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Taurine fails to protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced striatal dopamine depletion in mice

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Summary. Taurine, a known antioxidant and neuroprotector has been investigated for its free radical scavenging action in vitro in isolated mitochondria, and tested whether it protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in mice. Taurine (0.1–10 mM) did not affect 1-methyl-4-phenyl pyridinium-induced hydroxyl radical production in isolated mitochondria. Systemic administration of taurine (250 mg/kg, i.p.) caused a small, but significant loss of dopamine levels in the striatum of mice. Taurine failed to reverse MPTP-induced striatal dopamine depletion, but caused significant increase in dopamine turnover in these animals. In the light of the present study it may be suggested that consumption of taurine may neither help in scavenging of neurotoxic hydroxyl radicals in the brain mitochondria, nor would it help in blocking the process of neurodegeneration.

Keywords: Taurine – Striatum – Hydroxyl radical – Parkinson's disease – Mitochondria – MPTP

Introduction

The potent parkinsonian neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) through its oxidized metabolite, 1-methyl-4-phenylpyridinium (MPP^+) causes lesion specifically to the dopaminergic system of the substantia nigra pars compacta (SNpc) of the brain (Burns et al., 1983). This ability of MPTP and MPP^+ has been exploited to develop animal models for Parkinson's disease (PD). MPTP crosses the blood–brain barrier, gets converted to its neurotoxically active metabolite MPP^+ by monoamine oxidase-B in astrocytes and is selectively taken up into dopaminergic neurons by dopamine (DA) transporters (Javitch et al., 1985; Ransom et al., 1987), where it is sequestered into the mitochondria (Ramsay and Singer, 1986). MPP^+ causes mitochondrial complex I inhibition, leading to an out surge of free radical species as the lethal process in the neuronal death (Tieu et al., 2003).

Taurine, 2-aminoethane sulfonic acid is one of the most abundant amino acids found in the central nervous system (Palkovits et al., 1986). Taurine has been implicated in the normal development of the brain, calcium homeostasis, antioxidant action, and neuromodulation especially at GABAergic and glycinergic neurons (Huxtable, 1989; Saransaari and Oja, 2000; Foos and Wu, 2002; Dominy et al., 2004; Albrecht and Schousboe, 2005).

One of the basic mechanisms of dopaminergic neurotoxicity by MPTP is increased production of hydroxyl radical ($^{\bullet}OH$) in the SNpc and striatal areas of the brain (Thomas et al., 2000). It has also been demonstrated that $^{\bullet}OH$ scavengers provide commendable protection against MPTP or MPP^+ mediated neurotoxicity in mice or rats (Mohanakumar and Muralikrishnan, 2000; Sairam et al., 2003; Thomas and Mohanakumar, 2004), and $^{\bullet}OH$ generators fail to do so, or exacerbate the neurotoxic insults (Knaryan et al., 2005, 2006). Taurine has been widely used to rescue oxidative stress and as a neuroprotective agent in disparate models (Khan et al., 2000; Mankovskaya et al., 2000; Shuaib, 2003; El Idrissi, 2006).

Cerebrospinal fluid (CSF) levels of different amino acids including taurine decreases significantly in PD patients in comparison to age and sex matched controls (Jimenez-Jimenez et al., 1996; Molina et al., 1997). However treatment with L-DOPA restored normal levels of all amino acids except taurine, which maintained a significant decline with respect to the controls (Engelborghs et al., 2003). This result has been corroborated in rat models for the disease where the loss of dopaminergic markers was correlated with a decline in striatal taurine

(Dominy et al., 2004). These reports suggest the possibility that taurine supplementation may protect against MPTP-induced dopaminergic neuronal degeneration mice, and provide foundation for a novel therapeutic strategy for PD. Therefore, we examined in the present study whether taurine could scavenge MPP^+-induced $^{\bullet}OH$ generation in vitro in the mitochondria and attenuate MPTP-induced striatal DA depletion in mice.

Materials and methods

Animals and treatments

Adult male Balb/c mice (22–25 g) were used for generating chemical model of PD in the present study. They were housed under standard conditions of temperature (22 ± 1 °C), humidity (60 ± 5%) and illumination (12 h light/dark cycles). Water and food were made available to them ad libitum. MPTP and taurine were dissolved in normal saline. Control animals received normal saline. Out of the three groups of animals, the first, second and third group received MPTP alone, taurine alone, and MPTP and taurine, respectively. Animals were injected with MPTP (30 mg/kg body weight, i.p.) twice, 16 h apart. Thirty minutes after the second dose of MPTP, taurine (250 mg/kg, i.p.) was injected followed by a second dose 8 h later. Animals received two more doses of taurine on the third day with an eight-hour interval. Another group of animals received four doses of taurine alone (250 mg/kg). The animal ethics committee of Indian Institute of Chemical Biology, strictly following the national CPCSEA guidelines, approved all the animal experiments.

Electrochemical detection of biogenic amines

Animals were sacrificed by cervical dislocation 48 h after the last MPTP or taurine injection. The left and right striata were microdissected and processed for the analysis of biogenic amines employing an HPLC-electrochemical procedure (Mehta et al., 2001). The tissue was sonicated in 10 volume (v/w) ice-cold 0.1 M $HClO_4$ containing 0.01% EDTA. Ten µl of the supernatant collected after centrifugation at 10,000 × *g* for 5 min was resolved in an HPLC system using a mobile phase (8.65 mM heptane sulfonic acid, 0.27 mM EDTA, 13% acetonitrile, 0.43% triethylamine and 0.32% *O*-phosphoric acid). The flow rate was 0.7 ml/min and the electrochemical detection was performed at +0.74 V. The HPLC system consisted of an isocratic pump (Bioanalytical Systems, West Lafayette, USA), an amperometric detector (Epsilon, Bioanalytical Systems) and C18, ion pair, analytical column (4.6 × 250 mm; Ultrasphere IP; Beckman, USA), with a particle size of 5 µ and pore size of 80A°. The values were calculated against standards containing 4 pmol of norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), DA, 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT).

Measurement of hydroxyl radicals in submitochondrial particles

In vitro $^{\bullet}OH$ generation in P_2 fraction was assayed by trapping them in salicylate and measuring the adduct, 2,3-dihydroxybenzoic acid (2,3-DHBA) (Halliwell et al., 1991). Normal adult male Balb/c mice (22–25 g) were sacrificed to collect cerebral cortices, which were homogenized in ten volumes of ice-cold 10 mM potassium phosphate buffer (pH 7.2) containing 0.32 M sucrose. The homogenate was centrifuged at 1,000 × *g* for 10 min at 4 °C. The supernatant was collected and centrifuged again at 15,000 × *g* for 30 min at 4 °C. The pellet thus obtained was resuspended in ice-cold potassium phosphate buffer (10 mM, pH 7.2) containing 50 mM Tris. The pellet formed when this solution was centrifuged at 15,000 × *g* for 30 min at 4 °C was resuspended in 10 mM potassium phosphate buffer, pH 7.2. Fifty µl of mitochondrial fraction obtained was incubated with or without MPP^+ (100 µM) and/or taurine (100 µM, 1 or 10 mM) at 37 °C for 30 min along with 0.75 mM sodium salicylate. Reaction was terminated by adding equal volume of 0.1 M perchloric acid containing 0.01% EDTA. Ten µl supernatant after centrifuging at 10,000 × *g* for 5 min was injected into the HPLC to detect salicylate adducts 2,3-DHBA using the same mobile phase used for the detection of neurotransmitters. Readings were normalized to total protein content in the P_2 fraction, measured by the method of Lowry et al. (1951).

Statistical analysis

Results are given as mean ± SEM. All data were analyzed for significance using paired two-tailed Student's *t*-test. Value of $p \leq 0.05$ was considered significant.

Results

Taurine fails to attenuate MPTP-induced striatal DA depletion

MPTP or taurine administration in mice respectively caused 55 and 8.5% reduction of striatal DA content as compared to the control. Taurine treatment failed to alter MPTP-induced striatal DA loss (Fig. 1A). MPTP administration resulted in highly significant increase in the biogenic amine turnover, which was potentiated in animals treated with taurine (Fig. 1B). Two doses of MPTP in mice resulted in significant decrease in the levels of NE, 5-HT and 5-HIAA in the striatum (Fig. 2). Taurine caused a significant decrease in the level of striatal 5-HIAA (Fig. 2). Repeated taurine administration in MPTP treated animals significantly attenuated the neurotoxin-induced decline in the levels of NE and 5-HIAA, but not in the level of 5-HT (Fig. 2).

Effect of taurine on $^{\bullet}OH$ generation in submitochondrial particles

There are previous reports of spontaneous production of $^{\bullet}OH$ and reactive oxygen species by isolated mitochondrial preparation (Dykens, 1994; Knaryan et al., 2006). MPP^+ (100 µM), the metabolite of MPTP, causes an increase in $^{\bullet}OH$ production in sub-mitochondrial particles prepared from normal mouse brain cortex as evidenced by a significant increase in 2,3-DHBA level (about 3-fold; Fig. 3). Addition of taurine (100 µM–10 mM) to the normal mitochondria or that has been incubated with MPP^+ (100 µM), caused no change in the production of 2,3-DHBA level in the mitochondria (Fig. 3).

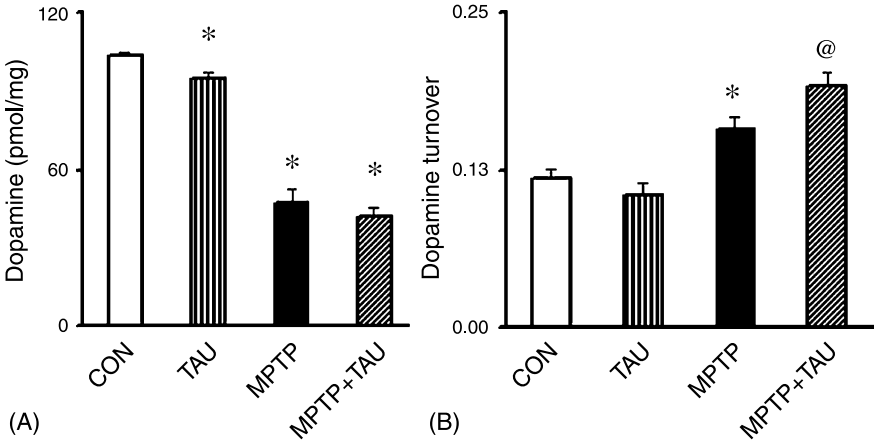

Fig. 1. Taurine treatment reduces striatal dopamine levels. Mice were treated with MPTP (30 mg/kg, i.p.) and/or 0.9% saline twice, 16 h apart followed by 4 doses of either taurine (*TAU*; 250 mg/kg, i.p.) or saline in a span of two days. Animals were sacrificed on the fourth day and dopamine (*DA*), and its metabolites homovanillic acid (*HVA*) and 3,4-dihydroxyphenylacetic acid (*DOPAC*) were estimated in the microdissected striata by a sensitive HPLC-electrochemical procedure. **A** Levels of DA in the striatum of mice are given as pmol/mg tissue. **B** DA turnover, obtained as the ratio of the metabolites to the neurotransmitter [(HVA + DOPAC):DA]. The data are represented as mean ± SEM. $^{*}p \leq 0.001$ as compared to control, @ $p \leq 0.05$ as compared to MPTP group. $n = 6$

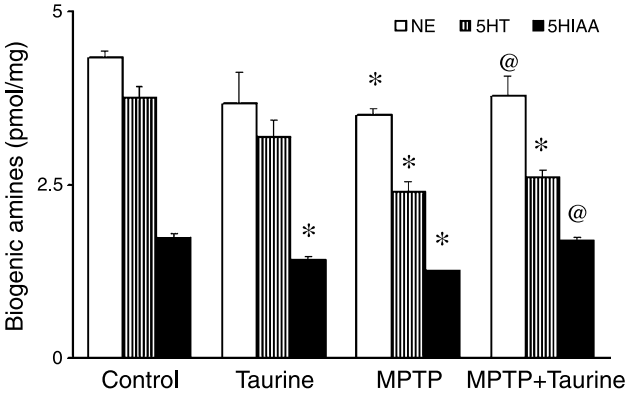

Fig. 2. Taurine affects striatal concentrations of 5-HIAA, but not serotonin and norepinephrine. Mice were treated with MPTP and taurine as described in Fig. 1. Animals were sacrificed on the fourth day and striatal levels of serotonin (*5-HT*), norepinephrine (*NE*) and 5-hydroxyindole acetic acid (*5-HIAA*) were estimated employing a sensitive HPLC-electrochemical procedure. The values are given in pmol/mg tissue and are represented as mean ± SEM. $^{*}p \leq 0.001$ as compared to control, @ $p \leq 0.05$ as compared to MPTP group. $n = 6$

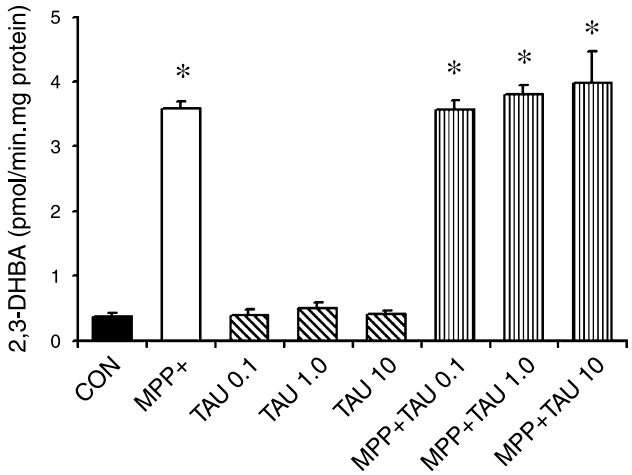

Fig. 3. MPP^{+} but not taurine causes increase in hydroxyl radical formation in sub-mitochondrial particles. Mitochondria prepared from normal rat brain cortices were incubated with 100 μM MPP^{+} or 0.1–10 mM taurine along with 100 μM MPP^{+} at 37 °C in microcentrifuge tubes for 30 min with sodium salicylate (0.75 mM). Levels of 2,3-dihydroxybenzoic acid (2,3-DHBA) formed were measured employing an HPLC coupled with electrochemical detector. Results given are mean ± SEM of the salicylate adducts formed (in pmol) per min per mg protein. ($^{*}p \leq 0.05$, compared to control data, $n = 6$)

Discussion

Taurine, although reported to be a potent antioxidant and neuroprotector, has failed to protect against MPTP-induced striatal DA loss in mice. The present result that taurine did not influence the level of $^{\bullet}OH$ production caused by MPP^{+} in mitochondria explain such a finding in mice. Yet another feature of the present study is the revelation of taurine's potential to decrease striatal DA concentrations following its repeated administration in mice. This is an observation consistent to earlier reports that intranigral infusion of taurine caused decline in striatal DA levels in cats (Leviel et al., 1979) and rats (Ruotsalainen et al., 1996).

Interestingly, taurine has been considered a potent free radical scavenger and antioxidant. Previously, it has been reported that three intraperitoneal injections of 200 mg/kg taurine are sufficient to prevent hypoxia-induced lactate accumulation and lipid peroxidation in rat brain (Mankovskaya et al., 2000). There are other reports

of successful protection of neurodegeneration in animal models of Alzheimer's and Huntington's diseases by this amino acid (Sola et al., 2003; Louzada et al., 2004; Tadros et al., 2005) and in rat coronal slides incubated with MPP^+ (O'Byrne and Tipton, 2000). However, in our experiments, when animals were treated with taurine at comparable doses that has been used in earlier studies that showed neuroprotection (Mankovskaya et al., 2000), the animals suffered about 18% reduction in striatal DA, which has been found to be consistently significant. Not only taurine alone caused decrease in striatal DA levels, but it also caused augmentation of the effects of MPTP on striatal DA turnover in mice. However, other biogenic amines such as 5-HT or NE from this region of the brain showed no significant variation. This implied that the effect of taurine is by and large limited to dopaminergic system in the brain. An earlier study has indicated a significant correlation between striatal DA and taurine level (Dawson et al., 1999), which corroborates well with the present findings. The increase in DOPAC and HVA in taurine treated animals is an indicator of higher catabolism of DA in these animals, and hence the increase in DA turnover.

Taurine has long been speculated to have neurological functions. It has been found that taurine levels plunge in the brain or plasma as ageing take place (Strolin Benedetti et al., 1991; Dawson et al., 1999). The CSF samples of PD patients contained low levels of this amino acid (Jimenez-Jimenez et al., 1996; Molina et al., 1997), and is not corrected by L-DOPA therapy (Engelborghs et al., 2003). These reports taken together with the present results that systemic administration of taurine failed to protect against striatal DA loss in MPTP-induced parkinsonism indicates that taurine may not be of any beneficial use in patients with PD. Furthermore, the present finding that systemic administration of taurine causes decrease in striatal DA further suggests that taurine should be avoided in the treatment of PD. At a time when taurine is being widely prescribed and consumed as an agent for general well being of the body (Della Corte et al., 2002; Bouckenooghe et al., 2006), the present findings have considerable relevance to its general use as a prophylactic agent. Apparently there is a need for a serious review on prescription of taurine especially in persons predisposed to develop PD.

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