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MONOAMINERGIC DYSREGULATION IN GLUTATHIONE-DEFICIENT MICE: POSSIBLE RELEVANCE TO SCHIZOPHRENIA?

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Abstract—Several lines of research have implicated glutathione (GSH) in schizophrenia. For instance, GSH deficiency has been reported in the prefrontal cortex of schizophrenics *in vivo*. Further, in rats postnatal GSH-deficiency combined with hyperdopaminergia led to cognitive impairments in the adult. In the present report we studied the effects of 2-day GSH-deficiency with L-buthionine-(*S*,*R*)-sulfoximine on monoaminergic function in mice. The effect of GSH-deficiency per se and when combined with the amphetamine and phencyclidine (PCP) models of schizophrenia was investigated. GSH-deficiency significantly altered tissue levels of dopamine (DA), 5-hydroxytryptamine (5-HT) and their respective metabolites homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in a region-specific fashion. The effects of GSH-deficiency on tissue monoamines were distinct from and, generally, did not interact with the effects of amphetamine (5 mg/kg; i.p.) on tissue monoamines. Microdialysis studies showed that extracellular DA-release after amphetamine (5 mg/kg, i.p.) was two-fold increased in the nucleus accumbens of GSH-deficient mice as compared with control mice. Basal DA was unaltered. Further, extracellular levels of HVA in the frontal cortex and hippocampus and 5-HIAA in the nucleus accumbens were elevated by GSH-deficiency per se. Spontaneous locomotor activity in the open field was unchanged in GSH-deficient mice. In contrast, GSH-deficiency modulated the locomotor responses to mid-range doses of amphetamine (1.5 and 5 mg/kg, i.p.). Further, GSH-deficient mice displayed an increased locomotor response to low (2 and 3 mg/kg, i.p.) doses of phencyclidine (PCP). In conclusion, the data presented here show that even short-term GSH-deficiency has consequences for DA and 5-HT function. This was confirmed on both neurochemical and behavioral levels. How GSH and the monoamines interact needs further scrutiny. Moreover, the open field findings suggest reduced or altered *N*-methyl-D-aspartate (NMDA) receptor function in GSH-deficient mice. Thus, GSH-deficiency can lead to disturbances in DA, 5-HT and NMDA function, a finding that may have relevance for schizophrenia.

Key words: locomotor, NMDA, glutamate, dopamine, 5-HT, microdialysis.

In the brain, the tri-peptide glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) plays a central role in the defense against reactive oxygen species (ROS; Dringen, 2000). GSH also protects against dopamine (DA)-induced toxicity (Grima et al., 2003; Hastings et al., 1996; Hirrlinger et al., 2002) and may modulate glutamate receptor function (Janaky et al., 2000; Oja et al., 2000). Recently, GSH was reported to be decreased by 51% in the prefrontal cortex of non-medicated schizophrenic patients *in vivo*, as assessed with nuclear magnetic resonance imaging (NMR; Do et al., 2000). This is intriguing since prefrontal deficits (Harrison, 1999; Shenton et al., 2001) as well as oxidative stress (Mahadik and Mukherjee, 1996; Reddy and Yao, 1996) and dopaminergic and glutamatergic dysfunction (Laruelle et al., 2003; Moghaddam, 2003) have been associated with the disorder. Conceivably, GSH-deficiency could compromise neuronal function. For instance, GSH-deficiency sensitizes neurons to DA-induced dendritic degeneration (Grima et al., 2003) and this could relate to the reduced dendritic arborization reported in schizophrenics postmortem (Glantz and Lewis, 2000; Selemon and Goldman-Rakic, 1999). The postmortem findings imply that neuronal integrity is compromised in schizophrenia. This was also suggested by *in vivo* NMR studies where *N*-acetyl aspartate (NAA), a marker of neuronal integrity, was decreased in schizophrenics (Callicott et al., 2000; Deicken et al., 2000; Yamasue et al., 2002). Interestingly, GSH-deficiency decreases NAA in the rat brain (Heales et al., 1995; Jain et al., 1991). Thus, it is possible that GSH-deficiency and neuronal impairment in schizophrenia is functionally linked.

GSH protects neurons against DA-induced toxicity *in vitro* (Berman and Hastings, 1997; Grima et al., 2003; Hirrlinger et al., 2002; Offen et al., 1996) and *in vivo* (Hastings et al., 1996; Klivenyi et al., 2000). The antioxidative effect of GSH could be one mechanism behind this protective action since DA metabolism is a source of free radicals (Hirrlinger et al., 2002; Napolitano et al., 1995; Offen et al., 1996). Additionally, GSH can conjugate DA and thereby counter its toxicity (Dagnino-Subiabre et al., 2000; Grima et al., 2003; Hastings et al., 1996). This is also intriguing because it creates the possibility that GSH directly influences DA transmission by extracellular conjugation and inactivation. This point, however, needs further studies. In any case, reductions in GSH may sensitize the

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; COMT, cathecol *O*-methyltransferase; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; GSH, glutathione; HPLC-ECD, high-pressure liquid chromatography-electrochemical detection; HVA, homovanillic acid; L-BSO, L-buthionine-(*S*,*R*)-sulfoximine; NA, noradrenaline; NAA, *N*-acetylaspartate; NAT, noradrenaline transporter; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance imaging; PCP, phencyclidine; ROS, reactive oxygen species; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine.

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brain to the toxic effects of DA. Conceivably, this could contribute to the pathogenesis of schizophrenia.

Changes in GSH levels could also affect the function of the *N*-methyl-D-aspartate (NMDA) receptor. *In vitro*, the NMDA receptor is positively modulated by reductants, including GSH (Aizenman et al., 1989; Janaky et al., 1993; Jiang et al., 2000; Kohr et al., 1994; Regan and Guo, 1999), and negatively regulated by oxidants (Aizenman et al., 1989). *In vivo*, mice with reduced activity of GSH peroxidase, a key enzyme in GSH function (Dringen, 2000), display sub-functional NMDA receptors correlating with increased NMDA-receptor oxidation (Jiang et al., 2000). Further, GSH can interact, via its glutamyl moiety, with the glutamate-binding site on the NMDA receptor. Here it can serve, at least in some cases, as an agonist (Leslie et al., 1992; Varga et al., 1997). Thus, although the exact significance of the interaction is not yet clear, GSH may support NMDA function (Jacobsson and Fowler, 1999; Janaky et al., 1999). Interestingly, reduced NMDA receptor function has been proposed as a cause of schizophrenia because NMDA antagonists, such as phencyclidine (PCP) and ketamine, can induce schizophreniform psychotic and cognitive symptoms in healthy humans (Moghaddam, 2003). It has been proposed that NMDA antagonist treatment in humans and rodents mimic a hypothesized hypocortical state in schizophrenia. In this model, decreased cortical glutamatergic regulation of midbrain dopaminergic neurons results in hyperdopaminergia in the basal ganglia and hypodopaminergia in the frontal cortex (Laruelle et al., 2003; Moghaddam, 2003). In fact, NMDA-antagonists modestly increases DA release (Steinpreis and Salamone, 1993) and also potentiates amphetamine-induced DA release in the striatum (Balla et al., 2001; Miller and Abercrombie, 1996). However, recent studies have shown that NMDA-antagonists rather induce frontocortical hyperdopaminergia, not hypodopaminergia, in rats (Balla et al., 2003; Javitt et al., 2004). Further, there is, as yet, little evidence for hypodopaminergia in the frontal cortex of schizophrenics (Laruelle et al., 2003). Thus, while an interaction between the NMDA and DA systems is well established, further scrutiny of the nature of this interaction and its relevance for schizophrenia is warranted. In any case, it seems plausible that GSH-deficiency could affect DA transmission by changing NMDA receptor function.

Cognitive deficits are frequent in schizophrenia (Coyle et al., 2003). GSH-deficiency *in vivo* induces cognitive deficits in rodents. Central GSH-deficiency using L-buthionine-(*S*,*R*)-sulfoximine (L-BSO) sensitizes rats to DA-induced psychomotor and spatial learning deficits (Shukitt-Hale et al., 1997, 1998). L-BSO is a selective inhibitor of γ-glutamylcysteine synthase, the rate limiting enzyme in GSH synthesis (Broquist, 1992; Griffith, 1982). Independent reports found spatial learning deficits as well as decreased long-term potentiation in rats *in vivo* following GSH-deficiency with diethylmaleate, a GSH-chelator (Almaguer-Melian et al., 2000; Cruz-Aguado et al., 2001; Shukitt-Hale et al., 1998). Further, administration of L-BSO in combination with GBR 12909, a DA transporter (DAT) antagonist, during early postnatal development led to cognitive deficits in the adult rat (Castagne et al., 2004a). These latter findings show that increased dopaminergic tone coinciding with GSH deficiency can result in lasting deficits in higher brain function.

To summarize, there is circumstantial evidence that impaired GSH function could lead to increased oxidative stress, increased susceptibility to the toxic sequelae of DA transmission, possibly increased or altered DA transmission, reduced NMDA receptor function and impaired neuronal development and integrity. All these phenomena may have relevance for the pathogenesis of schizophrenia.

The aim of the present study was to determine whether GSH-deficiency by L-BSO altered the function of the monoaminergic systems, with special focus on DA. We combined L-BSO-induced GSH-deficiency in mice *in vivo* with the amphetamine model of schizophrenia (Kokkinidis and Anisman, 1981) and screened for changes in tissue monoamines, extracellular monoamines and for altered locomotor activity in the open field. Additionally, we investigated the effect of GSH-deficiency on PCP-induced locomotor activity in the open field and monoamine release in the nucleus accumbens.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice (Jackson Laboratory, Ben Harbor, ME, USA) 7–9 weeks old (24–27 g) were used in all experiments. The mice were housed four to five per cage except after implantation of microdialysis guide probes, when after the mice were housed individually. Standard laboratory chow and water were available *ad libitum*. Mice were housed in a humidity- and temperature controlled room with a 14 h/10 h light/dark cycle (lights on at 07:00 h). All studies were conducted with an approved protocol from the Duke University Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of mice used and their suffering.

Surgery and i.c.v. injections of L-BSO

The mouse was anesthetized using a saline solution (10 ml/kg; i.p.) of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic frame equipped with a mouse adapter (Kopf, Tujunga, CA, USA). A guide cannula (cat.no. C313GS-5; Plastics One, Roanoke, VA, USA) was implanted into the lateral ventricle (AP: −0.3 mm; ML: 0.9 mm; DV: 2.0 mm; Paxinos and Franklin, 1997) and fixed in place with two anchor screws (CMA, North Chelmsford, MA, USA) and dental cement. The guide cannula was protected with a dummy cannula (cat.no. C313DC; Plastics One) until use. All mice recovered for at least 1 week before experimentation. All mice were treated with antibiotics (1.2 mg sulfamethoxazole/ml and 0.24 mg trimethoprim/ml) and analgesics (aspirin; 1 mg/ml) in the drinking water for the first 4 days after surgery; thereafter only antibiotics.

The mice were injected i.c.v. twice, on 2 consecutive days, with L-BSO (Toronto Research Chemicals, North York, ON, Canada; 500 μg/mouse in 5 μl) or sterile saline. This paradigm has previously been shown to provide maximal GSH-deficiency (Abe et al., 2000; Pileblad and Magnusson, 1989). The internal cannula (cat.no. C313I; Plastics One) was inserted into the guide and L-BSO or saline was injected (1 μl/min, 5 min) using a A-99 Razel syringe pump (Stamford, CT, USA). The internal cannula was left in place for 10 min after injection to allow for dispersion away from