Biochemical Pharmacology, Vol. 38, No. 9, pp. 1499–1505, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

SELECTIVE POTENTIATION BY L-CYSTEINE OF APPARENT BINDING ACTIVITY OF [^{3}H]GLUTATHIONE IN SYNAPTIC MEMBRANES OF RAT BRAIN

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(*Received* 27 *January* 1988; *accepted* 12 *September* 1988)

Abstract—Significant apparent binding activity of [^{3}H]glutathione was detected in synaptic membranous preparations of the rat brain. *In vitro* addition of sucrose (50–1000 mM) and Triton X-100 (0.02–0.1%) significantly diminished the apparent binding activity, whereas pretreatment of the membranes with Triton X-100 (0.01–0.4%) did not affect the activity. A slight but statistically significant reduction of the apparent binding activity was induced by the *in vitro* addition (1 mM) of two constituent amino acids, L-glutamic acid and glycine. In contrast, another constituent amino acid, L-cysteine, potently enhanced the binding activity at a concentration higher than 0.1 mM. No prominent alteration of the activity occurred following the inclusion of structurally-related amino acids, dithiothreitol, dithioerythritol and numerous other amino acids. Scatchard analysis revealed that the apparent binding consisted of two independent separate components with K_d values of 0.76 and 11.0 μM, and B_{max} values of 4.00 and 27.0 pmol/mg protein respectively. *In vitro* addition of 1 mM L-cysteine resulted in a single component with a K_d of 8.5 μM and a B_{max} of 105 pmol/mg protein. Pretreatment of the membranes with 1 mM L-cysteine potentiated the apparent binding, with a further addition of L-cysteine having no effect. The retina had the highest activity followed by the hypothalamus, striatum, spinal cord, midbrain, hippocampus, medulla-pons, cerebellum and cerebral cortex, which occurred independently of the incubation temperature. In peripheral organs examined, the pituitary possessed higher activity than the retina, with progressively lower activities in the adrenal, liver, spleen, skeletal muscle and heart. No significant activity was detected in the kidney. Addition of 1 mM L-cysteine significantly potentiated the activities at 30°, but not at 2°, in the hippocampus and cerebral cortex without affecting those in other central structures. In contrast, a profound inhibition of the activity was induced by the addition of L-cysteine in the pituitary, adrenal, intestinal mucosa, skeletal muscle and retina independently of the temperature. These results suggest that L-cysteine may selectively potentiate the apparent binding activity of [^{3}H]glutathione in particular regions of the brain, while eliminating that in the peripheral excitable tissues.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine) is an endogenous tripeptide consisting of L-glutamic acid (Glu), L-cysteine (CySH) and glycine (Gly). Although two of these constituent amino acids are supposed to be neurotransmitters in the mammalian central nervous system (CNS), relatively little attention has been paid to the specific functional role of this peptide in synaptic neurotransmission in the brain. The rodent brain contains a large amount of glutathione in synaptosomes [1]. The reduced form (GSH) of the peptide is shown to play an important physiological role in the maintenance of cellular integrity [2, 3]. In addition, evidence is accumulating that some endogenous peptides enriched in acidic amino acids are involved in the neurotransmission at central Glu-ergic synapses. For example, *N*-acetyl-L-aspartyl-L-glutamic acid (NAAG) potently inhibits [^{3}H]Glu binding [4] with concomitant convulsive seizures similar to those produced by an agonist for Glu receptors, quisqualic acid, when injected into the rat hippocampus [5]. This endogenous dipeptide has been proposed to be the neurotransmitter in the lateral olfactory tract [6].

On the other hand, we have demonstrated recently that both GSH and oxidized glutathione (GSSG) potently displace Na^+-dependent and -independent binding of [^{3}H]Glu in synaptic membranous preparations of the rat brain [7]. This inhibition occurs independently of the incubation temperature in a concentration-dependent manner [8]. In accord with these findings, we have also raised the possibility that synaptic membranes may contain specific binding sites of [^{3}H]GSH which exhibit protein-dependency, structure-selectivity, temperature-sensitivity, high affinity and saturability [9, 10]. This apparent binding activity is displaced significantly by some GSH derivatives without SH-moieties such as GSSG, *S*-methyl-glutathione and *S*-hexyl-glutathione, suggesting no participation of the disulfide bonding between membranous SH-residues and SH-moieties of the ligand in the apparent binding [9]. Similarly prominent inhibition is induced by the addition of various α- and γ-peptides containing L-Glu, but not by those containing D-Glu [9]. The apparent binding sites seem to consist of two independent separate components, such as temperature-independent high-affinity sites and temperature-dependent low-affinity sites [10]. In the present study, we have attempted

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to characterize further the apparent binding activity of [^{3}H]GSH in order to elucidate its functional significance.

MATERIALS AND METHODS

Materials. GSH, GSSG, NAAG and other amino acids were all obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{3}H]GSH (glycine-2-[^{3}H]-glutathione, 1 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Other chemicals used were all of the highest purity commercially available.

[^{3}H]*GSH binding.* Extensively washed synaptic membranes obtained from the brains of male Wistar rats weighing 200–250 g [11] were frozen at −80° in 0.32 M sucrose. Frozen suspensions were thawed at room temperature and washed twice with 50 vol. of 50 mM Tris–acetate buffer (pH 7.4) by centrifuging at 50,000 *g* for 30 min at 2° before each use. The final suspensions (approximately 350–400 μg protein) were incubated with 100 nM [^{3}H]GSH in 500 μl of 50 mM Tris–acetate buffer (pH 7.4) at 30° for 60 min unless indicated otherwise. Incubation was terminated by the addition of 3 ml of ice-cold buffer and subsequent rapid filtration through a Whatman GF/B glass filter under a constant vacuum of 15 mm Hg. The filter was washed four times with 3 ml of cold buffer within 10 sec, and radioactivity trapped on the filter was measured by a liquid scintillation spectrometer (LSC 900, Aloka, Japan) using 5 ml of modified Trition–toluene scintillant at a counting efficiency of 40–42% [11].

For Scatchard analysis, membrane suspensions were incubated with 100 or 200 nM [^{3}H]GSH in the presence of various concentrations of nonradioactive GSH over the range from 0 to 9,800 nM. Kinetic parameters such as K_d and B_{max} were calculated from the equation estimated by a personal computer (PC9801, NEC, Japan) with a nonlinear regression analysis program. Nonspecific and nonsaturable binding was obtained from the radioactivity found in the presence of 1 mM GSH and subtracted from each experimental value to calculate the specific binding as described previously [9]. Binding assays were carried out in triplicate with a variation of less than 10%. Protein content was determined by the method of Lowry *et al.* [12]. More than 90% (95.7 ± 3.7%) of the radioactivity in the incubation medium after incubation at 30° for 60 min co-migrated with the authentic GSH when analyzed by thin-layer chromatography on cellulose-coated plates [9].

Pretreatment of membranes. Membranes were preincubated with 1 mM L-CySH at 30° for 30 min, and the preincubation was terminated by centrifugation at 50,000 *g* for 30 min. The resultant pellets were suspended in the buffer and the suspensions were again centrifuged as above. The final pellets were resuspended in the buffer, and the suspensions were subjected to the routine [^{3}H]GSH binding assay.

Regional distribution. Each brain region was disssected on a chilled plastic plate according to the procedures described by Glowinski and Iversen [13]. These central structures and peripheral tissues were homogenized individually in 50 vol. of ice-cold, distilled, deionized and sterilized water with the aid of a Polytron homogenizer at setting 6 for 1 min. Homogenates were washed three times with the buffer by centrifuging as mentioned above. The final pellets were suspended in 0.32 M sucrose and frozen at −80° for 14–16 hr. Frozen suspensions were thawed and washed as above before the assay.

Statistical analysis. Results were usually expressed as the mean ± SE and statistical significance was determined by Student's *t*-test.

RESULTS

Biochemical characteristics. Significant apparent binding activity of [^{3}H]GSH was detected in synaptic membranous preparations of the rat brain to a lesser extent at 2° than that found at 30° (2°, 337 ± 26 fmol/mg protein; 30°, 888 ± 104 fmol/mg protein). To evaluate the functional significance of the apparent binding, the effect of *in vitro* addition of sucrose and Triton X-100 was examined (Fig. 1). Increasing concentrations of sucrose gradually decreased the activity up to 200 mM. A potent inhibition occurred following the addition of sucrose at concentrations higher than 200 mM (Fig. 1A). Triton X-100 at 0.02–0.1% also significantly reduced the apparent binding activity (Fig. 1B). However, the detergent was unable to induce complete abolition even at the highest concentration employed.

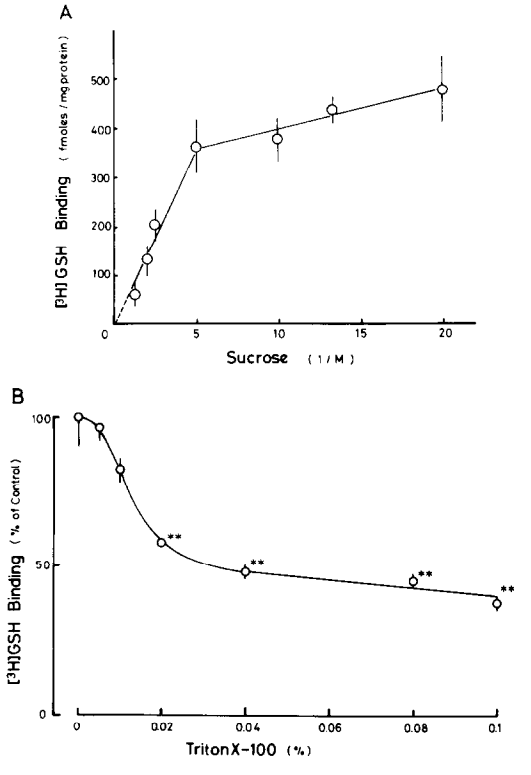

Fig. 1. Effect of *in vitro* addition of (A) sucrose and (B) Triton X-100 on apparent binding of [^{3}H]GSH. Synaptic membranes were incubated with [^{3}H]GSH in the presence and absence of various concentrations of (A) sucrose or (B) Triton X-100. Values (mean ± SE) were obtained from six separate experiments. Key: (**) $P < 0.01$, compared with each control value. Control binding: 810 ± 80 fmol/mg protein.

Membranes were preincubated with various concentrations (0.01–0.4%) of Triton X-100 at 2° for 10 min, and the preincubation was terminated by centrifugation. The resultant pellets were again washed with the buffer. This pretreatment of the synaptic membranes with Triton X-100 did not affect significantly the apparent binding of [^{3}H]GSH at 30° (Fig. 2). In addition, no profound change was caused by pretreatment with 0.4% Triton X-100 in the binding activity found at 2° (337 ± 26 vs 370 ± 32 fmol/mg protein). These results suggest that apparent binding of [^{3}H]GSH may reflect, at least in part, the association of ligand with the detergent-insensitive binding sites in the brain synaptic membranes.

Effect of constituent amino acids. The apparent binding was not affected significantly by the *in vitro* addition of NAAG at concentrations from 10^{-6} to 10^{-3} M (data not shown). No prominent change occurred following the inclusion of some inorganic ions, including 20 mM NH_4Cl, 2.5 mM $Ca(CH_3COO)_2$, 20 mM NH_4Cl + 2.5 mM $Ca(CH_3COO)_2$ and 100 mM CH_3COONa (data not shown). Figure 3A shows the effect of three constituent amino acids on the apparent binding of [^{3}H]GSH. Both L-Glu and Gly significantly inhibited the apparent binding at the highest concentration used. In contrast, L-CySH potently augmented the activity in the concentration range higher than 10^{-4} M. No profound change was induced by the inclusion of similar concentrations of other SH-containing compounds, such as L-cysteinesulfinic acid, L-cysteic acid, L-cystine, hypotaurine, taurine, L-cystathionine, DL-homocysteine, L-methionine, dithiothreitol and dithioerythritol (Fig. 3B). None of numerous other amino acids had a significant effect on the apparent binding at similar concentrations: these included L-alanine, γ-aminobutyric acid, L-arginine, L-aspargine, L-aspartic acid, L-glutamine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine (data not shown). These results suggest that L-CySH may selectively potentiate the apparent binding of [^{3}H]GSH in the synaptic membranes.

L-CySH-induced potentiation. In the absence of added L-CySH, Scatchard analysis did not give a straight line but rather a hyperbola ($K_{d_1} = 0.56 \pm 0.11\ \mu M$, $B_{max_1} = 2.5$ pmol/mg protein; $K_{d_2} = 12.6 \pm 3.7\ \mu M$, $B_{max_2} = 20.5$ pmol/mg protein). In the presence of 1 mM L-CySH, however, a straight line was obtained from the Scatchard analysis ($K_d = 8.5 \pm 0.8\ \mu M$, $B_{max} = 105 \pm 13$ pmol/mg protein). L-CySH at 1 mM apparently abolished the high-affinity sites with a concomitant increment of the number of the low-affinity sites (Fig. 4). Synaptic membranes were preincubated in the presence and absence of 1 mM L-CySH accompanied by centrifuging twice. Resultant pellets were resuspended in the buffer and the suspensions were incubated with [^{3}H]GSH in the presence of various concentrations of L-CySH. L-CySH significantly potentiated the apparent binding at a concentration higher than 10^{-4} M in the membranes not treated previously with 1 mM L-CySH. Preincubation with 1 mM L-CySH resulted in a significant potentiation of the apparent binding in the absence of added L-CySH, and *in vitro* addition of L-CySH failed to induce an additional enhance-

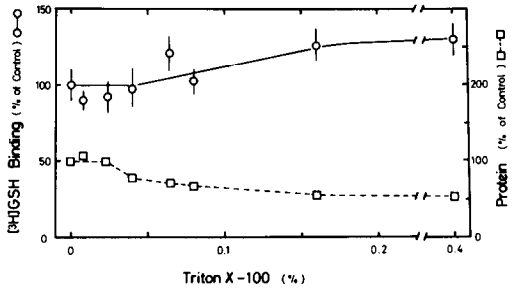

Fig. 2. Effect of pretreatment with Triton X-100 on apparent binding of [^{3}H]GSH. Synaptic membranes were treated with the indicated concentrations of Triton X-100 at 2° for 10 min. Binding assays were carried out as described in the text. Values (mean ± SE) were obtained from four to six separate experiments. Control binding: 830 ± 86 fmol/mg protein.

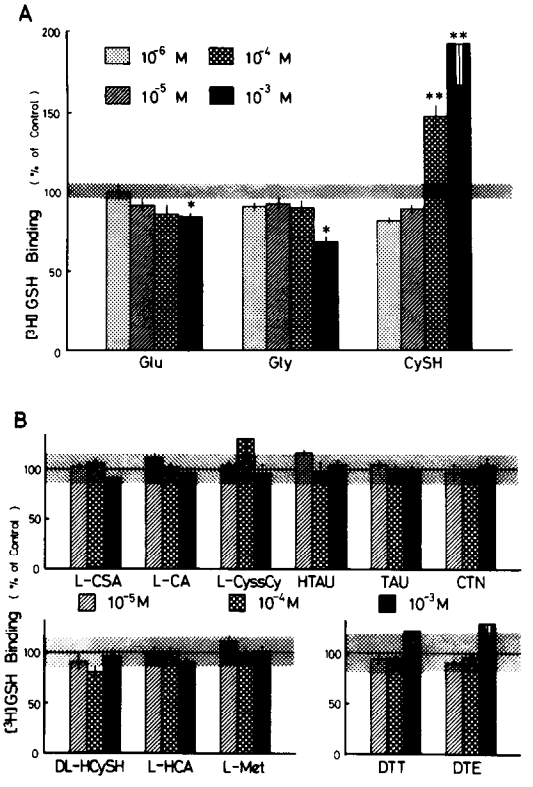

Fig. 3. Effect of (A) three constituent amino acids and (B) various sulfur-containing compounds on apparent binding of [^{3}H]GSH. Synaptic membranes were incubated in the presence of various concentrations of the compounds indicated. Each value (mean ± SE) was obtained from four to six separate experiments. Key: (*) $P < 0.05$ and (**) $P < 0.01$, compared with each control value. Control binding: 1033 ± 53 fmol/mg protein. Abbreviations: L-CA, L-cysteic acid; L-CSA, L-cysteinesulfinic acid; CTN, cystathionine; CySH, L-cysteine; CyssCy, L-cystine; DTE, dithioerythritol; DTT, dithiothreitol; Glu, L-glutamic acid; Gly, glycine; L-HCA, L-homocysteic acid; DL-HCySH, DL-homocysteine; HTAU, hypotaurine; L-Met, L-methionine; and TAU, taurine.

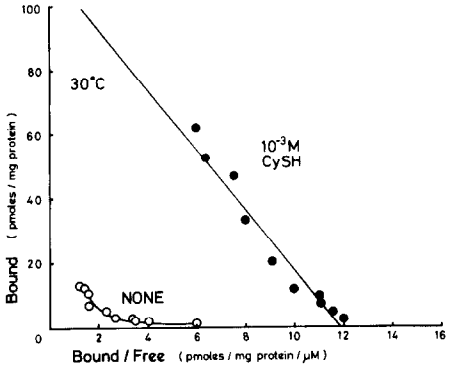

Fig. 4. Scatchard analysis of apparent binding of [^{3}H]GSH. Membranes were incubated with 100 or 200 nM [^{3}H]GSH in 50 mM Tris–acetate buffer containing various concentrations of nonradioactive GSH over the concentration range from 0 to 9800 nM, in the presence and absence of 1 mM L-CySH. Values were obtained from six independent experiments.

ment in these membranes pretreated with 1 mM L-CySH (Fig. 5A). Figure 5B shows the effect on the CySH-induced potentiation of three amino acids which are taken up by the same transport carriers as L-CySH. These amino acids were found not to have any significant effect on the apparent binding at a concentration range from 10^{-5} to 10^{-3} M. In addition, none of these amino acid substrates affected the CySH-induced augmentation (Fig. 5B). These data suggest that the transport system for L-CySH may not be responsible for the L-CySH-induced potentiation which is an irreversible phenomenon.

To characterize further the CySH-induced potentiation, the effect of Triton pretreatment was examined. Membranes were preincubated with 0.4% Triton X-100 for various periods, as indicated in Fig. 6. It was found that this Triton treatment gradually increased the basal activity with a concomitant loss of the stimulatory effect of L-CySH. Therefore, the effect of L-CySH seems to be sensitive to a detergent.

Regional variations. Apparent binding activity of [^{3}H]GSH was unevenly distributed in both central and peripheral structures. In the CNS, the retina possessed the highest apparent binding activity with progressively lower activities in the hypothalamus, striatum, spinal cord, midbrain, hippocampus, medulla-pons, cerebellum and cerebral cortex, which occurred independently of the incubation temperature (Fig. 7). *In vitro* addition of 1 mM L-CySH significantly enhanced the apparent binding activity determined at 30° in the hippocampus and cerebral cortex, while inhibiting that in the retina. At 2°, L-CySH failed to potentiate the binding activity in the former two central regions with a concurrent inhibition of that found in the retina and hypothalamus. L-CySH at 1 mM did not induce any detectable changes of the activities at both temperatures in the other central structures (Fig. 7). In addition to the CNS, an apparent binding activity was also detected in some peripheral tissues. The pituitary was found to contain much higher activity than that in the retina independently of the incu-

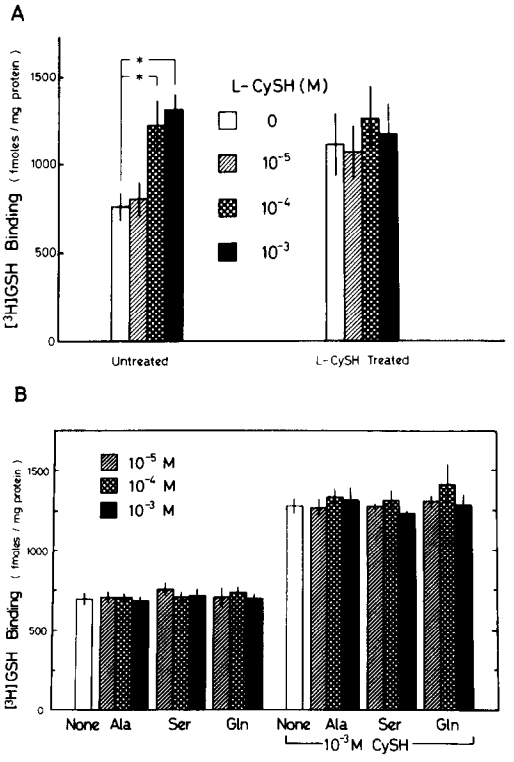

Fig. 5. Effect of (A) pretreatment with CySH and (B) *in vitro* addition of ASC transport substrates on apparent binding of [^{3}H]GSH. (A) Synaptic membranes were pretreated with 1 mM L-CySH as described in the text. Apparent binding activity of [^{3}H]GSH was determined in the presence of various concentrations of L-CySH as indicated. Each value (mean ± SE) was obtained from four separate experiments. Key: (*) $P < 0.05$, compared with each control value. (B) [^{3}H]GSH binding assays were carried out in the buffer containing various concentrations of L-alanine (Ala), L-serine (Ser) or L-glutamine (Gln) in the presence and absence of 1 mM L-CySH. Values (mean ± SE) were obtained from four separate experiments.

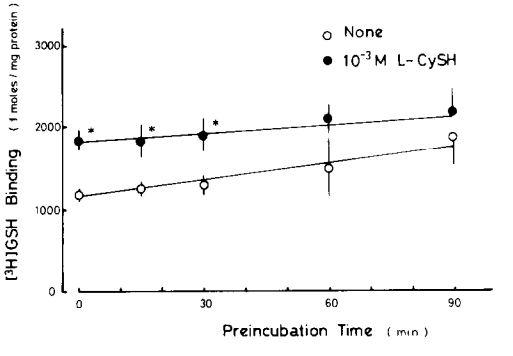

Fig. 6. Effect of pretreatment with Triton X-100 on CySH-induced potentiation of apparent binding of [^{3}H]GSH. Membranes were pretreated with 0.4% Triton X-100 at 2° for various periods indicated. Routine binding assays were performed by using these membranes in the presence and absence of 1 mM L-CySH. Values (mean ± SE) were obtained from four independent experiments. Key: (*) $P < 0.05$, compared with each control value.

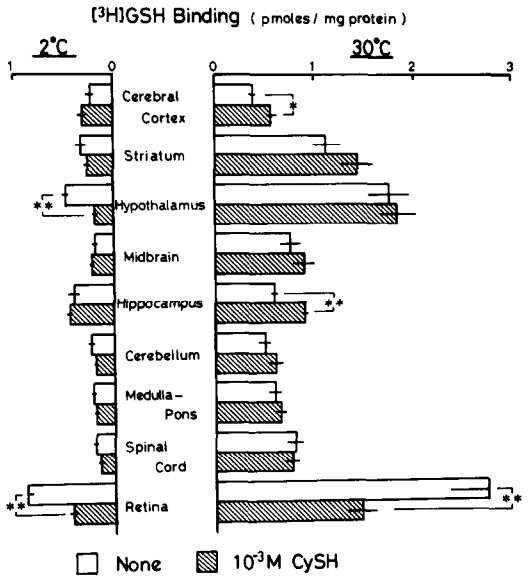

Fig. 7. Effect of CySH on distribution profile of apparent binding of [3H]GSH in central structures. Homogenate particulate matter (200–300 μg protein) of each central region was incubated with [3H]GSH at 2° or 30° in the presence and absence of 1 mM CySH. Each value (mean ± SE) was obtained from eight independent experiments. Tissues from four animals were used to detect the activity in the retina. Key: (*) $P < 0.05$, and (**) $P < 0.01$, compared with each control value.

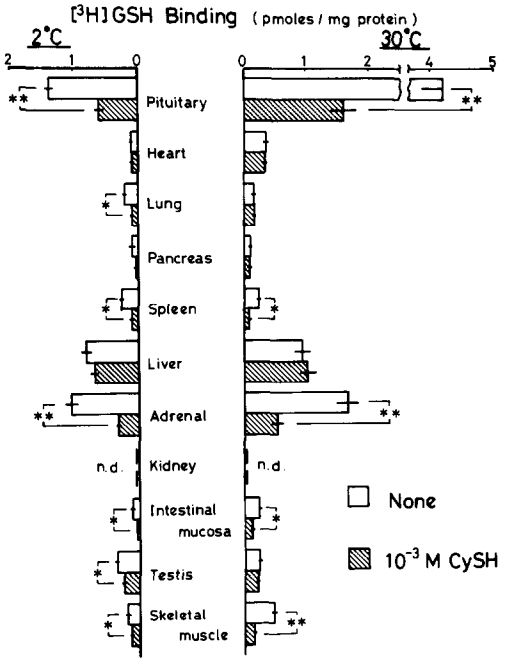

Fig. 8. Effect of CySH on the distribution profile of apparent binding of [3H]GSH in peripheral structures. Homogenate particulate matter (200–300 μg protein) of individual organs was incubated with [3H]GSH at 2° or 30° in the presence and absence of 1 mM CySH. Values (mean ± SE) were obtained from eight separate experiments. Tissues from four rats were used for detecting the activities in the small structures such as the pituitary and adrenal. Key: (*) $P < 0.05$ and (**) $P < 0.01$, compared with each control value; n.d., not detectable.

bation temperature, followed by the adrenal, liver, spleen, skeletal muscle and heart (Fig. 8). Relatively low activities were found in the lung, intestinal mucosa, testis and pancreas, but the kidney had no detectable activity. Inclusion of 1 mM L-CySH drastically diminished the apparent binding activities at both temperatures in the pituitary, adrenal, intestinal mucosa and skeletal muscle (Fig. 8). Binding activities detected at 2° in the lung and testis were also inhibited significantly by the addition of 1 mM L-CySH. These results suggest that the effect of L-CySH may be distinctly different in the central and peripheral structures.

DISCUSSION

A couple of findings support the proposal that apparent binding of [3H]GSH, at least in part, reflects the association of ligand with the binding sites in the brain synaptic membranes. For instance, the apparent binding activity was detectable under experimental conditions unfavorable to the temperature- and energy-dependent transport process. The apparent binding was absent from the kidney which undoubtedly possesses GSH transport capacity [14]. The fact that [3H]GSH was not converted into [3H]GSSG does not support the possibility that the apparent binding may represent the association of [3H]GSH with its metabolizing enzyme(s) in the preparations. The lack of a need for γ-peptide structure [9] is also unfavorable to the possible involvement of GSH-metabolizing enzymes in the apparent binding.

Several lines of evidence have suggested the possible physiological importance of GSH in the mammalian CNS, such as (1) enrichment in the synaptosomal fractions [1], (2) possible involvement in convulsive seizures [15, 16] and Parkinson's disease [17], (3) reduction of brain content by ischemia [18] and (4) mental retardation in an inherently deficient patient [19]. Both GSH and GSSG also exhibit a concentration-dependent and temperature-independent displacement of [3H]Glu binding in brain synaptic membranes [8], which coincides well with the association of this putative excitatory amino acid neurotransmitter with its physiologically relevant synaptic receptors. Considering these previous findings along with the present results, it seems possible to speculate that glutathione may play some specific and positive functional role other than maintenance of cellular integrity in the central synaptic neurotransmission through association with the apparent binding sites demonstrated here.

It is noteworthy that L-CySH selectively potentiated the apparent binding, with L-cystine having no effect. This selective potentiation seems to be derived not from the SH-moiety, but from the molecular structure of L-CySH itself. L-CySH has been proposed to be transported into various cells via the ASC transport system [20, 21]. Since none of other substrates for this transport system, including L-alanine, L-serine and L-glutamine, affected the poten-

tiation by L-CySH, it seems unlikely that the ASC transport system may be responsible for the selective and irreversible potentiation of the apparent binding by *in vitro* L-CySH. L-CySH may directly augment the apparent binding by way of acting at the membranous surfaces without being oxidized into its dimeric form. The presence of an exchange system between L-cystine and L-Glu across plasma membranes has been demonstrated recently in human fibroblasts [22]. L-Cystine has also been shown to potently inhibit the Cl^--dependent binding of [^{3}H]Glu, which may reflect the amino acid transport system, x_c^- [23]. These previous observations, together with the fact that neither L-cystine nor L-Glu potently diminished the apparent binding of [^{3}H]GSH, raise the possibility that apparent [^{3}H]GSH binding sites may interact with [^{3}H]Glu binding sites. In fact, a significant inhibition of apparent [^{3}H]GSH binding occurs following the addition of various displacers of [^{3}H]Glu binding, such as L-Glu-containing peptides and agonists and antagonists of Glu receptors, but not of peptides containing D-Glu [9]. Functional significance of endogenous sulfur-containing amino acids, including L-CySH, L-cystine and glutathione, in the brain should be re-evaluated in future studies.

L-CySH at 1 mM drastically increased the number of apparent low-affinity binding sites, with a concomitant elimination of the high-affinity sites. The high-affinity sites exhibited a temperature-independency, whereas the low-affinity sites were entirely dependent on the incubation temperature. In addition, *S*-methyl-glutathione eliminates the high-affinity sites without significantly affecting the low-affinity sites [9]. These results all support the assumption that the low-affinity sites may play a physiological role distinctly different from that of the high-affinity sites. It is still conceivable that the low-affinity and temperature-dependent apparent binding sites may represent an energy- and temperature-dependent low-affinity uptake process of GSH as observed in the kidney [14]. GSH may play dual specific functional roles in the CNS through association with the temperature-independent high-affinity binding sites, in addition to interacting with the CySH-sensitive and temperature-dependent low-affinity transport sites. In fact, the latter sites exhibited typical biochemical characteristics similar to those of a sequestration process, such as temperature dependency, detergent and osmolarity sensitivity and substantially high capacity.

One of the interesting findings obtained in this study is that L-CySH differentially affected the apparent binding in central and peripheral structures. A 1 mM concentration of CySH potentiated the apparent binding in the brain, possibly through acting on the low-affinity sites, while inhibiting that in the peripheral tissues. L-CySH-induced potentiation was detected exclusively in the cortex and hippocampus amongst various brain regions. A similar distribution profile was observed with [^{3}H]Glu binding. The cerebral cortex and hippocampus have a relatively low basal binding activity of [^{3}H]Glu, whereas sodium ions most potently enhance [^{3}H]Glu binding in these two structures [24]. These findings again support the possible interaction of apparent binding sites of [^{3}H]GSH with [^{3}H]Glu binding sites. The L-CySH-induced potentiation might be related, at least in part, to the Na^+-dependent binding of [^{3}H]Glu which is a biochemical measure for an energy-dependent uptake process of this neurotransmitter [25].

On the other hand, L-CySH significantly diminished the apparent binding in the peripheral excitable tissues including the pituitary, adrenal, intestinal mucosa and skeletal muscle. Several independent lines of evidence have suggested the possible presence of [^{3}H]Glu binding sites in some peripheral excitable tissues such as the pituitary [26], adrenal [27] and pineal [28]. We have also demonstrated that sodium ions prominently suppress these peripheral bindings of [^{3}H]Glu [29]. These previous findings, along with the present results, suggest a potential relationship between L-CySH-induced potentiation of apparent binding of [^{3}H]GSH and Na^+-dependent binding of [^{3}H]Glu. These aforementioned findings all maintain the hypothesis that glutathione may play some important specific functional role in the central and/or peripheral synaptic neurotransmission through associating with its own binding site, in addition to interacting with [^{3}H]Glu binding sites. It seems likely that central apparent binding of [^{3}H]GSH may be distinctly different from that in the peripheral excitable tissues in terms of the effect of L-CySH.

In summary, it appears that apparent binding of [^{3}H]GSH is unevenly distributed in the central and peripheral structures. L-CySH may be useful in differentiating these apparent bindings. The functional role of endogenous sulfur-containing compounds such as L-CySH and glutathione may need to be re-evaluated.

Acknowledgements—This work was supported in part by a Grant-in-Aid for Scientific Research (63772026) to K. O. from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Reichelt KL and Fonnum F, Subcellular localization of *N*-acetyl-aspartyl-glutamate, *N*-acetyl-glutamate and glutathione in brain. *J Neurochem* **16**: 1409–1416, 1969.
2. Christopherson BO, The inhibitory effect of reduced glutathione on the lipid peroxidation of the microsomal fraction and mitochondria. *Biochem J* **106**: 515–522, 1968.
3. Orlowski M and Karkowsky A, Glutathione metabolism and some possible functions of glutathione in the nervous system. *Int Rev Neurobiol* **19**: 75–121, 1976.
4. Koller KJ and Coyle JT, Characterization of the interactions of *N*-acetyl-aspartyl-glutamate with L-[^{3}H]glutamate receptors. *Eur J Pharmacol* **98**: 193–199, 1984.
5. Zaczek R, Koller K, Cotter R, Heller D and Coyle JT, *N*-Acetylaspartylglutamate: An endogenous peptide with high affinity for a brain "glutamate" receptor. *Proc Natl Acad Sci USA* **80**: 1116–1119, 1983.
6. Ffrench-Mullen JMH, Koller K, Zaczek R, Coyle JT, Hori N and Carpenter DO, *N*-Acetylaspartylglutamate: Possible role as the neurotransmitter of the lateral olfactory tract. *Proc Natl Acad Sci USA* **82**: 3897–3900, 1985.
7. Ogita K, Kitago T and Yoneda Y, Possible involvement of glutathione in central glutamatergic neuro-

transmission. In: *Contemporary Themes in Biochemistry, Proceeding of the Fourth FAOB Congress, Singapore, 30 Nov.–5 Dec. 1986.* (Eds. Kon OL, Chung MCM, Hwang PLH, Leong SF, Loke KH, Thiyagarajah P and Wong PTH), pp. 580–581. ICSU Press, Cambridge, 1986.

8. Ogita K, Kitago T, Nakamuta H, Fukuda Y, Koida M, Ogawa Y and Yoneda Y, Glutathione-induced inhibition of Na^+-independent and -dependent bindings of L-[^{3}H]glutamate in rat brain. *Life Sci* **39**: 2411–2418, 1986.
9. Ogita K and Yoneda Y, Possible presence of [^{3}H]glutathione (GSH) binding sites in synaptic membranes from rat brain. *Neurosci Res* **4**: 486–496, 1987.
10. Ogita K and Yoneda Y, Temperature-dependent and -independent apparent binding activities of [^{3}H]glutathione in brain synaptic membranes. *Brain Res* **463**: 37–46, 1988.
11. Ogita K and Yoneda Y, Differentiation of the Ca^{2+}-stimulated binding from the Cl^--dependent binding of [^{3}H]glutamate in synaptic membranes from rat brain. *Neurosci Res* **4**: 129–142, 1986.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
13. Glowinski J and Iversen 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 brain. *J Neurochem* **13**: 655–669, 1966.
14. Inoue M and Morino Y, Direct evidence for the role of membrane potential in glutathione transport in renal brush border membranes. *J Biol Chem* **260**: 326–331, 1985.
15. Berl S, Purpura DP, Girado M and Waelsch H, Amino acid metabolism in epileptogenic and non-epileptogenic lesions of the neocortex (cat). *J Neurochem* **4**: 311–317, 1959.
16. Hiramatsu M and Mori A, Reduced and oxidized glutathione in brain and convulsions. *Neurochem Res* **6**: 301–306, 1981.
17. Perry TL, Godin DV and Hansen S, Parkinson's disease: A disorder due to nigral glutathione deficiency? *Neurosci Lett* **33**: 305–310, 1982.
18. Rehncrona S, Folbergrova J, Smith DS and Siesjo BK, Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical concentrations of oxidized and reduced glutathione in brain. *J Neurochem* **34**: 477–486, 1980.
19. Skullerud K, Marstein S, Schrader H, Brundelet PJ and Jellum E, The cerebral lesions in a patient with generalized glutathione deficiency and pyroglutamic acidurea (5-oxoprolinemia). *Acta Neuropathol (Berl)* **52**: 235–238, 1980.
20. Kilberg MS, Handlogten ME and Christensen HN, Characteristics of system ASC for transport of neutral amino acids in the isolated rat hepatocyte. *J Biol Chem* **256**: 3304–3312, 1981.
21. Franchi-Gazzola R, Gazzola GC, Dall'Asta V and Guidotti GG, The transport of alanine, serine and cysteine in cultured human fibroblasts. *J Biol Chem* **257**: 9582–9587, 1982.
22. Bannai S, Exchange of cystine and glutamate across plasma membrane of human fibroblasts. *J Biol Chem* **261**: 2256–2263, 1986.
23. Kessler M, Baudry M and Lynch G, Use of cystine to distinguish glutamate binding from glutamate sequestration. *Neurosci Lett* **81**: 221–226, 1987.
24. Yoneda Y, Ogita K, Nakamuta H, Koida M, Ohgaki T and Meguri H, Possible interaction of [^{3}H]glutamate binding sites with anion channels in rat neutral tissues. *Neurochem Int* **9**: 521–531, 1986.
25. Ogita K and Yoneda Y, Characterization of Na^+-dependent binding sites of [^{3}H]glutamate in synaptic membranes from rat brain. *Brain Res* **397**: 137–144, 1986.
26. Yoneda Y and Ogita K, [^{3}H]Glutamate binding sites in the rat pituitary. *Neurosci Res* **3**: 430–435, 1986.
27. Yoneda Y and Ogita K, Localization of [^{3}H]glutamate binding sites in rat adrenal medulla. *Brain Res* **383**: 387–391, 1986.
28. Govitrapong P, Ebadi M and Murrin LC, Identification of a Cl^-/Ca^{2+}-dependent glutamate (quisqualate) binding site in bovine pineal organ. *J Pineal Res* **3**: 223–234, 1986.
29. Yoneda Y and Ogita K, Enhancement of [^{3}H]glutamate binding by *N*-methyl-D-aspartic acid in rat adrenal. *Brain Res* **406**: 24–31, 1987.