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Thioctic acid does not restore glutathione levels or protect against the potentiation of 6-hydroxydopamine toxicity induced by glutathione depletion in rat brain

T. A. Seaton[1], **P. Jenner**[1], and **C. D. Marsden**[2]

[1] Neurodegenerative Disease Research Centre, Pharmacology Group, Biomedical Sciences Division, King's College, London, and
[2] University Department of Clinical Neurology, Institute of Neurology, National Hospital for Neurology and Neurosurgery, London, United Kingdom

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Summary. Decreased reduced glutathione (GSH) levels are an early marker of nigral cell death in Parkinson's disease. Depletion of rat brain GSH by intracerebroventricular administration of buthionine sulphoximine (BSO) potentiates the toxicity of 6-hydroxydopamine (6-OHDA) to the nigrostriatal pathway. We have investigated whether thioctic acid can replenish brain GSH levels following BSO-induced depletion and/or prevent 6-OHDA induced toxicity.

Administration of BSO (2 × 1.6 mg ICV) to rats depleted striatal GSH levels by upto 75%. BSO treatment potentiated 6-OHDA (75 μg ICV) toxicity as judged by striatal dopamine content and the number of tyrosine hydroxylase immunoreactive cells in substantia nigra. Repeated treatment with thioctic acid (50 or 100 mg/kg i.p.) over 48 h had no effect on the 6-OHDA induced loss of dopamine in striatum or nigral tyrosine hydroxylase positive cells in substantia nigra. Also thioctic acid treatment did not reverse the BSO induced depletion of GSH or prevent the potentiation of 6-OHDA neurotoxicity produced by BSO.

Thioctic acid (50 mg or 100 mg/kg i.p.) alone or in combination with BSO did not alter striatal dopamine levels but increased dopamine turnover. Striatal 5-HT content was not altered by thioctic acid but 5-HIAA levels were increased.

Under conditions of inhibition of GSH synthesis, thioctic acid does not replenish brain GSH levels or protect against 6-OHDA toxicity. At least in this model of Parkinson's disease, thioctic acid does not appear to have a neuroprotective effect.

Keywords: Thioctic acid, 6-OHDA toxicity, buthionine sulphoximine, glutathione depletion, substantia nigra.

Introduction

Parkinson's disease is primarily due to a progressive loss of nigral dopamine containing cells. The cause of nigral cell destruction is unknown but recent postmortem studies suggest that free radical formation and the onset of oxidative stress may contribute to the progression of the disease (Jenner et al., 1992). In addition, these studies show that nigral cell loss is associated with increased iron levels (Dexter et al., 1989) and impaired mitochondrial complex I (Schapira et al., 1990) and α-ketoglutarate dehydrogenase (α-KGDH; Mizuno et al., 1994). There is a decrease in the levels of reduced glutathione (GSH) in substantia nigra in Parkinson's disease which does not occur in other brain areas or in other neurodegenerative diseases and which is not due to drug treatment (Perry et al., 1982; Riederer et al., 1989; Sofic et al., 1992; Sian et al., 1994a). This change may be particularly important because it occurs in the early presymptomatic stage of the disease (incidental Lewy body disease) at a time when other indices of oxidative stress are unaltered (Dexter et al., 1994). The decrease in GSH levels may therefore be closely related to the primary pathological process occurring in Parkinson's disease.

Treatment strategies aimed at raising brain GSH levels may be useful in preventing the onset or slowing the progression of Parkinson's disease. However, it is difficult to manipulate brain GSH levels using either precursors (Weiner et al., 1988; Mesina et al., 1989; Anderson and Meister, 1989) or prodrugs of glutathione (Pileblad and Magnusson, 1992; Aw et al., 1991; Jain et al., 1991). For this reason, attention has turned to the actions of thioctic acid (α-lipoic acid) which in conjunction with its reduced form, dihydrolipoic acid, forms a thiol-disulphide redox couple and possesses actions highly relevant to Parkinson's disease. Thioctic acid is an essential endogenous cofactor for not only α-KGDH but also for pyruvate dehydrogenase and dehydrogenases for branched chain amino acids (Ehrenthal and Preilwitz, 1986). Thioctic acid posseses a wide range of antioxidant activity (Suzuki et al., 1991; Kagen et al., 1992; Scott et al., 1994). Thioctic acid scavenges hydroxyl and peroxyl radicals. In addition, thioctic acid replenishes brain GSH levels induced by irradiation of mice (Busse et al., 1992), by cadmium intoxication in rats (Sumathi et al., 1994) or by buthionine sulphoximine-induced inhibition of GSH synthesis to rat neonates (Maitra et al., 1995).

For these reasons, we have examined the actions of thioctic acid in a model of brain glutathione deficiency relevant to Parkinson's disease. Previous studies, showed that the intracerebroventricular (ICV) administration of BSO, an inhibitor of the rate limiting enzyme for GSH formation, namely γ-glutamylcysteine synthetase (Griffith, 1982), decreased brain GSH levels and potentiated the ability of the neurotoxin 6-hydroxydopamine (6-OHDA) to deplete striatal dopamine content (Pileblad and Magnusson, 1989; Pileblad et al., 1989). These results suggested that under normal conditions, GSH scavenges radicals produced from 6-OHDA and acts to limit its toxicity.

In the present study, we have examined the ability of thioctic acid to prevent the BSO-induced potentiation of 6-OHDA toxicity in rat brain. The results confirm that BSO increases 6-OHDA toxicity, but show no protective

effect of thioctic acid despite its ability to alter monoamine function in the striatum.

Materials and methods

Surgical procedures

Male Wistar rats (Bantin & Kingman, UK) weighing 180–200 g were allowed food and water ad libitum and were housed under a 12 h light/dark cycle at 20 ± 1°C and approximately 50% humidity. Animals were anaesthetised (Sagatal; sodium pentobarbitone; 60 mg/kg i.p.) and placed in a Kopf stereotaxic frame with the nosebar raised 2.5 mm above horizontal. The skin was cut longtitudinally to expose the skull and to visualise the bregma. A Hamilton syringe was positioned over the lateral ventricle (A − 1.1 mm and L ± 1.2 mm, Konig and Klippel, 1963) and a burr hole made in the skull.

Drug administration

BSO (80 mg/ml) was dissolved in distilled water and was administered bilaterally (1.6 mg/20 μl) into the lateral ventricles (V-3.3 mm) on day 1 and 3. Control animals received an equivalent volume of 0.9% saline (20 μl). Animals (n = 6–7) were killed 48 h after the second ICV administration of BSO. The effect of BSO-induced depletion of striatal GSH content was examined in these groups of animals. 6-OHDA hydrobromide (3.75 mg/ml) dissolved in 0.01% ascorbate was administered bilaterally (75 μg/20 μl) into the lateral ventricles, 48 h following the second injection of BSO. Control animals again received 0.9% saline (20 μl) at the same time point followed by 6-OHDA (75 μg/20 μl) 48 h after the second saline injection. Rats (n = 6–7) were killed on day 8 following 6-OHDA administration. The effect of BSO on 6-OHDA toxicity in the striatum was investigated in these groups of animals.

Thioctic acid treatment

R,S-Thioctic acid (50 mg/ml) was dissolved in sodium hydroxide (0.1 M) and neutralised with hydrochloric acid (0.1 M) to approximately pH 7. Animals (n = 6–7) were treated with thioctic acid (50 or 100 mg/kg i.p.) 24, 36 and 45 h following the second ICV injection of BSO or saline. The actions of thioctic acid were examined in animals killed 48 h following BSO administration and in BSO and 6-OHDA treated animals killed on day 8 following 6-OHDA administration.

Brain dissection

The whole brain was quickly removed over ice and cut coronally at the site of the optic chiasm. The posterior cerebral hemispheres were separated and the striata were dissected out and rapidly frozen over cardice.

Measurement of GSH

Brain GSH content was determined according to a modification of the method of Reed et al. (1980). This method of extraction is based on the reaction between GSH and iodoacetic acid to produce S-carboxymethyl derivatives which are then labelled with a chromophore and measured by HPLC (model 501, Waters, UK) with a UV detector (model 411, Waters, UK) at a wavelength of 365 nm.

The left striatum was homogenised in 18 volumes of cold 0.4 M perchloric acid containing 40 mg of diethylenetriamine penta-acetic acid using a microprobe. After centrifugation for 10 min at 12,000 rpm and 4°C, the supernatant was removed. Iodoacetic acid (0.44 μM), cysteic acid (1 mM) and an excess of sodium bicarbonate were added to

the supernatant (0.5 ml), mixed and left for 1 h in the dark at room temperature. After 1 h, 1.5% alcoholic fluorodinitrobenzene (0.5 ml) was added, mixed and samples were left for a further 4 h in the dark at room temperature. After this time, diethyl ether (1.0 ml) was added to the sample, mixed and centrifuged for 20 min at 4,000 rpm. The top ethereal layer was discarded and the lower aqueous layer was analysed by HPLC.

Samples (10 µl) were injected onto a Spherisorb S-5 amino ODS column (25 cm × 4.6 cm, 5 µ particle size, Phase Separations, UK) and eluted along a continuous gradient of methanol (80%) in combination with glacial acetic acid, methanol and water (pH 5.05; flow rate 1.0–1.25 ml/min at 2,700 psi). GSH content was determined using a Waters ultraviolet detector (model 411) at a wavelength of 365 nm. The internal standard, cysteic acid, was used to quantify the GSH content of the samples.

Measurement of monoamines and metabolites by HPLC

Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured by HPLC with electrochemical detection (Rose et al., 1989).

The right striatum was homogenised in 9 volumes of cold 0.4 N perchloric acid containing 0.01% sodium metabisulphite and ethylenediaminetetra-acetic acid (1 mM) using a microprobe. The homogenate was added to the internal standard, 3,4-dihydroxybenzylamine (DHBA), to give a final concentration of DHBA of 100 ng/ml. The homogenates were centrifuged at 12,000 rpm at 4°C for 15 min.

The sample supernatant (30 µl) was injected onto a Spherisorb ODS-2 reverse phase column (25 cm × 0.46 cm, 5 µ particle size: Phase Separations, UK) using a Waters Wisp 710 B automated sampler with a 100 µl sample loop. Sodium dihydrogen phosphate (0.1 M) containing octane sulphonic acid (0.65 mM), ethylenediaminetetra-acetic acid (1 mM) and 18% methanol (pH 3.1 adjusted with 3 M phosphoric acid). Chromatographic peaks were measured with a BAS LC-4B amperometric detector with a thin layer electrochemical cell fitted with a glassy carbon working electrode and Ag/AgCl reference electrode. The working potential was 0.76 V, pressure 2,750 psi and the temperature was maintained at 9–10°C to prevent sample degradation. Integration of the chromatographic peaks was performed using a Waters data module. Tissue levels of the monoamines were quantified against the internal standard.

Tyrosine hydroxylase immunocytochemistry

Brain sections were cut using a Bright's cryostat (20 µm) and washed in 0.1 M phosphate buffered saline, pH 7.4 (PBS) and immersed in PBS containing 20% normal goat serum (NGS) for 1 h, before they were rinsed in PBS containing 1% NGS and 0.05% Triton X-100. The sections were left in 0.05% anti-rabbit tyrosine hydroxylase polyclonal antisera overnight at room temperature on a gently shaking platform. The sections were rinsed in PBS containing 1% NGS.

An avidin-biotinylated rabbit vector stain kit was used to label the primary antibody. Briefly, this involved incubation in PBS (10 ml) containing 1 drop of biotinylated goat anti-rabbit IgG antibody for 1 h; washing in PBS and finally incubating the sections in PBS (10 ml) containing two drops of both avidin and biotinylated enzyme, for 1 h. The slices were rinsed in PBS and then in 0.05 M Tris-buffer, pH 7.4. The sections were immersed in 0.05 M Tris buffer, containing 0.05% 3,3′-diaminobenzidine (DAB) for 10 min. After this time, 0.01% hydrogen peroxide was added. The reaction was stopped by immersing the sections in fresh Tris buffer. The slides were dehydrated in graded alcohols, cleared in histoclear and mounted in DPX.

The number of tyrosine hydroxylase positive cells were counted at five different levels of the substantia nigra corresponding to −4.8 mm, −5.1 mm, −5.4 mm, 5.7 mm and −6.0 mm from bregma (Carman et al., 1991) using an Axostop microscope (Zeiss, Germany).

Statistical analysis

The effect of thioctic acid, BSO and 6-OHDA treatment on GSH levels, monoamine content and TH positive cells were compared using the Mann Whitney U test.

Results

Effects of BSO and thioctic acid on striatal GSH levels

At 48h following the final bilateral intracerebroventricular administration of BSO (2 × 1.6mg), striatal GSH levels were reduced to 30–40% of control values (Fig. 1A,B).

Following administration of thioctic acid (50 or 100mg/kg i.p.) to vehicle treated animals, striatal GSH levels were not different from those of control animals. The repeated administration of thioctic acid (50 or 100mg/kg i.p.)

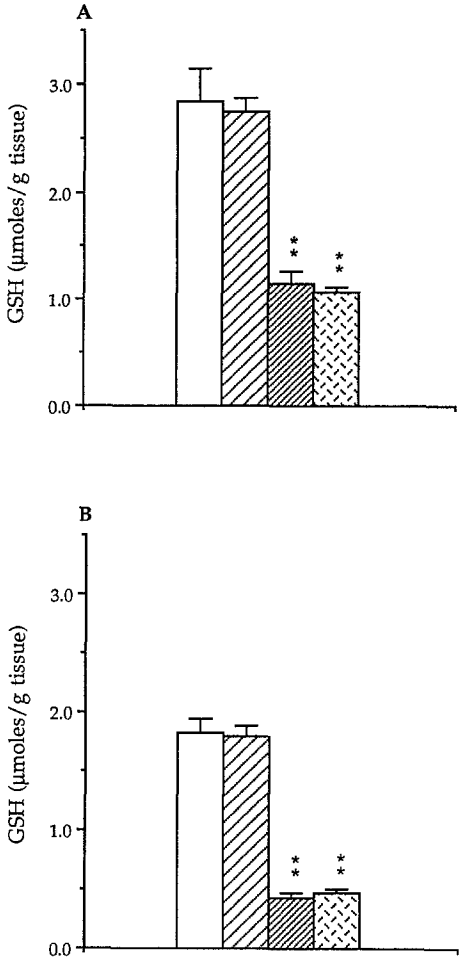

Fig. 1. Effect of bilateral administration of BSO (2 × 1.6mg ICV) and/or thioctic acid (**A** 50mg/kg i.p; **B** 100mg/kg i.p) treatment on striatal GSH levels 48h following the last injection of BSO. The data shown are mean ± sem (N = 6). **p < 0.01 compared to saline treated controls using the Mann Whitney U-Test. □ control, ▨ thioctic acid, ▩ BSO, ▩ BSO + thioctic acid

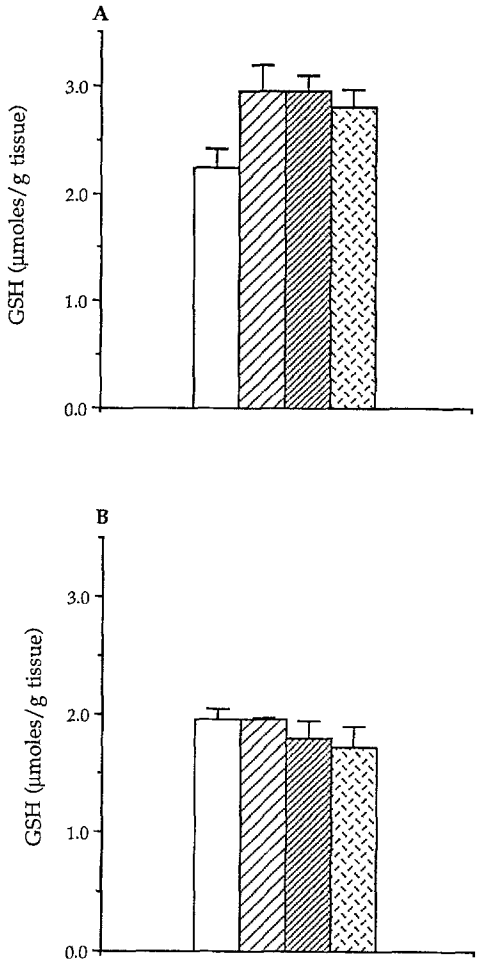

Fig. 2. Effects of bilateral administration of 6-OHDA (75μg ICV), BSO (2 × 1.6mg ICV) and thioctic acid (**A** 50mg/kg i.p; **B** 100mg/kg i.p) treatment on striatal GSH levels 8 days following the injection of 6-OHDA. The data shown are mean ± sem (N = 6) using the Mann Whitney U-Test. There were no significant differences in GSH levels following 6-OHDA treatment alone or with thioctic acid and/or BSO. □ 6-OHDA, ▨ thioctic acid + 6-OHDA, ▩ BSO + 6-OHDA, ▩ BSO + thioctic acid + 6-OHDA

had no effect on the striatal levels of GSH in rats treated with BSO 48h previously (Fig. 1A,B).

At 8 days following 6-OHDA administration and with BSO pretreatment, striatal GSH values were not different from control values. In addition, administration of thioctic acid alone or following BSO treatment at either 50 or 100mg/kg dose, did not alter striatal GSH levels at this time point in 6-OHDA-lesioned animals (Fig. 2A,B).

Effects of thioctic acid on BSO potentiation of dopamine depletion induced by 6-OHDA

Administration of 6-OHDA (75μg bilateral ICV) caused a decrease in striatal dopamine content to approximately 60% of control values when measured 8

days following toxin treatment. Administration of BSO (2 × 1.6mg) alone had no effect on striatal dopamine levels when measured 8 days later. However, treatment with BSO potentiated the 6-OHDA induced depletion of dopamine in striatum (Tables 1 and 2).

Administration of thioctic acid (50 or 100mg/kg i.p.) alone had no effect on striatal dopamine levels when measured 8 days later. Treatment with thioctic acid (50 or 100mg/kg i.p.) had no effect on the depletion of striatal dopamine caused by 6-OHDA or on the potentiation of the 6-OHDA-induced dopamine loss produced by prior treatment with BSO (Tables 1 and 2).

Treatment with 6-OHDA decreased striatal levels of HVA and DOPAC when measured 8 days following toxin application (Tables 1 and 2). BSO treatment alone had no effect on striatal HVA and DOPAC levels at this time point. However, BSO potentiated the losses of HVA and DOPAC produced by 6-OHDA administration. Thioctic acid (50 or 100mg/kg i.p.) did not alter the decrease in striatal HVA and DOPAC concentration in 6-OHDA treated animals or the potentiation of these reductions produced by prior BSO treatment (Tables 1 and 2).

Alterations in tyrosine hydroxylase immunoreactive cell numbers in substantia nigra

Bilateral intracerebroventricular administration of 6-OHDA (75μg/20μl) resulted in a loss of immunoreactive tyrosine hydroxylase cells throughout the

Table 1. Effects of bilateral administration of BSO (2 × 1.6mg ICV) followed by thioctic acid (50mg/kg i.p.) and/or 6-OHDA (75μg ICV) administration on striatal dopamine, DOPAC and HVA levels

	Dopamine	DOPAC (μg/g wet weight tissue)	HVA
Control	10.89 ± 0.86	0.58 ± 0.06	0.47 ± 0.04
Thioctic acid	11.97 ± 1.20	0.72 ± 0.10	0.73 ± 0.12 *
BSO	11.55 ± 1.20	0.63 ± 0.08	0.54 ± 0.07
BSO + thioctic acid	10.85 ± 0.90	0.63 ± 0.07	0.64 ± 0.08 **
6-OHDA	6.00 ± 0.50 **	0.45 ± 0.10	0.44 ± 0.08
Thioctic acid + 6-OHDA	6.69 ± 0.58 **	0.43 ± 0.04	0.42 ± 0.04
L-BSO + 6-OHDA	2.33 ± 0.67 **##	0.16 ± 0.04 **##	0.19 ± 0.03 **##
L-BSO + thioctic acid + 6-OHDA	2.66 ± 0.68 **##	0.19 ± 0.06 **##	0.25 ± 0.05 **##

The data shown are mean ± sem (N = 6). *$p < 0.05$ and **$p < 0.01$ compared to saline treated controls and ##$p < 0.01$ compared to 6-OHDA treatment using the Mann Whitney U-Test

Table 2. Effects of bilateral administration of BSO (2 × 1.6 mg ICV) followed by thioctic aicd (100 mg/kg i.p.) and/or 6-OHDA (75 μg ICV) administration on striatal dopamine, DOPAC and HVA levels

	Dopamine	DOPAC (μg/g wet weight tissue)	HVA
Control	14.23 ± 1.07	0.82 ± 0.05	0.64 ± 0.08
Thioctic acid	14.61 ± 1.04	1.02 ± 0.06 *	0.84 ± 0.12 *
BSO	13.34 ± 0.60	0.72 ± 0.04	0.66 ± 0.07
BSO + thioctic acid	13.99 ± 0.90	0.87 ± 0.06	0.68 ± 0.09
6-OHDA	8.17 ± 0.5 **	0.48 ± 0.03 **	0.41 ± 0.03 *
Thioctic acid + 6-OHDA	6.32 ± 0.90 **	0.40 ± 0.05 **	0.38 ± 0.03 **
L-BSO + 6-OHDA	3.38 ± 0.70 **##	0.23 ± 0.04 **##	0.26 ± 0.03 **##
L-BSO + thioctic acid + 6-OHDA	3.45 ± 1.27 **##	0.22 ± 0.07 **##	0.29 ± 0.03 **##

The data shown are mean ± sem (N = 6). *$p < 0.05$ and **$p < 0.01$ compared to saline treated controls and ##$p < 0.01$ compared to 6-OHDA treatment using the Mann Whitney U-Test

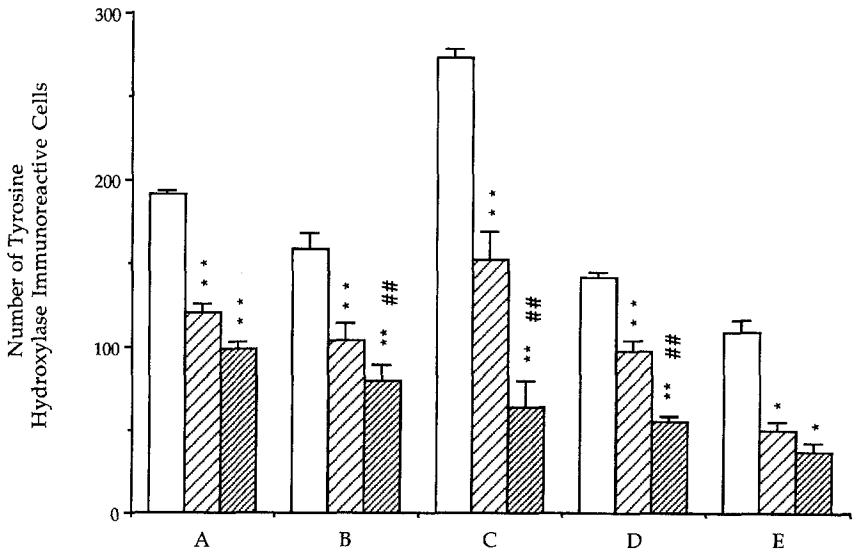

Fig. 3. Effects of 6-OHDA (75 μg ICV) administration and/or BSO pretreatment (2 × 1.6 mg ICV) on the number of nigral tyrosine hydroxylase immunoreactive cells 8 days following the injection of 6-OHDA. The data shown are mean ± sem (N = 6). *$p < 0.05$ and **$p < 0.01$ compared to saline treated controls and ##$p < 0.01$ compared to 6-OHDA treatment using the Mann Whitney U-Test. The levels of substantia nigra, A–E, correspond to −4.8 mm, −5.1 mm, −5.4 mm, −5.7 mm and −6.0 mm from bregma (Carman et al., 1991). □ control, ▨ 6-OHDA, ▩ BSO + 6-OHDA

substantia nigra when assessed 8 days following toxin treatment (Fig. 3). Administration of BSO (2 × 1.6 mg) potentiated this loss of immunoreactive tyrosine hydroxylase cells in sections of the substantia nigra correlating to −5.1 mm, −5.4 mm and −5.7 mm from bregma, but not at those sites −4.8 mm and −6.0 mm from bregma (Fig. 3).

Thioctic acid (50 or 100 mg/kg i.p.) treatment of 6-OHDA lesioned rats had not effect on the number of immunoreactive tyrosine hydroxylase cells in the substantia nigra compared to 6-OHDA treatment alone. Similarly, thioctic acid at either 50 or 100 mg/kg doses did not prevent BSO potentiating the effect of 6-OHDA (data not shown).

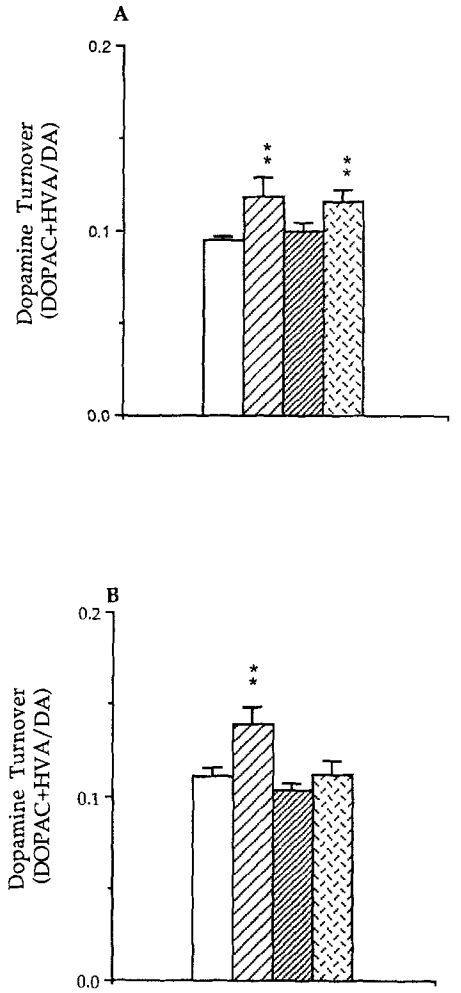

Fig. 4. Effects of bilateral administration of BSO (2 × 1.6 mg ICV) and/or thioctic acid (**A** 50 mg/kg i.p; **B** 100 mg/kg i.p) treatment on striatal dopamine turnover, 48 h following the last injection of BSO. The data shown are mean ± sem (N = 6). **$p < 0.01$ compared to saline treated controls using the Mann Whitney U-Test. □ control, ▨ thioctic acid, ▩ BSO, ▩ BSO + thioctic acid

Effects of thioctic acid on monoamine turnover

When measured at 3 h following the final administration of thioctic acid (50 or 100 mg/kg i.p.) in control or BSO-treated animals, changes in monoamine turnover occurred.

Treatment with BSO alone had no effect on striatal dopamine, HVA or DOPAC levels or on dopamine turnover (HVA + DOPAC/DA). Administration of thioctic acid (50 mg/kg i.p.) had no effect on striatal dopamine levels, but increased both HVA content in striatum of control and BSO-treated animals and DOPAC levels in control animals (Table 1A). However, dopamine turnover was increased in both control and BSO-treated animals following treatment with thioctic acid (50 mg/kg; Fig. 4A). Similarly, thioctic

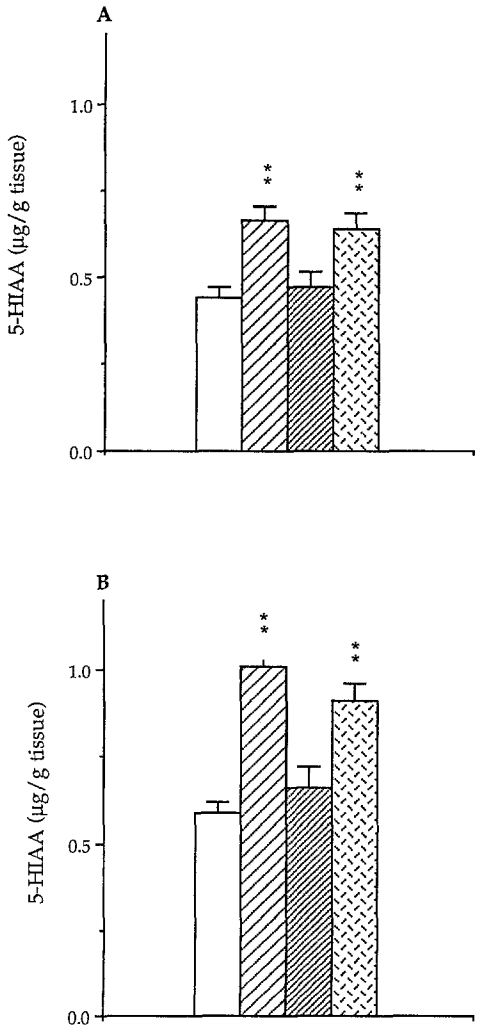

Fig. 5. Effects of bilateral administration of BSO (2 × 1.6 mg ICV) and/or thioctic acid (**A** 50 mg/kg i.p; **B** 100 mg/kg i.p) treatment on striatal 5-HIAA levels, 48 h following the last injection of BSO. The data shown are mean ± sem (N = 6). **$p < 0.01$ compared to saline treated controls using the Mann Whitney U-Test. □ control, ▨ thioctic acid, ▩ BSO, ▩ BSO + thioctic acid

acid (100mg/kg i.p.) did not alter striatal dopamine levels in control or BSO-treated animals and increased DOPAC and HVA levels in control tissues, but not in BSO-treated animals (Table 1B). However, thioctic acid (100mg/kg i.p.) increased dopamine turnover in control, but not BSO treated rats (Fig. 4B).

Treatment with BSO had no effect on striatal 5-HT (data not shown) or 5-HIAA levels, compared to those of controls. In contrast, although 5-HT levels were unchanged, 5-HIAA levels in striatum were increased following administration of both 50 and 100mg/kg thioctic acid in control and BSO-treated animals (Fig. 5A,B).

Discussion

The regional and disease specific early decrease in GSH levels in substantia nigra in Parkinson's disease may contribute to the progression of dopamine cell loss. There are no obvious means of raising brain (GSH) levels since glutathione itself does not readily penetrate cell membranes. For this reason, compounds such as thioctic acid, a thiol-disulphide couple which, like glutathione, might replenish brain GSH levels and which possesses potential antioxidant activity may be worth considering as a treatment.

The mechanism by which thioctic acid increases GSH levels is unclear, but may involve other redox couples such as ascorbate/dehydroascorbate, succinate/α-ketoglutarate, NAD^+/NADH and $NADP^+$/NADPH. Oxidation of dihydrolipoic acid to thioctic acid regenerates ascorbate from dehydroascorbate which in turn reduces chromanoxyl radicals to regenerate membrane bound vitamin E (Kagen et al., 1992). Furthermore, since dihydrolipoic acid/thioctic acid has a greater redox potential than NAD^+/NADH, $NADP^+$/NADPH and succinate/α-ketoglutarate, it can substitute for these important electron transport substrates which are also involved in vitamin E recycling. Thus, thioctic acid is likely to enhance decreased GSH levels indirectly by bolstering cellular antioxidant levels of vitamin E and ascorbate.

In this study, we utilised a model of brain glutathione deficiency and increased 6-OHDA toxicity described previously (Pileblad and Magnusson, 1989; Pileblad et al., 1989). Our studies confirmed the ability of intracerebroventricular administration of BSO to deplete brain (GSH) by approximately 60–70% 48h following treatment. We also confirmed the ability of BSO treatment to potentiate the decrease in striatal dopamine levels produced by 6-OHDA administration into the lateral ventricles. Our data show that GSH levels return to normal 10 days following the last administration of BSO, indicating that γ-glutamylcysteine synthetase is no longer inhibited. Indeed, striatal GSH levels are only maximally depleted for 36–60h following ICV administration of BSO (3.2mg) and thereafter slowly return to control levels (Pileblad and Magnusson, 1989).

The action of BSO in potentiating 6-OHDA induced striatal dopamine depletion presumably reflects the loss of GSH and, consequently, a decrease in antioxidant defences. However, BSO treatment of neonatal rats leads to mitochondrial damage in brain (Jain et al., 1991). This may render cells more

sensitive to the actions of 6-OHDA and may be relevant to Parkinson's disease where reduced activity of complex I (Schapira et al., 1990) and α-ketoglutarate dehydrogenase (Mizuno et al., 1994) occurs in substantia nigra. However, in a subsequent study we found that rat cerebral cortex mitochondrial enzyme activity was not affected by the schedule of BSO administration employed in the present investigation (Seaton et al., 1995a).

Administration of thioctic acid over a 2 day period in high doses did not have any effect on striatal GSH levels in normal animals, or in those where GSH levels had been depleted by BSO treatment. It is not surprising that thioctic acid had no effect in normal animals where glutathione levels in brain are high and almost entirely in the reduced form. However, thioctic acid has been claimed to replenish depleted levels of GSH content following irradiation of mice (Busse et al., 1992), cadmium intoxication in rats (Sumathi et al., 1994) or BSO administration to neonatal rats (Maitra et al., 1995) presumably by bolstering cellular antioxidant levels. The use of BSO to inhibit γ-glutamylcysteine synthetase (γ-GCS), the rate limiting enzyme for GSH synthesis (Griffith, 1982), may limit the ability of thioctic acid to stimulate GSH formation. However, Maitra and colleagues (1995) showed that thioctic acid could partially reverse the BSO induced decrease in GSH content of the lens in neonatal rats. So, the failure of thioctic acid to replenish striatal GSH may be perculiar to the adult rat brain. Indeed, it may be necessary to explore the actions of thioctic acid in other models of altered glutathione function in brain. In particular, it may be relevant to assess its actions in models of oxidative stress, such as the increase in GSSG levels induced by haloperidol treatment of mice (Cohen and Spina, 1989) and rats (Shivakumar and Ravindranath, 1992). Indeed, models of this type may be more relevant to Parkinson's disease where γ-GCS levels are normal but oxidative stress occurs (Sian et al., 1994b).

Even though thioctic acid did not raise brain GSH levels, it exerts antioxidant activity (Suzuki et al., 1991; Kagen et al., 1992; Scott et al., 1994) and so theoretically might reduce 6-OHDA toxicity, which is believed to be free radical mediated (Heikkila and Cohen, 1973; Cohen and Heikkila, 1974). However, this was not the case in the present experiments. Thioctic acid did not prevent the 6-OHDA induced depletion of dopamine or the BSO induced potentiation of this effect. Recently, the effects of thioctic acid on MPTP toxicity were examined (Götz et al., 1994). Thioctic acid was not able to prevent the decrease in striatal dopamine content produced by MPTP and which was presumably due to neuronal toxicity as a result of mitochondrial impairment and free radical formation. The failure of thioctic acid to influence 6-OHDA or MPTP toxicity could be because the drug does not penetrate into the basal ganglia. However, in the striatum of mice, thioctic acid alters the ratio of oxidised to reduced coenzyme Q towards the reduced form (Götz et al., 1994). In rats, we have shown thioctic acid to alter glucose utilisation in basal ganglia (Seaton et al., 1995b,c,d) and, in the present study, thioctic acid had effects on brain monoamine function. Of particular interest is the recent report that thioctic acid and dihydrolipoic acid protect against NMDA and malonic acid-induced excitotoxic lesions in rat striatum (Greenamyre et al.,

1994). So it may be the nature of the toxic insult which dictates whether thioctic acid exerts a neuroprotective action.

In conclusion, we have confirmed the potentiation of 6-OHDA toxicity in rats produced by BSO induced glutathione depletion, but we were unable to demonstrate a protective effect of the antioxidant molecule, thioctic acid. However, thioctic acid did alter dopamine and 5-HT neuronal function which may be related to it's ability to alter glucose utilisation in brain.

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Authors' address: Prof. P. Jenner, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London SW3 6LX, United Kingdom.

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