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Minireview

Multiple molecular and neuropharmacological effects of MDMA (Ecstasy)

Rabi Simantov*

Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA), commonly referred to as Ecstasy, is a widely abused, psychoactive recreational drug, which induces short- and long-term neuropsychiatric behaviors. This drug is neurotoxic to serotonergic neurons in vivo, and induces programmed cell death in cultured human serotonergic cells and rat neocortical neurons. Over the years it has been shown that MDMA alters the release of several neurotransmitters in the brain, it induces recompartmentation of intracellular serotonin and c-fos, and modifies the expression of a few genes. Recently, we observed changes in gene expression in mice treated with MDMA, and cloned and sequenced 11 cDNAs thus affected (4 correspond to known and 7 to unknown genes). The effect of MDMA on two of these genes, GABA transporter 1 and synaptotagmin IV was studied in detail. Characterization of the relationship between a given gene and certain physiological or behavioral effects of MDMA could shed light on the mechanism of the drug's action. However, establishing such a connection is difficult for several reasons, including that serotonergic neurons are not the only cells affected by MDMA. In this review, molecular and neurochemical events that occur in the brain following exposure to MDMA, and link between the observed molecular changes with known physiological effects of the drug are discussed. It is indicated that MDMA alters the expression of several proteins involved in GABA neurotransmission, thus having critical effect on thermoregulation and MDMA acute toxicity. This analysis should facilitate development of novel approaches to prevent deleterious effects, especially mortality induced by MDMA and other abused psychostimulants.

Keywords: Serotonin; Drug abuse; Gene expression; Protein trafficking; Dopamine; GABA; Neuronal cell death; Plasticity; Hyperthermia

* Tel.: +972-8934-2110; fax: +972-8934-4108.
E-mail address: lgrabi@wicc.weizmann.ac.il (R. Simantov).

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Introduction

The abused psychoactive drug MDMA induces psychostimulation, hallucination, and long-term neuropsychiatric behaviors, such as depression and psychosis (for review see Green et al., 1995; Ricaurte and McCann, 2001). Initially, MDMA was found to be neurotoxic primarily to serotonergic neurons, and to induce degeneration of neuronal fibers (Ricaurte et al., 1985; Schmidt, 1987; O'Hearn et al., 1988). However, it is now known that MDMA neurotoxicity is less selective in some species, and often affects the dopaminergic pathway (Stone et al., 1987; Logan et al., 1988; Ricaurte and McCann, 1992; Cadet et al., 1994; Scheffel et al., 1998; Ricaurte et al., 2002). Briefly, the physiological effects of MDMA in animals include enhanced motor activity, hyperthermia, and a set of behaviors referred to as serotonin behavioral syndrome (SBS; Slikker et al., 1988; Spanos and Yamamoto, 1989; Green et al., 1995; Colado et al., 1997a; Lyles and Cadet, 2003). When administered experimentally at a high dose, and occasionally in drug users, MDMA can cause convulsions, acute toxicity, and death (Schmidt et al., 1990; Gordon et al., 1991; Henry et al., 1992; Drafters, 1994; Hegadoren et al., 1999; McCann et al., 2000; Gill et al., 2002). Long-term memory deficits occur in rats exposed to the drug during brain development (Broening et al., 2001). In humans, MDMA impairs verbal, visual, and recall memories (Bolla et al., 1998; Morgan, 1999; Zakzanis and Young, 2001). In vitro, the drug is also toxic, and induces programmed cell death in human JAR cells (Simantov and Tauber, 1997), rat neocortical neurons (Stumm et al., 1999), and other cell types (Montiel-Duarte et al., 2002). Increased free radical production appears to be associated with MDMA toxicity (Colado et al., 1997b; Cadet et al., 2001; Camarero et al., 2002), and possibly also nitric oxide (NO) (Taraska and Finnegan, 1997; Simantov and Tauber, 1997; Zheng and Laverty, 1998; Itzhak et al., 2003).

Primary effects of MDMA on serotonin neurotransmission

Binding of MDMA to its paramount target in the brain, the presynaptic serotonin transporter (SERT), inhibits serotonin reuptake, and enhances SERT-mediated exchange and release of serotonin (Nichols et al., 1982; Rudnick and Wall, 1992; Gudelsky and Nash, 1996; Iravani et al., 2000). Serotonin thus released must be swiftly removed from synapses to maintain properly controlled neurotransmission. Since SERT is blocked by MDMA, the neurotransmitter accumulates in the synaptic cleft, and will eventually degrade or possibly taken up by other transporters (see below). Following the early release of serotonin, there is a long-term depletion of the neurotransmitter in the brain, severity of which depends on the various regions and animal species. Mutant mice deficient in SERT were insensitivity to MDMA (Bengel et al., 1998), confirming a major role of the transporter in the mode of action of MDMA.

While the early effects of MDMA on serotonin homeostasis (release, uptake, and exchange) are relatively well understood, the events following them are still unascertained. Evidently, the MDMA-enhanced release of serotonin activates both pre- and postsynaptic serotonin receptors (Sprague et al., 1998; Bankson and Cunningham, 2001), and induces intracellular pathways, such as adenylate cyclase and IP_3, or a direct ligand-gated Na/Ca current. Activation of multiple systems will probably cause a variety of neurochemical and molecular events. Indeed, MDMA influences expression of several genes, including bcl-xl, bcl-xs (Stumm et al., 1999), c-fos (Stephenson et al., 1999; Erdtmann-Vourliotis et al., 1999), egr-1 (Shirayama et al., 2000), and 5-HT1C, glucocorticoid and mineralo-

corticoid receptors (Yau et al., 1994, 1997). However, until recently a relationship between a specific gene and a certain physiological or behavioral effect of MDMA was not found. Difficulties in establishing such relationships are probably due to the ability of MDMA to also affect cells other than serotonergic neurons.

Alteration of the release of several neurotransmitters by MDMA

In the brain, interplay between serotonergic and dopaminergic pathways exists at several levels and has various implications (Gasper et al., 1993; Ichikawa and Meltzer, 1999; Gainetdinov et al., 1999). Crosstalk between the two systems is also relevant to MDMA mode of action. Ample evidence indicate that the effect of MDMA on the serotonergic system is tightly associated with activation of dopaminergic pathways and release of dopamine (Brodkin et al., 1993; Gudelsky and Nash, 1996; Shankaran and Gudelsky, 1998; Yamamoto et al., 1995; Ricaurte et al., 2002). The enhanced release of dopamine by MDMA may partially result from reversal of dopamine transport (Nash and Brodkin, 1991), in addition to the more common Ca^{++}-dependent release (Crespi et al., 1997; Camarero et al., 2002). It is worth indicating also that MDMA itself has a low affinity to dopamine transporters, thus not excluding involvement of other mechanisms (see Iravani et al., 2000).

Neurotransmitters other than serotonin and dopamine are also affected by MDMA. Activation of serotonin receptors upon treatment with the drug decreased the efflux of γ – amino butyric acid (GABA) in the substantia nigra (Yamamoto et al., 1995; Sprague et al., 1998; Bankson and Cunningham, 2001). This lower activity of GABAergic cells is crucial to the enhanced activity of dopaminergic neurons, and dopamine release. Also, involvement of glutamate in MDMA activity was shown in release experiments and by using glutamate receptor antagonists (Farfel et al., 1992; Nash and Yamamoto, 1992; Finnegan and Taraska, 1996).

Plasticity in serotonin reuptake: involvement of dopamine transporters and relevancy to MDMA toxicity

Mutants lacking a particular neuronal property are effective research tools for studying aspects of the nervous system. Using this approach, an important role of the serotonergic system was revealed in dopamine transporter knockout mice (Gainetdinov et al., 1999). We used a SERT knockout mice model to elucidate plasticity at the level of serotonin transport (Pan et al., 2001). As expected, uptake of serotonin in cultured neuronal cells from wild-type mice was completely blocked by fluoxetine, a selective SERT inhibitor. In contrast, uptake of serotonin in cultured neuronal cells from SERT knockout mice, although very weak, was inhibited more by nomifensine, a dopamine uptake inhibitor, than by fluoxetine (Prozac). Immunocytochemical staining of cultured cells from SERT knockout mice with anti-serotonin antibodies was blocked by nomifensine, again suggesting that serotonin was taken up by dopamine transporters. These and other findings imply that in cultures of SERT knockout mice, non-serotonergic, apparently dopaminergic neurons can acquire the capacity to take up serotonin (Pan et al., 2001). This is in agreement with in vivo voltametry studies in which striatal dopaminergic terminals exhibited "false transmission", release of serotonin after 5-HTP pretreatment (Stamford et al., 1990). This functional plasticity in serotonin uptake suggests that when SERT is blocked or absent, by MDMA

or in SERT knockout animals, respectively serotonin can be taken up by non-serotonergic neurons, such as dopaminergic ones. Likewise, an increased extracellular dopamine accumulated upon MDMA treatment, at a time of severe serotonin depletion, could end up with some dopamine uptake in serotonergic terminals, and thus contribute to the neurotoxic effect of the drug (see also Sprague et al., 1998).

Effect of MDMA on intracellular compartmentalization of serotonin and c-fos

MDMA is cytotoxic to serotonergic human JAR cells, with apoptotic particles being observed within 24 hours (Simantov and Tauber, 1997; Meissner et al., 2001). This MDMA activity is associated with alterations in the intracellular distribution of serotonin and c-fos (Meissner et al., 2001). In untreated JAR cells, the immunoreactivity of both serotonin and c-fos is evenly distributed in the cytoplasm. Upon treatment with an MDMA concentration inducing apoptosis (0.8 mM), this distribution changes within 30 min; serotonin starts to concentrate in small regions near the center of the cell in a time dependent way, finally resulting with a few and very strongly labeled vesicle-like organelles at 24 hours after treatment. c-fos distribution takes a similar pattern of a more-strongly labeled spots within the first 30–60 minutes after MDMA treatment. However, the effect of MDMA on c-fos subsides with time and immunoreactivity of the transcription factor returns within 24 hours to the distribution in untreated cells. Since redistribution of c-fos occurs prior to MDMA-induced cell death, this transcription factor may play a role during the early stages of the drug action, and one cannot exclude the possibility that it has a protective role. Interestingly, the serotonin transporters fluoxetine and imipramine, as well as amphetamine and fenfluramine, also induce redistribution of serotonin (Meissner et al., 2001). Yet, at low dose the effect of these four compounds on serotonin redistribution was transient, and did not induce cell death. Therefore, while blocking the serotonin transporter appears to be sufficient to alter the intracellular distribution of serotonin, this by itself does not appear to be sufficient to induce toxicity.

Using a model system involving rat organotypic striatal slices, Schatz et al. (2000) found that MDMA induced both c-fos protein and mRNA. In vivo analysis showed that MDMA increased by 3–5 fold the number of neurons labeled with both c-fos and serotonin in the raphe nucleus (Stephenson et al., 1999), being in agreement with our finding with cultured JAR cells (Meissner et al., 2001). It appears also that induction of c-fos by several psychoactive compounds correlates negatively with the addictive potency of the drugs (Erdtmann-Vourliotis et al., 1999). Analyzing the relationship between c-fos and apoptotic or suppressor genes should facilitate elucidation of early events responsible for MDMA-induced cell death.

Identification of genes induced or down regulated by MDMA: the GAT family

Over the last decade effort has been made to identify the genes involved in the action of MDMA (Yau et al., 1994, 1997; Erdtmann-Vourliotis et al., 1999; Stephenson et al., 1999; Stumm et al., 1999; Shirayama et al., 2000). Using the differential display PCR (DD-PCR) approach to elucidate the gene expression profile of MDMA-treated mice, we observed altered expression of several cDNAs (Peng et al., 2002b; Peng and Simantov, 2003). We isolated, cloned and sequenced some of

these cDNAs, and the sequence of one such MDMA-induced mRNA corresponds to the mouse GABA transporter 1 (mGAT1). In light of the interaction between serotonergic and GABAergic systems in the brain, we analyzed the effect of MDMA on the GABA transporter (GAT) gene family. Using RT-PCR analysis, we confirmed the DD-PCR observation that MDMA increased the expression of mGAT1 in the frontal cortex and midbrain. MDMA effect on expression of two other GATs was determined as well. While MDMA did not effect the expression of mGAT2, it caused an increase in the mRNA of mGAT4 in both the frontal cortex and midbrain. Time course experiments revealed that the effect of MDMA on mGAT1 was sustained for 7 days, whereas the increase in mGAT4 was transient (Peng and Simantov, 2003). Quantitative Real-time PCR further proved the MDMA-associated increase in expression of mGAT1 and mGAT4, and Western immunoblotting with anti-GAT1 antibodies revealed an MDMA-associated increase in GAT1 protein. Anti-GAT1 antibodies were used to determine the effect of MDMA on the expression of mGAT1 in SERT knockout mice, which are behaviorally nonresponsive to MDMA (Bengel et al., 1998). MDMA did not induce expression of GAT1 protein in SERT knockout mice (Peng and Simantov, 2003). Taken together, these findings are in line with the notion that MDMA induce alteration in GABA uptake and neurotransmission in the brain.

The essential role of GABAergic neurons in MDMA-induced dopamine release and loss of serotonergic axons is well established (Yamamoto et al., 1995; Sprague et al., 1998). Furthermore, in the cerebellum serotonin regulates both the activity and expression of glial GABA transporters (Voutsinos et al., 1998). Therefore, serotonin released upon MDMA treatment could directly regulate expression of mGAT1 and mGAT4. However, since GABA itself regulates turnover of GATs (Bernstein and Quick, 1999), whether the MDMA-associated enhanced expression of mGAT1 and mGAT4 in mice result from changes in GABA levels or reflects a regulatory role of serotonin is not yet clear. It will be of interest to determine whether nontoxic serotonin releasers or SERT blockers affect the expression of mGAT1 and mGAT4.

GAT inhibitors attenuate MDMA acute toxicity

Whether activation of GAT by MDMA is involved in the acute toxicity of the drug was analyzed with inhibitors of GAT. Mice were treated with a lethal dose of MDMA after pretreatment with different compounds. Tiagabine, GAT inhibitor which is also anti-epileptic drug, significantly reduced MDMA toxicity, while another GAT inhibitor, NO-711, exerted a partial protective effect (Peng and Simantov, 2003). The anti-epileptic/anti-convulsive compounds, vigabatrin and valproate, which are not GAT inhibitors, and guvacine, which does not readily cross the blood brain barrier, were ineffective in blocking the acute toxicity of MDMA (Peng and Simantov, 2003). These recent observations are in agreement with previous reports that pentobarbitone (Colado et al., 1999) and the GABA agonist baclofen (Kanthasamy and Nichols, 2000) attenuate MDMA-induced neurotoxicity.

MDMA has profound effect on body temperature of experimental animals and humans, occasionally resulting in death (Schmidt et al., 1990; Gordon et al., 1991; Henry et al., 1992; Drafters, 1994; Malberg and Seiden, 1998; Hegadoren et al., 1999; Gill et al., 2002). An increased expression of GAT1 and GAT4, followed by an enhanced removal of extracellular GABA, should influence various physiological systems controlled by this neurotransmitter, including thermoregulation. Since the toxicity of MDMA was decreased upon pretreatment with tiagabine or NO-711, compounds that

selectively block GABA transporters, these compounds may be useful in preventing harmful effects of MDMA (see below).

Involvement of MDMA in protein and neurotransmitter trafficking

One of the genes we recently identified (Peng and Simantov, 2003), the expression of which was decreased after MDMA treatment, was a cDNA that corresponds to the synaptic vesicle protein synaptotagmin IV (Peng et al., 2002b). Since synaptotagmin IV is functionally related to synaptotagmin I, the effect of MDMA on the expression of the two proteins was studied further. The decrease in synaptotagmin IV expression after MDMA treatment was confirmed at the level of mRNA and protein. In parallel, expression of synaptotagmin I in the midbrain increased after administration of MDMA. Additional analysis indicated that the effect of MDMA was selective and differential, since it depended on the particular synaptotagmin and brain region. As with the effect of MDMA on expression of GAT1, MDMA did not induce down- or up-regulation of synaptotagmin IV and I, respectively in SERT knockout mice. This indicates that the drug-associated alterations in the amounts of the two synaptotagmins is associated with the pharmacological and behavioral effects of the drug (Peng et al., 2002b).

Synaptotagmins, a family containing about a dozen proteins (Schiavo et al., 1998), regulate neurotransmitter secretion, vesicle trafficking, and vesicle docking to the plasma membrane (Ullrich et al., 1994; Robinson and Martin, 1998; Littleton et al., 1999). Herschman, Baudry and colleagues have shown that synaptotagmin IV has a role as an immediate early gene (Vician et al., 1995; Ferguson et al., 1999). Both synaptotagmin I and IV are also involved in neurite outgrowth, and the ratio between the two regulates synaptic activity (Littleton et al., 1999). Interestingly, activation of dopamine D-1 receptors or exposure to cocaine increases expression of synaptotagmin IV (Denovan-Wright et al., 1998; Glavan et al., 2000), whereas chronic treatment with haloperidol, a dopamine receptor antagonist, causes the opposite effect (Nakahara et al., 1998). Since MDMA activates both dopamine D-1 and D-2 receptors as a result of the release of dopamine, whether the decrease in synaptotagmin IV and increase in synaptotagmin I in MDMA-treated mice (Peng et al., 2002b) are due to MDMA activation of dopamine D-2 receptors should be considered. However, since extended blocking of serotonin uptake affects phosphorylation of synaptotagmin (Popoli et al., 1997), involvement of the serotonergic or other neuronal systems in modulating expression of synaptotagmin I and IV in MDMA-treated mice should not ruled out.

Modulation by MDMA of several proteins involved in GABA neurotransmission

Gene profiling of the cerebral cortex and midbrain of MDMA-treated mice identified several genes, including GABA transporters and synaptotagmin I and IV, as discussed above. More recent analysis revealed cloned cDNAs homologous to other genes, including septin and dystrophin (Simantov et al., to be published). Although these two proteins belong to different gene families and are structurally different from the GABA transporters and synaptotagmins, they all are involved in neurotransmission of GABA. The role of GABA transporters is obvious (see above). Synaptotagmins can bind to presynaptic plasma membrane proteins, such as syntaxin; syntaxin 1A interacts with and regulates the activity of

GAT1 (Beckman et al., 1998; Deken et al., 2000). Studies with molecular constructs of syntaxin 1A suggested that trafficking of GAT1 is controlled by other proteins interacting with synthaxin 1A (Horton and Quick, 2001), possibly also synaptotagmins.

Cytoskeletal proteins of the septin family are involved in neurotransmitter release (Peng et al., 2002a), and some are abundant in inhibitory terminals and associated with GABAergic vesicles (Kinoshita et al., 2000). As for dystrophin, several isoforms of this protein, an abundant cytoskeletal component in muscles, are expressed in the brain. Mutant mice devoid of dystrophin (mdx mice) show altered synaptic clustering and stabilization of GABAA receptors (Knuesel et al., 1999). Moreover, mdx mice also exhibit abnormal responses to inhibition of GABAA receptors (Vallend and Billard, 2002). Recently, a complex of dystrophin and a glycoprotein (dystroglycan) was observed in a subset of inhibitory synapses, co-localized with GABAA receptors (Levi et al., 2002). Dystorphin also interacts with the cytoskeleton protein gephrin, and knockout mice devoid of gephrin are deficient in GABAA receptors (Kneussel et al., 1999).

MDMA-induced depletion of serotonin was shown to be attenuated by drugs that enhance GABAergic neurons (Colado et al., 1993; Colado and Green, 1994). More recently, pentobarbitone (Colado et al., 1999) and the GABA agonist baclofen (Kanthasamy and Nichols, 2000) were shown to attenuate MDMA-induced neurotoxicity. Our current finding that MDMA alters the expression of several genes involved in GABA neurotransmission is in agreement with these previous studies, thus emphasizing the essential role of GABA in MDMA activity in the brain.

Novel approaches for inhibiting or alleviating neurotoxicity of MDMA

The effort of the last two decades to elucidate the molecular and neuropharmacological mode of action of MDMA provided important new insight, yet effective methods to inhibit or alleviate the damaging effects of this widely abused drug are insufficient. Three recently emerging approaches to address this issue will be discussed here.

Considering the central role of the dopaminergic system in MDMA neurotoxicity, whether blocking dopamine receptors or inhibiting dopamine uptake with drugs could protect against MDMA toxicity was examined, but numerous difficulties and drawbacks were encountered in these studies. Recently, this issue was addressed by infusing dopamine transporter antisense oligonucleotide into rat brain for 7 days, and then monitoring serotonergic deficits and hyperthermia (Kanthasamy et al., 2002). The antisense oligonucleotide decreased dopamine transporter density by 70%, and attenuated MDMA-induced neurotoxicity. Interestingly, the antisense did not block all effects of MDMA, thus suggesting that this molecular approach will be useful for differentiating between various neurochemical, physiological and behavior activities of the drug.

MDMA-induced expression of GABA transporters may provide another opportunity to restrict deleterious effects of MDMA. In mice, two GABA transporter inhibitors, tiagabine and NO-711 attenuated MDMA acute toxicity and death. Since this effect was dose dependent, and compounds that do not inhibit GABA transport did not have such effect (Peng and Simantov, 2003), developing more potent and selective inhibitors of GABA transporters is desirable. Whether the other MDMA-regulated genes involved in GABA transmission, discussed above, might be useful to inhibit MDMA effects should be further studied.

Finally, MDMA induce loss of serotonergic and possibly other neurons was observed in rodents and primates, and could occur also in human (McCann et al., 2000; Ricaurte and McCann, 2001; Lyles and

Cadet, 2003). Cell loss may contribute to some of the long lasting psychiatric effects of the drug, such as hallucination, depression, psychotic attacks, and paranoia. Recently, efforts to develop new methods for cell replacement therapy in the brain particularly with stem cells, were intensified (Gage, 2000; Tsai et al., 2002). Cellular and molecular approaches are being developed to produce dopaminergic and serotonergic neurons, and cell replacement therapy is being examined for neurodegenerative diseases, such as Parkinson's disease (Lee et al., 2000; Isacson et al., 2003). Whether such stem cells could be used to correct the damaging effects of neurotoxic chemicals, such as MDMA, remains to be seen.

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