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Short communication

Methamphetamine selectively alters brain glutathione

Christine Harold [a], Tanya Wallace [b], Ross Friedman [a], Gary Gudelsky [c], Bryan Yamamoto [a,*]

[a] *Program in Basic and Clinical Neuroscience, Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106, USA*
[b] *Neuroscience Graduate Program, University of Cincinnati, Cincinnati, OH 45267, USA*
[c] *College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA*

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Abstract

As methamphetamine-induced neurotoxicity has been proposed to involve oxidative stress, reduced and oxidized glutathione (GSH and GSSG, respectively), vitamin E and ascorbate were measured in the striata of rats killed 2 or 24 h after a neurotoxic regimen of methamphetamine. At 2 h, methamphetamine increased GSH and GSSG (32.5% and 43.7%, respectively) compared to controls at 2 h. No difference was seen in glutathione at 24 h, and in vitamin E and ascorbate at either time point. These findings indicate selectivity of methamphetamine for the glutathione system and a role for methamphetamine in inducing oxidative stress.

Keywords: Methamphetamine; Glutathione; Oxidative stress

1. Introduction

Methamphetamine produces long-term depletion of dopamine and serotonin (5-hydroxytryptamine; 5-HT) tissue contents (Seiden et al., 1975–76), a reduction in tyrosine hydroxylase and tryptophan hydroxylase activity (Hotchkiss et al., 1979; Hotchkiss and Gibb, 1980), and decreases in the density of dopamine and 5-HT uptake sites (Wagner et al., 1980). While many long-lasting toxic effects have been demonstrated, the mechanisms underlying this toxicity have yet to be determined.

Recent studies indicate that oxidative stress and alterations in cellular metabolism mediate methamphetamine toxicity. Striatal ATP is reduced following high doses of methamphetamine (Chan et al., 1994). Moreover, methamphetamine increases the production of free radicals and produces oxidative damage (Yamamoto and Zhu, 1998), presumably via the excess release of dopamine and/or glutamate (Yamamoto et al., 1998). Additionally, the administration of antioxidants and spin trapping agents has been reported to protect against methamphetamine-induced toxicity (DeVito and Wagner, 1989; Cappon et al., 1996; Yamamoto and Zhu, 1998). Therefore, concentrations of endogenous antioxidants, such as glutathione, ascorbate, and vitamin E, may be altered as a consequence of methamphetamine-induced changes.

Moszczynska et al. (1998) demonstrated a modest but significant reduction in total glutathione in the brain following subchronic dosing of methamphetamine. However, it is unknown if the decrease in total glutathione is a consequence of increased oxidation. The purpose of the present study is to extend these findings to include parallel measurement of oxidized glutathione (GSSG) and reduced glutathione (GSH). We hypothesized that GSSG in the striatum will increase, while GSH will decrease after methamphetamine. We also compared the effects of methamphetamine on another water-soluble antioxidant, ascorbate, and compared it to these effects on vitamin E, a lipid soluble antioxidant.

2. Methods

2.1. Materials

Methamphetamine HCl, dinitrofluorobenzene, iodoacetic acid, glutathione ethyl ester, and GSSG were purchased from Sigma (St. Louis, MO).

* Corresponding author. Tel.: +1-216-844-5849; fax: +1-216-844-5840.
E-mail address: bky2@po.cwru.edu (B. Yamamoto).

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2.2. Drug treatment

Male Sprague–Dawley rats (190–350 g) were treated over an 8 h period at 2-h intervals with either methamphetamine (10 mg/kg) dissolved in 0.9% saline or saline alone. The rats were killed by decapitation at 2 or 24 h following the fourth injection. The striata were dissected, immediately frozen on dry ice, and stored at −80°C until assayed. All animal procedures were conducted in compliance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the local Institutional Animal Care and Use Committee.

2.3. Biochemical assays

2.3.1. Glutathione analysis

Striata were analyzed by a modification of the method of Reed et al. (1980). The striatum was homogenized in 0.1N $HClO_4$ (800 μl) and centrifuged at 14 000 × *g* for 6 min at 4°C. The supernatant (100 μl) was incubated with 0.88 M iodoacetic acid (20 μl) and excess sodium bicarbonate (approximately 15 mg) for 1 h in the dark, at room temperature. The solution was incubated with 100 μl of 1.5% dinitrofluorobenzene (in methanol) for 4 h in the dark, at room temperature. Diethyl ether (500 μl) was then added and the solution was centrifuged for 20 min at 2000 × *g*. The aqueous layer was separated and stored at 0°C. Protein content was analyzed according to the method of Bradford.

The aqueous layer was analyzed by high pressure liquid chromatography (HPLC) with ultraviolet spectroscopy (λ = 355 nm). GSH and GSSG were separated chromatographically on a Luna 3 μ C18, 150 × 4.6 mm column (Phenomenex, Torrance, CA). The mobile phase (pH 3.5) consisted of 0.8 M sodium acetate trihydrate, 15% glacial acetic acid and 20% methanol. Column temperature was maintained at 33°C. The retention times of GSH and GSSG under isocratic conditions were 14 and 96 min, respectively.

2.3.2. Vitamin E assay

Vitamin E was measured by HPLC, coupled with electrochemical detection. The striatum was homogenized in ethyl acetate (600 μl) and centrifuged at 14 000 × *g* for 6 min. The supernatant (20 μl) was injected into a 4.6 × 250 mm Ultrasphere C18 reverse phase column (5 μm particle size; Beckman, San Ramon, CA). The mobile phase was 96% methanol: 4% ddH_2O and 40 mM sodium perchlorate.

2.3.3. Ascorbate assay

The striatum was homogenized in 1 ml of 0.1 N $HClO_4$ and centrifuged for 6 min at 14 000 × *g*. The supernatant (20 μl) was assayed for ascorbate by HPLC with electrochemical detection. The mobile phase (pH 4.5) was 21.7 mM sodium acetate, 2.5 nM tridecylamine, 3.3% glacial acetic acid and 6% methanol. Ascorbate was separated from biogenic amines and metabolites using a C18 reverse phase column (2 × 100 mm, 3 μm) (Phenomenex). The potential of the glassy carbon electrode was maintained at 0.6 V vs. an Ag/AgCl reference electrode.

3. Results

The total striatal GSH and GSSG content 2 h after the fourth injection of methamphetamine was increased by 33% ($P < 0.01$), and 44% ($P < 0.02$), respectively (Fig. 1). The GSH content was 273.7 ± 21.1 μg/mg protein in saline control rats and 362.8 ± 26.6 μg/mg protein in methamphetamine treated rats. The GSSG content was 10.1 ± 0.9 ng/mg protein in the saline group and 14.5 ± 1.4 ng/mg protein in the methamphetamine-treated rats. The total GSH in striatum of the rats killed 24 h following the last injection was not different in saline and in methamphetamine-treated rats (saline: 263.3 ± 21.6 μg/mg protein; methamphetamine: 276.2 ± 18.0 μg/mg protein; $P > 0.30$) (Fig. 1). Similarly, the total GSSG content in methamphetamine-treated rats and in the con-

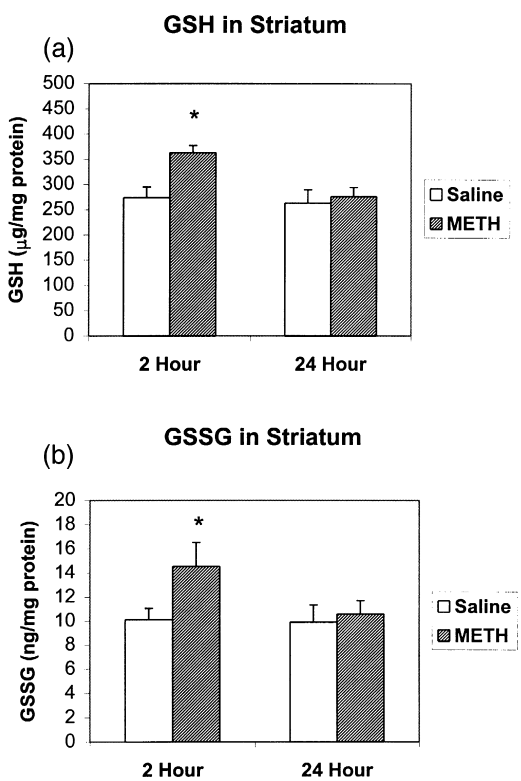

Fig. 1. (a) GSH ($n = 10$ for 2 and 24 h saline and 2 h methamphetamine; 13 for 24 h methamphetamine) and (b) GSSG ($n = 6$ for 2 h saline; 7 for 2 h methamphetamine and 24 h saline; and 9 for 24 h methamphetamine) in striatum 2 or 24 h after methamphetamine administration. Results are expressed as means ± S.E.M. Data was analyzed with a Student's *t*-test. * $P < 0.05$ compared to saline controls.

Table 1
Vitamin E and ascorbate in striatum following methamphetamine or saline administration. Results are shown as means ± S.E.M. ($n = 6$/group)

	Saline	Methamphetamine	
		2 (h)	24 (h)
Vitamin E (ng/mg wet tissue weight)	8.57 ± 0.34	9.02 ± 0.46	8.53 ± 0.40
Ascorbate (ng/μg protein)	5.66 ± 0.42	6.11 ± 0.37	5.76 ± 0.42

trols was not different (saline: 9.9 ± 1.9 ng/mg protein; methamphetamine: 10.6 ± 1.1 ng/mg protein; $P > 0.30$).

The total content of vitamin E was the same after methamphetamine as after saline administration (Table 1). The average total vitamin E content in control rat striatum was 8.5 ± 0.3 ng/mg wet tissue weight, and in the methamphetamine-treated rats, it was 9.0 ± 0.4 and 8.5 ± 0.4 ng/mg wet tissue weight after 2 and 24 h, respectively. Similarly, total ascorbate in the striatum of methamphetamine-treated animals was not different ($P > 0.05$) from that in the controls at either 2 or 24 h after the fourth injection (Table 1). The mean ascorbate content in control rats was 5.6 ± 0.4 ng/μg protein, and 6.1 ± 0.3 and 5.7 ± 0.4 ng/μg protein for the 2 and 24 h methamphetamine groups, respectively.

4. Discussion

Antioxidants in rat striatum were measured after the administration of methamphetamine. Total GSH and GSSG were both increased at 2 h following the fourth injection of methamphetamine, but returned to their control values at 24 h. No differences in vitamin E and ascorbate in striatum were observed at either 2 or 24 h after methamphetamine.

Methamphetamine produces oxidative stress in the striatum through the production of hydroxyl free radicals (Yamamoto and Zhu, 1998). GSH reacts non-enzymatically with hydroxyl radicals to produce GSSG (Griffith, 1999). The enzymatic formation of GSSG has also been shown to be a consequence of an oxidation (Han et al., 1999) and of a neuroprotective response to an oxidative stressor (Iwata-Ichikawa et al., 1999). However, Moszczynska et al. (1998) found that glutathione peroxidase and glutathione reductase activities were unaltered following repeated systemic administrations of methamphetamine. It follows that the increase in GSSG at 2 h after methamphetamine could be the result of elevated accumulation due to the combination of increased non-enzymatic oxidation of GSH to GSSG and lack of an increase in enzymatic reduction. The alteration of GSSG in the presence of oxidative stress is evidence for the oxidative effects of methamphetamine on the glutathione system.

GSH was also elevated 2 h after the fourth injection of methamphetamine. This increase could have been due to an increased synthesis of GSH in glial cells. It has been shown that the rate-limiting enzyme in GSH synthesis, γ-glutamylcysteine synthetase, is up-regulated in glial cells, as well as in other organs, in the presence of oxidative stress (Iwata-Ichikawa et al., 1999; Moellering et al., 1998; Woods et al., 1999). Therefore, the increased synthesis of GSH may be responsible for its increase at 2 h following methamphetamine. By 24 h after methamphetamine, however, the activity of the glutathione system has returned to its control values (Fig. 1). An alternative, but less likely explanation, is that the increase in glutamate caused by methamphetamine administration (Nash and Yamamoto, 1992) disrupts the feedback inhibition by GSH on γ-glutamylcysteine synthetase (Richman and Meister, 1975), an effect observed in the rat kidney, but yet to be observed in the brain.

The increase in GSH after methamphetamine contrasts with the decrease in GSH observed earlier (Moszczynska et al., 1998). The twofold increase in the total dose of methamphetamine used in the Moszczynska et al (1998) study could have produced greater oxidative stress and, consequently, long-term depletion of total glutathione stores. In contrast, the oxidative stress produced with the neurotoxic doses of methamphetamine in the present study may not have been sufficient to saturate the glutathione-related enzyme systems to produce measurable depletions of GSH in tissue. The acute increase in both GSH and GSSG in the striatum, however, supports the conclusion that methamphetamine alters the glutathione system, presumably in response to oxidative stress.

The present study also extended the examination of the effects of methamphetamine to include its effects on other antioxidants. Vitamin E is lipid soluble and primarily prevents free radical-induced lipid peroxidation (Chow, 1991). Vitamin E requires ascorbate and glutathione as co-factors to prevent lipid peroxidation and depends on ascorbate to reduce the oxidized vitamin E (Chow, 1991; Cardoso et al., 1998). Consequently, greater changes in glutathione and ascorbate concentrations may be necessary before vitamin E content is affected.

Ascorbate content also appeared unaffected by methamphetamine. Ascorbate functions as both a pro-oxidant and antioxidant. As a pro-oxidant, ascorbate reacts with iron to generate reactive oxygen species and produce oxidative damage (Chow, 1991), the effects of which can be reversed by GSH (Burk, 1982). In contrast, the antioxidant activity of ascorbate is mediated through the reduction of oxidized vitamin E and water-soluble oxidants (Chow, 1991). Therefore, because of these opposing actions, changes in total brain ascorbate concentrations may be difficult to detect after methamphetamine. Future studies differentiating between reduced and oxidized forms of ascorbate after methamphetamine should permit a clearer interpretation of the role of ascorbate in methamphetamine toxicity.

In conclusion, methamphetamine selectively affects the glutathione antioxidant system without influencing ascor-

bate and vitamin E. The sensitivity of glutathione to methamphetamine may be due to its diversified functions as a primary antioxidant and as a co-factor in the actions of other antioxidants. Regardless, alterations in total glutathione in the striatum provide further evidence that methamphetamine produces oxidative stress.

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