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2,5-*bis*-(Glutathion-*S*-yl)-α-methyldopamine, a putative metabolite of (±)-3,4-methylenedioxyamphetamine, decreases brain serotonin concentrations

R. Timothy Miller [1], Serrine S. Lau, Terrence J. Monks [*]

Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas at Austin, Austin, TX 78712, USA

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Abstract

3,4-(±)-Methylenedioxyamphetamine (MDA) and 3,4-(±)-methylenedioxymethamphetamine (MDMA) are serotonergic neurotoxicants. However, when injected directly into brain, MDA and MDMA are not neurotoxic, suggesting that systemic metabolism plays an important role in the development of neurotoxicity. The nature of the metabolite(s) responsible for MDA- and MDMA-mediated neurotoxicity is unclear. α-Methyldopamine is a major metabolite of MDA and is readily oxidized to the *o*-quinone, followed by conjugation with glutathione (GSH). Because the conjugation of quinones with GSH frequently results in preservation or enhancement of biological (re)activity, we have been investigating the role of quinone-thioethers in the acute and long-term neurochemical changes observed after administration of MDA. Although intracerebroventricular (i.c.v.) administration of 5-(glutathion-*S*-yl)-α-methyldopamine (4 × 720 nmol) and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine (1 × 7 nmol) to Sprague-Dawley rats produced overt behavioral changes similar to those seen following administration of MDA (93 μmol/kg, s.c.) they did not produce long-term decreases in brain serotonin (5-hydroxytryptamine, 5-HT) concentrations. In contrast, 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (4 × 475 nmol) decreased 5-HT levels by 24%, 65% and 30% in the striatum, hippocampus and cortex, respectively, 7 days after injection. The relative sensitivity of the striatum, hippocampus and cortex to 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine was the same as that observed for MDA; the absolute effects were greater with MDA. The effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine were also selective for serotonergic nerve terminal fields, in that 5-HT levels were unaffected in regions of the cell bodies. Because 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine caused long-term depletion in 5-HT without adversely affecting the dopaminergic system, it also mimics the selectivity of MDA/MDMA. The data imply a possible role for quinone-thioethers in the neurobehavioral and neurotoxicological effects of MDA/MDMA.

Keywords: MDA (3,4-Methylenedioxyamphetamine); MDMA (3,4-Methylenedioxymethamphetamine); α-Methyldopamine; Glutathione; 5-HT (5-hydroxytryptamine, serotonin); Neurotoxicity

1. Introduction

The ring-substituted amphetamines, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), are well established serotonergic neurotoxicants (Ricaurte et al., 1985; Commins et al., 1987; Battaglia et al., 1988). Peripheral administration of MDA or MDMA causes decreases in markers of serotonergic function, including decreases in levels of serotonin (5-hydroxytryptamine, 5-HT) and in the 5-HT carrier protein in serotonergic nerve terminal fields (Ricaurte et al., 1985; Commins et al., 1987). The serotonergic deficits are long-lasting since recovery of these parameters is not observed for up to 18 months following administration of MDMA to nonhuman primates (Ricaurte et al., 1988, 1992). These observations are of concern to the general population because non-human primates appear to be especially susceptible to the toxicity of these agents (Ricaurte et al., 1988). Moreover, the recreational use of MDMA (Peroutka, 1987) appears to be increasing in both the

[*] Corresponding author. Tel.: (1-512) 471-6699; Fax: (1-512) 471-5002; e-mail: scouser@mail.utexas.edu

[1] Present address: University of Texas Health Science Center at San Antonio, Department of Biochemistry, 7703 Floyd Curl Drive, San Antonio, TX 78284-7760, USA.

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United States (Cuomo et al., 1994; McDowell and Kleber, 1994; Newmeyer, 1994) and Europe (Henry, 1992; Randall, 1992).

The neurotoxic component(s) of MDA and MDMA is not known, but roles for endogenous dopamine (Stone et al., 1988), and for the 5-HT_2 receptor (Schmidt et al., 1990, 1991) have been proposed. However, it appears that systemic metabolism is important for eliciting the neurotoxic response, because direct injection of MDA or MDMA into brain does not cause long-term serotonergic deficits (Molliver et al., 1986; Schmidt and Taylor, 1988), and administration of α-methyldopamine or 3-*O*-methyl-α-methyldopamine, major metabolites of MDA and MDMA, into brain also fails to produce long-term serotonergic neurotoxicity (McCann and Ricaurte, 1991). Although direct central injection of 2,4,5-trihydroxyamphetamine or 2,4,5-trihydroxymethamphetamine, putative in vivo metabolites of MDA and MDMA, are toxic to the serotonergic neurotransmitter system, they also target the dopaminergic system (Johnson et al., 1992; Elayan et al., 1992; Zhao et al., 1992), and thus do not exhibit the selectivity of the parent amphetamines. In addition, mechanisms by which these metabolites gain access to the brain have not been determined.

MDA is metabolized to α-methyldopamine (Marquardt et al., 1978; Midha et al., 1978) and MDMA to *N*-methyl-α-methyldopamine and α-methyldopamine (Lim and Foltz, 1988; Kumagai et al., 1991) both of which are catechols that can undergo oxidation to the corresponding *ortho*-quinones, followed by the reductive addition of glutathione (GSH) to form GSH conjugates (Hiramatsu et al., 1990; Patel et al., 1991). Since quinone-thioethers retain their biological (re)activity (Monks and Lau, 1992), we have been investigating the potential role of thioether metabolites of α-methyldopamine in the neurotoxicity of MDA. 5-(Glutathion-*S*-yl)-methyldopamine is metabolized via the mercapturic acid pathway within the central nervous system (CNS), forming 5-(cystein-*S*-yl)-α-methyldopamine and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine (Miller et al., 1995). 5-(Glutathion-*S*-yl)-α-methyldopamine is also readily oxidized to the corresponding quinone-GSH conjugate and undergoes addition of a second molecule of GSH to form 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine. The brain uptake of peripherally administered 5-(glutathion-*S*-yl)-α-methyldopamine has also been demonstrated (Miller et al., 1996). However, long-term deficits in brain 5-HT levels were not observed following a single i.c.v. infusion of 720 nmol 5-(glutathion-*S*-yl)-α-methyldopamine (Miller et al., 1996). Because multiple dose regimens have been employed in models of MDMA and MDA neurotoxicity, and because such regimens might produce shifts in the metabolism and/or accumulation of a neurotoxic metabolite in brain tissue, the present study was undertaken to determine the effects of 5-(glutathion-*S*-yl)-α-methyldopamine, 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine and 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine on rat brain dopamine and 5-HT concentrations following multiple-dose administration.

2. Materials and methods

2.1. Chemicals

Dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxyindole acetic acid (5-HIAA), GSH and mushroom tyrosinase (5600 U/mg) were obtained from Sigma (St. Louis, MO, USA). 5-HT-hemihydrate and *N*-acetyl-L-cysteine were obtained from Aldrich (Milwaukee, WI, USA). α-Methyldopamine was a generous gift from Anthony Y.H. Lu at Merck Research Laboratories (Rahway, NJ, USA). (±)-MDA was provided by the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD, USA). 5-(Glutathion-*S*-yl)-α-methyldopamine and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine were synthesized according to standard procedures (Ito and Prota, 1977; Hiramatsu et al., 1990), with modifications (Miller et al., 1995). All other chemicals were of the highest grade commercially available.

2.2. Synthesis of 2,5-bis-(glutathion-S-yl)-α-methyldopamine

2,5-*bis*-(Glutathion-*S*-yl)-α-methyldopamine was synthesized by dissolving 5-(glutathion-*S*-yl)-α-methyldopamine (approx. 95 mg) in 100 ml of 10% formic acid with stirring and adding 50 mg sodium periodate. The color of the solution changed from light golden to deep purple. GSH was then added, in aliquots, to excess until the color of the solution changed from deep purple back to light golden. The resulting reaction mixture was concentrated by rotary evaporation, frozen over dry ice/acetone and lyophilized to dryness. The resulting product was purified by high pressure liquid chromatography (HPLC; Shimadzu, LC-6A) by dissolving in 20 ml of 1% formic acid (approx. 2 mg/ml) and injecting 1 ml aliquots onto a Beckman Ultrasphere ODS-5 reverse-phase semipreparative column. The product was eluted using water (pH adjusted to 3.0 by addition of formic acid)/methanol (95:5) at a flow rate of 3 ml/min. Fractions containing the major UV absorbing product ($\lambda = 280$ nm; retention time 11 min) were collected, concentrated by rotary evaporation, frozen over dry ice/acetone and lyophilized to dryness. The powder resulting from the fractions containing 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine, when reanalyzed by HPLC with UV and coulometric electrode array detection (CEAS), gave rise to a single peak. The UV spectrum of the purified compound was recorded on a Shimadzu UV 160U spectrophotometer and the structure was confirmed by ^{1}H-nuclear magnetic resonance (NMR) (Bruker AM-500Hz) spectroscopy.

Table 1
Behaviors observed following administration of (±)-3,4-MDA, α-methyldopamine (α-MeDA), 5-(glutathion-*S*-yl)-α-MeDA, 5-(*N*-acetyl-L-cystein-*S*-yl)-α-MeDA (5-(NAC)-α-MeDA) and 2,5-*bis*-(glutathion-*S*-yl)-α-MeDA

	Hyperactivity	Forepaw treading	Straub tail	Low posture	Profuse salivation
(±)-3,4-MDA (93 μmol/kg, s.c.)	Yes	Yes	Yes	Yes	Yes
α-MeDA (3 μmol, i.c.v.)	No	No	No	No	No
5-(Glutathion-*S*-yl)-α-MeDA (720 nmol, i.c.v.)	Yes	Yes	Yes	Yes	No
5-(NAC)-α-MeDA (7 and 100 nmol, i.c.v.)	Yes	Yes	Yes	Yes	No
2,5-*bis*-(Glutathion-*S*-yl)-α-MeDA (475 nmol, i.c.v.)	Yes	Yes	Yes	Yes	Yes

2.3. ^{1}H-NMR and UV characterization of 2,5-bis-(glutathion-S-yl)-α-methyldopamine

2,5-*bis*-(Glutathion-*S*-yl)-α-methyldopamine was obtained as a white powder. In 1% formic acid, 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine exhibited a UV spectrum with λmax, nm (log εmax, M^{-1} cm^{-1}) at 301(s, 3.42), 275(3.90) and 237(3.90). ^{1}H-NMR (D_2O) δ 6.86 (s, H6), 4.25 (m, Cys-α), 3.62 (d, Glu-α), 3.63 (s, Gly-α), 3.44 (m, CH), 3.26, (m, Cys-β), 3.02 (m, Cys-β), 2.38 (m, CH_2), 2.32 (t, Glu-γ), 1.98 (m, Glu-β), 1.14 (t, CH_3).

2.4. Animals

Male Sprague-Dawley rats (200–225 g) (Harlan Sprague-Dawley, Houston, TX, USA) were used for all experiments. The animals were maintained on a 12 h light/dark cycle and were allowed free access to food and water before and during the experiments.

2.5. Dosing and analysis

The procedure for surgical implantation of cannulas has been previously described (Miller et al., 1995). Dosing solutions were prepared in artificial cerebrospinal fluid (ACSF; NaCl 147 mM, KCl 4 mM, $CaCl_2$ 1.2 mM and $MgSO_4$ 1.2 mM) at concentrations which would yield 5-(glutathion-*S*-yl)-α-methyldopamine (720 nmol), 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine (100 nmol) and 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (475 nmol) in a volume of 10 μl. This volume was infused into the left lateral ventricle of awake animals at a rate of 2 μl every 30 s. Animals were dosed every 12 h (6 a.m./6 p.m.) for a total of four consecutive doses. Control animals received four i.c.v. doses of ACSF. The doses of 5-(glutathion-*S*-yl)-α-methyldopamine and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine represented the maximum tolerated dose (higher doses were lethal). The dose of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine was the lowest dose at which behaviors were observed. Since the toxicity profile of MDA is well documented, MDA (93 μmol/kg, s.c.) was employed as a positive control. Seven days after the last dose, animals were killed by decapitation. Brains were dissected, processed, and analyzed by HPLC-CEAS as previously described (Miller et al., 1996).

2.6. Statistics

The Student's *t*-test was used to compare control to treated groups and a confidence level of at least 0.05 was used to determine significant differences.

3. Results

3.1. Acute behavioral response to MDA, α-methyldopamine, 5-(glutathion-S-yl)-α-methyldopamine, 5-(N-acetylcystein-S-yl)-α-methyldopamine and 2,5-bis-(glutathion-S-yl)-α-methyldopamine

Following multiple i.c.v. administration of 5-(glutathion-*S*-yl)-α-methyldopamine and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine, animals became hyperactive, aggressive, displayed forepaw treading, Straub tails, and most displayed splayed hind limbs (Table 1). Some animals began circling away from the side of the i.c.v. injection of 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine. These behaviors appeared abruptly (within 1–2 min following administration) and continued for approximately 30 min. With each successive dose, behaviors became less apparent. Following multiple i.c.v. administrations of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine, animals became hyperactive and displayed Straub tails (beginning 1–2 min post-dose). Five to ten minutes post-dose the animals

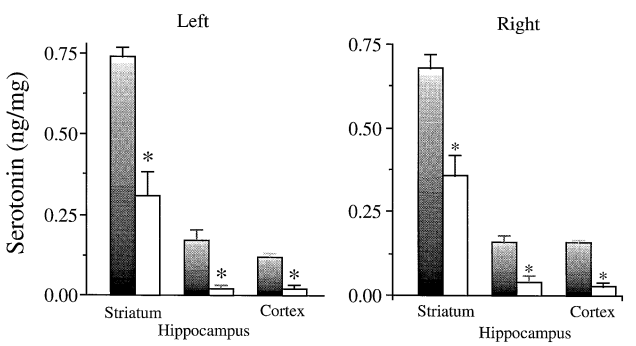

Fig. 1. Effects of (±)-3,4-methylenedioxyamphetamine (93 μmol/kg, s.c.) on serotonin (5-HT) concentrations in the striatum, hippocampus and cortex 7 days after drug administration. Black bars represent control animals, open bars represent drug-treated animals. Values are expressed as the mean ± S.E. of 4–7 animals, and are significantly different from controls at * $P < 0.05$.

Table 2
The lack of effect of 5-(glutathion-*S*-yl)-α-methyldopamine and 5-(*N*-acetyl-L-cystein-*S*-yl)-α-methyldopamine on serotonin concentrations in the striatum, hippocampus and cortex, 7 days after administration

	Ipsilateral		Contralateral	
	Control	Treated	Control	Treated
5-(Glutathion-*S*-yl)-α-methyldopamine				
Striatum	0.59 ± 0.08	0.62 ± 0.05	0.56 ± 0.05	0.59 ± 0.05
Hippocampus	0.17 ± 0.03	0.17 ± 0.02	0.21 ± 0.03	0.19 ± 0.02
Cortex	0.18 ± 0.03	0.16 ± 0.01	0.17 ± 0.02	0.16 ± 0.02
5-(*N*-Acetylcystein-*S*-yl)-α-methyldopamine				
Striatum	0.81 ± 0.06	0.76 ± 0.03	0.70 ± 0.03	0.59 ± 0.06
Hippocampus	0.28 ± 0.03	0.22 ± 0.01	0.28 ± 0.01	0.29 ± 0.02
Cortex	0.22 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.23 ± 0.01

5-(Glutathion-*S*-yl)-α-methyldopamine (4×720 nmol) and 5-(*N*-acetyl-L-cystein-S-yl)-α-methyldopamine (4×100 nmol) were adminstered by i.c.v. injection and 5-HT concentrations determined in brain tissue ipsilateral and contralateral to the site of injection, as described in Section 2. The data are expressed as ng 5-HT/mg wet weight, and represent the mean ± S.E. of 3–5 animals.

exhibited behaviors similar to 'wet dog shakes' and approximately 30 min post-dose the animals were salivating profusely (Table 1). After approximately 30 min following the initial dose, 3 of the 5 treated animals were standing on their hind legs for prolonged periods of time and moving their heads in circular motions. With each successive dose the hyperactivity decreased and by the third and fourth dose, only a few animals were displaying 'wet dog shakes'. With each successive dose, behaviors became less apparent. Similar overt changes in behavior were seen following the subcutaneous administration of MDA (Table 1).

3.2. Effects of MDA, 5-(glutathion-S-yl)-α-methyldopamine, 5-(N-acetyl-L-cystein-S-yl)-α-methyldopamine and 2,5-bis-(glutathion-S-yl)-α-methyldopamine on brain 5-HT and dopamine concentrations

Hippocampal, striatal and cortical 5-HT concentrations were significantly decreased 7 days after subcutaneous administration of MDA (93 μmol/kg), consistent with previously published data (Fig. 1). However, no such decreases were seen following either 5-(glutathion-*S*-yl)-α-methyldopamine (4 × 720 nmol) or 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine (4 × 100 nmol) administration (Table 2). In contrast, 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (4 × 475 nmol) produced serotonergic neurotoxicity 7 days after the last dose (Fig. 2). In cortex, the concentration of 5-HT was decreased by 29 and 40% (ipsilateral, $P < 0.001$; and contralateral, $P < 0.005$), respectively. In hippocampus, ipsilateral 5-HT concentrations were decreased by 65% ($P < 0.001$) whereas contralateral 5-HT concentrations were unaffected. In addition, 5-HIAA concentrations were significantly ($P < 0.004$) decreased by 37% in ipsilateral, but not contralateral hippocampus (Fig. 3). The striatum was less effected by 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine; 5-HT concentrations were 24 and 16% of control (ipsilateral, $P < 0.07$; and contralateral, $P < 0.08$), respectively. Further support for a potential role for 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine in MDA-mediated neurotoxicity is based on its selectivity. Thus, 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine caused long-term depletions in 5-HT without ad-

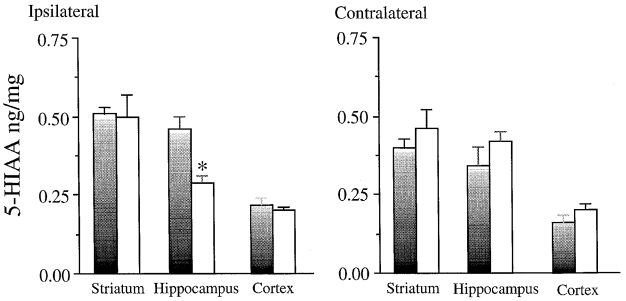

Fig. 3. Effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (4×475 nmol, i.c.v.) on 5-hydroxyindole acetic acid (5-HIAA) concentrations in the striatum, hippocampus and cortex 7 days after drug administration. Black bars represent control animals, open bars represent drug-treated animals. Values are expressed as the mean ± S.E. of 4–7 animals and are significantly different from controls at * $P < 0.05$.

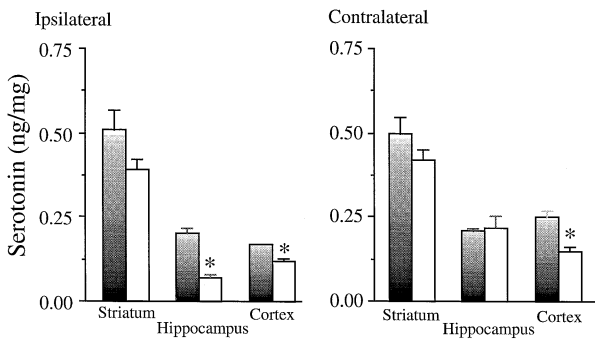

Fig. 2. Effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (4×475 nmol, i.c.v.) on serotonin (5-HT) concentrations in the striatum, hippocampus and cortex 7 days after drug administration. Black bars represent control animals, open bars represent drug-treated animals. Values are expressed as the mean ± S.E. of 4–7 animals and are significantly different from controls at * $P < 0.05$.

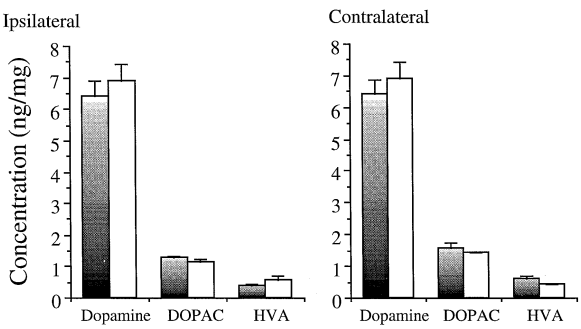

Fig. 4. Effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (4×475 nmol, i.c.v.) on dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) concentrations in the striatum, 7 days after drug administration. Black bars represent control animals, open bars represent drug-treated animals. Values are expressed as the mean ± S.E. of 4–7 animals and are significantly different from controls at * $P < 0.05$.

versely affecting the dopaminergic system (Fig. 4), again mimicking the selectivity of the parent drugs (Commins et al., 1987; O'Hearn et al., 1988). The effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine were also selective for 5-HT nerve terminal fields (striatum, hippocampus, cortex) in that 5-HT levels were unaffected in regions of the cell bodies (0.59 ± 0.01 vs. 0.55 ± 0.02 ng/mg in the pons/medulla and 0.81 ± 0.12 vs. 0.69 ± 0.06 ng/mg in the mid-brain, in control and 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine-treated groups, respectively; mean ± S.E. of 4–7 animals). This is also a characteristic of MDA-mediated neurotoxicity.

4. Discussion

Administration of MDA subcutaneously, or 5-(glutathion-*S*-yl)-α-methyldopamine by i.c.v. injection, to rats, produces a very similar behavioral response (Table 1). This behavioral syndrome, termed the 'serotonin syndrome', typically occurs following administration of 5-HT releasers such as MDA, and consists of forepaw treading, Straub tail, head weaving, hyperactivity and 'wet dog' shakes. Although both 5-(glutathion-*S*-yl)-α-methyldopamine and MDA produced this behavioral syndrome, the time-course for the appearance of the behaviors was different between the different compounds. 5-(Glutathion-*S*-yl)-α-methyldopamine-treated animals began to exhibit changes in behavior within 1–2 min following drug administration, while the onset of behaviors for MDA-treated animals was delayed for approximately 15 min. The difference in the time of onset of behaviors and the similarity in behavioral profiles between 5-(glutathion-*S*-yl)-α-methyldopamine and MDA is perhaps indicative of a requirement for absorption, metabolism and distribution processes.

5-(Glutathion-*S*-yl)-α-methyldopamine crosses the blood–brain barrier (Miller et al., 1996) and is rapidly metabolized within the CNS to 5-(cystein-*S*-yl)-α-methyldopamine and 5-(*N*-acetyl-L-cystein-*S*-yl)-α-methyldopamine (Miller et al., 1995), the latter being the final redox active metabolite with an apparent ability to persist in brain tissue. Remarkably, 5-(*N*-acetyl-L-cystein-*S*-yl)-α-methyldopamine was able to reproduce the overt behaviors of MDA at a dose of only 7 nmol (0.03% dose of MDA) (Table 1). Based on these observations, we examined a multiple dose regimen with 5-(*N*-acetyl-L-cystein-*S*-yl)-α-methyldopamine with the expectation that such a protocol might result in the accumulation of this metabolite to toxic concentrations. However, no long-term serotonergic neurotoxicity was observed with this metabolite (Table 2) implying that the acute behavioral changes can be dissociated from the long-term neurotoxicity.

Since 5-(glutathion-*S*-yl)-α-methyldopamine is able to reoxidize and form 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine, and since the potency of polyphenolic-GSH conjugates increases with the degree of GSH substitution (Lau et al., 1988), we examined the effects of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine on brain 5-HT concentrations. 2,5-*bis*-(Glutathion-*S*-yl)-α-methyldopamine decreased serotonin levels by 24%, 65% and 30% in the striatum, hippocampus and cortex, respectively (Fig. 2). The relative sensitivity of the striatum, hippocampus and cortex to 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine was the same as that observed for MDA, although the absolute effects were greater with MDA (Fig. 1). The greater sensitivity of the hippocampal serotonergic system to 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine compared to the cortex may be a consequence of the proximity of the lateral ventricles to the hippocampus, thus permitting greater access to 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine. Consistent with this view, concentrations of 5-(glutathion-*S*-yl)-α-methyldopamine, at the earliest time-point (15 min) measured following i.c.v. injection, were highest in the hippocampus > striatum > cortex (Miller et al., 1995). Potential differences in exposure to 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine however cannot fully explain the lower sensitivity of the striatal system, since it is also close to the ventricles. Experiments on the effects of direct intrastriatal and intracortical administration of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine may answer these questions.

5-(Glutathion-*S*-yl)-α-methyldopamine is rapidly cleared from the brain (Miller et al., 1995) and it is therefore likely that the neurotoxicity of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine is mediated by downstream metabolites such as 2,5-*bis*-(cystein-*S*-yl)-α-methyldopamine and 2,5-*bis*-(*N*-acetylcystein-*S*-yl)-α-methyldopamine. The toxicity of these metabolites can be regulated by intramolecular cyclization reactions that occur subsequent to oxidation (Monks et al., 1990; Miller et al., 1995), therefore it may be important that there are regional differences in the distribution of cysteine conjugate *N*-acetyl transferase and *N*-acetylcysteine conjugate deacetylase in the brain (Miller et al., 1995). Cyclization of 2,5-*bis*-(cystein-*S*-yl)-α-methyldopamine may occur in one of two ways. Following oxidation, the side chain (alanine-derived) amino group can cyclize to give the 5,6-dihydroxyindole or the cysteinyl amino group can condense with the quinone carbonyl to give a benzothiazolyl-like compound. Only the latter reaction removes the reactive quinone function, since the dihydroxyindole can undergo further oxidation. Because the cysteinyl amino groups are blocked in 2,5-*bis*-(*N*-acetylcystein-*S*-yl)-α-methyldopamine it can no longer undergo cyclization following oxidation, and this metabolite will maintain redox activity. The ratio of *N*-acetylation to *N*-deacetylation in the hippocampus is more than double that in the striatum and 5-(*N*-acetylcystein-*S*-yl)-α-methyldopamine appears to persist in the brain after i.c.v. administration of 5-(glutathion-*S*-yl)-α-methyldopamine (Miller et al., 1995). Experiments on the metabolism and distribution of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine following i.c.v. administration are required to address these questions.

Whether 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine produces decreases in 5-HT concentrations by a similar mechanism as MDA and MDMA is not known. The combination of the polyphenolic (catechol) and peptide (γ-glutamylcysteinylglycine; GSH) structure of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine, and its metabolites, probably confers both pharmacological and toxicological properties on this class of metabolites (Miller et al., 1996). Thus, GSH possesses diverse neuropharmacological properties (Ogita and Yoneda, 1988; Pileblad et al., 1989; Zangerle et al., 1990; Guo et al., 1992; Kubo et al., 1992; Leslie et al., 1992; Liu and Quirion, 1992; Strupp et al., 1992), cysteine is neurotoxic in young animals (Olney, 1971; Olney et al., 1990; Fonnum et al., 1992) and the electrophilic and redox properties of the conjugates may also initiate neurotoxic effects. Quinone-thioethers retain the ability to redox cycle, with the concomitant generation of reactive oxygen species (Wefers and Sies, 1983; Brown et al., 1991; Mertens et al., 1995) a role for which has been described in methamphetamine-induced serotonergic neurotoxicity (Hirata et al., 1995). Studies with Cu/Zn-superoxide dismutase transgenic mice also implicate $O_2^{\cdot -}$ in MDMA-mediated dopaminergic neurotoxicity (Cadet et al., 1995) and in the lethal effects of MDA and MDMA in mice (Cadet et al., 1994). *Ortho*-quinones are also electrophiles, and readily react with nucleophiles, particularly protein sulfhydryls. Reduced sulfhydryls appear important in maintaining neuronal 5-HT transporter function (Wolf and Kuhn, 1992) and either alkylation or oxidation of this transporter may contribute to MDA and MDMA-mediated neurotoxicity. 5-HT nerve terminal fields are the most severely affected by 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine (Fig. 2), with regions closer to the serotonergic nerve cell bodies being less severely affected. This pattern is similar to the changes seen after MDA and MDMA administration (Ricaurte et al., 1985; Harvey et al., 1993). Multiple doses of 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine also decrease levels of 5-HIAA in the hippocampus (Fig. 3) consistent with the effects of MDA and MDMA. In contrast, decreases in 5-HT concentrations in the cortex were not accompanied by similar decreases in 5-HIAA (Fig. 3). It is possible that undamaged neurons in this region are compensating for the loss of function of the damaged neurons. In support of this view, immunocytochemical studies by O'Hearn et al. (1988) revealed a loss of 5-HT in small variscosities and fine terminals caused by MDMA, accompanied by an increase in diameter and staining of the larger preterminal axon fibers. In addition, although tryptophan hydroxylase activity decreases in the cortex, hippocampus, and striatum of MDMA-treated animals, it is elevated in the raphe nucleus (Stone et al., 1988). Interestingly, increases in 5-HIAA concentrations, in the presence of decreased 5-HT levels, were seen in animals treated with trihydroxymethamphetamine i.c.v. (Johnson et al., 1992).

The spectrum of behavioral and toxicological sequelae that occur following MDMA and MDA administration make it highly unlikely that just one metabolite produces such complex effects. Thioether metabolites of α-methyldopamine reproduce the overt behavioral changes caused by MDA at remarkably low doses, and 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine reproduces the long-term deficits in brain 5-HT concentrations. However, there remain a sufficient number of subtle differences in the response to MDA and 2,5-*bis*-(glutathion-*S*-yl)-α-methyldopamine that most likely require the participation of other, perhaps as yet unidentified, metabolites.

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