (allodynia) and increased sensitivity to mechanical stimulation (hyperalgesia) of the hindpaw skin (1, 2, 61). The precise mechanisms leading to these functional changes are, however, uncertain. Increased activity or damage to small-diameter C-fibers has been implicated in the development of pain (1, 5, 11), producing a central
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membrane depolarization and reductions in the amplitude of caffeine and ATP-induced Ca\textsuperscript{2+} transients.

The downregulation of functional purinoreceptors and reduced amplitude of DRG neuron Ca\textsuperscript{2+} transients in diabetic rats in response to agents that normally promote nociceptive signaling may seem paradoxical. However, this impaired response is consistent with other reports in this animal model (26, 66) and is consistent with depletion or downregulation of caffeine- and ATP-sensitive intracellular Ca\textsuperscript{2+} stores or transport pathways, respectively, secondarily to excessive stimulation. Resting [Ca\textsuperscript{2+}], levels were not altered in diabetic rats, which is consistent with some reports in spinal dorsal horn neurons (66), primary sensory neurons (31), and nonneuronal diabetic tissues (71) but not with another report in neurons isolated from L\textsubscript{4}–L\textsubscript{6} DRG (26). The reasons for these discrepancies are unclear and cannot be accounted for by duration of diabetes or the population of DRG neurons studied. In contrast, the delay of recovery of the Ca\textsuperscript{2+} transients to the resting level after depolarization has been a consistent finding in diabetic rodents (26, 66) and may reflect a number of mechanisms that have been disrupted by diabetes, including impaired Ca\textsuperscript{2+} uptake in the endoplasmic reticulum (26, 66) or mitochondria (62) or decreased activity of the plasmalemmal Ca\textsuperscript{2+} pumps (47) or the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (70).

In diabetic rats, recovery of [Ca\textsuperscript{2+}], in response to KCl was 73% corrected by taurine, and the amplitude of caffeine and ATP-induced [Ca\textsuperscript{2+}], transients was partially or completely corrected by taurine replacement, respectively. The salutary effects of taurine on diabetes-induced alterations of Ca\textsuperscript{2+} homeostasis are consistent with other reports under euglycemic, nondiabetic conditions. Taurine has been reported to lower cytosolic Ca\textsuperscript{2+} by stimulating mitochondrial uptake (29, 35), by inhibiting phosphoinositide turnover (35, 56), by altering Ca\textsuperscript{2+} binding to membrane phospholipids (54), and by decreasing internal Ca\textsuperscript{2+} flux (29, 35). The protective effects of taurine against glutamate-induced neurotoxicity in cultured neurons are mediated by its ability to lower intracellular Ca\textsuperscript{2+} via an effect on the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (10, 17). The reduced amplitude of the caffeine-mediated [Ca\textsuperscript{2+}], transient, and its partial correction by taurine, is consistent with an effect of taurine to enhance endoplasmic reticulum Ca\textsuperscript{2+} storage. The complete correction of ATP-induced [Ca\textsuperscript{2+}], transients by taurine could potentially be mediated by a wide range of mechanisms involving P\textsubscript{2\textsubscript{x}} receptors, plasmalemmal Ca\textsuperscript{2+} channels (26, 29, 34, 35, 56), and endoplasmic reticulum stores (63). It has previously been reported that nerve growth factor (NGF) can upregulate P\textsubscript{2\textsubscript{x}(3)} expression in sensory neurons (50) and that a corrective effect of neurotrophin-3 on ATP-induced Ca\textsuperscript{2+} transients may be mediated via the tyrosine kinase A-expressing, NGF-dependent sensory neurons (26). Therefore, the ability of taurine replacement to correct NGF protein deficits in the sciatic nerve of diabetic rats (43) would also be consistent with this mechanism of action. Finally, correction of neuronal taurine deficits would also provide a mechanistic explanation for the ability of aldose reductase inhibition to ameliorate pain thresholds (6) and altered Ca\textsuperscript{2+} signaling (23, 24) in experimental diabetes.

Oxidative stress has also recently been invoked as a contributing factor to painful DPN, and the ability of taurine to function as an important endogenous antioxidant (25, 43, 44) would be consistent with this being an important mechanism of action. The chain-breaking antioxidant DL-α-lipoic acid (ALA) and dietary GSH supplementation can attenuate thermal hyperalgesia and mechanical allodynia in experimental DPN (9, 65; ALA also partially corrects nerve taurine content (see Ref. 60)). In humans, intravenous ALA can ameliorate major neuropathic symptoms (i.e., pain and paraesthesias) in type 2 DPN subjects (72). The mechanisms whereby oxidative stress could promote nociception in diabetes are unclear but could involve activation of PKC (32, 39), stimulation of Ca\textsuperscript{2+} influx (12), or alterations of nerve, ganglion, or cutaneous blood flow (9, 13, 60). In any event, whatever the precise mechanism(s) involved, the ability of taurine replacement to decrease nerve oxidative stress (43), improve nerve perfusion (49), exert anti-PKC effects (36, 37), and correct DRG neuron Ca\textsuperscript{2+} signaling suggests that taurine’s effects could be mediated at multiple levels.

In conclusion, our findings implicate an important role for taurine depletion in the development of nerve conduction deficits, tactile allodynia, thermal hyperalgesia, and abnormal DRG neuron Ca\textsuperscript{2+} signaling in experimental DPN. These data, together with the ability of taurine to function as a central neuromodulator or inhibitory neurotransmitter (15), its interactions with the spinal endogenous opiate system (33), and its ability to inhibit substance P-induced nociception (55), suggest that perturbations of taurine metabolism may contribute to increased pain perception by both central and peripheral mechanisms (59). Ongoing studies are aimed at identifying the critical loci of taurine depletion and at addressing whether taurine therapy can reverse, rather than prevent, established neurological deficits. In any event, the findings reported herein, together with the identification of taurine depletion in patients with diabetes (19), suggest that taurine replacement may provide a novel mechanistically based approach to the treatment or prevention of DPN.

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