Biochemical Pharmacology, Vol. 44, No. 5, pp. 895–903, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

INCREASE IN RAT BRAIN GLUTATHIONE FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF γ-GLUTAMYLCYSTEINE

ERIK PILEBLAD* and TOR MAGNUSSON
Department of Pharmacology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden

(*Received* 25 *November* 1991; *accepted* 5 *June* 1992)

Abstract—The effects of intracereboventricularly (i.c.v.) administered γ-glutamylcysteine (γ-GC) and glutathione (GSH) monoethyl ester, subcutaneously (s.c.) injected L-2-oxo-4-thiazolidinecarboxylic acid (OTC) and intraperitoneally (i.p.) administered cysteine on the concentration of GSH in rat brain were investigated. The brain content of GSH, cysteine and γ-GC was determined by HPLC with electrochemical detection (gold/mercury electrode) using *N*-acetylcysteine as internal standard. A dose-dependent increase in the GSH concentration (145–170% of controls) was found in the substantia nigra (SN) and in the rest of the brain stem after injection of γ-GC, whereas no significant alterations in GSH were observed in the striatum and in the cerebral cortex. High levels of γ-GC could be detected in the brain tissue after the administration, and the concentration of cysteine did also increase markedly after γ-GC injection in all brain regions assessed. I.c.v. administration of L-buthionine sulfoximine (L-BSO) reduced the brain concentration of GSH by 50–70% within 24 hr. Injection of γ-GC 24 hr after L-BSO resulted in an increase in GSH up to control values within 1–3 hr in the SN and the rest of the brain stem, whereas only a slight increase in GSH was observed in the striatum and the cerebral cortex. The concentration of GSH in the striatum and SN did not change after i.p. injection of cysteine, but a slight increase in the GSH concentration in the limbic region was observed. GSH monoethyl ester (i.c.v.) and OTC (s.c.) did not produce any significant increase in the GSH concentration in the brain. When the GSH concentration had been reduced by administration of L-BSO (i.c.v.; 24 hr) subsequent injection of GSH monoethyl ester led to a slight increase in the striatal and limbic GSH levels. These data show that, of the drugs studied, γ-GC was the most effective in increasing brain GSH. It could thus serve as a valuable tool in future studies regarding metabolism and function of GSH in the brain. The observed difference in the effects of γ-GC in different brain regions indicate that the brain tissue is not homogeneous with regard to GSH synthesizing capacity.

The function and metabolism of the tripeptide glutathione (GSH†) have been studied extensively in peripheral organs. GSH is known to participate in a variety of biochemical reactions, e.g. enzymatic elimination of hydrogen peroxide, organic peroxides by GSH peroxidase (see e.g. Ref. 1) and detoxification of foreign compounds [2]. It also acts to maintain the thiol-disulfide status of the cell, preventing oxidation of protein sulfhydryl groups [3]. However, comparably few investigations on the function and metabolism of brain GSH have been performed. In view of the fact that inherited disorders in GSH metabolism cause neurological defects (see e.g. Ref. 4), and with respect to the possible involvement of toxic oxygen species in neuronal degeneration, e.g. in Parkinsons disease, such studies seem warranted.

In order to perform these kind of experiments, specific tools to modify the metabolism of GSH are needed. Several drugs known to interfere selectively with certain steps in the GSH metabolism exist [5]. Their actions have mainly been evaluated in peripheral organs but in recent years several studies regarding their effects in the brain have been initiated. Thus, a couple of ways to reduce brain GSH are now described. In newborn and preweanling animals systemic administration of the γ-glutamylcysteine synthetase inhibitor L-buthionine sulfoximine (L-BSO) [5, 6] does effectively decrease brain GSH [7, 8]. The drug does not readily cross the blood–brain barrier and the effects in preweanling animals have been ascribed to the immature barrier in these animals [7]. The problem with the poor distribution of L-BSO to the brain of adult animals after systemic administration could partly be overcome by estrification of L-BSO and by administering the drug in combination with dimethyl sulfoxide [9]. However, probably the most effective way to use L-BSO in adult animals is to administer the drug intracerebroventricularly (i.c.v.). Using this method a very effective and long-lasting reduction of the brain concentration GSH was observed [10]. The GSH concentration could be even further reduced when L-BSO was combined with systemically administered 2-cyclohexene-1-one [11].

Using the method of i.c.v. administered L-BSO we found that when the GSH concentration was reduced, the dopaminergic neurons in the brain were more vulnerable [12]. An interesting question is of course whether an increased concentration of GSH could decrease the vulnerability of nerve cells. However, no method for producing a reliable

* Corresponding author. Tel. (46) 31-853425; FAX (46) 31-821795.

† Abbreviations: GSH, glutathione; γ-GC, γ-glutamylcysteine; OTC, L-2-oxo-4-thiazolidinecarboxylic acid; L-BSO, L-buthionine sulfoximine; DTPA, diethylenetriaminepentaacetic acid; DTT, dithiothreitol; i.c.v., intracerebroventricular; SN, substantia nigra.

increase in the concentration of GSH in the brain has been described yet, even though several drugs are known to increase GSH in peripheral organs [5]. Amongst these is the cysteine precursor L-2-oxo-4-thiazolidinecarboxylic acid (OTC) [5, 13]. Some authors have found an increase in brain GSH after systemic administration of OTC [14, 15], others have been unable to reproduce these data [16]. GSH monoethyl ester is another drug known to produce increased GSH levels in peripheral organs [17]. GSH monoethyl ester appears to partly counteract L-BSO-induced reduction in the cortical GSH concentration in newborn rats [8], but it has not been examined whether the drug has the ability to produce an actual increase in the brain concentration of GSH. The GSH precursor γ-glutamylcysteine (γ-GC) is also known to increase GSH [18] but its effect on brain GSH has to our knowledge not been evaluated. Thus, in an attempt to find a method to increase the concentration GSH in the brain, the effects of the following drugs were examined: γ-GC, GSH monoethyl ester, OTC and cysteine.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Alab, Södertälje, Sweden) weighing 200–260 g were used.

Drugs used in the animal experiments. The following agents were used in the animal experiments: cysteine (Merck, Darmstadt, Germany), OTC (Sigma Chemical Co., St Louis, MO, U.S.A.), L-BSO (Sigma) GSH monoethyl ester (synthesized in these laboratories, see below) and γ-GC (synthesized in these laboratories, see below).

Drugs were administered either systemically or intracerebroventricularly (i.c.v.). For i.c.v. injections the animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and polyethylene cannulae were permanently implanted into each lateral ventricle as described previously [19]. The rats were then allowed to recover from anaesthesia for 1 day before injections were made. The positions of the cannulae were checked at autopsy.

For i.c.v. injections, γ-GC, GSH monoethyl ester and L-BSO were dissolved in distilled water and the pH of the solution was adjusted to 7.0–7.4 with $NaHCO_3$. Twenty microlitres of drug solution followed by 5 μL of 0.9% NaCl was slowly administered through each cannulae. Control animals received the same volume of 0.9% NaCl.

Cysteine was added to 0.9% NaCl and 6 M HCl was added in small portions until the cysteine had totally dissolved. Before i.p. injection the pH of the solution was adjusted to 5 with $NaHCO_3$. Injection volume was 5 mL/kg. OTC was dissolved in 0.9% NaCl and injected s.c. (injection volume was 5 mL/kg).

The rats were killed by decapitation and their brains were rapidly taken out and put on an ice-chilled petri dish. The corpus striatum, the limbic forebrain (containing i.a. the nucleus accumbens and the olfactory tubercles) and the remaining hemispheral parts (mainly cortex) were dissected out as described by Carlsson and Lindqvist [20] and were immediately placed on dry ice. The cerebellum was removed and the brain stem was then turned up-side down and the crura cerebri were removed. The two substantia nigra (SN) could then be identified and dissected out. The SNs and the remaining part of the brain stem (in the following referred to as brain stem) were then placed on dry ice.

Immediately after the experiments the brain parts were homogenized in 0.1 M perchloric acid containing 4.3 mM diethylenetriaminepentaacetic acid (DTPA). After centrifugation for 10 min (10,000 *g*, 0°) the samples were filtered and *N*-acetylcysteine (internal standard) was added, and cysteine, GSH and γ-GC in the supernatant were determined by HPLC with electrochemical detection (gold/mercury electrode; oxidation potential: +0.15 V) [21–23]. The mobile phase used was 0.15 M monochloroacetic acid, containing 4.3 mM DTPA and 3.75% (v/v) methanol, pH 2.8–2.9. The column (stainless steel 0.45 × 15 cm) was packed with Nucleosil, RP-18, 5 μm (Macherey-Nagel, Düren, Germany). For retention times of the compounds see Fig. 1.

Synthesis of γ-GC. The compound was synthesized by a procedure described by Strumeyer and Bloch [24] with some modifications. Oxidized GSH (4 g; GSSG; Sigma) was dissolved in 100 mL H_2O. Fifty milligrams of carboxypeptidase A (Boeringer, Mannheim, Germany), washed twice with distilled water, was added to the solution and the pH was set to 8 with 25% ammonia solution. The solution was incubated for 20 hr at 37° under gentle shaking. The solution was centrifugated at 10,000 *g* for 10 min and the supernatant was decanted. Twenty-five milligrams of carboxypeptidase A (washed twice) was added to the supernatant and the solution was incubated at 37° for another 6 hr. Thereafter the solution was again centrifuged and the supernatant was then passed through a column (diameter 14 mm, length 160 mm) with an anionic exchanger (BioRad AG 1-X8, 100–200 mesh, formate form). Glycine was eluted with water (200–300 mL) until ninhydrin negative. Thereafter the γ-GC disulfide was eluted with 4 M formic acid until the eluate was ninhydrin negative. The eluate (*ca.* 200 mL) was then lyophilized. γ-GC was prepared by reducing the disulfide with dithiothreitol (DTT) as described by Anderson and Meister [18] (see also Ref. 25): 3.3 g of DTT and 24.5 mg EDTA were dissolved in 70 mL H_2O. γ-GC disulfide (3.3 g) was added and the pH was adjusted to 8 with NaOH. The mixture was placed at 20° for 1 hr. The pH was then adjusted to 2 with HCl and DTT was extracted by shaking the mixture four times with 200 mL of water-saturated ethylacetate. The aqueous phase was then lyophilized. In the reduction procedure, pH had been adjusted twice which means that NaCl had been formed/added to the mixture. In order to purify γ-GC further the product was resuspended in 50 mL of ethanol. After shaking the suspension for 5 min the undissolved salt was separated by filtration. The remaining salt was suspended in 50 mL ethanol and the procedure was repeated three times. Ethanol was then evaporated by flushing argon gas over the solution. The γ-GC was dried and stored under reduced pressure at 4°. Four grams of oxidized GSH yielded 1.8 g of γ-GC. The synthesized γ-GC gave rise to a single peak in the HPLC chromatogram (see Fig. 1). The product was found to contain less than 1% of cysteine and GSH. Injection of the product into an

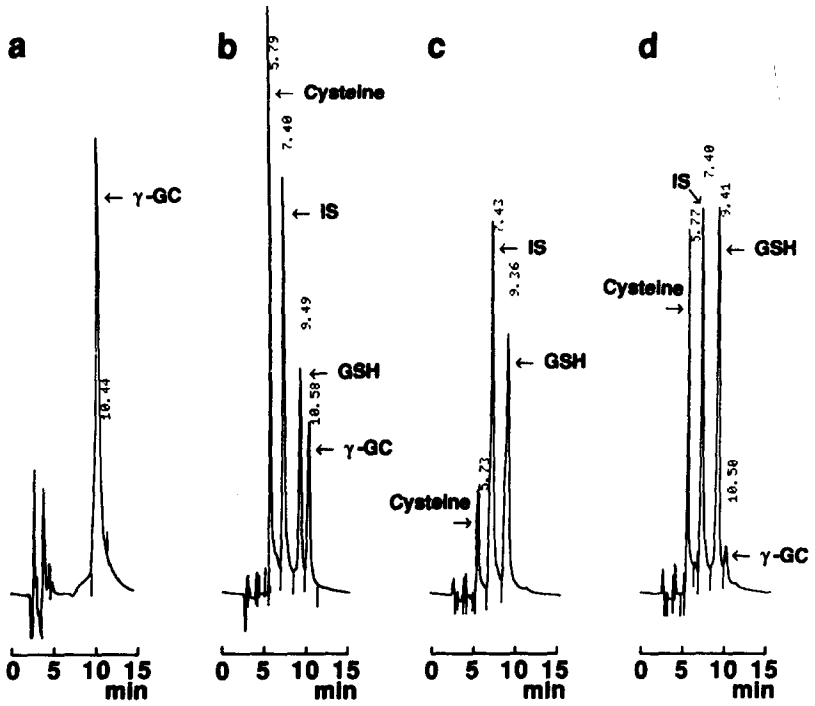

Fig. 1. HPLC chromatograms. For details regarding the HPLC system see Materials and Methods. The compounds indicated in the chromatograms are: IS, *N*-acetylcysteine; GSH, glutathione; γ-GC, γ-glutamylcysteine. Figures in connection to the peaks indicate retention times in minutes. (a) The single peak of γ-GC. The sensitivity here is 2.5 times higher than for the other runnings. (b) Standard solution; each compound in a concentration of 40 nmol/mL. (c) Extract from SN of a control animal. (d) Extract from SN of an animal which has received 2 mg of γ-GC i.c.v. 2 hr before death.

amino acid analyser revealed that the amount of glycine and glutamate was below 1% of the amount of γ-GC (Dr Mats Sandberg, Department of Histology, University of Göteborg, personal communication).

Synthesis of GSH monoethylester. The compound was synthesized exactly as described by Anderson *et al.* [26]. The product was dried under reduced pressure at 4° over $CaCl_2$ and KOH for 3 days; 2 g of GSH yielded 1.8 g of product. Analysis of the product with HPLC (see above) revealed that the product contain less than 3% GSH. In the estrification reaction small amounts of GSH diethyl ester are also formed. We could detect the diethyl ester with TLC as a small yellow spot with an R_f value of *ca.* 0.7, as compared to *ca.* 0.4 for the pink spot representing the monoethyl ester. Anderson *et al.* [26] found that the content of monoethyl ester in the product varied between 90 and 98% and that the GSH content varied between 0 and 5%. The amount of diethyl ester would then not be expected to exceed a few per cent.

RESULTS

Figure 1 shows examples of chromatograms from HPLC analyses. Figure 1a illustrates the single peak following injection of our synthesized γ-GC (10 μg/mL, equivalent to 40 nmol/mL assuming that the product contains 100% γ-GC). The sensitivity here was 2.5 times higher than in the other analyses. Cysteine and GSH could not be detected in this chromatogram. Analyses where higher sensitivity was applied (not shown) revealed that that amount of cysteine and GSH was less than 1% of the product. Figure 1b shows the HPLC chromatogram when a standard containing cysteine, *N*-acetylcysteine (internal standard), GSH and γ-GC had been injected. All compounds were injected in concentrations of 40 nmol/mL. Figure 1c shows a chromatogram from an analysis of a SN extract from a control rat. The γ-GC peak could not be detected at this sensitivity. However, with higher sensitivity γ-GC could be detected in the brains of control rats (see Fig. 2). Figure 1d shows a chromatogram from an analysis of a SN extract of a rat injected with γ-GC 2 hr before death. As can be seen, the peaks of cysteine and GSH have increased and the peak of γ-GC now appears in the chromatogram.

Figure 2 shows the effect of i.c.v. injected γ-GC (8 mg/rat) on the concentrations of γ-GC, GSH and cysteine in various brain regions at various times. In the SN and in the brain stem γ-GC produced a relatively large increase in GSH (Fig. 2a and b). Three hours after injection GSH had increased by 70% in the SN and by 55% in the brain stem. In contrast, no significant increase in GSH was observed in the striatum and in the hemispheres after injection of γ-GC (Fig. 2c and d). Very large amounts of γ-GC could

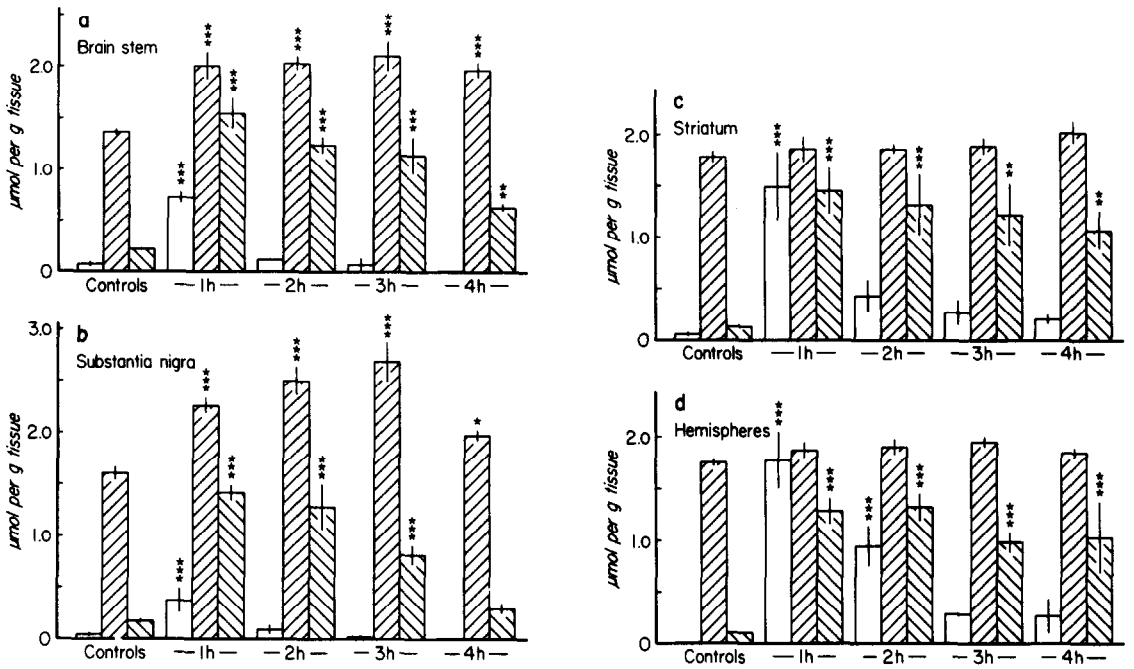

Fig. 2. Effects of i.c.v. administration of γ-GC (8 mg) at various times on the concentration of GSH in (a) rat brain stem; (b) SN; (c) striatum and (d) cerebral hemispheres. Data are means ± SEM in μmol/g wet tissue; (□) γ-GC, (▨) GSH, (▩) cysteine; number of animals = 5–8. The low control values of γ-GC (μmol/g) are here given with the exact figures: brain stem, 0.077 ± 0.037; SN, 0.063 ± 0.031; striatum, 0.047 ± 0.018; hemispheres, 0.023 ± 0.01. Statistical significances were calculated by one-way analysis of variance followed by Fisher protected least significant difference. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

be detected in the brain tissue following administration of 8 mg of γ-GC (Fig. 2); control values of γ-GC were found to be very low (the exact figures are given in the legend to Fig. 2). The highest concentrations were found 1 hr after the injection; thereafter the concentrations decreased rapidly. The level of γ-GC reached much higher concentrations in the striatum and in the hemispheres than in the SN and in the brain stem. Also the concentration of cysteine increased markedly following administration of γ-GC (Fig. 2). The highest cysteine values were found 1 hr after the administration of γ-GC, except in the hemispheres where the cysteine concentration reached maximum 2 hr after the administration. In the striatum and in the hemispheres the cysteine concentration decreased more slowly than in the SN and in the brain stem.

Figure 3 illustrates the effects of various doses of γ-GC on the GSH concentration in the brain stem and in the SN 2 hr after administration. A maximal increase in GSH of approximately 50% in the SN and approximately 45% in the brain stem was observed. In the SN, maximal effect of γ-GC on the GSH level was observed after 4 mg of γ-GC, whereas maximal GSH values in the brain stem were reached after 2 mg.

Figure 4 shows the effects of γ-GC on the concentration of GSH in the SN, the brain stem, the striatum and the hemispheres after pretreatment with L-BSO. Twenty-four hours after the administration of L-BSO the concentration of GSH had decreased to 30–45% of controls in all brain regions. Administration of 4 mg γ-GC 24 hr after L-BSO resulted in control levels of GSH at 1 hr after injection in the SN and at 3 hr after injection in the brain stem. In the striatum and in the hemispheres γ-GC administration produced only slight increases in the GSH concentration 24 hr after L-BSO: from approximately 45% of controls to approximately 60% of controls 3 hr after the γ-GC administration. It should be noted that the GSH concentration is constantly low between 24 and 48 hr after administration of L-BSO alone [10, 11].

Table 1 illustrates the effects of GSH monoethyl ester, OTC and cysteine on the concentrations of GSH and cysteine in various regions of rat brain. GSH monoethyl ester (6 mg/rat i.c.v.) had no significant effect on the concentration of GSH and cysteine in the striatum and SN. In a separate experiment the HPLC system was changed in order to determine the concentration of GSH monoethyl ester in the brain tissue. This could be performed by increasing the concentration of methanol in the buffer from 4 to 25%. High concentrations of the compound were found (1.1 ± 0.15 μmol/g; N = 3) in the striatum 2 hr after injection of 8 mg/rat i.c.v.; after 4 hr the striatal level had declined to 0.36 ± 0.05 μmol/g (N = 4).

OTC (0.6 or 1.2 g/kg s.c.) produced a significant increase in cysteine in the striatum but not in the SN 4 hr after the injection (Table 1). In contrast,

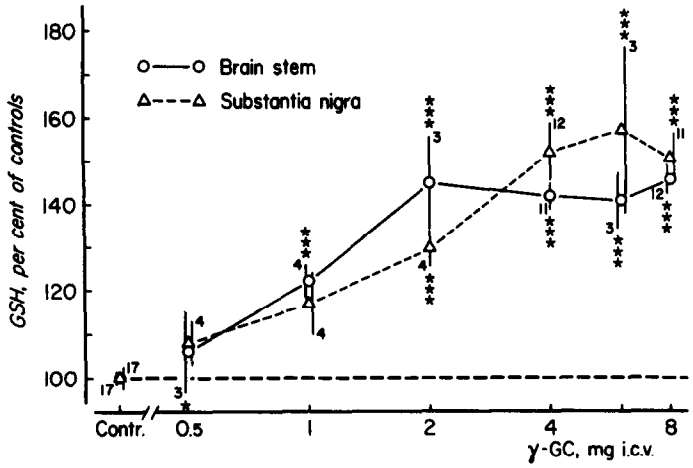

Fig. 3. Effects of various doses of i.c.v. injected γ-GC on the concentration of GSH in the brain stem (○) and the SN (△) 2 hr after administration. Data are means ± SEM in per cent of controls; the number of animals is indicated in the figure. Control values of GSH in μmol/g were: brain stem, 1.18 ± 0.06 (N = 17); SN, 1.32 ± 0.05 (N = 17). Statistical significances were calculated by one-way analysis of variance followed by Fisher protected least significant difference. *P < 0.05, **P < 0.01, ***P < 0.001.

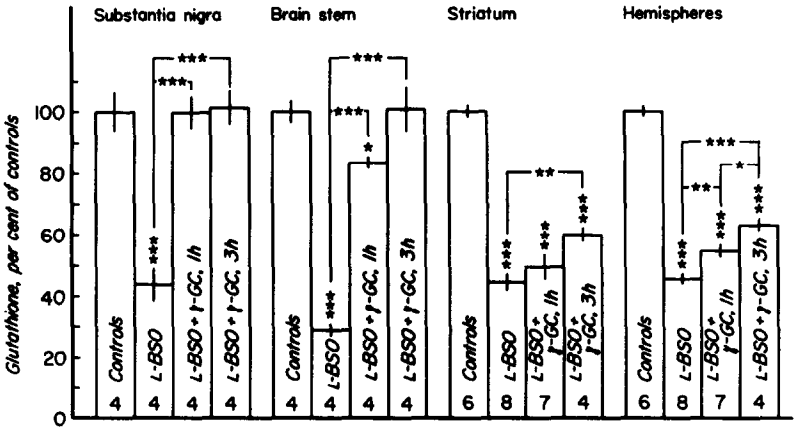

Fig. 4. Effects of γ-GC on the concentration of GSH in the SN, the brain stem, the striatum and the hemispheres after pretreatment with L-BSO. L-BSO (3.2 mg) was injected i.c.v. and after 24 hr the rats were injected with γ-GC (4 mg). One or three hours after the γ-GC injection the animals were killed. Data are means ± SEM in per cent of controls; figures in the bars indicate the number of animals. Control values of GSH in μmol/g were: brain stem, 1.22 ± 0.06; SN, 1.38 ± 0.09; striatum, 2.06 ± 0.15; hemispheres, 2.37 ± 0.10. Statistical significances were calculated by one-way analysis of variance followed by Fisher protected least significant difference. *P < 0.05, **P < 0.01, ***P < 0.001.

OTC had no significant effect on the concentration of GSH in the striatum or in the SN.

After i.p. injection of 500 mg/kg of cysteine, a massive increase in its concentration was observed in the striatum and in the limbic region but not in the SN (Table 1). In the striatum and in the SN a slight insignificant increase in the GSH concentration was observed. In the limbic region a significant increase of approximately 20% was observed 2 and 4 hr after the injection (Table 1).

Table 2 shows the effects of GSH monoethyl ester on the concentration of GSH in the striatum and in the limbic region after pretreatment with L-BSO. Twenty-four hours after the administration of L-BSO the concentration of GSH had decreased to approximately 45% of controls in both brain regions. Subsequent administration of GSH monoethyl ester (6 mg/rat; 24 hr after L-BSO) led to a slight increase in the GSH concentration (8%). In the limbic region, GSH monoethyl ester produced a somewhat larger increase in GSH (29%; Table 2).

No behavioral changes were observed after

Table 1. Effects of GSH monoethyl ester, OTC and cysteine on the concentrations of GSH and cysteine in rat brain

Drug	Brain area	1 hr GSH	1 hr Cysteine	2 hr GSH	2 hr Cysteine	4 hr GSH	4 hr Cysteine
GSH monoethyl ester (6 mg/rat i.c.v.)	Striatum	107 ± 6.5 N = 8	129 ± 6.5 N = 8	100 ± 4.5 N = 8	164 ± 23.7 N = 8	—	—
	SN	109 ± 3.9 N = 8	85 ± 17.6 N = 8	123 ± 7.3 N = 8	123 ± 27.8 N = 8	—	—
OTC (0.6 g/kg s.c.)	Striatum	—	—	—	—	111 ± 4.0 N = 4	190 ± 16.8‡ N = 4
	SN	—	—	—	—	102 ± 2.2 N = 4	109 ± 6.0 N = 4
OTC (1.2 g/kg s.c.)	Striatum	—	—	—	—	106 ± 0.9 N = 4	174 ± 5.8‡ N = 4
	SN	—	—	—	—	107 ± 2.1 N = 4	91 ± 17.1 N = 4
Cysteine (500 mg/kg i.p.)	Striatum	114 ± 6.1 N = 5	875 ± 126† N = 5	114 ± 7.5 N = 5	848 ± 229† N = 5	117 ± 5.5 N = 6	250 ± 48.4 N = 6
	Limbic	109 ± 6.0 N = 5	872 ± 63.6‡ N = 5	119 ± 1.6† N = 5	942 ± 109‡ N = 5	121 ± 4.8† N = 6	257 ± 32.8* N = 6
	SN	—	—	113 ± 6.0 N = 6	170 ± 29.6 N = 6	—	—

GSH monoethyl ester was administered i.c.v. (6 mg/rat) at various times before death. OTC was administered s.c. (0.6 or 1.2 g/kg) 4 hr before death and cysteine was injected i.p. (500 mg/kg) at various times before death. Data are means ± SEM in per cent of controls; the number of animals in each group is indicated in the table.

Control values in μmol/g wet tissue were:

GSH monoethyl ester experiment: GSH (striatum), 1.93 ± 0.11 (N = 8); GSH (SN), 1.48 ± 0.08 (N = 8); cysteine (striatum), 0.15 ± 0.02 (N = 8); cysteine (SN), 0.35 ± 0.08 (N = 8).

OTC experiment: GSH (striatum), 1.88 ± 0.04 (N = 4); GSH (SN), 1.50 ± 0.04 (N = 4); cysteine (striatum), 0.13 ± 0.05 (N = 4); cysteine (SN), 0.32 ± 0.06 (N = 4).

Cysteine experiment: GSH (striatum), 2.11 ± 0.11 (N = 7); GSH (limbic), 1.93 ± 0.06 (N = 7); GSH (SN), 1.29 ± 0.08 (N = 7); cysteine (striatum), 0.16 ± 0.04 (N = 7); cysteine (limbic region), 0.11 ± 0.03 (N = 7); cysteine (SN), 0.21 ± 0.04 (N = 7).

Statistical significances were calculated by one-way analysis of variance followed by Fisher protected least significant difference. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

Table 2. Effects of GSH monoethyl ester on the concentration of GSH in the striatum and in the limbic region after pretreatment with L-BSO

Brain area	L-BSO, 24 hr	L-BSO, 24 hr, plus GSH monoethyl ester, 0.5 hr	L-BSO, 24 hr, plus GSH monoethyl ester, 2 hr	L-BSO, 24 hr, plus GSH monoethyl ester 4 hr
Striatum	43.2 ± 1.4 N = 6	44.7 ± 0.3 N = 3	50.6 ± 1.3* N = 5	51.3 ± 2.2* N = 3
Limbic region	46.5 ± 1.9 N = 6	53.3 ± 1.5 N = 3	72.2 ± 1.4† N = 5	75.3 ± 2.3† N = 3

L-BSO (3.2 mg) was injected i.c.v. and after 24 hr the rats were injected with GSH monoethyl ester (6 mg; i.c.v.). Thirty minutes, 2 hr and 4 hr after the GSH monoethyl ester injection the animals were killed. Data are means ± SEM in per cent of controls; the number of animals is indicated in the table.

Control values of GSH in μmol/g wet tissue were: striatum, 2.26 ± 0.07; limbic region, 2.09 ± 0.13.

Statistical significances were calculated by one-way analysis of variance followed by Fisher protected least significant difference. *$P < 0.01$, †$P < 0.001$.

administration of GSH monoethyl ester, OTC or cysteine. For the first 30 min after injection of 8 mg of γ-GC short periods of convulsions were noted, but lower doses of the drug did not produce any behavioral effects.

DISCUSSION

In the present study we observed a marked and dose-dependent increase in the concentration of GSH in the brain stem and in the SN following i.c.v. injection of the GSH precursor γ-GC. This is in line with previous studies showing a marked increase in kidney GSH following systemic administration γ-GC [18]. The increase in GSH appears to be explained by conversion of γ-GC to GSH via addition of glycine, a reaction catalysed by GSH synthetase [18]. The activity of this enzyme appears not to be rate-limiting in the GSH synthesis. This is indicated by the very low concentration of γ-GC in the brain (Fig. 2), and it appears most likely that the increase in GSH observed in the present experiments is due to direct conversion of γ-GC to GSH.

A large increase in the concentration of cysteine was found after administration of γ-GC, which actually exceeded the increase in GSH found in the SN and brain stem (Fig. 2a and b). The increase in cysteine is probably explained by the fact that γ-GC is a good substrate for γ-glutamyl cyclotransferase [27, 28]. This enzyme catalyses cleavage of the dipeptide leading to formation of cysteine and 5-oxoproline; the latter is formed by cyclization of the γ-glutamyl moiety. It is via this pathway that vast amounts of 5-oxoproline is formed in patients who have GSH synthetase deficiency (5-oxoprolinuria; [29, 30]). γ-Glutamyl cyclotransferase is widely distributed in animal tissue including the brain [31]. Even though γ-GC is a substrate for both GSH synthetase and for γ-glutamyl cyclotransferase, the synthesis of GSH appears to be favoured under normal conditions [27, 32, 33]. However, when the concentration of γ-GC is increased, a relatively large percentage of the dipeptide becomes degraded by γ-glutamyl cyclotransferase. This would probably explain the vast increase in cysteine found in the present experiments.

In contrast to what was observed in the SN and in the brain stem, the GSH concentration in the striatum and in the cortical region did not increase significantly following γ-GC administration (Fig. 2). The concentration of γ-GC, on the other hand, was found to be much higher in the striatum and the cerebral hemispheres than in the SN and in the brain stem. It appears that the differences between the γ-GC values in the striatum or cortical region and the γ-GC values in the SN or brain stem 1 hr after the injection are equivalent to the amounts of GSH formed in the SN and in the brain stem. The concentration of cysteine reached approximately the same value in all four brain areas 1 hr after administration. Thus, the sum of the increase in the major thiol-containing compounds (cysteine, GSH, γ-GC) was approximately equal in all brain regions assessed. These data would argue against an uneven distribution of i.c.v. administered γ-GC, or that the uptake of the compound is different in the different brain regions. The results would rather suggest that the striatum and the hemispheres have a poorer GSH-synthesizing capacity; perhaps the concentration of GSH synthetase is lower in these regions. That γ-GC is more readily metabolized by γ-glutamyl cyclotransferase in the striatum and in the hemispheres seems unlikely because the cysteine concentration did not increase more in these brain parts than in the brain stem and SN the first hour after the administration. The difference between the brain regions in GSH-synthesizing capacity is not evident under normal conditions because similar low values of γ-GC were found in all brain regions (Fig. 2). The data in Fig. 4, showing the effect of γ-GC after the GSH concentration had been reduced by L-BSO, do also support the notion of a poorer GSH-synthesizing capacity in the striatum and in the cerebral hemispheres.

These differences between the brain regions are interesting and indicate that the brain is not homogeneous regarding GSH metabolism. We have previously noted that the GSH concentration is lower in the brain stem and in the SN than in the striatum, and that the turn-over rate of GSH is more rapid in the SN than in the striatum [11]. A lower GSH concentration in the brain stem and in the SN

than in the striatum and the hemispheres was also observed in the present experiments (Figs. 2 and 4; Table 1).

Other drugs that are known to increase the GSH concentration in peripheral tissues were also tested on their ability to increase GSH in the brain. Cysteine is a precursor of GSH and a rise in its concentration might be expected to increase the concentration of GSH. However, in line with the findings by Anderson and Meister [16], administration of cysteine did not result in more than a slight increase in GSH in one of the brain regions assessed, even though an almost 10-fold increase in the cysteine concentration was observed in the striatal and limbic regions (Table 1). The reason for this is probably that brain γ-glutamyl synthetase is almost fully saturated with cysteine under normal conditions. The activity of this enzyme, which catalyses the formation of γ-GC from cysteine and glutamate, appears to be rate-limiting for the GSH synthesis and is feed-back inhibited by GSH [34]. Surprisingly, Vina *et al.* [35] found that the GSH concentration in the brain actually decreased after cysteine administration; this is not confirmed by the present study.

OTC is another drug known to be able to increase the tissue concentration of GSH [5, 13]. OTC appears to be a substrate for 5-oxoprolinase and could be converted to cysteine and thereafter to GSH. In the present experiments we did not find any significant increase in GSH after OTC in the striatum or in the SN. The only effect of OTC that was found was an increase in the concentration of cysteine in the striatum. These data are in line with the data by Anderson and Meister [16] but they are in contrast to the results of other authors who found a slight to moderate increase in brain GSH following administration of OTC [14, 15]. The reason for this discrepancy is unknown. With respect to the observed poor effect of administered cysteine on the concentration of GSH (see above), it seems logical that a precursor of cysteine is also ineffective in this regard.

GSH monoethyl ester, administered intraperitoneally or orally, has been shown to increase the GSH content in many peripheral organs [17, 36]. The agent appears to be transported into the cell and GSH is released through hydrolysis of the ester, a reaction catalysed by intracellular esterases [17]. GSH monoethyl ester appears to partly counteract L-BSO-induced reduction in the cortical GSH concentration in newborn rats [8], but it has not been examined whether the drug has the ability to produce an actual increase in the brain concentration of GSH. To avoid problems with peripheral hydrolysis and poor passage across the blood–brain barrier, GSH monoethyl ester was injected directly into the lateral ventricles. Although high levels of the compound were detected in striatal tissue 2 hr after the injection (see Results) no significant increase in the GSH concentration in the striatum or SN was observed after the administration (Table 1). When the GSH concentration had been decreased by pretreatment with L-BSO a slight increase in the brain concentration of GSH was observed after injection of GSH monoethyl ester (Table 2), a finding in line with the data by Jain *et al.* [8]. However, the fact that GSH monoethyl ester does not increase the GSH concentration from a normal value indicates a marked difference between brain tissue and peripheral tissue. The reason for this is unclear, but perhaps the esterase activity in brain tissue is the limiting factor. In any event, the present data clearly show that GSH monoethyl ester is not at all as effective as γ-GC in increasing brain GSH.

Acknowledgements—The skilful technical assistance of Gun Andersson, Mia Eriksson and Maria Falkenberg is gratefully acknowledged. We thank Dr Håkan Wikström for advice regarding the preparation of γ-GC. This study was supported by grants from The Swedish Medical Research Council (No. 9076), "Åke Wibergs Stiftelse", "Loo och Harald Ostermanns Fond", "Stiftelsen Lars Hiertas Minne", "Torsten och Ragnar Söderbergs Stiftelser", "Stiftelsen Sigurd och Else Goljes Minne" and "Stiftelsen Handlanden Hjalmar Svenssons Forskningsfond".

REFERENCES

1. Cohen G, The pathobiology of Parkinson's disease: biochemical aspects of dopamine neuron senescence. *J Neural Transm.* (suppl) **19**: 89–103, 1983.
2. Booth J, Boyland E and Sims P, An enzyme from rat liver catalysing conjugations with glutathione. *Biochem J* **79**: 516–524, 1961.
3. Kosower NS and Kosower EM, The glutathione status of cells. *Int Rev Cytol* **54**: 109–160, 1978.
4. Orlowski M and Karkowsky A, Glutathione metabolism and some possible functions of glutathione in the nervous system. *Int Rev Neurobiol* **19**: 75–121, 1976.
5. Meister A, Methods for the selective modification of glutathione metabolism and study of glutathione transport. *Methods Enzymol* **113**: 571–585, 1985.
6. Griffith OW and Meister A, Glutathione: interorgan translocation, turnover, and metabolism. *Proc Natl Acad Sci USA* **76**: 5606–5610, 1979.
7. Slivka A, Spina MB, Calvin HI and Cohen G, *J Neurochem* **50**: 1391–1393, 1988.
8. Jain A, Mårtensson J, Stole E, Auld PA and Meister A, Glutathione deficiency leads to mitochondrial damage in brain. *Proc Natl Acad Sci USA* **88**: 1913–1917, 1989.
9. Steinherz R, Mårtensson J, Wellner D and Meister A, Transport into brain of buthionine sulfoximine, an inhibitor of glutathione synthesis, is facilitated by estrification and administration of dimethylsulfoxide. *Brain Res* **518**: 115–119, 1990.
10. Pileblad E and Magnusson T, Intracerebroventricular administration of L-buthionine sulfoximine; a method for depleting brain glutathione. *J Neurochem* **53**: 1878–1882, 1989.
11. 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.
12. Pileblad E, Fornstedt B and Magnusson T, Reduction of brain glutathione by L-buthionine sulfoximine potentiates the dopamine-depleting action of 6-hydroxydopamine in rat striatum. *J Neurochem* **52**: 978–980, 1989.
13. Williamson JM and Meister A, Stimulation of hepatic glutathione formation by administration of L-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. *Proc Natl Acad Sci USA* **78**: 936–939, 1981.
14. Weiner HJ, Hashim A, Lajtha A and Sershen H, (−)-2-Oxo-4-thiazolidine-carboxylic acid attenuates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced

neurotoxicity. *Res Commun Subst Abuse* **9**: 53–60, 1988.
15. Mesina JE, Page RH, Hetzel FW and Chopp M, Administration of L-2-oxo-4-thiazolidine-carboxylate increases glutathione levels in rat brain. *Brain Res* **478**: 181–183, 1989.
16. Anderson ME and Meister A, Marked increase in cysteine levels in many regions of the brain after administration of 2-oxothiazolidine-4-carboxylate. *FASEB J* **3**: 1632–1636, 1989.
17. Meister A, Anderson ME and Hwang O, Intracellular cysteine and glutathione delivery systems. *J Amer Coll Nutr* **5**: 137–151, 1986.
18. Anderson ME and Meister A, Transport and direct utilization of γ-glutamylcyst(e)ine for glutathione synthesis. *Proc Natl Acad Sci USA* **80**: 707–711, 1983.
19. Garcia-Sevilla JA, Ahtee L, Magnusson T and Carlsson A, Opiate-receptor mediated changes in monoamine synthesis in rat brain. *J Pharm Pharmacol* **30**: 613–621, 1978.
20. Carlsson A and Lindqvist M, *In-vivo* measurements of tryptophan and tyrosine hydroxylase activities in mouse brain. *J Neural Transm* **34**: 79–91,1973.
21. Bioanalytical Systems Inc., LCEC application note 46: glutathione in whole blood, 1982.
22. Allison L and Shoup R, Dual electrode liquid chromatography for thiols and disulfides. *Anal Chem* **55**: 8–12, 1983.
23. Demaster E, Shirota F, Redfern B, Goon D and Nagasawa H, Analysis of hepatic reduced glutathione, cysteine and homocysteine by cation-exchange high-performance liquid chromatography with electrochemical detection. *J Chromatogr* **308**: 83–91, 1984.
24. Strumeyer D and Bloch K, γ-L-Glutamyl-L-cystein from glutathione. In: *Biochemical Preparations* (Eds. Swenson RT and Hill RL), Vol. 9, pp. 52–58. Wiley, New York, 1962.
25. Butler J, Spielberg SP and Schulman JD, Reduction of disulfide-containing amines, amino acids, and small peptides. *Anal Biochem* **75**: 674–675, 1976.
26. Anderson ME, Powrie F, Puri RN and Meister A, Glutathione monoethyl esters: preparation, uptake by tissues and conversion to glutathione. *Arch Biochem Biophys* **239**: 538-548, 1985.
27. Orlowski M and Meister A, Isolation of highly purified gamma glutamyl cysteine synthetase from rat kidney. *Biochemistry* **10**: 372–380, 1971.
28. Palekar AG, Tate SS and Meister A, Formation of 5-oxoproline from glutathione in erythrocytes by the γ-glutamyltranspeptidase-cyclotransferase pathway. *Proc Natl Acad Sci USA* **71**: 293–297, 1974.
29. Wellner VP, Sekura R, Meister A and Larsson A, Glutathione synthetase deficiency, an inborn error of metabolism involving the γ-glutamyl cycle in patients with 5-oxoprolinuria (pyroglutamic aciduria). *Proc Natl Acad Sci USA* **71**: 2505-2509, 1974.
30. Larsson A and Mattsson B, On the mechanism of 5-oxoproline overproduction in 5-oxoprolinuria. *Clin Chim Acta* **67**: 245–253, 1976.
31. Orlowski M and Meister A, γ-Glutamyl cyclotransferase: distribution, isozymic forms, and specificity. *J Biol Chem* **248**: 2836-2844, 1973.
32. Orlowski M and Wilk S, Intermediates in the γ-glutamyl cycle in mouse tissues: influence of administration of amino acids and pyrrolidone carboxylate and γ-glutamyl amino acids. *Eur J Biochem* **53**: 581–590, 1975.
33. Smith JE, Moore K and Board PG, Regulation of γ-glutamylcysteine utilization in erythrocytes. *Enzyme* **25**: 236–240, 1980.
34. Richman PG and Meister A, Regulation of γ-glutamylcysteine synthetase by nonallosteric feedback inhibition of glutathione. *J Biol Chem* **250**: 1422–1426, 1975.
35. Vina J, Romero FJ, Saez GT and Pallardo FV, Effects of cysteine and *N*-acetyl cysteine on GSH content of brain of adult rats. *Experentia* **39**: 164–165, 1983.
36. Anderson ME and Meister A, Glutathione monoesters. *Anal Biochem* **183**: 16–20, 1989.