Hypothesis

MDMA (Ecstasy) controls in concert a group of genes involved in GABA neurotransmission

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Abstract In several countries, 3,4-methylenedioxymethamphetamine (MDMA) is currently the most abundant psychoactive recreational drug. MDMA induces numerous neuropsychiatric behaviors, serotonergic neuron degeneration, programmed death of cultured cells, hyperthermia and occasional fatality. Using gene expression analysis in MDMA-treated mice, we identified changes in γ-amino butyric acid (GABA) transporters and synaptotagmins I and IV. Additional experiments showed decreases in mRNAs encoding septin and dystrophin. Although belonging to different gene families, it is striking that these four protein groups are implicated in neurotransmission of GABA, a major inhibitory neurotransmitter involved in thermoregulation. MDMA may control these genes in a combined fashion, assigning GABA a pivotal role in MDMA activities.

Key words: Gene profiling; Psychoactive compound; Transporter; Serotonin; Drug abuse

1. Introduction: profiling gene expression after MDMA treatment

3,4-Methylenedioxymethamphetamine (MDMA) (also called Ecstasy) enhances in the brain the release of the amine neurotransmitters, serotonin, noradrenaline and dopamine, and generates multiple short- and long-term neuropsychiatric behaviors, including memory deficits (see [1,2] for recent reviews). In animals, MDMA is neurotoxic to brain serotonergic neurons, and it induces programmed cell death in cultured human cells [3], cortical neurons [4] and liver cells [5]. It was therefore of interest to investigate whether MDMA alters the expression of certain genes in the brain. Initial studies showed that MDMA alters the expression of serotonin and corticoid receptors [6], as well as genes coding for Bcl-2 proteins, c-fos and egr-1 (NGFI-A) [4,7,8]. More recently, Cadet and colleagues have used cDNA array analysis, and observed MDMA-induced changes in expression of 28 genes associated with diverse activities [9].

The first set of genes we identified as being affected by MDMA was the γ-amino butyric acid (GABA) transporter (GAT) family; MDMA had a differential effect on expression of three GAT isoforms ([10] and Table 1). The significance of activation of mGAT1 and mGAT4 expression by MDMA, along with no change in mGAT2 expression, was discussed recently [11]. Involvement of GATs in GABA neurotransmission is readily explicable, as neuronal and glial GATs are the major players in maintaining proper synaptic GABA levels [12]. The increase in GAT expression and protein [10] is likely to result in an enhanced GABA re-uptake and a lower extracellular GABA level. This should re-modulate GAT expression, since GABA itself regulates the expression of GATs [13,14]. It is worth noting, however, that expression of the glial GABA transporter is also under the regulation of serotonin [15]. The initial MDMA-induced release of serotonin can therefore directly regulate the expression of GATs.

It was also found that MDMA altered the expression of synaptotagmins I and IV, the effect being in an opposite direction for the two genes [16]. These two proteins belong to a family of about a dozen members, having multiple functions such as acting as immediate-early genes and controlling of neurite outgrowth [17]. A major role of synaptotagmins is the control of presynaptic vesicle trafficking, docking and fusion to the plasma membrane, and neurotransmitter secretion [18,19]. Interestingly, synaptotagmins I and IV can form heterooligomers, and the ratio of the two proteins modulates synaptic function [20]. It is hypothesized herein that the cross-talk between synaptotagmins and GABA neurotransmission is indirect, as follows: synaptotagmins bind to presynaptic plasma membrane proteins such as syntaxin [21,22]. In a series of studies Quick and associates have shown that syntaxin 1A interacts with GAT1 [23] and regulates the transport rate of GABA [24]. Moreover, later experiments from the same laboratory confirmed that syntaxin 1A induces redistribution of GAT, resulting in an enhanced GABA transport [25]. This later work also showed that other presynaptic proteins, possibly synaptotagmins, are involved in controlling GAT1 activity. Taking into account the effect of MDMA on the expression of two GATs and two synaptotagmins, and the central role of GATs in GABA transport, it is tempting to conclude that syntaxin 1A may act as a link between synaptotagmins and the control of GABA transport. However, one cannot exclude additional activities of these proteins in the MDMA-treated brain, e.g. since MDMA induces neurodegeneration of serotonergic fibers, synaptotagmins and syntaxin may participate in membrane repair processes in the damaged neurons, as they do in PC12 neurites [26] and squid and crayfish axons [27].

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Table 1
Summary of MDMA effect on expression of genes associated with GABA neurotransmission

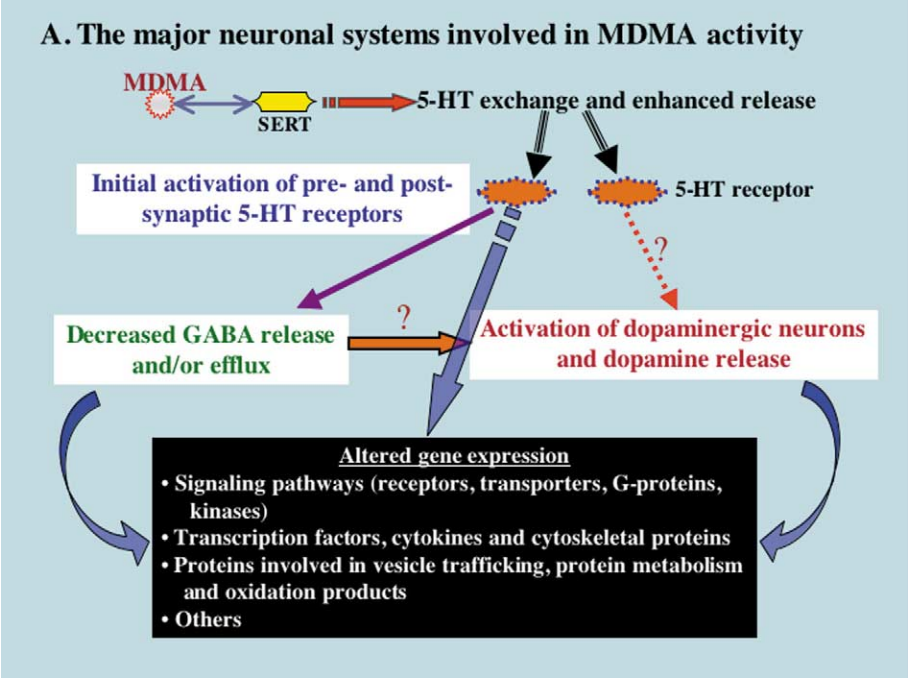
Gene	DD PCR expression			Reverse transcription (RT)-PCR	Real-time PCR	Chromosomal location	
	cDNA cloned (bp)	Cerebral cortex	Midbrain			Mouse	Human
mGAT1	298[a]	increase	increase	increase	increase	6	3p25.3
mGAT4 (hGAT-3)	–	increase	increase	increase	increase	6	3p25.3
Synaptotagmin I	–	UL	UL	increase	increase	10	12q21.2
Synaptotagmin IV	356[b]	decrease	decrease	decrease	decrease	18	18q12.3
Septin[c]	421	UL	decrease	NT	NT	1/16	2q37.3/22q11.21
Dystrophin[d]	498	UL	decrease	NT	NT	X/9	Xp21.2/3p21

UL, undetectable level; NT, not tested.
[a]Details of the DD PCR, RT-PCR and real-time PCR methods, and the data regarding MDMA effect on mGAT1, mGAT2 and mGAT4 expression in the brain were reported before [10]. mGAT4 is a homolog of rGAT-3 and hGAT-3.
[b]The data regarding synaptotagmins I and IV were reported before [16].
[c]Chromosomal localizations of septin 2 (Nedd 5) and septin 5 (CDCrel-1) are shown.
[d]Chromosomal localizations of dystrophin and dystrophin-associated glycoprotein 1 (DAG1 = dystroglycan 1) are shown. For comparison, the chromosomal locations of SERT and DAT are mouse 11 and 13 and human 17q11.2 and 5p15.33, respectively.

2. Recent findings

Using differential display polymerase chain reaction (DD PCR) analysis, it was found that MDMA decreased in the midbrain the expression of two additional cDNAs of 421 and 498 nucleotides (Table 1). Sequencing these cDNAs demonstrated that they encode septin and dystrophin, respectively. Surprisingly, a series of recent studies conducted by a number of laboratories point out that both septins and dystrophins are associated in the brain with the GABA system. Septins are cytoskeletal proteins with guanosine triphosphatase (GTPase) activity, and they are involved in cytokinesis and secretion [28]. Different members of the septin family have distinct but overlapping distribution in various tissues and in the brain. Septin 5, also called CDCrel-1, is predominantly expressed in the nervous system, and it is abundant in presynaptic inhibitory GABAergic terminals [29]. Interestingly, in several brain regions CDCrel-1 [30] as well as Nedd 5 (septin 2) interact with syntaxin. The crosstalk between synaptotagmin, syntaxin, and septin proteins certainly needs further assessment, yet it seems probable that they cooperate presynaptically to monitor GABA transport (Scheme 2).

Dystrophin is a major cytoskeletal component of muscles but also expressed in the brain, colocalized postsynaptically with GABAA receptor subunits [31]. Furthermore, this last study showed that mutant mice lacking dystrophin (*Mdx* mice) have an altered clustering and stabilization of GABAA receptor subunits in several brain regions. Experiments with hippocampal slices from *Mdx* mice displayed abnormally enhanced synaptic plasticity, which was inhibited by bicuculline,



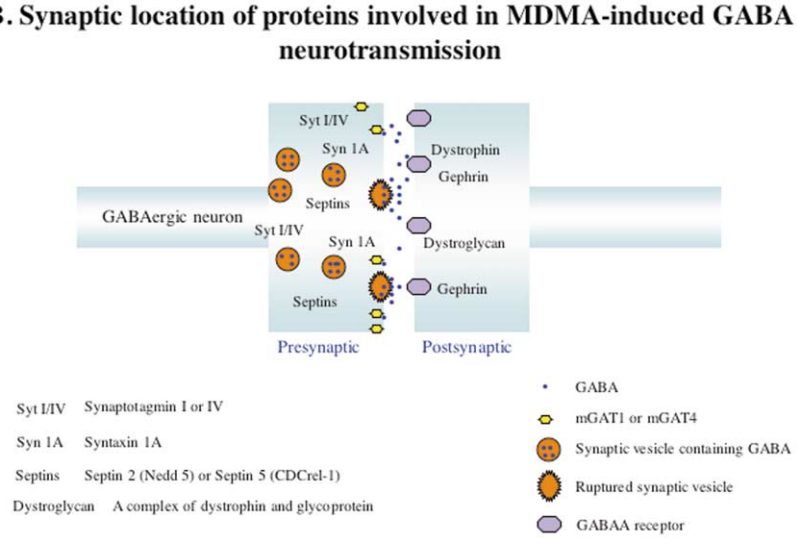
Scheme 1. The three major neuronal systems involved in MDMA activity. The serotonergic, dopaminergic, and GABAergic neurons are shown in a schematic form. All or part of these systems, as well as other neuronal pathways not shown (such as the noradrenergic and glutamatergic) may contribute to the MDMA-induced altered gene expression profile as described over the years [1,2,4–10,16]. SERT, 5-HT transporter.

a selective GABAA receptor antagonist [32]. Dystrophin also interacts with the tubulin-binding protein gephrin, an important player in the assembly of the GABAergic postsynaptic membrane [33]. Knockout mice devoid of gephrin are deficient in GABAA receptor subunits, but they show a normal distribution of another (excitatory) postsynaptic receptor [34]. Finally, a complex of dystrophin and a glycoprotein (dystroglycan) was observed in a subset of inhibitory synapses, colocalized with GABAA receptors [35].

3. Summary and conclusions

Thiriet et al. [9] took a wide-ranging approach to unravelling MDMA effects on the brain by using the cDNA array method, and analyzed the temporal changes in gene expression in the rat cortex. They found changes in genes encoding proteins active in nine functional systems, including signal transduction (receptors, G-proteins, kinases), transcription factors, cytoskeleton/matrix proteins, proteins reacting to oxidative stress, and metabolism. This study opened new avenues in elucidating the repertoire of MDMA activities, and also confirmed some early findings, using more conventional methods [6–8]. Likewise, the observation that MDMA decreased expression of Bcl-2 in the cortex [9] is in line with experiments with cultured cells, indicating activation of programmed cell death by MDMA [3–5]. Yet, there are many open questions as to the specific neuronal system responsible for each altered gene (see Scheme 1), initial versus late effects resulting from adaptive processes, and the causal relationship between a certain gene and some drug-induced neuropsychiatric behavior(s) or memory loss.

In mice, we found that MDMA altered the expression of the GABA transporters mGAT1 and mGAT4, synaptotagmins I and IV, septin and dystrophin. A common feature of these proteins, as reported by a number of laboratories, is that all of them are associated with GABA neurotransmission. It is noteworthy that they belong to four different gene families, and lack any obvious relation in chromosomal localization in mice or human (Table 1). Nevertheless these proteins were found in GABAergic synapses, either presynaptically, thus able to modulate GABA release or re-uptake, or postsynaptically, accessible for regulation of GABAA receptors (Scheme 2). To what extent is this observation relevant to the wide repertoire of MDMA effects in vivo, which comprises serotonergic neuron degeneration, psychostimulation, hyperthermia and occasional death? Studies by Yamamoto and associates [36] have shown that activation of serotonin receptors by serotonin after treatment with MDMA results in a diminished efflux of GABA, followed with an enhanced activity of dopaminergic neurons and dopamine release. Ample evidence suggests that activation of the dopamine system is central to MDMA-induced psychoactivation, oxidative stress, and serotonergic degeneration ([1,2] and references included). Yet, GABAergic neurons are key players in MDMA-induced loss of serotonergic axons [37,38]. The crosstalk between the serotonin, dopamine and GABA systems is therefore crucial to the multiple behavioral and gene expression changes induced by the drug (illustrated in Scheme 1). Focusing on the role of GABA, it is worth noting the early work of Green and associates that drugs enhancing GABAergic neurons attenuate the later depletion in serotonin, which accompanies serotonergic nerve terminal degeneration [39,40]. Later studies confirmed that pentobarbitone [37], acting on ion channels associated with GABA receptors, and the GABA agonist baclofen [41] diminish MDMA-induced neurotoxicity. Furthermore, recent experiments have shown that GAT inhibitors reduce MDMA toxicity in mice [10]. By and large, the recognition that MDMA alters the expression of several genes involved in GABA neurotransmission emphasizes the essential role of GABA in MDMA activity in the brain. GABA plays a role in thermoregulation [42,43], and MDMA-induced fatality in drug users is coupled with severe hyperthermia [1,2]. Undoubtedly more rigorous assessment of the multiple molecular pathways that participate in controlling the expression of the genes involved in GABA neurotransmission should provide important insights in the mechanism of action of MDMA and similar psychostimulants. GABA neurotransmission at pre- or postsynaptic sites could also serve as a useful drug



Scheme 2. Hypothetical synaptic location of proteins involved in MDMA-induced GABA neurotransmission. This illustration is based on our recent findings [10,16] and studies conducted by several other laboratories [17–25,28–35].

target to alleviate some of the deleterious effects of MDMA and similar psychoactive drugs, including sporadic fatality among drug users.

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