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Comparison of taurine and pantoyltaurine as antioxidants *in vitro* and in the central nervous system of diabetic rats

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ABSTRACT

This study has comparatively evaluated the antiradical and antilipid peroxidizing actions of taurine (TAU) and its *N*-pantoyl analog pantoyltaurine (PTAU) *in vitro*, and has determined the extent to which these findings agree with the *in vivo* ability of these compounds to prevent changes in plasma glucose and in indices of oxidative stress in the plasma, brain and spinal cord induced by the diabetogen streptozotocin (STZ) in Sprague-Dawley rats. Using free radical-generating and oxidizing systems, PTAU was found more effective than TAU in scavenging DPPH, hydroxyl, peroxyl, and superoxide anion radicals and peroxynitrite, and in preventing lipid peroxidation of a brain homogenate by iron (III)-dopamine and the oxidation of dopamine by iron (III). On the other hand, when administered intraperitoneally (*i.p.*) at a 1.2 mM/kg dose, 75 min and 45 min before a single *i.p.*, 60 mg/kg, dose of (STZ), TAU was about equipotent with PTAU in attenuating STZ-induced increases in glucose, malondialdehyde (MDA) and nitric oxide (NO), and the loss of reduced glutathione (GSH) in plasma collected at 24 h post STZ. Moreover, the analysis of concurrently collected brain and spinal cords samples revealed that both TAU and PTAU were able to equally reverse the increases in MDA and NO concentrations and to effectively counteract the decrease in the GSH/GSSG ratio caused by STZ. Likewise, both compounds were very effective in preventing the losses of tissue catalase, glutathione peroxidase and superoxide dismutase activities. A comparison of the results for spinal cord and for brain parts such as the cerebellum, cortex and brain stem suggested the existence of regional differences in antioxidant potency between TAU and PTAU, especially in terms of antioxidant enzymes. In general, differences in antiradical and antioxidant potencies between TAU and PTAU derived from *in vitro* test are not reliable indicators of the antioxidant potencies these compounds may subsequently manifest in a living organism.

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1. Introduction

Taurine (TAU) is a ubiquitous nonprotein amino acid that has been extensively investigated as an antioxidant both *in vitro* and *in vivo*. In spite of lacking a readily oxidizable functional group and having a demonstrable low free radical scavenging action, this 2-aminosulfonate compound has consistently been found to protect cultured cells, organs and mammalian species against the deleterious consequences of oxidative stress fostered by a myriad of chemical agents (Das and Sil, 2012; Sayed et al., 2012), biochemicals (Kalaz et al., 2013), drugs (Das et al., 2012; Hagar et al., 2006; Shao et al., 2012), toxins (Bhavsar et al., 2010; Das et al., 2012; McCarty, 2013; Turkez and Aydin, 2012) and disease states (Ito et al., 2012; Manna et al., 2013; Rikimaru et al., 2012; Raschka and Daniel, 2005). Mechanisms such as elevation of the activities of the antioxidant enzymes superoxide dismutase (Vohra and Hui, 2001), glutathione peroxidase (Anand et al., 2011; Hagar, 2004; Vohra and Hui, 2001), glutathione reductase (Anand et al., 2011) and catalase (Hagar, 2004), preservation of the expression and secretion of extracellular superoxide dismutase (Nonaka et al., 2001), protection of reduced glutathione stores (Oudit et al., 2004) and of the intracellular redox status (Acharya and Lau-Cam, 2013), prevention or reduction of intracellular calcium increase (Chen et al., 2001; Yamauchi-Takihara et al., 1988) and movement (Timbrell et al., 1995), membrane stabilization (Chen et al., 2001; Timbrell et al., 1995), and direct binding to reactive aldehydes (Ogasawara et al., 1993) are some of the explanations that have been put forth to account for the seemingly unexpected beneficial antioxidant actions of this amino acid.

Previous studies in this laboratory have suggested that N-substitution of TAU can impact on the antioxidant properties

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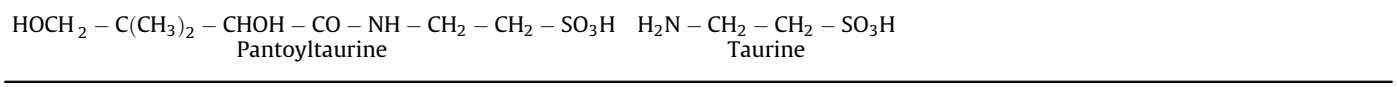
of this compound, with *N*-methylation having a marked negative effect (Clark and Lau-Cam, 2006; Pokhrel and Lau-Cam, 2000a,b), and *N*-carbamoyl substitution providing an enhancing effect (Clark and Lau-Cam, 2008; Pokhrel and Lau-Cam, 2000a,b). To further establish the significance of N-substitution on the antioxidant properties of TAU, the present study was undertaken to specifically compare TAU with PTAU, to determine the impact of introducing the *N*-2,4-dihydroxy-3,3-dimethylbutyryl (pantoyl) group into the TAU molecule on the ability of TAU to scavenge various types of free radicals *in vitro* and to protect the brain and spinal cord against lipid peroxidation (LPO) and changes in enzymatic and nonenzymatic antioxidant defenses brought about by the diabetogen streptozotocin in the rat. To our knowledge this subject has not been previously investigated.



2. Materials and methods

1.1. In vitro test systems

Free radical scavenging activity was evaluated using (a) the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) test as described by Cotelle et al. (1996) and which measures the decrease in absorbance at 517 nm due to radical depletion, (b) 2,2′-azobis-2-methyl-propanimidamide (AAPH) as a source of peroxyl radicals to induce the LPO of a suspension of lecithin liposomes to MDA using the experimental conditions of Nakamura et al. (1993); (c) the deoxyribose assay method of Gutteridge (1984) in which a ferric chloride-H_2O_2 system generates hydroxyl radical for the degradation of deoxyribose to MDA; (d) an aerobic mixture of NADH and phenazine methosulfate to generate the superoxide anion radical for the reduction of nitro blue tetrazolium as reported by Ewing and Janero (1995); (e) the spectrophotometric method of Biasetti and Dawson (2002) to follow the iron-induced oxidation of dopamine (DA) to dopachrome; (f) the spectrophotometric method of Biasetti and Dawson (2002) to follow the rate of oxidation of DA by peroxynitrite; and (g) a LPO test relying on a DA-iron system to cause the degradation of a brain homogenate to MDA as reported by Dawson et al. (1998). All the tests were performed in triplicate using a concentration of test compound of 20 mM in the final volume of reaction mixture. For comparative purposes, *N*-acetylcysteine (NAC), an established antioxidant, was tested alongside TAU and PTAU.

1.2. Animals

Male Sprague-Dawley rats, 225–250 g, 7–8 weeks old, were obtained from a commercial source (Taconic Farms Inc., Germantown, NY), housed in a temperature- (23 ± 1°C) and humidity-controlled room on a 12 h light-12 h dark cycle, and used after a 5 day acclimation period during which they had free access to a commercial rodent diet (LabDiet® 5001, PMI Nutrition International, Brentwood, MO) and filtered tap water.

1.3. Treatment solutions and treatments

The solutions of streptozotocin (STZ), the diabetogen, and of TAU and PTAU, the test compounds, were prepared in 10 mM citrate buffer pH 4.5. STZ was administered as a single, 60 mg/kg, intraperitoneal (*i.p.*) dose. The test compounds were administered by the same route in two equal divided doses at 75 min and 45 min before STZ. TAU was evaluated at a dose of 1.2, 2.4 and 3.6 mM/kg, and PTAU at a dose of 2.4 mM/kg. None of the treatment volumes exceeded 2 mL. A preliminary evidence of diabetes was obtained by measuring the blood glucose levels of rats treated only with STZ using a blood glucose meter and test strip system (ACCU-CHEK® Active, Roche Diagnostics, Indianapolis, IN) and a drop of tail vein blood. Only rats exhibiting a blood glucose level in excess of 300 mg/dL were used in the study. Rats receiving neither STZ nor a sulfur-containing compound served as controls. All the experimental groups consisted of 6 rats each.

1.4. Samples

The animals were sacrificed by decapitation at 24 h post-STZ to collect their blood and brains. The blood samples were immediately mixed with EDTA disodium and then centrifuged at $1300 \times g$ for 10 min to separate the plasma fractions. The skulls were cut open with a long Friedman rongeur to expose the brains and spinal cords, which were removed by the freeze clamp technique. The brains were divided into two portions, one was dissected into their cerebellum, cortex, brain stem and spinal cord components, and the other was left intact for total brain assays.

1.5. Assays

The plasma was used to measure glucose by a commercial colorimetric assay kit (Procedure No. 510, Sigma Chemical Co., St. Louis, MO), malondialdehyde (MDA) by the colorimetric method of Buege and Aust (1978) after conjugation with thiobarbituric acid, reduced glutathione (GSH) by the fluorometric method of Akerboom and Sies (1981) after reaction with *ortho*-phthalaldehyde, and nitric oxide (NO) as nitrite using the Griess reagent and the conditions described by Fox et al. (1981). The brain, its parts or the spinal cord were made into suitable extracts for biochemical assays by homogenization with 0.05 M EDTA disodium in 0.01 M phosphate buffered saline, pH 7.4, in a 1:30 (w/v) ratio, using an electric hand-held blender (Tissue-Tearor®, Bio Spec Products, Inc., Bartlesville, OK) followed by centrifugation at $3000 \times g$ and 4 °C for 30 min. The clear supernatants were assayed for their contents of MDA, GSH and NO as described for the plasma, of glutathione disulfide (GSSG) as described for the plasma GSH after removing any preformed GSH with *N*-ethylmaleimide using the conditions of Guntherberg and Rost (1966); and for their activities of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) by the methods of Aebi (1984),Günzler and Flohé (1985) and Misra (1985), respectively.

1.6. Statistical analysis of the data

The experimental results are expressed as the mean ± SEM for $n = 6$. They were statistically analyzed by one-way ANOVA and Tukey's multiple comparisons test. Differences were considered to be significant at $p \leq 0.05$.

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