The Anticonvulsive Effect of Glutathione in Mice

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To explore the role of glutathione as a neuromodulator, we investigated effects of reduced (GSH) and oxidized glutathione (GSSG) on drug-induced convulsions in mice. Intracerebroventricular administration of GSH or GSSG (10—300 nmol) did not produce convulsions. When GSH was administered prior to subcutaneous administration of pentylenetetrazol (80 mg/kg), it significantly inhibited pentylenetetrazol-induced convulsions. The inhibitory effect of GSH was dose dependent, and mimicked by GSSG. In addition, neither GSH nor GSSG affected convulsions induced by subcutaneous administration of *N*-methyl-DL-aspartate (400 mg/kg). These findings suggest that glutathione has a specific anticonvulsive effect.

Key words glutathione; convulsion; pentylenetetrazol; *N*-methyl-DL-aspartate; mice

The tripeptide glutathione (L- γ -glutamyl-L-cysteinyl-glycine; GSH) is present in most mammalian tissues in millimolar concentrations. GSH plays an important role in the storage and interorgan transport of cysteine and in cellular defenses against free radicals, peroxides and electrophilic xenobiotics as a cofactor of glutathione peroxidase and glutathione-S-transferase. Glutathione peroxidase converts GSH into oxidized glutathione (GSSG) in the presence of hydrogen peroxides, and GSH is regenerated from GSSG via a process catalyzed by glutathione reductase. In the brain, GSH is localized almost exclusively in astroglial cells, though it may also exist in nerve terminals and some neurons.^{1–3} The concentration of total GSH in the brain is approximately 2 mM, most of which is the reduced form GSH and only 1.2% or less is the oxidized form GSSG.^{4–6}

GSH is also found in the cerebrospinal fluid, where its concentration was reported to be approximately 5 μ M in rats⁷ and 200 nM in humans.⁸ However, its origin is not fully understood. Yudkoff *et al.* demonstrated that GSH is spontaneously effluxed from cultured astrocytes⁹; this efflux from astrocytes is likely to be mediated by a carrier.¹⁰ On the other hand, Zangerle *et al.* reported that GSH is released from rat brain slices upon high K⁺-induced depolarization in a Ca²⁺-dependent manner, suggesting that GSH release originates from a neuronal compartment.¹¹ It is also known that GSH is transported from the plasma to the cerebrospinal fluid via epithelial cells of the choroid plexus, the major sites of cerebrospinal fluid formation.⁷ The fact that GSH is released from brain cells and is present at a significant level in the cerebrospinal fluid implies that it also functions as a neurotransmitter or neuromodulator in the brain.

To explore the possible role of GSH in brain neurotransmission, we undertook to investigate the effect of GSH in a number of behavioral experiments using the whole animal. Here we report that GSH acutely affects drug-induced convulsions in mice, suggesting its novel role in the brain.

MATERIALS AND METHODS

Chemicals GSH, GSSG, *N*-methyl-DL-aspartate (NMDLA) and 2-amino-5-phosphonovaleate were purchased from Sigma Chemicals Co. (St Louis, MO, U.S.A.). Pentylenetetrazol was purchased from Tokyo Chemical Industries Ltd. (Tokyo, Japan). All drugs were dissolved in

saline. If necessary, the pH was adjusted to approximately 7.0—7.5 using 1 N NaOH.

Implantation of Intracerebroventricular (i.c.v.) Guide Cannula Male *ddY* mice 5 weeks old were obtained from Japan SLC, Inc. (Shizuoka, Japan) and housed in aluminum cages for one week with food and water available *ad libitum*. The implantation of an i.c.v. guide cannula was done as described.¹² In brief, mice were anesthetized with an intramuscular injection of ketamine and xylazine (41 and 3.5 mg/kg, respectively) and fixed in a stereotaxic apparatus. A stainless steel cylindrical cannula (0.35 mm i.d., 5 mm length) was implanted in each animal with the tip set in the left lateral ventricle (1.3 mm lateral to the midline, 0.3 mm posterior to the bregma, 2.0 mm ventral to the skull surface). The implanted cannulas were fixed with dental cement and served as guide cannulas for i.c.v. injection of drug solution. The operated mice were allowed to recover for one week before experiments.

Behavioral Observation GSH and GSSG were directly administered into the brain through the implanted i.c.v. guide cannula. An injection cannula connected to a micrometer syringe was very gently inserted into the implanted guide cannula, and 5 μ l of GSH or GSSG dissolved in saline was injected over a 3 min period. Pentylenetetrazol and NMDLA were subcutaneously (s.c.) administered at the volume of 10 ml/kg. The animals were observed for 30 min following drug administration, and the following 4 parameters were measured to evaluate drug-induced convulsions: 1) latency to appearance of clonic convulsion; 2) latency to appearance of tonic convulsion; 3) the number of tonic convulsion episodes; and 4) lethality. Animals not showing a convulsion within 30 min were given a maximum latency score of 30 min. Lethality was defined as the percentage of the animals that died within 30 min after drug injection. All efforts were made for the care and use of animals according to the Guidelines for Animal Experiments of the Faculty of Pharmaceutical Sciences, the University of Tokyo.

RESULTS

I.c.v. administration of GSH or GSSG (10—300 nmol) did not cause any convulsions (data not shown, *n*=8). To test the possibility that GSH or GSSG affects drug-induced convulsions, 2 convulsants, pentylenetetrazol and NMDLA, were

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Fig. 1. Pentylentetrazol- or NMDLA-Induced Convulsions in Mice

The mice were observed for 30 min following s.c. administration of pentylentetrazol (60–120 mg/kg, A) or NMDLA (200–500 mg/kg, B), and the latencies to appearance of clonic (●) or tonic convulsions (○) were measured. The data represent the means $\pm$ S.E.M. of values obtained from 7 mice.

Fig. 2. Effects of GSH and GSSG on Pentylentetrazol-Induced Convulsions in Mice

Saline, GSH (10–300 nmol) or GSSG (10–100 nmol) was i.c.v. administered 20 min prior to s.c. administration of pentylentetrazol (80 mg/kg). The mice were observed for 30 min following administration of pentylentetrazol, and the latency to appearance of clonic convulsion (A), the latency to appearance of tonic convulsion (B) and the number of tonic convulsion episodes (C) were measured. Since lethality was zero in all groups, the result is not shown here. The data represent the means $\pm$ S.E.M. of values obtained from 7 to 8 mice. * p < 0.05, ** p < 0.01 vs. saline; Mann-Whitney's U -test.

used. S.c. administration of pentylentetrazol (60–120 mg/kg) induced both clonic and tonic convulsions in a dose-dependent manner (Fig. 1A). In most cases, clonic convulsions were followed by tonic convulsions. NMDLA (200–500 mg/kg, s.c.) also induced clonic and tonic convulsions in a dose-dependent manner (Fig. 1B). The NMDLA (mg/kg, s.c.)-induced convulsions were completely abolished by in-

Fig. 3. Effects of GSH and GSSG on NMDLA-Induced Convulsions in Mice

Saline, GSH (100 or 300 nmol) or GSSG (100 nmol) was i.c.v. administered 20 min prior to s.c. administration of NMDLA (400 mg/kg). The mice were observed for 30 min following administration of NMDLA, and the 4 parameters were measured. The data represent the means $\pm$ S.E.M. from 7 to 8 mice.

tracerebroventricular administration of the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovalerate (50 nmol/brain; data not shown, n = 7), confirming that the response is mediated by NMDA receptors. To detect the proconvulsive or anticonvulsive effect of GSH or GSSG, modest doses of pentylentetrazol (80 mg/kg) and NMDLA (400 mg/kg, s.c.) were used in the following experiments.

When GSH (10–300 nmol, i.c.v.) was administered 20 min prior to pentylentetrazol (80 mg/kg, s.c.), it significantly prolonged the latency and decreased the number of episodes of tonic convulsion induced by pentylentetrazol (Fig. 2B, C). The effects of GSH were dose dependent. The latency to appearance of clonic convulsion also tended to be prolonged, but the change was not statistically significant (Fig. 2A). Lethality was zero in all groups. GSSG (10–100 nmol, i.c.v.) was also effective in inhibiting pentylentetrazol-induced convulsions (Fig. 2). The effect of GSSG was dose dependent and very similar to that of GSH.

NMDLA-induced convulsions, however, were not affected by GSH (100–300 nmol, i.c.v.) nor by GSSG (100 nmol, i.c.v.; Fig. 3). Lethality was slightly increased by concomitant administration of NMDLA and GSH or GSSG, though the effect was not statistically significant (Fig. 3D).

DISCUSSION

Pinelli *et al.* previously reported that GSH inhibits isoniazid-induced seizures in rats.¹³ However, in their study, GSH was administered s.c. twice a day for 5 d. In the present study, we found that single i.c.v. administration of GSH or GSSG dose-dependently inhibits pentylenetetrazol-induced convulsions in mice, demonstrating that they have acute, anticonvulsive effects. Furthermore, neither GSH nor GSSG affected NMDLA-induced convulsions, indicating that their effects are specific for certain types of convulsions.

The intracerebroventricular dose of GSH effective in inhibiting pentylenetetrazol-induced convulsions was 10–300 nmol/brain. The wet weight of adult mouse brain used in our present study was 0.459 ± 0.005 g ($n=10$). On the assumption that 1 g of wet weight of tissue corresponds to 1 ml of water and that i.c.v. injected GSH is equally distributed in the mouse brain, 10–300 nmol GSH injected into the brain is estimated to reach 22–654 μ M in the cerebrospinal fluid. The GSH concentration showing anticonvulsive effects is higher than the extracellular GSH level under normal conditions (5 μ M)⁷ and lower than the intracellular GSH level (2 mM).^{4–6} Thus, modulation of seizure activity by GSH is likely to occur under a physiological or pathological condition.

Although cellular mechanisms by which GSH inhibits pentylenetetrazol-induced convulsions are not clear, several possibilities can be argued. First, GSH is known to act as an antioxidant. However, pentylenetetrazol-induced convulsions were not affected by other antioxidants such as *N*-(2-mercaptopropionyl)glycine (200 mg/kg, i.p.; our unpublished data). Furthermore, the anticonvulsive effect of GSH was shared by GSSG, which lacks antioxidant activity. Therefore, the anticonvulsive effect of GSH is probably independent of antioxidant activity. Second, disulfide bonds play a role in modulating the function of many proteins. GSH or GSSG has been reported to modulate the function of a variety of receptors and channels by changing the redox state of proteins. For example, GSSG, but not GSH, inhibited NMDA receptor-mediated Ca^{2+} influx in cultured brain neurons.^{14–16} GSH inhibited the ligand binding to μ -opioid, neurokinin-1 and kainate receptors.¹⁷ Glycine-induced currents in rat retinal ganglion cells were suppressed by GSH and enhanced by GSSG, while γ -aminobutyrate-induced currents were enhanced by GSH.¹⁸ However, very high concentrations (5–10 mM) are required for GSH or GSSG to exert these effects. Therefore, the anticonvulsive effect observed in the present study is unlikely to result from the redox modulation of receptors or channels. Third, both GSH and GSSG have been reported to work as agonists for the NMDA receptor at concentrations of 100 nM–100 μ M.^{19,20} However, in the present study, GSH did not affect convulsions induced by NMDA receptor activation;

therefore, the anticonvulsive effect of GSH is probably independent of the NMDA receptor. Slight increase in lethality by concomitant administration of NMDLA and GSH or GSSG (Fig. 3D) may be related to the potentiating effects of GSH and GSSG on NMDA receptor functions. NMDLA-induced convulsions and death may be mediated by different types of NMDA receptors, which are discriminated by the sensitivity to GSH and GSSG. Taken together, it is possible that GSH and GSSG exert anticonvulsive effects through novel mechanisms different from any others reported. Further investigations are underway in our laboratory to elucidate the molecular target of GSH and GSSG.

In conclusion, we demonstrated for the first time that GSH and GSSG exert acute, anticonvulsive effects in mice. GSH may function as a determinant of neuronal excitability in the brain. Our present findings will give new insight into the possible role of GSH as a neurotransmitter or neuromodulator.

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