3,4-Methylenedioxymethamphetamine (MDMA, ecstasy)-mediated production of hydrogen peroxide in an in vitro model: the role of dopamine, the serotonin-reuptake transporter, and monoamine oxidase-B

Sandra L. Hrometz a, Andrew W. Brown a, David E. Nichols b, Jon E. Sprague a,∗

a The Department of Pharmaceutical and Biomedical Sciences, The Raabe College of Pharmacy, Ohio Northern University, Ada, OH 45810, USA
b The Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, USA

Abstract

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) has been shown to induce long-term deficits in serotonergic function in animal models. Several studies have suggested that dopamine (DA) uptake into serotonin (5-HT) terminals by the 5-HT reuptake transporter (SERT) and subsequent deamination by monoamine oxidase-B (MAO-B) leads to the formation of hydrogen peroxide and may be major contributors to this serotonergic toxicity. In the present study, when human choriocarcinoma (JAR) cells were exposed to MDMA (1.2 mM) for 6 h, followed by treatment with DA (0.1 mM), hydrogen peroxide production increased over a 24 h period, peaking at 420% over baseline and decreasing cell viability by 30%. DA alone increased hydrogen peroxide production 84% over baseline, but did not significantly decrease cell viability. Incubation of MDMA treated cells with the SERT inhibitor, fluoxetine (500 nM) or the MAO-B inhibitor, l-deprenyl (0.1 mM) for 30 min prior to DA, significantly blocked free radical production and cell death. These findings support the hypothesis that the deamination of DA by MAO-B within the serotonergic cell can lead to hydrogen peroxide formation and ultimately cell death.

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a recreational drug widely abused by large numbers of teenagers and young adults [11,12]. Chronic abusers of MDMA have been reported to suffer from serotonergic neurotoxicity [16] and memory impairment [1] that is ostensibly linked to alterations in serotonergic function. The formation of hydroxyl free radicals [3,28] and subsequent lipid peroxidation [32] appear to be critical events in the loss of serotonergic function seen following MDMA treatment. We previously suggested that MDMA-induced serotonin neurotoxicity within the striatum involves multiple steps [30]. In general, monoamine oxidase (MAO) catalyzes the deamination of monoamine neurotransmitters such as dopamine (DA) and serotonin (5-HT). Of the two isoforms (monoamine oxidase-A (MAO-A) and MAO-B), monoamine oxidase-B (MAO-B) is found primarily in 5-HT nerve terminals [13]. Deamination of DA by MAO-B results in the production of hydrogen peroxide and hydroxyl free radicals [2,15]. The detection of lipid peroxidation products in the striatum of rats administered MDMA was taken as evidence for the formation of this hydrogen peroxide and ultimately hydroxyl free radicals [32]. The cellular mechanisms involved in this neurotoxic process in other susceptible brain regions (i.e. hippocampus) may involve other catecholamines [27]. Furthermore, these neurotoxic effects are greatly affected by the thermogenic response seen following MDMA administration [14], and studies of many pharmacologic agents that have been shown to protect against MDMA-induced free-radical formation and lipid peroxidation may have been inadvertently confounded by drug induced attenuation of MDMA-mediated hyperthermia.

In vivo, MDMA administration induces the immediate release of neuronal 5-HT, and inhibition of tryptophan hydroxylase, resulting in depletion of neuronal 5-HT [26,34].
The initially released 5-HT activates 5-HT2A postsynaptic receptors that lead to the upregulation of DA biosynthesis and release [20,24,25]. We have hypothesized that within the striatum the resulting excess DA is then transported into the 5-HT-depleted terminals [20,32] by the serotonin reuptake transporter (SERT) [7]. We propose that serotonergic terminals must first be depleted of 5-HT in order for significant DA transport to occur. For example, tryptophan or 5-hydroxytryptophan administered before MDMA increase 5-HT levels [10] and also attenuate its neurotoxicity [31]. Our contention is also supported by recent data demonstrating that DA can be efficiently utilized as a substrate by the SERT in a temperature-dependent fashion [23]. Furthermore, fluoxetine, a SERT inhibitor, is capable of protecting against MDMA-induced serotonergic neurotoxicity, even when administered up to 6 h after MDMA [24]. Once concentrated within the serotonergic terminal, the DA then could be deaminated by MAO-B, ultimately generating hydrogen peroxide and other potential reactive oxygen species. Consistent with this idea, pharmacologic or genetic reductions in MAO-B activity have also been shown to protect against MDMA-induced loss of serotonergic function in the striatum [6,8,32].

The aim of the present study was to investigate the role of DA as the potential source of the hydrogen peroxide formed after MDMA treatment, using a human choriocarcinoma cell line (JAR) that constitutively expresses a functional SERT [4,22]. The JAR cell line stably expresses high affinity 5-HT transporters with characteristics similar to those of the human platelet, brain, and placenta, and also possesses monoamine oxidase activity [4]. JAR cells were plated at a density of 5.0–7.5 × 10^4 cells/well (150 μL) into 96-well plates (Nunc Nalge) and left to adhere for 12 h before experimentation. Cells were treated with 1.2 mM MDMA for 6 h. This dose of MDMA was utilized based on previous dose response characterizations of MDMA in the JAR cell line [29]. The media was then removed to eliminate excess or unbound MDMA and any 5-HT released from the cells in response to treatment. Next, media containing 0.1 mM DA was introduced and left to adhere for 12 h before experimentation. Cells were treated with either fluoxetine (a SERT inhibitor) or l-deprenyl (a MAO-B inhibitor) for 30 min prior to treatment with DA. These studies were run in triplicate.

MDMA was synthesized following the method of Nichols et al. [21]. Hydrogen peroxide production was measured using the Amplex® Red Monoamine Oxidase Activity Assay Kit from Molecular Probes® (Eugene, OR) [18,37]. Cell viability was assessed using an MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) cell proliferation assay (American Type Culture Collection, Rockville, MD) [9,19]. RPMI-1640 media and penicillin-streptomycin were purchased from VWR International (West Chester, PA). The MTT cell proliferation assay and the JAR cells were obtained from American Type Culture Collection (Rockville, MD). All other reagents were purchased from Sigma (St. Louis, MO).

Cell treatment was comprised of: 1. control media; 2. MDMA alone; 3. MDMA prior to fluoxetine, followed by DA; or 4. MDMA prior to l-deprenyl, followed by DA, and resulted in 7, 9, 35 and 23% increases, respectively, in hydrogen peroxide production over baseline after 24 h (Fig. 1A). Treatment with DA alone led to a larger 84% increase and, most importantly, MDMA treatment followed by DA resulted in a very marked 420% increase in hydrogen peroxide production over baseline after 24 h. Although cells treated with DA alone had hydrogen peroxide production significantly above baseline after 12, 18 and 24 h, the cells treated with MDMA followed by DA had hydrogen peroxide production significantly above baseline after all time points: 1, 6, 12, 18, and 24 h and the extent of peroxide elevation was markedly greater. There were no significant increases in hydrogen peroxide production in cells treated with MDMA alone, fluoxetine or l-deprenyl alone, fluoxetine followed by l-deprenyl, or MDMA alone with fluoxetine, followed by DA. These results suggest that the peroxide formed by MDMA treatment is dependent on the presence of DA, which is consistent with our previous observations that the hydrogen peroxide formed by MDMA is a by-product of the autooxidation of DA [26].

The differences between groups were analyzed using a one-way ANOVA followed by a Student-Newman-Keuls Multiple Comparison Test.

![Graph](image-url)
by DA (Fig. 1A), or 1-deprenyl followed by DA (data not shown).

When 24 h cell viability was assessed, treatment with media, DA only, MDMA only, and MDMA followed by treatment with fluoxetine or 1-deprenyl prior to the addition of DA had no effect. In stark contrast, treatment with MDMA followed by DA resulted in a 30% decrease in cell viability over 24 h (Fig. 1B).

The blockade of hydrogen peroxide production and cell death by fluoxetine suggests that DA uptake by SERT is important in this neurotoxic process. The ability of 1-deprenyl to reduce hydrogen peroxide production and cell death induced by DA in MDMA treated cells confirms a role for DA deamination. Thus, DA uptake and deamination within the serotonergic cells is an important event in the formation of hydrogen peroxide after MDMA treatment.

The lack of cell death with MDMA alone in this in vitro model is consistent with in vivo studies that have demonstrated a lack of neurotoxicity with direct administration of MDMA into the striatum [5]. Although the direct administration of MDMA into the striatum has been shown to induce a neurochemical response that includes the acute release of DA and 5-HT, neurotoxicity was not induced [5]. Those authors thus concluded that the ultimate source of the toxic insult must be a metabolite of MDMA. Much controversy surrounds this conjecture, however. When the potential MDMA metabolite 2,4,5-trihydroxymethamphetamine was directly injected into the striatum local serotonergic and dopaminergic toxicity were both induced, but no toxicity was seen after an intracerebroventricular (ICV) dose [36]. Therefore, the pattern of toxicity differed from that typically seen with systemic doses of MDMA in the animal model. Furthermore, α-methyldopamine, a metabolite of the MDMA deamination product 3,4-methylenedioxyamphetamine (MDA), when injected into the striatum was devoid of serotonergic toxicity [33]. By contrast, a putative dose of α-methyldopamine generated a serotonergic toxicity [35] and the glutathione thioester of α-methylidopamine induced a serotonergic toxicity after ICV administration [17].

In the present study, the incubation of JAR cells with MDMA did not increase hydrogen peroxide formation or cell death until an exogenous source of DA was also added. Although this observation does not eliminate the possibility of a MDMA metabolite being involved in the neurotoxic process, it does suggest that MDMA primes the cells for the toxic effect of DA.

The primary focus of the present study was to delineate further the role of DA as the source of hydrogen peroxide produced during the MDMA-induced serotonergic neurotoxicity process. In a human cell line endogenously expressing both the SERT and MAO, MDMA treatment followed by DA induced a significant increase in both hydrogen peroxide production and cytotoxicity. Neither MDMA nor DA alone was able to induce the same degree of hydrogen peroxide production and cell death. Cells treated with MDMA followed by either fluoxetine or 1-deprenyl before adding DA were protected from the excessive hydrogen peroxide production and cytotoxicity produced by MDMA followed only by DA. These findings are consistent with in vivo pharmacologic studies that have demonstrated the ability of fluoxetine [24] and 1-deprenyl [32] to protect against MDMA induced neurotoxicity. Our results are consistent with the hypothesis that SERT-mediated uptake of DA into 5-HT terminals and subsequent deamination by MAO-B contribute to free radical formation.

Acknowledgements

This work was supported in part by grants from Pfizer Inc., and Bowyer, J. Bennett, H. Bennett Pharmacy Research Foundation.

References


