Biochemical Pharmacology, Vol. 43, No. 2, pp. 263–269, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

SELECTIVE MODULATION OF GLUTATHIONE IN MOUSE BRAIN REGIONS AND ITS EFFECT ON ACRYLAMIDE-INDUCED NEUROTOXICITY

BANGALORE R. SHIVAKUMAR and VIJAYALAKSHMI RAVINDRANATH*
Department of Neurochemistry, National Institute of Mental Health and Neuro Sciences, Bangalore 560 029, India

(*Received* 2 *May* 1991; *accepted* 12 *September* 1991)

Abstract—Selective modulation of brain glutathione (GSH) may assist the elucidation of the role of GSH in the central nervous system. Subcutaneous administration of diethyl maleate (DEM) depleted both cerebral and hepatic GSH in a dose- and time-dependent manner. While hepatic GSH levels returned to control levels 6 hr after DEM administration, brain GSH levels remained significantly lowered for up to 12 hr after administration of DEM. However, intrathecal administration of DEM resulted in a selective lowering of brain GSH without altering hepatic levels. Intrathecal administration of L-buthionine sulfoximine (L-BSO; 1.0 mmol/kg body wt) also depleted the GSH content of the brain and the levels remained low 24 hr after L-BSO administration. The extent of GSH depletion varied in different regions of the brain; maximal depletion was observed in the brainstem, followed by the cerebellum, striatum, cortex and hippocampus. Intrathecal administration of L-2-oxothiazolidine 4-carboxylate (OTC) resulted in a marginal elevation of GSH levels in the brain. There was considerable regional variation. A maximal elevation of 134% was seen in the hippocampus, 6 hr following the intrathecal administration of 8.0 mmol of OTC/kg body wt. The effect of the modulation of brain GSH levels on acrylamide (ACR)-induced neurotoxicity was examined. Depletion of GSH by pretreatment of mice with L-BSO or DEM (administered intrathecally) enhanced the toxicity of ACR as measured by the inhibition of brain glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity. The inhibition of GAPDH by ACR was attenuated by pretreatment of animals with OTC. Thus, brain GSH may play an important role in the detoxification of xenobiotics, *in situ* within the central nervous system.

The tripeptide glutathione (GSH†) is the most abundant non-protein thiol in the cell and plays an important role as a cellular protectant [1]. Although it is widely distributed in the brain, its biological function in the central nervous system is not fully understood [2]. Selective modulation of brain GSH and observation of the effects of such modulation on xenobiotic metabolism might help in understanding the significance of GSH in the brain.

Various compounds have been used to modulate the levels of tissue GSH, namely, DEM which is known to deplete GSH in many organs by forming GSH conjugates [3]. More recent methods used for depleting tissue GSH involve the inhibition of GSH synthesis in the cell. Thus L-BSO, a selective inhibitor of γ-glutamyl cysteine synthetase, a key enzyme in GSH biosynthesis, has been used extensively to deplete GSH levels in tissues [4].

GSH concentration can also be increased by administration of compounds which are precursors of GSH synthesis. Administration of sulfur-containing precursors like cysteine or *N*-acetyl-cysteine results in toxicity to the central nervous system [5]. GSH by itself does not cross the blood–brain barrier. Although systemic administration of OTC results in an increase in hepatic GSH content [6], only a marginal increase is observed in brain GSH content.

The present work was undertaken to develop simple and easy methods for the selective modulation of GSH in the brain. Since the brain exhibits considerable regional heterogeneity, the effect of GSH modulation on various regions of the brain was examined. GSH is known to play an important role in the detoxification of xenobiotics and depletion of intracellular GSH leads to increased toxicity in liver and lung [1]. Earlier studies have shown that the potent neurotoxin ACR inhibits glycolytic enzymes by reacting with sulfhydryl groups of the enzyme moieties [7]; this can be overcome by elevating intracellular levels of GSH *in vitro* in brain slices [8]. In view of the above, the effect of GSH modulation on ACR-induced neurotoxicity was also examined.

MATERIALS AND METHODS

Materials. Oxidized glutathione, NADPH, 5,5′-dithiobis(2-nitrobenzoic acid), GSH reductase, ACR and maleic acid diethyl ester were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). OTC and L-BSO were obtained from the Chemical Dynamics Corporation (New Jersey, U.S.A.). All other reagents were of analytical grade.

Female Swiss albino mice, aged 3–4 months and weighing 20–30 g, were obtained from the stock

* Corresponding author: Vijayalakshmi Ravindranath, Department of Neurochemistry, NIMHANS, P.O. Box 2900, Bangalore 560 029, India.

† Abbreviations: GSH, glutathione; ACR, acrylamide; L-BSO, L-buthionine sulfoximine; DEM, diethyl maleate; OTC, L-2-oxothiazolidine-4-carboxylate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline.

colony of the Institute and used throughout the study. Animals had free access to pelletted diet (Lipton India Ltd, Calcutta, India) and water *ad lib*. The details of the dosage and the route of drug administration are given in the respective figure legends. Prior to killing, the animals were anaesthetized with ether and perfused transcardially with ice-cold saline (0.9% w/v) to prevent contamination of brain tissue with blood. Whole brain or different regions of the brain (cortex, cerebellum, brainstem, striatum, hippocampus and thalamus) were dissected out [9]. The main lobe of the liver was also removed. Tissues were frozen in liquid nitrogen immediately. Prior to analysis, the frozen tissue was weighed and homogenized in 9 vol. of 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA. One aliquot of the homogenate was immediately mixed with an equal volume of 10% perchloric acid (v/v) and centrifuged at 2000 rpm for 5 min. The acid supernatant was used for the assay of total glutathione by enzymatic recycling method using 5,5'-dithiobis-(2-nitrobenzonic acid), GSH reductase and NADPH [10]. Another aliquot of the brain homogenate was centrifuged at 1000 rpm for 5 min and the supernatant was used for the assay of GAPDH using DL-glyceraldehyde 3-phosphate diethyl acetal as substrate and monitoring the increasing absorbance at 340 nm due to conversion of NAD to NADH [7]. γ-Glutamyl cysteine synthetase was also assayed in the above supernatant using α-aminobutyric acid and ATP, and measuring the liberated phosphorus [11]. Protein content in the supernatant was estimated by dye-binding method using bovine serum albumin as standard [12].

Data was analysed statistically using Students *t*-test or one way analysis of variance with Duncan's test.

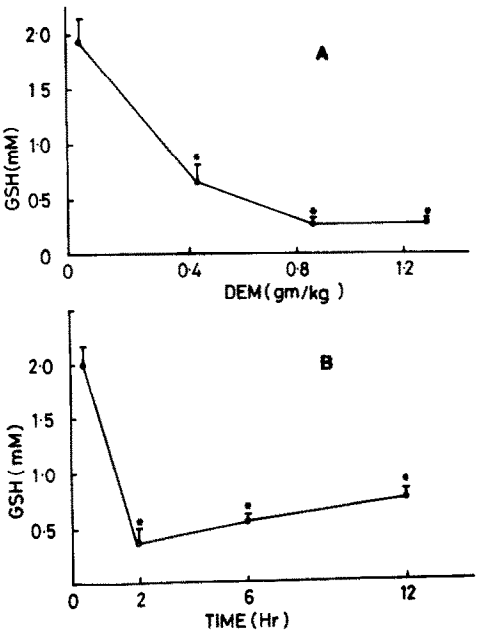

Fig. 1. Effects of DEM on brain GSH content. (A) DEM was administered in groundnut oil (0.43 g, 0.86 g and 1.29 g/kg body wt, s.c.) and the animals were killed 2 hr following administration. Control animals received the vehicle alone. Brain GSH content is expressed as mean ± SD (N = 5). Asterisks indicate values that are significantly different from controls ($P < 0.01$). (B) DEM was administered in groundnut oil (0.86 g/kg body weight, s.c.). Control animals received the vehicle alone. Mice were killed 2, 6 and 12 hr after administration and brain GSH content was analysed. Data are expressed as mean ± SD (N = 5). Asterisks indicate the values that are significantly different from controls ($P < 0.01$). Brain GSH content in control animals did not vary significantly during the time period of the experiment.

RESULTS

Effect of DEM treatment on brain and liver GSH

Cerebral GSH content was depleted significantly after subcutaneous administration of DEM (Fig. 1A). Brain GSH levels were depleted by 67%, 2 hr after a dose of 0.43 g/kg body wt of DEM. An increase in the dose of DEM (0.86 g/kg body wt) resulted in greater depletion of brain GSH (85% depletion) as compared with control animals. Administration of a higher dose of DEM (1.29 g/kg body wt) did not result in any further depletion of brain GSH. Subcutaneous administration of DEM also resulted in the depletion of hepatic GSH. The hepatic GSH levels 2 hr after administration of 1.29 g/kg body wt of DEM were only 31% of control levels (data not shown). Twelve hours following subcutaneous administration of DEM (0.86 g/kg body wt), brain GSH content was only 39% of controls (Fig. 1B). However, hepatic GSH had recovered in the DEM-treated animals and the levels were not significantly different from control animals 12 hr after administration (data not shown).

Effect of intrathecal BSO administration on brain and liver GSH

Brain GSH levels following a single intrathecal administration of L-BSO are depicted in Fig. 2. Administration of a single dose of L-BSO (0.5 mmol/kg body wt, intrathecal) resulted, 24 hr later, in a small but significant lowering of GSH levels in the hippocampus (84% of control) and thalamus (90.5% of control). There was no significant change in GSH levels in the cortex following the administration. However, in the brainstem GSH levels decreased significantly (28% decrease) compared to untreated controls. In the cerebellum and striatum, GSH levels were 67% and 75% of corresponding controls, respectively. Following the administration of a higher dose of L-BSO (1.0 mmol/kg body wt, intrathecal), GSH content was found to be significantly depleted in all regions of the brain examined, but the extent of depletion varied among the regions studied. Thus, 24 hr after administration of the dose maximum depletion was observed in the brainstem (42% depletion) followed by the cerebellum (33%), striatum (25%), cortex (20%) and hippocampus (16%). In the thalamus GSH levels were decreased by only 9% as compared to controls. There was no change in the hepatic GSH content after intrathecal administration of L-BSO (data not shown). Following intrathecal administration of a single dose of L-BSO

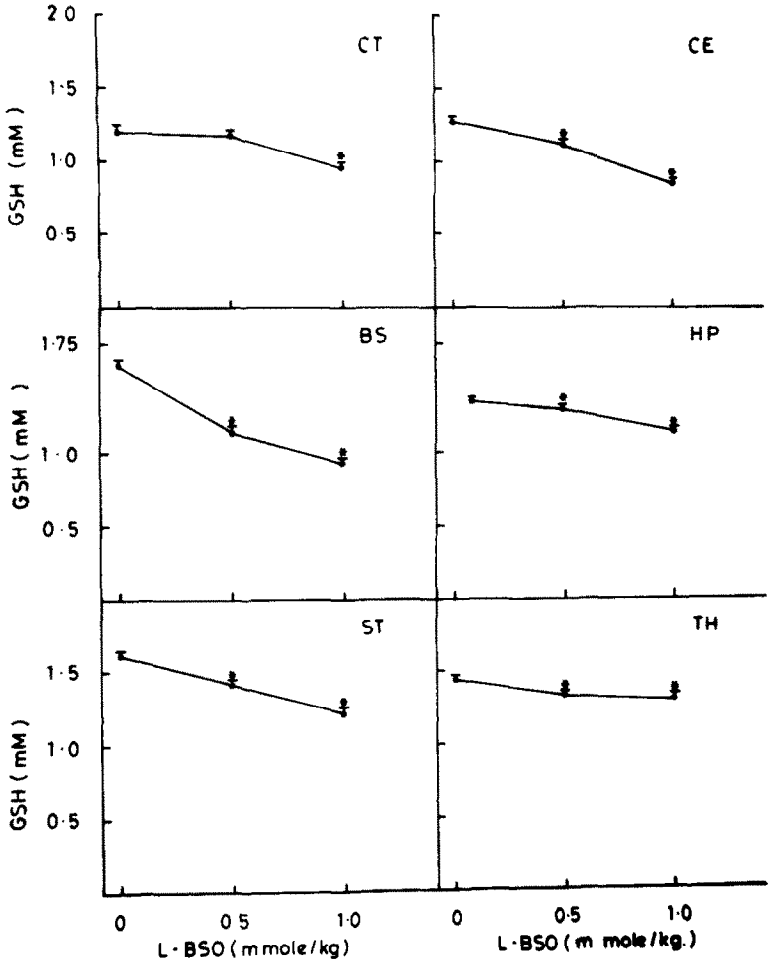

Fig. 2. Concentration of GSH in different regions of the mouse brain following administration of L-BSO. L-BSO was administered intrathecally (0.5 and 1.0 mmol/kg body wt) in PBS. Control animals received vehicle alone. Animals were killed 24 hr following administration and the brains were dissected into various regions, namely, the cortex (CT), cerebellum (CE), brainstem (BS), striatum (ST), hippocampus (HP) and thalamus (TH), prior to GSH estimation. Data are expressed as means ± SEM (N = 4). Values significantly different from controls are indicated by asterisks ($P < 0.001$).

(1.0 mmol/kg body wt), GSH levels remained significantly low in various regions of the brain for up to 24 hr. Only after 48 hr did the GSH content of the brain regions return to control levels, as shown in Fig. 3.

Effect of intrathecal administration of OTC on brain GSH

Brain GSH content did not increase significantly following intrathecal administration of a single dose of 4.0 mmol/kg body wt of OTC. However, administration of higher doses of OTC (8 and 16 mmol/kg body wt) resulted in a small but significant increase in GSH levels in the brain (data not shown). Estimation of the GSH content of regions of the brain at different time intervals following an intrathecal dose of OTC (8 mmol/kg body wt) revealed a differential effect in the various regions, as shown in Fig. 4. An initial depletion of GSH was observed in all regions of the brain. The period of the depletion varied from 2 to 8 hr depending on the region examined. An increase in GSH levels was observed in some regions following the initial depletion. Maximal increase of GSH level was seen in the hippocampus (134%) 6 hr after administration of the dose. In all other regions, the increase in GSH levels was small and varied between 3 and 15% as compared to controls. GSH content increased in various brain regions between 4 and 8 hr after administration, and the GSH content in OTC-treated animals was not significantly different from control, 24 hr after administration.

Effect of pretreatment with DEM on ACR-induced neurotoxicity

Intrathecal administration of a high dose of DEM (0.43 g/kg body wt) resulted in selective depletion of cerebral GSH content (75% depletion) while the hepatic GSH levels remained unaltered (data not shown). The effect of this depletion of brain GSH

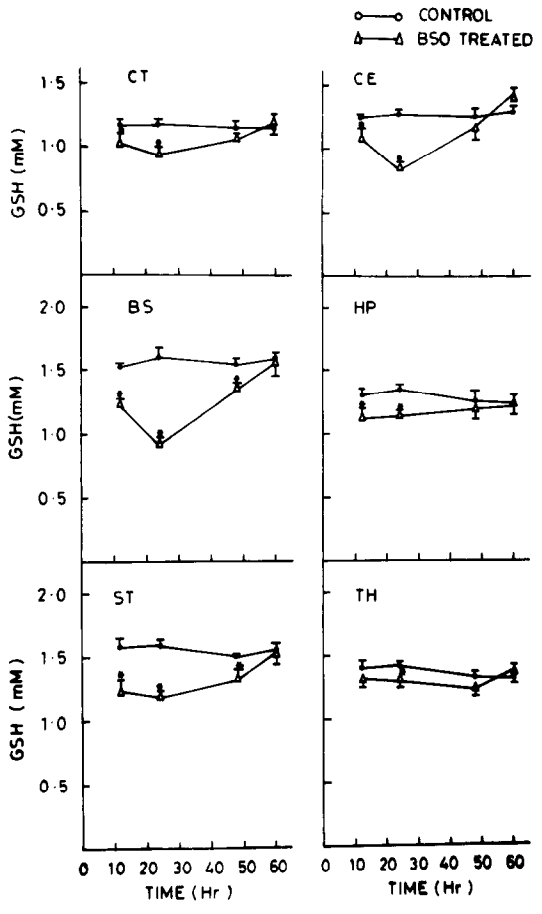

Fig. 3. Time course of depletion of brain GSH levels following intrathecal administration of L-BSO. L-BSO (1.0 mmol/kg body wt) was administered in PBS intrathecally to animals. Control animals received vehicle alone. Animals were killed at 12, 24, 48 and 60 hr after L-BSO administration. Different regions of the brain were dissected out (as indicated in Fig. 2) and later analysed for total GSH content. The data are means ± SEM (N = 4). Values significantly different from controls are indicated by asterisks ($P < 0.01$).

on ACR-induced neurotoxicity was examined. Administration of a non-toxic dose of ACR (25 mg/kg body wt, i.p.) did not result in any significant change in brain GAPDH activity (Fig. 5). However, in animals pretreated with DEM (0.1 g/kg body wt, intrathecal) administration of the same dose of ACR resulted in significant inhibition of brain GAPDH activity (10% inhibition) as compared to untreated control.

Effect of pretreatment with L-BSO on ACR-induced neurotoxicity

Administration of a non-toxic dose of ACR to mice (25 mg/kg body wt, i.p.) did not result in any significant alteration in brain GAPDH activity (Fig. 6). However, pretreatment of animals with L-BSO (1.0 mmol/kg body wt) followed by administration of ACR (25 mg/kg body wt, i.p.) resulted in significant inhibition (8% inhibition) of brain GAPDH activity. Administration of L-BSO alone (1.0 mmol/kg body wt, intrathecal) did not result in an alteration of brain GAPDH activity.

Effect of pretreatment with OTC on ACR-induced neurotoxicity

A neurotoxic dose of ACR (100 mg/kg body wt, i.p.) decreased brain GSH levels (88% of control), as shown in Fig. 7A, and also inhibited the glycolytic enzyme GAPDH significantly (30% inhibition) as shown in Fig. 7B. However, following pretreatment of animals with OTC, ACR-induced depletion of GSH and inhibition of GAPDH were attenuated: GSH content was not depleted as compared to untreated control and GAPDH activity was inhibited by only 10%. Administration of OTC alone had no effect on brain GAPDH activity but GSH levels were increased significantly (21% increase). There was a significant decrease in GSH levels in animals treated with OTC and ACR as compared to animals treated with OTC alone.

DISCUSSION

Subcutaneous administration of DEM (a well known depletor of tissue GSH) resulted in depletion of both brain and liver GSH levels (Fig. 1A). However, brain GSH levels remained depleted for up to 12 hr after administration while hepatic GSH levels returned to control levels within this period. Intrathecal administration of a lower dose of DEM (0.43 g/kg body wt) resulted in selective depletion of brain GSH while hepatic levels were unaffected (Fig. 5). In addition to depleting GSH, DEM is known to affect several enzymes including cytochrome P450 [13]. It is preferable to use selective inhibitors of GSH biosynthesis. L-BSO, an inhibitor of γ-glutamyl cysteine synthetase, has been used extensively for depleting tissue GSH levels. L-BSO does not cross the blood–brain barrier and is effective in depleting brain GSH only when the blood–brain barrier is non-functional, for example in preweanling animals [14]. Depletion of GSH by L-BSO requires intracerebroventricular administration using stereotaxic techniques [15, 16]. This is difficult to perform when using a large number of animals to study the effects of toxicants on the central nervous system. Therefore, we administered L-BSO intrathecally, since this method of administration does not require stereotaxic techniques and can be carried out easily. Since the brain exhibits considerable regional heterogeneity the effect of intrathecally administered L-BSO was examined in different regions of the brain. The extent of GSH depletion varied from region to region with maximal depletion being seen in the brainstem (Fig. 2). The GSH levels remained significantly lower in the various regions examined, up to 24 hr following L-BSO dose, indicating a low turnover of GSH or L-BSO in brain [17]. The time course of depletion of GSH by L-BSO was examined at earlier time points (2, 4, 8, 12 and 24 hr after L-BSO dosage). Maximum depletion was seen only 24 hr after L-BSO administration (data not shown). Hence, in the present study the GSH levels were monitored 12–48 hr after L-BSO administration. The decrease in

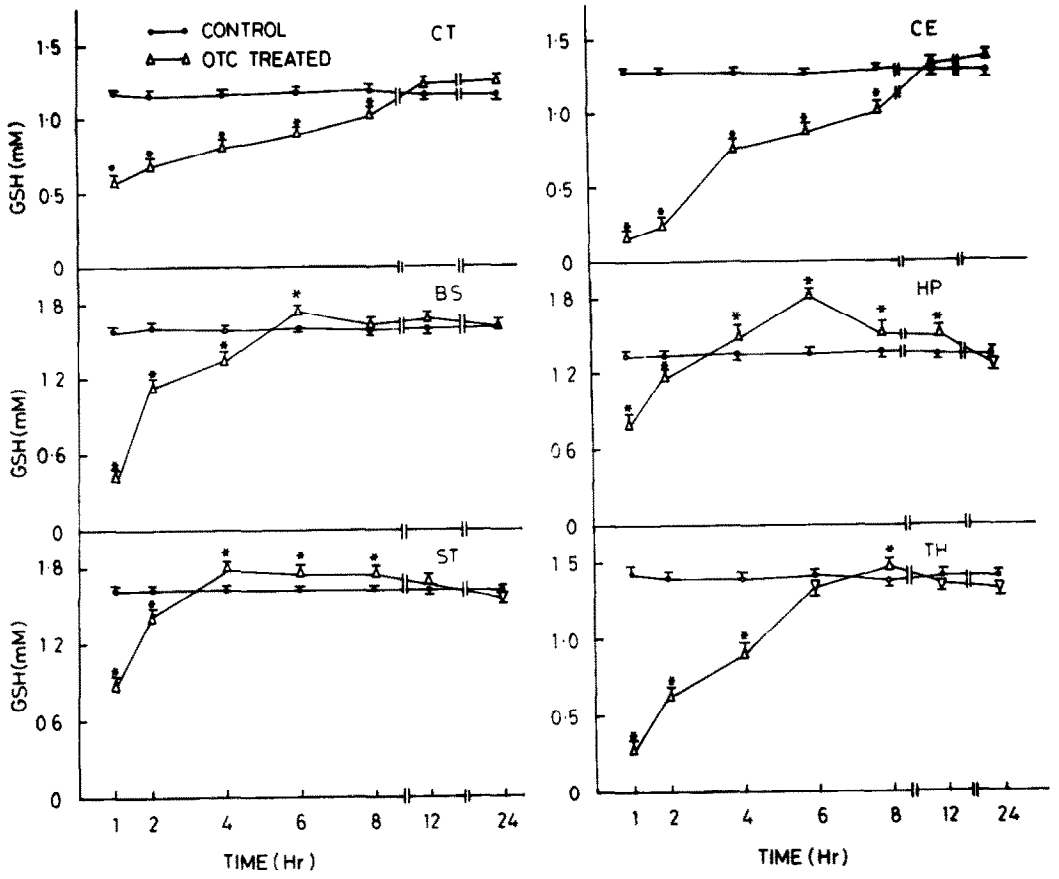

Fig. 4. GSH levels in mouse brain regions at various time intervals following a single dose of OTC. OTC (8.0 mmol/kg body wt) was administered intrathecally in PBS to mice. Control animals received vehicle alone. Animals were killed at indicated time intervals. Different regions of the brain were dissected out (as indicated in Fig. 2) and GSH content in these regions was analysed. Values are expressed as means ± SD (N = 4). Asterisks indicate values that are significantly different from controls ($P < 0.05$). The brain GSH levels in control animals did not vary significantly during the time period of the experiment.

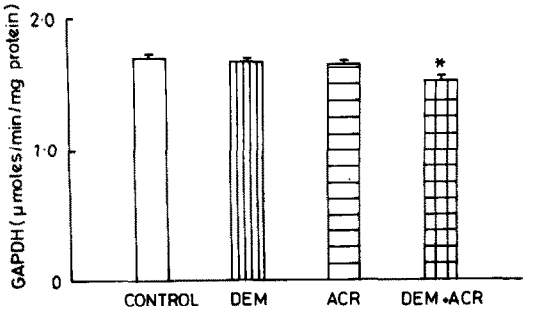

Fig. 5. Effect on DEM pretreatment on ACR-induced inhibition of brain GAPDH activity. DEM (0.10 g/kg body wt) was administered in groundnut oil intrathecally to animals. After 2 hr ACR (25 mg/kg body wt, i.p.) was administered in PBS. Control animals received vehicle alone. Animals were killed 4 hr after administration. Data are expressed as means ± SD (N = 3–8). Asterisks indicate values that are significantly different from untreated controls ($P < 0.01$).

GSH levels in certain regions of the brain was very small (e.g. 9% decrease in the thalamus, Figs 2 and 3), though statistically significant. Since brain exhibits considerable cellular heterogeneity, it is possible that the depletion of GSH within a particular cell type is more or less than the overall value determined. The physiological relevance of such depletion could be understood by examining the GSH levels in individual cell types following L-BSO treatment.

Intrathecal administration of OTC did not result in a massive increase in brain GSH levels, in contrast to that seen in hepatic tissue [6]. The increase in GSH levels was 3–15% in most regions of the brain examined except hippocampus where the GSH levels were elevated to 134% of controls. Intrathecal administration of OTC resulted in a significant decrease in GSH levels 2–8 hr after administration depending on the brain regions examined. One hour after OTC administration, GSH levels were significantly lowered in all regions of the brain examined. The reason for this is not known. Systemic administration of OTC has been shown to elevate cysteine levels in the brain while GSH levels remain

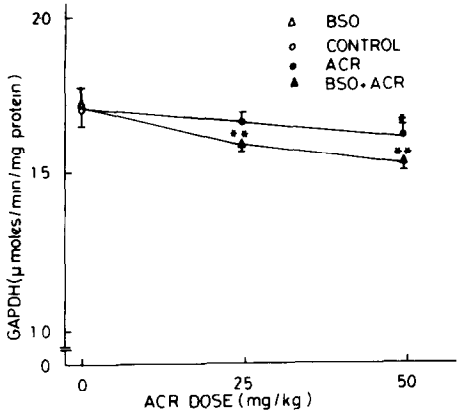

Fig. 6. Effect of L-BSO pretreatment on ACR-induced inhibition of brain GAPDH activity. L-BSO (1.0 mmol/kg body wt) was administered intrathecally to mice. After 12 hr ACR was administered in PBS (25 and 50 mg/kg body wt, i.p.). Control animals received vehicle alone. Animals were killed 2 hr following ACR administration. Data are expressed as means ± SD (N = 5). * Indicates values significantly different from untreated controls and ** indicates values significantly different from mice treated with L-BSO alone or corresponding doses of ACR alone ($P < 0.01$).

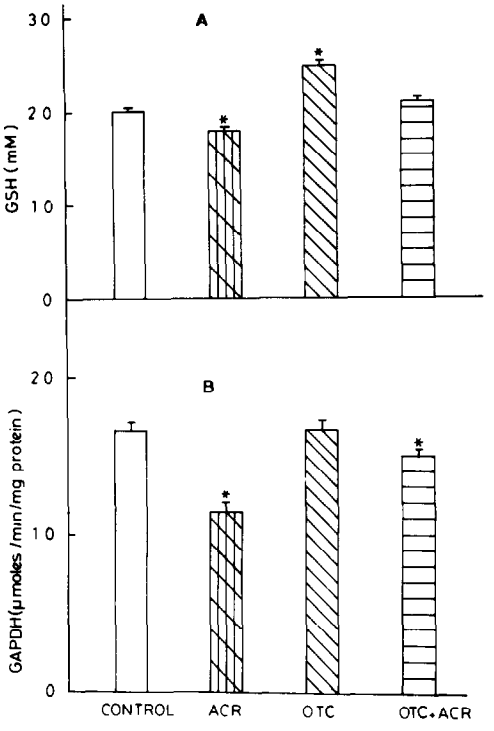

Fig. 7. Effect of OTC pretreatment on ACR-induced inhibition of brain GSH content and GAPDH activity. OTC (8.0 mmol/kg body wt) was administered intrathecally to mice. After 11 hr, ACR was administered (100 mg/kg body wt, i.p.). Control animals received vehicle alone. Two hours after administration of ACR, the animals were killed and the brain GSH levels (A) and GAPDH activity (B) were determined. Data are means ± SD (N = 5). Values that are significantly different from untreated controls are indicated by asterisks ($P < 0.01$).

unaffected [18]. However, in the present study intrathecal administration of OTC resulted in a small but significant increase in GSH levels in the brain regions at later time points which could be a direct effect of OTC or a compensatory synthesis due to an initial depletion by OTC. The increase in GSH was not uniform in all regions of the brain examined. OTC treatment may not, therefore, be the method of choice for modulation of GSH levels in the brain.

The effect of selective modulation of GSH on ACR-induced toxicity in the brain was examined with a view to determining whether brain GSH plays a role in the detoxification of xenobiotics. ACR is a potent neurotoxin and is known to exert its action by inhibiting the glycolytic enzyme GAPDH [7]. Pretreatment with DEM or L-BSO resulted in potentiation of ACR-induced toxicity. Non-toxic doses of ACR (25 and 50 mg/kg body wt) inhibited brain GAPDH activity when the animals were pretreated with DEM or L-BSO (Figs 5B and 6) while pretreatment with OTC attenuated the toxicity of ACR. Since OTC elevated brain GSH levels only marginally, total protection of GAPDH activity from ACR was not observed. Since the hepatic GSH level was unaffected by the pretreatment, it is unlikely that the ACR level in the liver is affected by the pretreatment. Decreased GSH levels in brain, thus, lead to potentiation of ACR toxicity while increased brain GSH levels lead to attenuation of ACR toxicity. These results indicate that cerebral GSH plays a role in the detoxification of ACR in brain.

In the present study the GSH content of the mouse brain showed considerable regional variation. The pattern of GSH distribution in various regions was different from that observed in rat brain [19]. It was observed that in the mouse brain, the brainstem region had a higher GSH content (1.6 ± 0.02 μmol/g tissue) as compared to the cortex (1.195 ± 0.009 μmol/g tissue). In the rat brain, the brainstem region contained significantly lower levels of GSH (0.79 ± 0.36 μmol/g tissue) as compared to the cortex (1.74 ± 0.02 μmol/g tissue). Thus, the regional distribution of GSH in brain seems to vary between species.

The present study demonstrates that brain GSH may indeed play a role in the detoxification of xenobiotics. Furthermore, the regional heterogeneity of brain GSH levels has to be taken into account when developing methods for the modulation of GSH involving either inhibition or stimulation of GSH biosynthesis. The differential effects of L-BSO and OTC on various brain regions examined indicate that regional differences could exist in the uptake of the modulators as well as in the turnover of brain GSH.

Acknowledgements—This work was funded by a research grant from NIMHANS. The authors thank Prof. S. M. Channabasavanna, Director, NIMHANS, and Prof. B. S. Sridhara Rama Rao, for encouragement and support. B.R.S. thanks CSIR for award of Senior Research Fellowship.

REFERENCES

1. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52**: 711–760, 1983.
2. Orlowski M and Karkowsky A, Glutathione metabolism and some possible functions of glutathione in the nervous system. *Int Rev Neurobiol* **19**: 75–121, 1976.
3. Boyland EL and Chasseaud LF, The effect of some carbonyl compounds on rat liver glutathione levels. *Biochem Pharmacol* **19**: 1526–1528, 1970.
4. Meister A, Methods for the selective modification of glutathione metabolism and study of glutathione transport. In: *Methods in Enzymology* (Ed. Meister A), Vol. 113, pp. 571–585. Academic Press, New York, 1985.
5. Olney JW, Ho OL and Rhee V, Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. *Exp Brain Res* **14**: 61–76, 1971.
6. Anderson ME and Meister A, Intracellular delivery of cysteine. In: *Methods in Enzymology* (Eds. Jakoby WB and Griffith OW), Vol. 143, pp. 313–325. Academic Press, New York, 1987.
7. Howland RD, Vyas IL, Lowndes HE and Argentieri TM, The etiology of toxic peripheral neuropathies: *in vitro* effects of acrylamide and 2,5 hexanedione on brain enolase and other glycolytic enzymes. *Brain Res* **202**: 131–142, 1980.
8. Ravindranath V and Pai KS, The use of rat brain slices as an *in vitro* model for mechanistic evaluation of neurotoxicity: studies with acrylamide. *Neurotoxicology* **12**: 225–234, 1991.
9. Glowinsky J and Iverson LL, Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the rat brain. *J Neurochem* **13**: 655–669, 1966.
10. Tietze F, Enzyme method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal Biochem* **27**: 502–522, 1969.
11. Chung AS, Maines MD and Reynolds WA, Inhibition of the enzymes of glutathione metabolism by mercuric chloride in the rat kidney: reversal by selenium. *Biochem Pharmacol* **31**: 3093–3100, 1982.
12. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein dye binding. *Anal Biochem* **72**: 248–254, 1976.
13. Bump EA, Yu NY, Taylor YC, Brown JM, Travis EL and Boyd MR, Radiosensitization and chemosensitization by diethyl maleate. In: *Radioprotectors and Anticarcinogens* (Eds. Nygaard OF and Simic MG), pp. 297–323. Academic Press, New York, 1983.
14. Slivka A, Spina MB, Calvin HI and Cohen G, Depletion of brain glutathione in preweanling mice by L-buthionine sulfoximine. *J Neurochem* **50**: 1391–1393, 1988.
15. Pileblad E and Magnusson T, Effective depletion of glutathione in rat striatum and substantia nigra by L-buthionine sulfoximine in combination with 2-cyclohexene-1-one. *Life Sci* **47**: 2333–2342, 1990.
16. Pileblad E and Magnusson T, Intracerebroventricular administration of L-buthionine sulfoximine: a method for depleting brain glutathione. *J Neurochem* **53**: 1878–1882, 1989.
17. Douglas GW and Mortensen RA, Rate of metabolism of brain and liver glutathione in rat studied with ^{14}C-glycine. *J Biol Chem* **222**: 581–585, 1956.
18. Anderson ME and Meister A, Marked increase of cysteine levels in many regions of the brain after administration of 2-oxothiazolidine-4-carboxylate. *FASEB J* **3**: 1632–1636, 1989.
19. Ravindranath V, Shivakumar BR and Anandatheerthavaradha HK, Low glutathione levels in brain regions of aged rats. *Neurosci Lett* **101**: 187–190, 1989.