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Neuroprotective effect of taurine in 3-nitropropionic acid-induced experimental animal model of Huntington's disease phenotype

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Abstract

An experimental animal model of Huntington's disease (HD) phenotype was induced using the mycotoxin 3-nitopropionic acid (3-NP) and was well characterized behaviorally, neurochemically, morphometrically and histologically. Administration of 3-NP caused a reduction in prepulse inhibition (PPI) of acoustic startle response, locomotor hyper- and/or hypoactivity, bilateral striatal lesions, brain oxidative stress, and decreased striatal γ-aminobutyric acid (GABA) levels. Taurine is a semi-essential β-amino acid that was demonstrated to have both antioxidant and GABA-A agonistic activity. In this study, treatment with taurine (200 mg/kg daily for 3 days) prior to 3-NP administration reversed both reduced PPI response and locomotor hypoactivity caused by 3-NP injection. Taurine pretreatment also caused about 2-fold increase in GABA concentration compared to 3-NP-treated animals. In addition, taurine demonstrated antioxidant activity against oxidative stress induced by 3-NP administration as evidenced by the reduced striatal malondialdehyde (MDA) and elevated striatal glutathione (GSH) levels. Histochemical examination of striatal tissue showed that prior administration of taurine ahead of 3-NP challenge significantly increased succinate dehydrogenase (SDH) activity compared to 3-NP-treated animals. Histopathological examination further affirmed the neuroprotective effect of taurine in 3-NP-induced HD in rats. Taken together, one may conclude that taurine has neuroprotective role in the current HD paradigm due, at least partly, to its indirect antioxidant effect and GABA agonistic action.

Keywords: Taurine; 3-NP; Histochemistry; GABA; Histopathology

1. Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder, in which progressive widespread neuropathological deficits result in behavioral, histological and neurochemical abnormalities (Ryu et al., 2004). 3-Nitropropionic acid (3-NP) is a mitochondrial toxin that has been found to effectively induce specific behavioral changes and selective striatal lesions in rats and non-human primates mimicking those in HD (Lee and Chang, 2004). The animal model of 3-NP-induced HD in rats was documented to manifest disruption of prepulse inhibition (PPI) of acoustic startle response, locomotor hyper- and/or hypoactivity, bilateral striatal lesions, elevated brain oxidative status, and decreased striatal γ-aminobutyric acid (GABA) levels (Kodsi and Swerdlow, 1997; Schulz et al., 1996; Beal et al., 1993).

The startle reflex is a contraction of the facial and skeletal muscles to sudden, relatively intense stimuli that is usually classified as a defensive response (Swerdlow et al., 1995). PPI is a very robust experimental phenomenon in which there is a normal suppression of the amplitude of startle reflex, when an intense startling stimulus is preceded 30–500 ms by a weak stimulus (Davis et al., 1982). The preceding stimulus "sets up an inhibitory network", which dampens the response to the second stimulus. This sensory gating mechanism is suggested to protect the brain from stimulus inundation, which could otherwise lead to cognitive fragmentation and disturbed thought (Braff and Geyer, 1990). The PPI paradigm thus offers an opportunity to evaluate the effects of 3-NP on a simple quantifiable reflex measure that is known to be abnormal in HD patient.

Many pharmacological agents have been attempted to protect against HD in experimental animals. Though, very

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few pharmacotherapies have been clinically used for the management of the disease. The most promising therapeutic agent in this setting is minocycline; an antibiotic with anti-inflammatory and antiapoptotic properties. In a 2-year study, minocycline was administered to 14 patients with genetically confirmed HD (Bonelli et al., 2004a). In this study, patients exhibited stabilization in general motor and neuropsychological function at endpoint assessed by the Mini-Mental State Examination, the Total Motor Score, the Total Functional Capacity Scale and the Independence Scale. In another double-blind, randomized, placebo-controlled study in 60 HD patients, tolerability and adverse event frequency were similar between minocycline-treated and placebo groups (Huntington Study Group, 2004). Other clinical data indicate that minocycline was well tolerated in 30 HD patients during 6-month period with no serious adverse events (Thomas et al., 2004). Therefore, long-term, double-blind, placebo-controlled large trials could further help in establishing the value of minocycline in management of HD.

Taurine is a semi-essential β-amino acid that is most abundant in brain, heart, retina, skeletal muscle and leukocytes of mammalian species (McCool and Botting, 2000). In a hypoxic rat model, taurine prevented hypoxia-induced lactate accumulation and lipid peroxidation in brain, liver, and heart tissues (Mankovskaya et al., 2000). Beside its reported antioxidant effect, taurine has a GABA-A agonistic activity (El Idrissi et al., 2003). Such pharmacological activities of taurine may suggest a potential therapeutic value in management of HD though yet to be investigated. Therefore, this study aimed at testing the possible protective effect of taurine against 3-NP-induced neurotoxicity in rats. The effect of both taurine and 3-NP alone and in combination was tested on PPI response and locomotor activity of rats. Experiments were also performed to investigate the effect of these treatments on striatal GABA, malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSHPx) and lactate dehydrogenase (LDH) levels/activities. Histochemical determination of striatal succinate dehydrogenase (SDH) activity along with histopathological evaluations was also conducted in this study.

2. Materials and methods

2.1. Animals

Male albino rats of Wistar strain weighing 250–300 g were used. They were housed in plastic cages in a room maintained at constant temperature (21 ± 2 °C) with alternating 12 h light/dark cycle where animal chow and water were provided ad libitum. On the day of the experiment, animals were brought to the experimental room and allowed to habituate to the environmental conditions for a period of approximately 60 min before the beginning of the experiment. All animal treatments adhered strictly to institutional and international ethical guidelines concerning the care and use of laboratory animals and the experimental protocol was approved by Ain Shams University Faculty of Pharmacy Review Committee for the use of animal subjects.

2.2. Drugs and chemicals

Taurine, 3-NP, sodium succinate dibasic hexahydrate, *o*-phosphoric acid, L-glutamic acid, ninhydrin solution, Ellman's reagent [5,5′-dithio-bis-2-nitrobenzoic acid; DTNB], ethylene diaminetetracetic acid disodium (Na_2EDTA), GABA, GSHPx, GSH, thiobarbituric acid (TBA) and 1,1′,3,3′-tetramethoxypropane were all purchased from Sigma-Aldrich, Chemie Gmbh, Germany. NitroBlue Tetrazolium (NBT) was obtained from Fluka AG, Buschs SG, Switzerland. Trifluoroacetic acid (TFA) was purchased from Reidel-de Haën (Germany). Sodium azide (NaN_3) was purchased from Merck-Darmstadt, Germany. Methanol (HPLC grade) was obtained as from Honil Limited, London, UK. Lactate dehydrogenase (LDH) kit was purchased from SGM, Rome, Italy. The rest of the chemicals used in this experimental work were of the highest commercial grade.

Taurine was dissolved in saline and was administered intraperitoneally in a dose of 200 mg/kg daily for three consecutive days (Mankovskaya et al., 2000). 3-NP was dissolved in saline and was administered subcutaneously in a dose of 20 mg/kg daily for five consecutive days (Beal et al., 1993). Drugs or vehicle were administered in a volume of 1 ml/kg body weight. Control animals received respective solvent injections, and they were run concurrently with drug-treated groups.

2.3. Experimental groups

Forty-eight animals were divided into four groups ($n = 12$). The first group received intraperitoneal saline injections once daily for three consecutive days followed at day 4 to 8 by daily injections of 3-NP (20 mg/kg, s.c.). The second group received taurine injections (200 mg/kg, i.p.) once daily for three consecutive days followed at day 4 to 8 by daily injections of 3-NP (20 mg/kg, s.c.). The third group received taurine injections (200 mg/kg, i.p.) once daily for three consecutive days followed at day 4 to 8 by daily subcutaneous injections of saline. The fourth group served as control animals receiving intraperitoneal saline injections once daily for three consecutive days followed at day 4 to 8 by daily subcutaneous saline injections. Locomotor activity was determined for all groups at 3.5 h after drug treatments at days 1, 4, 5, 6, 7 and 8 of the experiment. %PPI of acoustic startle response and startle amplitude were both assessed 215 min after drug treatments at day 5 of the experiment. At day 8 of the experiment, animals were decapitated and skulls were split on ice and salt mixture. Striata were dissected out, homogenized and allocated to two groups for estimation of GABA and oxidative stress indices' levels/activities. Another four groups of rats ($n = 12$) received the same treatment conditions as previously described. On day 8, animals were sacrificed and brains were divided into two groups for conduction of both histochemical and histopathological examinations.

2.4. PPI response measurement

Startle responses were measured using Responder X apparatus (Columbus, Ohio, USA) which consists of Plexiglas

cages and force platforms that are equipped with precise load cells to be used as sensors. Animal movement on the platform develops a transient force, which can be transduced by an accelerometer into a voltage that is proportional to the displacement velocity which is measured at its peak (negative or positive). These signals were amplified, digested, and fed into a data-acquisition board in a computer for further analysis. For acoustic startle measurements, animal cages were housed in a sound-attenuating chamber with a high-frequency speaker located on the side of each cage. The high-frequency speaker delivered the acoustic stimulus in a background noise level of 70 dB. After a 5 min acclimatization period, during which time there was no stimulus, each rat received 36 sessions of either pulse alone or prepulse/pulse sessions presented in a random order with an inter-stimulus interval of 29 s. In the pulse alone sessions, animals received 120 dB stimuli for 40 ms. In the prepulse/pulse sessions, animals received 76 dB prepulse for 20 ms followed 100 ms later by the stimulus (Seaman, 2000). Assessment of PPI response was performed after the second 3-NP injection since subacute injection of 20 mg/kg 3-NP was reported to cause motor slowing and dystonic posturing in rats by the third to fifth day after 3-NP injection (Schulz et al., 1996). PPI response was calculated according to Buuse and Eikelis (2001) where %PPI=[Average startle amplitude on pulse alone sessions − average startle amplitude on prepulse/pulse sessions]/Average startle amplitude on pulse alone sessions × 100. Startle amplitude was measured as the mean maximum value of pulse-alone trials measured in grams (Yamada et al., 2000).

2.5. Locomotor activity detection

Activity monitor (Opto-Varimex-Mini Model B, Columbus Instruments, Ohio, USA) was used to measure the locomotor activity of animals. Such activity was measured based on the traditional infrared photocell principle where interruption of 15 infrared beams (wavelength=875 nm, scan rate=160 Hertz, diameter=0.32 cm, spacing=2.65 cm) reflected total activity of the animal. The total locomotor activity of animals was expressed as counts/5 min. Assessments were carried out 3.5 h after drug treatments at days 1, 4, 5, 6, 7 and 8.

2.6. Estimation of striatal GABA and oxidative stress indices' levels/activities

After decapitation, striata were rapidly dissected out, weighed and homogenized for 1 min in 4 ml of 0.01 N HCl using ice-cold Teflon homogenizer (Glas-Col® Terre Haute, USA). Determination of striatal GABA level was carried out according to Sutton and Simmonds (1974). For determination of oxidative stress indices' levels/activities, 20% homogenate of the striata in ice-cold saline was prepared. Determination of MDA level was carried out according to Uchiyama and Mihara (1978). Estimation of both GSH and LDH level/activity were performed as described by Ellman (1959) and Kreutzer and Fennis (1964) respectively. For assessment of GSHPx activity, an HPLC method was carried out according to Xia et al. (1987).

2.7. Histochemical examination

An image analysis system was used which consisted of a colored video camera, monitor, hard disc of IBM personal computer connected to a microscope, and controlled by Leica Qwin 500 software, England. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Immediately after sacrificing the animals, brains were quickly dissected out, frozen, and stored at −80 °C until sectioning. Frozen sections were cut sagitally at 5 μm with a cryostat. Visualization of SDH location was performed according to Lillie and Fullmer (1976). Briefly, tissue sections were incubated in a 37 °C staining solution containing 0.81 g succinate disodium salt added as the enzyme substrate and 10 mg of NitroBlue Tetrazolium (NBT) in 60 ml of 0.05 M potassium phosphate buffer. Enzyme activity was visualized by the production of an insoluble blue product (a formazan) formed by reduction of NBT at sites of enzyme activity. The incubation period lasted for 45 min. Thereafter, the slides were

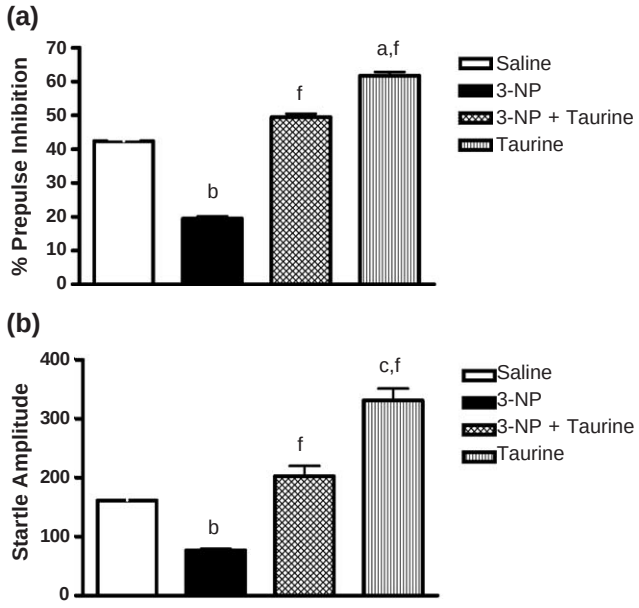

Fig. 1. (a) Effect of taurine on PPI of acoustic startle response in 3-NP treated rats. 3-NP was injected s.c. in a dose of 20 mg/kg for two consecutive days and PPI was assessed 215 min after the second injection. Taurine was given intraperitoneally in a dose of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means±S.E.M. ($n=12$). $^{a}P<0.05$, $^{b}P<0.01$ compared to control group; $^{f}P<0.001$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test). (b) Effect of taurine on startle amplitude in 3-NP treated rats. 3-NP was injected s.c. in a dose of 20 mg/kg for two consecutive days and startle amplitude was assessed 215 min after the second injection. Taurine was given intraperitoneally in a dose of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means±S.E.M. ($n=12$). $^{b}P<0.01$, $^{c}P<0.001$ compared to control group; $^{f}P<0.001$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test).

removed from the solution, air dried, and coverslipped. The image analysis system was used to measure the optical density of SDH reaction, using the grey measure menu in 10 measuring frames in each specimen with objective lens of magnification 40 (at a total magnification of 400 after grey calibration). The image was transformed into a grey image and then delineated to choose the areas exhibiting positive reactivity with accumulation of all grades of reactivity (minimum, maximum and median). The parameters chosen were the number of pixels, sum of grey and mean grey. The optical density was expressed in the form of mean grey parameter where mean grey = sum of grey / number of pixels measured.

2.8. Histopathological examination

Brains were kept in 10% formalin solution for 24 h using Hartz technique (1974). The fixed tissues were washed in tap water, dehydrated in a series of alcohol, cleared in xylene then embedded in paraffin blocks. 5-μm-thick sections were obtained from the blocks and stained by hematoxylin and eosin (Carelton et al., 1967). The tissue sections were then examined by light microscope. Histopathological examination of striatal sections was done blind to the group identity of the animal.

2.9. Statistical analysis

Statistical analysis was achieved using a software program (GraphPad Instat, version 2.0, Philadelphia, 1993). Comparisons between means of data were analyzed by one-way analysis of variance (ANOVA). If the overall F-value was found statistically significant, comparisons among groups were made according to Bonferroni test for selected pairs.

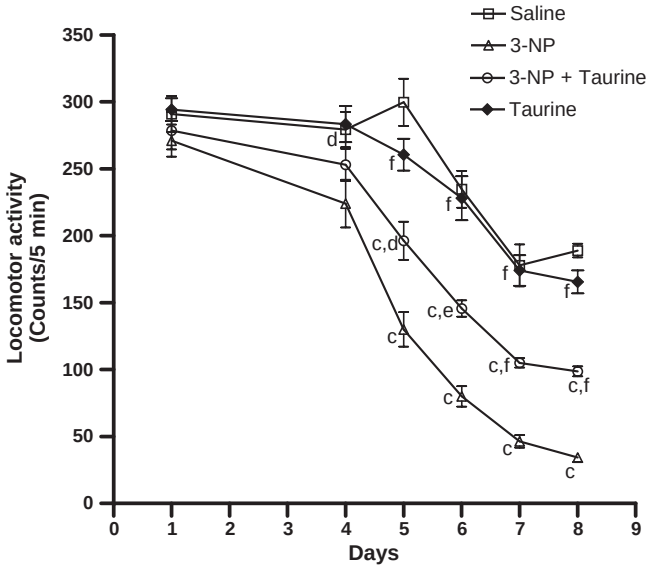

Fig. 2. Effects of 3-NP on the locomotor activity in 3-NP-treated rats. 3-NP was injected s.c. in a dose of 20 mg/kg for five consecutive days. Taurine was given by i.p. injection of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means ± S.E.M. (n = 12). $^{c}P < 0.001$ compared to control group; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test).

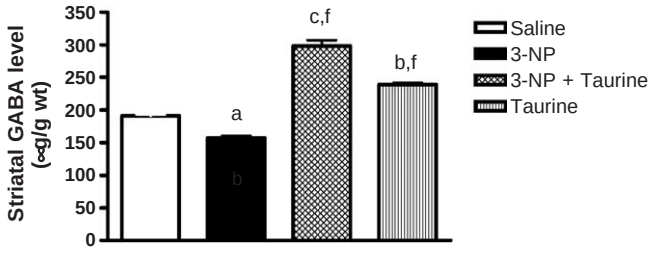

Fig. 3. Effect of taurine on striatal GABA level in 3-NP-treated rats. 3-NP was injected s.c. in a dose of 20 mg/kg for five consecutive days. Taurine was given by i.p. injection of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means ± S.E.M. (n = 6). $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ compared to control group; $^{f}P < 0.001$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test).

3. Results

3.1. Effect of taurine on both PPI of acoustic startle response and startle amplitude in 3-NP-treated rats

One-way ANOVA showed a significant effect of treatments on both PPI of acoustic startle response and startle amplitude [$F(3,188) = 19.42$; $P < 0.001$), $F(3,188) = 52.52$; $P < 0.001$) respectively] (Fig. 1). Comparisons among groups using Bonferroni test revealed that 3-NP administration produced 54% reduction in PPI response compared to control animals. Prior treatment of animals with taurine ahead of 3-NP challenge caused about 2.5-fold increase in PPI response compared to 3-NP-treated animals. Taurine by itself was found to increase the PPI response by about 46% compared to control value. Results also showed that subacute administration of 3-NP produced a significant reduction in startle amplitude by 52% compared to control value. Pretreatment of animals with taurine (200 mg/kg, i.p.) for 3 days before 3-NP administration caused about 2.5-fold increase startle amplitude compared to 3-NP-treated animals. Taurine by itself caused about 2-fold increase in startle amplitude compared to control value.

These results demonstrated very similar changes in both startle amplitude and prepulse inhibition after 3-NP treatment. Therefore, a subgroup comparison was performed between the PPI of the lowest startling saline animals and that of the

Table 1
Effects of taurine on striatal MDA, GSH, GSHPx and LDH levels/activities in 3-NP-treated rats

Treatment	MDA (nmol/g)	GSH (μmol/g)	GSHPX (U/ml)	LDH (μmol/min/g)
Saline	37.44 ± 2.30	0.272 ± 0.009	1.01 ± 0.05	29.69 ± 1.02
3-NP	67.34 ± 6.05^{c}	0.127 ± 0.011^{c}	1.06 ± 0.09	27.7 ± 1.58
3-NP + taurine	37.18 ± 3.31^{f}	0.186 ± 0.005^{c,d}	1.13 ± 0.09	28.6 ± 1.06
Taurine	30.75 ± 2.38^{f}	0.266 ± 0.005^{f}	1.17 ± 0.03	29.44 ± 0.61

3-NP was injected s.c. in a dose of 20 mg/kg for five consecutive days. Taurine was given by i.p. injection of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means ± S.E.M. (n = 6). $^{c}P < 0.001$ compared to control group; $^{d}P < 0.05$, $^{f}P < 0.001$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test).

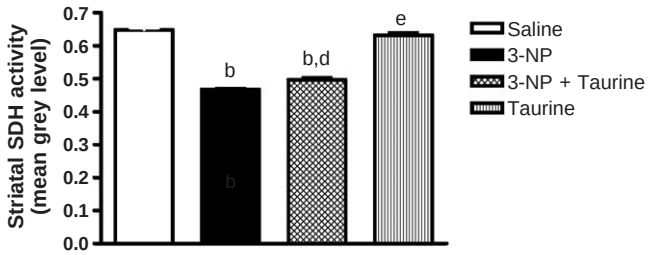

Fig. 4. Effects of taurine on striatal SDH activity in 3-NP-treated rats. 3-NP was injected s.c. in a dose of 20 mg/kg for five consecutive days. Taurine was given by i.p. injection of 200 mg/kg for 3 days alone or before 3-NP administration. Data are presented as means of grey levels ± S.E.M. ($n=6$). $^{b}P<0.01$ compared to control group; $^{d}P<0.05$, $^{e}P<0.01$ compared to 3-NP-treated group (one-way ANOVA followed by Bonferroni test).

highest startling 3-NP animals. It was found that these two groups still exhibited significant differences in both PPI and startle magnitude. Therefore, a comparison between the animals in the lowest quartile of startle amplitude for the saline group versus the highest quartile of startle amplitude for the 3-NP group using Cohen's *d* test was conducted. Such comparison aimed to detect the magnitude of treatment effect on both PPI and startle magnitude. The quartile comparisons were also conducted between 3-NP+taurine versus Taurine alone groups, where there are significant differences in startle amplitude. The value of effect size for the PPI response in saline versus 3-NP groups was 0.941. On the other hand, the value of effect size for startle amplitude in these two groups was 0.616. When Cohen's *d* test was conducted as a quartile PPI comparison between the animals in the lowest quartile of startle amplitude for taurine alone group versus the highest quartile of startle amplitude for the 3-NP+taurine group, the value of effect size for such comparison was 0.810 while that for startle amplitude was 0.521. In short, such statistical analyses demonstrated that the effect size of treatment on PPI is of large magnitude compared to a medium effect size on startle amplitude.

3.2. Effect of taurine on locomotor activity in 3-NP-treated rats

One-way ANOVA showed a significant effect of treatments on locomotor activity at days 4, 5, 6, 7 and 8 [$F(3,188)=3.67$; $P<0.05$, $F(3,188)=27.26$; $P<0.001$, $F(3,188)=38.88$; $P<0.001$, $F(3,188)=38.78$; $P<0.001$, $F(3,188)=50.31$; $P<0.001$ respectively] (Fig. 2). Bonferroni test revealed that treatment of animals with 3-NP caused a significant decline in locomotor activity by 56%, 66%, 74% and 82% at days 5, 6, 7 and 8 respectively, as compared to saline-treated animals. Pretreatment of animals with taurine before 3-NP administration caused a significant increase in locomotor activity by 51%, 82%, 126% and 188% at days 5, 6, 7 and 8 respectively, as compared to 3-NP-treated animals. Taurine alone did not change locomotor activity of animals beyond control values.

3.3. Effect of taurine on striatal GABA and oxidative stress indices' levels/activities in 3-NP-treated rats

One-way ANOVA showed a significant effect of treatments on striatal GABA, MDA and GSH levels [$F(3,92)=55.54$;

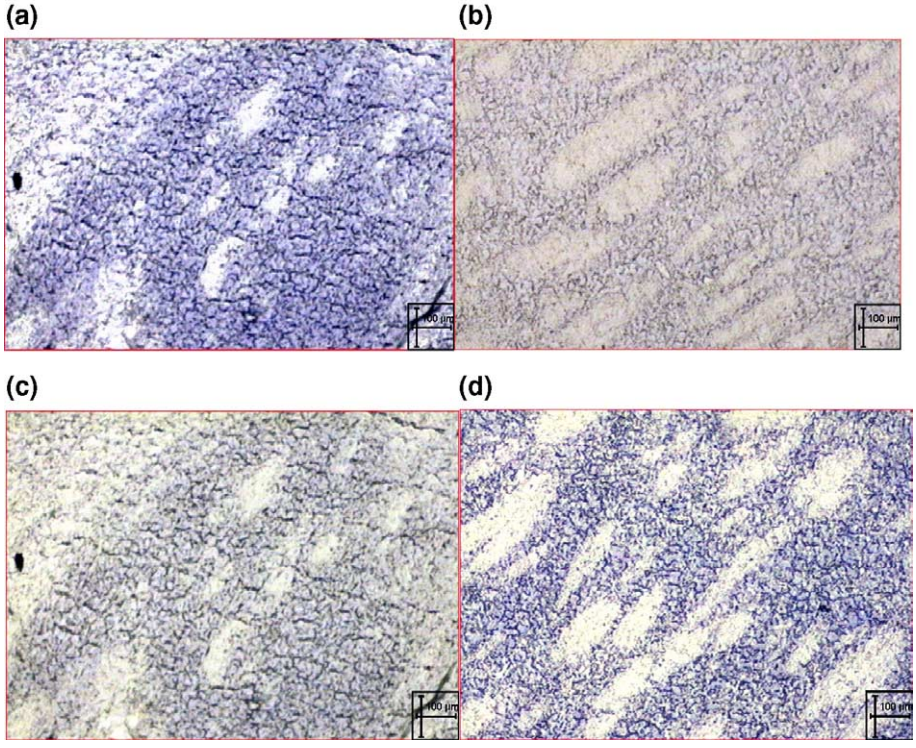

Fig. 5. (a, b, c, d) Photomicrographs of histochemical findings (×400) showing NBT stain intensity in striatum of (a) saline-treated animals, (b) 3-NP-treated animals, (c) 3-NP+taurine-treated animals. (d) Taurine alone-treated animals.

$P<0.001$, $F(3,92)=18.39$; $P<0.001$, $F(3,92)=27.47$; $P<0.001$ respectively]. Comparisons among groups using Bonferroni test revealed that administration of 3-NP significantly reduced striatal GABA concentration by 18% compared to saline-treated animals. Prior treatment with taurine ahead of 3-NP challenge significantly increased GABA concentration by almost 90% compared to 3-NP-treated animals. In animals treated with taurine alone there was an increase in striatal GABA level by 25% compared to their respective controls (Fig. 3). Results also showed that 3-NP administration for five consecutive days significantly increased striatal MDA level by 80% compared to saline-treated animals. Pretreatment with taurine before 3-NP administration restored striatal MDA level to normal value. In the taurine alone condition, striatal MDA level was not altered compared to control value. In addition, a significant decline of striatal GSH concentration resulted in 3-NP treated animals compared to the control group ($P<0.001$). Pretreatment with taurine significantly elevated GSH level by 46% compared to 3-NP-treated group. Taurine alone treatment did not affect striatal GSH content compared to saline-treated animals. From another perspective, none of the treatment modalities affected striatal GSHPx or LDH activity [$F(3,92)=0.95$; $P=0.43$, $F(3,92)=0.64$; $P=0.61$ respectively] (Table 1).

3.4. Histochemical examination

Histochemical findings of striatal SDH activity in brain sections of rats are shown in Fig. 4. Photomicrographs (a, b, c, d) in Fig. 5 illustrate the different optical densities in the striatum following staining with NBT. Sections from saline-treated animals showed normal optical density. 3-NP administration (20 mg/kg, s.c.) for five consecutive days caused a significant decrease in optical density by 28% compared to control value. Pretreatment with taurine (200 mg/kg, i.p.) for 3 days before 3-NP administration increased optical density by 6% compared to 3-NP-treated animals. Taurine alone group, however, was not significantly different from saline-treated group.

3.5. Histopathological examination

The histopathological findings in the striata of rats are illustrated in photomicrographs (a, b, c, d) of Fig. 6. Tissue sections from animals treated with saline showed normal histological structure. Treatment of rats with 20 mg/kg 3-NP for 5 days produced apparent striatal focal degeneration and necrosis with complete loss of the cell detail and architecture and appearance of basophilic nuclear remnants in the central

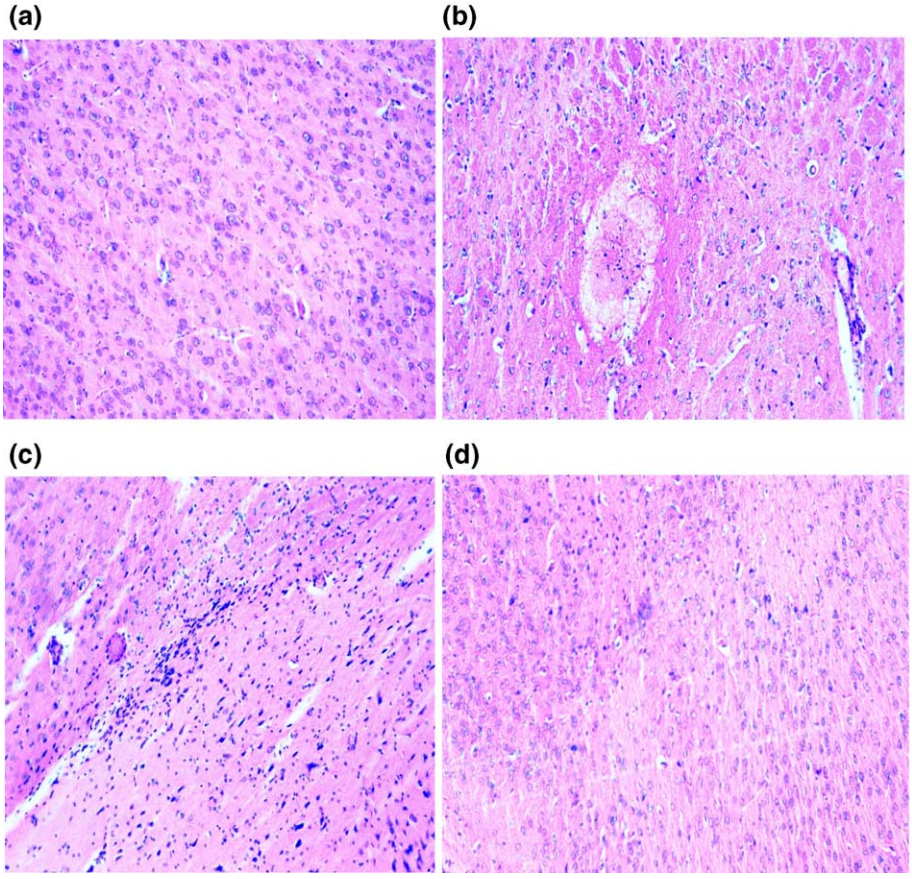

Fig. 6. Photomicrographs (a, b, c, d): Histopathological findings (×40) demonstrating striatal section of (a) saline-treated rats showing the normal histological structure (b) 3-NP-treated rats showing apparent focal degeneration and necrosis with complete loss of cell details and architecture, with the appearance of basophilic remnants of the nuclei in the central zone. (c) 3-NP+taurine-treated rats showing mild focal gliosis with swelling in the endothelial lining of the blood capillaries. (d) Taurine-treated rats showing normal histological structure with no pathological alterations.

zone. In animals pretreated with 200 mg/kg taurine for 3 days before 3-NP administration there was a mild focal gliosis associated with swelling in the endothelial lining of blood capillaries. Striata of taurine-treated rats showed no histopathological alterations compared to normal animals.

4. Discussion

In this study, administration of 3-NP for 2 days (20 mg/kg, s.c.) caused a significant reduction in PPI response and mean startle amplitude when compared to control value. Cohen's *d* test revealed that the effect size of 3-NP on PPI is of large magnitude compared to a medium effect size on startle amplitude. These results are consistent with previous research findings demonstrating reduced PPI after two daily systemic injections of 3-NP in rats (Kodsi and Swerdlow, 1997). Results also showed a significant decrease in locomotor activity starting from day 5 up to day 8 of the experiment following 3-NP administration. This finding is in accordance with those previously reported by Koutouzis et al. (1994) who demonstrated a gradual decline in locomotor activity in rats challenged with 3-NP (10 mg/kg) for 28 days. Likewise, long-term systemic administration of 3-NP to 6-, 10-, 14- and 28-week-old male Sprague–Dawley rats exhibited significant hypoactivity compared to age-matched animals treated with saline (Borlongan et al., 1995). Such compromise in locomotor behavior was suggested to reflect a combination of lowered energy levels resulting from SDH inhibition by 3-NP and consequent short-term changes in neural processing (Seaman, 2000). Our findings revealed that pretreatment with taurine before 3-NP injection significantly increased the locomotor activity of 3-NP-treated animals. Thus, taurine may correct the motor function deficits provoked by 3-NP. These data are partially in line with previous data reported by Aragon et al. (1992) who demonstrated that taurine-treated mice displayed higher motor activity scores when the drug was given before alcohol. It is to be noted that when the drug was given alone in this study, it did not affect the locomotor activity of animals. This is in harmony with earlier findings, which demonstrated no alterations in motor activity following taurine administration to rats (Sanberg and Fibigier, 1979). This finding was also supported thereafter by Rivas-Aranciba and co-workers (2000).

Neurochemical analysis of striatal GABA levels revealed that 3-NP administration significantly reduced striatal GABA concentration compared to normal values. Beal et al. (1993) reported that 3-NP administration over 1 month produced selective striatal lesions with concomitant loss of GABAergic neurons. In another study, in vitro incubation of rat mesencephalic cells for 24 h with 3-NP produced an apparent damage to GABAergic neurons leading to GABA depletion (Zeevalk et al., 1995). A significant decrease in striatal GABA level was also observed in 4-month-old rats treated with 10 mg/kg/day 3-NP for 7 days (Tsai et al., 1997). Pretreatment with taurine ahead of 3-NP challenge significantly increased the compromised striatal GABA content. This can be attributed to the inhibitory effect of taurine on GABA transaminase, the enzyme which catalyses the degradation of GABA (Sulaiman et al., 2003). Though, this effect of taurine on disturbed neurochemistry caused by 3-NP could alternatively be explained through other mechanisms of action. The drug was reported to have a slight direct effect on GABA receptors, but is most likely to act as an indirect neuromodulator of GABAergic neurotransmission in the brain (Liljequist, 1993). Other researchers also documented taurine as a low affinity agonist for GABA(A) receptors in different brain areas, thus, enhancing the GABAergic transmission (McCool and Botting, 2000).

Oxidative stress is a ubiquitously observed hallmark of neurodegenerative disorders. In this study, administration of 3-NP markedly increased the striatal level of MDA. This finding is in line with a previous study that demonstrated increased lipid peroxidation, secondary to free radical generation, in neuronal tissues following oral administration of 80 mg/kg of 3-NP to rats (Fu et al., 1995). Very recently, a significant rise in MDA levels in the striatum, cortex and cerebellum of rats challenged with 3-NP has been demonstrated (Yang et al., 2005). In another study, administration of 3-NP increased the production of hydroxyl free radicals in rat striatum suggesting that free radicals play a substantial role in the neurotoxicity of 3-NP (Schulz et al., 1996). Such peroxidation processes and the production of free radicals may lead to the consumption of detoxifying endogenous antioxidants such as GSH. In the present work, 3-NP produced a marked decline in striatal GSH content.

The abovementioned oxidative stress consequences may be counteracted by certain enzymatic antioxidants like GSHPx. Nevertheless, compensatory increase in striatal GSHPx activity was not evidenced in the present study. Such outcome was suggested to be dependent on dosimetry of 3-NP applied and the possibility that 3-NP might alter other defense mechanisms, other than GSHPx, such as catalase or superoxide dismutase (Desagher et al., 1996). The inhibition of oxidative phosphorylation as a sequelea to the effect of 3-NP may lead to increased in vitro lactate accumulation in brain tissues (Beal et al., 1993). However, in the present study, 3-NP had no effect on the LDH activity in striatal tissues. Such finding may be ascribed to the implementation of in vivo rather than in vitro experimental procedures in this study.

Taurine administration before 3-NP had an apparent antioxidant effect in the current study. It significantly reduced striatal MDA content, while elevating the level of GSH. Indeed, taurine has been demonstrated in different biological systems to act as a direct antioxidant that scavenges free radicals and as indirect antioxidant that stabilizes biomembranes preventing cell membrane damage (Raschke et al., 1995; Wright et al., 1986). It was reported that taurine played an antioxidant role in neurotoxicity induced by excitotoxins such as glutamate and kainic acid (El Idrissi and Trenkner, 1999). Intraperitoneal injection of taurine prevented hypoxia-induced lactate accumulation and lipid peroxidation in rat brain suggesting a neuroprotective role of the amino acid (Mankovskaya et al., 2000). Mahalakshmi et al. (2003) have also concluded that taurine treatment counteracted the oxidative stress induced by acrylonitrile in rat brain by reducing the levels of peroxides and enhancing the activities of enzymatic

and non-enzymatic antioxidant defense mechanisms. Hilgier et al. (2003) have documented that taurine reduced NMDA-induced accumulation of hydroxyl free radicals in rat striatum. Recently, administration of antioxidant combination containing taurine, vitamin C and vitamin E partially protected from oxidant damage induced by 3-NP in hippocampal tissues (Rodriguez-Martinez et al., 2004). Although the exact mechanism of the antioxidant effects of taurine is not fully elucidated yet, it was suggested to be attributed to its conversion to a more stable and less toxic compound, taurine chloramine, after its reaction with hypochlorous acid produced by the myeloperoxidase enzyme (Schuller-Levis and Park, 2003).

Morphometric analysis of striatal sections for the visual localization of SDH activity revealed that 3-NP produced an obvious decrease in SDH staining intensity reflecting an apparent decrease in the enzymatic activity. It is well established that inhibition of SDH, a mitochondrial complex II enzyme, would result in inhibition of oxidative phosphorylation and impairment of cellular energy metabolism that ultimately leads to neurotoxicity (Scallet et al., 2001). Pretreatment with taurine before 3-NP exhibited significant improvement in histochemical staining of striatal SDH activity compared to 3-NP alone condition. This, however, could have been expected given the extreme potency of the irreversible blockade of SDH by 3-NP for at least a week following challenge with the mycotoxin (Seaman, 2000).

In this study, histological examination of striatal lesions following challenge with 3-NP resulted in pathological lesions characterized by focal degeneration and necrosis with complete loss of cell integrity and architecture. These histologic findings have been well characterized in a number of previous reports (Beal et al., 1993; Brouillet et al., 1993). Animals pretreated with taurine ahead of 3-NP challenge demonstrated milder degenerating changes and swelling of endothelial lining of capillaries compared to those treated with 3-NP alone, further suggesting a protective role of the amino acid in striatal tissue. This finding is in line with the other biochemical, behavioral and histochemical findings in our study that when taken together would support the hypothesis that taurine have a neuroprotective role in 3-NP-induced excitotoxicity in rat brain. Such neuroprotective effect imparted by taurine in the current study could be attributed at least partly to its antioxidant effect, GABAergic stimulant action and its preservation of energy-producing systems.

In conclusion, taurine was demonstrated in this study to mitigate behavioral, neurochemical, histochemical and histologic abnormalities caused by the mitochondrial toxin 3-NP in rats. In humans, despite the increasing knowledge of the pathophysiology of Huntington's disease, there has been no cure available to completely cease or reverse the progressive neurodegeneration and behavioral consequences of the disease (Bonelli et al., 2004b). Symptomatic relief of the motor and depressive symptoms of the disease is the only currently approached interventions. Future research should therefore be directed to investigate whether our findings using an experimental animal model of HD phenotype would have clinical implications in management of Huntington's disease patients.

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