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Research report

Antidepressant-like responses in the forced swimming test elicited by glutathione and redox modulation

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HIGHLIGHTS

- GSH induces antidepressant-like effect in mice.
- Acivicin, a γ-GT inhibitor, abolishes antidepressant-like effect of GSH.
- Thiol/disulfide reagents modulate antidepressant-like effect of GSH.
- Extracellular redox milieu participates in the antidepressant-like effect of GSH.

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ABSTRACT

Glutathione (GSH) displays a broad range of functions, among them a role as a neuromodulator with some neuroprotective properties. Taking into account that oxidative stress has been associated with depressive disorders, this study investigated the possibility that GSH, a major cell antioxidant, elicits an antidepressant-like effect in mice. Thus, GSH was administered by i.c.v. route to mice that were tested in the forced swimming test and in the tail suspension test, two predictive tests for antidepressant drug activity. In addition, GSH metabolism and the redox environment were modulated in order to study the possible mechanisms underlying the effects of GSH in the forced swimming test. The administration of GSH decreased the immobility time in the forced swimming test (300–3000 nmol/site) and tail suspension test (100–1000 nmol/site), consistent with an antidepressant-like effect. GSH depletion elicited by L-buthionine sulfoximine (3.2 μmol/site, i.c.v.) did not alter the antidepressant-like effect of GSH, whereas the inhibition of extracellular GSH catabolism by acivicin (100 nmol/site, i.c.v.) prevented the antidepressant-like effect of GSH. Moreover, a sub-effective dose (0.01 nmol/site, i.c.v.) of the oxidizing agent DTNB (5,5′-dithiobis(2-nitrobenzoic acid)) potentiated the effect of GSH (100 nmol/site, i.c.v.), while the pretreatment (25–100 mg/kg, i.p.) with the reducing agent DTT (DL-dithiothreitol) prevented the antidepressant-like effect of GSH (300 nmol/site, i.c.v.). DTNB (0.1 nmol/site, i.c.v.), produced an antidepressant-like effect, per se, which was abolished by DTT (25 mg/kg, i.p.). The results show, for the first time, that centrally administered GSH produces an antidepressant-like effect in mice, which can be modulated by the GSH metabolism and the thiol/disulfide reagents. The redox environment may constitute a new venue for future antidepressant-drug development.

1. Introduction

The tripeptide γ-glutamyl-L-cysteinyl-glycine, or glutathione (GSH), is a ubiquitous antioxidant thiol, which plays a major role in maintaining intracellular reduction–oxidation (redox) balance. It is synthesized in the brain by both neurons and glial cells, being more abundant within astrocytes [1,2]. Besides its role as an antioxidant, GSH is essential for cell proliferation, modulation of signal transduction, immune response and regulation of apoptosis [2–6].

GSH is synthesized in the cell by the consecutive action of glutamate cysteine ligase (GCL) and glutathione synthetase. GCL, the rate limiting enzyme in GSH synthesis, uses glutamate and cysteine as substrates to form the dipeptide γ-glutamylcysteine, whereas glutathione synthetase catalyzes the synthesis of GSH by adding glycine to γ-glutamylcysteine [7]. The intracellular synthesis of GSH can be inhibited in vivo by buthionine sulfoximine (BSO), a specific GCL inhibitor [8].

Extracellular GSH is a substrate for the ectoenzyme γ-glutamyl transpeptidase (γ-GT), which catalyzes the transfer of the γ-glutamyl moiety from GSH to an acceptor molecule, thereby

Abbreviations: BSO, L-buthionine-[S,R]-sulfoximine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, DL-dithiothreitol; FST, forced swimming test; GCL, glutamate cysteine ligase; γ-GT, γ-glutamyl transpeptidase; GSH-t, total glutathione (GSH plus GSSG in GSH-equivalents); GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-*S*-transferase; TST, tail suspension test.

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generating the dipeptide Cys-Gly. The dipeptide Cys-Gly can be further hydrolyzed by ectopeptidases to cysteine and glycine, which are subsequently taken up by cells to serve again as substrates for de novo cellular GSH synthesis [2,9,10]. The degradation of extracellular GSH can be inhibited by acivicin, an irreversible inhibitor of γ-GT that has been used to study the role of γ-GT in GSH homeostasis [11].

The role of GSH as an important antioxidant agent in the central nervous system has been indicated by several studies. A decrease in the tissue levels of GSH has been associated with aging and the pathogenesis of neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's disease and Parkinson's disease [12,13]. Moreover, the levels of GSH are also decreased in the cerebrospinal fluid and prefrontal cortex of schizophrenic patients [14,15]. In line with these findings, GSH was effective against pentylenetetrazol-induced convulsions [16]. Thus, besides its antioxidant function GSH displays a neuroprotective role in the brain [17].

Several substances that exhibit neuroprotective effects have been reported to produce antidepressant effects in animal models predictive of antidepressant action and/or in clinical studies [18,19]. Considering that major depressive disorder has recently been linked to impairments in signaling pathways that regulate neuroplasticity and cell survival [20,21], the neuroprotective role of these antioxidant agents can be of pharmacological significance for the modulation of depression [22–26].

The development of an animal model of GSH deficiency, by knocking out GCL, the rate limiting enzyme in GSH synthesis, brought new insights on the importance of GSH in mood disorders [9,27]. Chronic GSH deficiency induces hyperlocomotion and higher responses to amphetamine, indicating that GSH may be an important component of bipolar disorders [9,27]. Thus, besides its antioxidant function, GSH appears also to be strongly linked with mood-related behavior. In line with this idea, the enzyme glyoxalase 1, the main pathway for disposal of the toxic aldehyde methylglyoxal [28], has been liked to anxiety-like behavior in rodents, which appears to present comorbidity with depressive-like behavior [29,30]. Since detoxification of methylglyoxal by glyoxalase 1 depends on GSH, its direct participation in these behavioral findings may not be excluded.

The present study was designed to investigate whether GSH, an endogenous and neuroprotective antioxidant, produces an antidepressant-like effect in the forced swimming test (FST) and in the tail suspension test (TST), two animal models predictive for antidepressant drug activity [31,32]. Moreover, to give some insight into the possible mechanisms underlying the effect of GSH in the FST, we investigated the interference of GSH metabolism and thiol/disulfide reagents.

2. Materials and methods

2.1. Animals

Swiss mice, either sex, weighing 30–40 g (55–60 days-old), were maintained at 21 ± 1 °C with free access to water and food, under a 12:12 h light/dark cycle (light onset at 07.00 h). All manipulations were carried out between 09.00 h and 17.00 h, with each animal used only once. The experiments were performed after approval of the protocol by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering, which are in conformity to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health.

2.2. Drugs and treatment

The following chemicals were used: acivicin ((α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid); L-buthionine-[S,R]-sulfoximine (BSO); 1-chloro-2,4-dinitrobenzene; DL-dithiothreitol (DTT); 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), bovine serum albumin; glutathione reductase (GR); nicotinamide adenine dinucleotide phosphate (reduced form, NADPH); oxidized glutathione (GSSG); *tert*-butyl hydroperoxide, perchloric acid; 1-chloro-2,4-dinitrobenzene; 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (from Sigma Chemical Co., St. Louis, MO, USA).

Drugs were administered by intracerebroventricularly (i.c.v.) in a constant volume of 5 μl/site, over 30 s, except for DTT that was administered intraperitoneally (i.p.) in a constant volume of 10 ml/kg body weight. i.c.v. injections were given under light ether anesthesia, directly into the lateral ventricle with the bregma fissure as a reference. GSH and BSO were dissolved in phosphate-buffered saline (PBS) 0.75 M, pH 7.4. All other drugs were dissolved in saline. Appropriate vehicle-treated groups were assessed simultaneously.

A microsyringe (25 μl, Hamilton) was used to perform the i.c.v. administration of drugs. Previous to drug injection, a 26-gauge stainless-steel needle that was inserted perpendicularly 2 mm deep through the skull, following previously published methodology by Laursen and Belknap [33]. Briefly, mice were gently restrained by hand after being lightly anesthetized with ether (i.e. just that necessary for loss of the postural reflex). The sterilization of the injection site was carried out using gauze embedded in 70% ethanol. Under light anesthesia, the needle was inserted unilaterally 1 mm to the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. Drugs were injected directly into the lateral ventricle using published coordinates taken from the atlas of Franklin and Paxinos [34], taking the bregma as a reference: anterioposterior −0.1 mm; mediolateral 1 mm; and dorsoventral −3 mm. A volume of 5 μl was used, based on literature data, and delivered in 30 s as previously employed [35]. Mice exhibited normal behavior within 1 min after injection.

2.3. Behavioral tests

The FST was carried out based on the method previously described by Porsolt et al. [31] with minor modifications. Briefly, mice were dropped individually into cylinders (height 25 cm, diameter 10 cm) containing 19 cm of water, maintained at 25 ± 1 °C, and left there for 6 min. In such a situation, from which they cannot escape, animals rapidly became immobile, that is, floating in an upright position and making only small movements to keep their heads above water. The total duration of immobility was scored.

The total duration of immobility induced by tail suspension was measured according to the method of Steru et al. [32]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period.

The ambulatory behavior was assessed in an open-field test as described previously [36]. The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossings) was counted in a 6-min session. Mice received an i.c.v. injection of GSH (dose range 100–3000 nmol/site) and the open-field test was performed after 20 min.

2.4. Pharmacological treatment

To investigate the antidepressant-like effect of GSH animals received an i.c.v. injection of GSH 20 min before the FST (dose range 100–3000 nmol/site) and TST (dose range 10–1000 nmol/site). This dose-range can be taken as of pharmacological nature, considerably surpassing the physiological levels of GSH.

To investigate the influence of GSH metabolism on the antidepressant-like effect of GSH, mice were pretreated with BSO (3.2 μmol/site, i.c.v., an inhibitor of the intracellular synthesis of GSH) or acivicin (100 nmol/site, i.c.v., an inhibitor of the degradation of extracellular GSH) and 24 h or 20 min later, respectively, animals received GSH (300 nmol/site, i.c.v., an active dose in the FST) and were tested in the FST after 20 min.

In a separate series of experiments, we also investigated the possible influence of thiol/disulfide reagents in the anti-immobility effect of GSH. To this end, mice received a sub-effective dose of DTNB (0.01 nmol/site, i.c.v.), an oxidizing agent, 20 min before administration of GSH (100 nmol/site, i.c.v., a sub-active dose in the FST) and were tested in the FST 20 min later. We also tested the influence of DTNB (10 nmol/site, i.c.v., a dose that produced no effect in the FST) on the antidepressant-like effect of GSH (300 nmol/site, i.c.v., an active dose in the FST). To this end, mice were pretreated with DTNB 20 min before GSH administration. FST was carried out after 20 min. The doses of DTNB were chosen from a dose–response curve (0.01–10 nmol/site, i.c.v.) in the FST, in which this oxidizing agent was administered 20 min before the FST.

The effect of DTT, a thiol reagent, on the antidepressant-like effect of GSH was also investigated. The animals were pretreated with DTT (25–100 mg/kg, i.p., doses that produced no effect in the FST) and after 20 min they received an i.c.v. injection of GSH (300 nmol/site). A further 20 min elapsed before the animals were tested in the FST.

In order to investigate whether DTT would be able to prevent the anti-immobility effect of DTNB (0.1 nmol/site, i.c.v.), mice were pretreated with DTT (25 mg/kg, i.p.). Twenty min later DTNB was injected and the FST was carried out after a further 20 min.