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Short- and long-term effects of MDMA ("ecstasy") on synaptosomal and vesicular uptake of neurotransmitters in vitro and ex vivo

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a commonly abused drug which has been shown to be neurotoxic to serotonergic neurons in many species. The exact mechanism responsible for the neurotoxicity of MDMA is, however, poorly understood. In this study, the effects of MDMA on the synaptosomal and vesicular uptake of neurotransmitters were investigated. Our results show that MDMA (0.5–20 μM) reduces both synaptosomal and vesicular uptake of serotonin and dopamine in a dose dependent manner in vitro, while the uptake of glutamate and γ-aminobutyric acid (GABA) remains unaffected. Ex vivo experiments support the importance of the monoamines, with predominant dopaminergic inhibition at short-term exposure (3 × 15 mg/kg; 2-h intervals), and exclusively serotonergic inhibition at long-term exposure (2 × 10 mg/kg per day; 4 days). This study also compares MDMA and the structurally related antidepressant paroxetine, in an attempt to reveal possible cellular mechanisms for the serotonergic toxicity of MDMA. One important difference between paroxetine and MDMA is that only MDMA has the capability of inhibiting vesicular uptake of monoamines at doses used. We suggest that inhibition of the vesicular monoamine transporter-2, and a following increase in cytoplasmatic monoamine concentrations, might be crucial for the neurotoxic effect of MDMA.

Keywords: 3,4-Methylenedioxymethamphetamine; Paroxetine; Synaptosomes; Synaptic vesicles; Uptake; Monoamines; Serotonin; Dopamine

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), popularly known as ecstasy, is an amphetamine derivative widely used as a recreational drug. According to the Norwegian Institute for Alcohol and Drug Research, 5.7% of youths in Oslo (aged 15–20 years) took MDMA at least once in 2000. The mechanisms underlying the pharmacological and toxicological effects of MDMA are not clear at present, but there is considerable evidence that MDMA acts by increasing the extracellular concentrations of the monoamine neurotransmitters serotonin, noradrenaline and dopamine (Iravani et al., 2000; Kalant, 2001). The increase in monoamine concentrations in the synaptic cleft is caused by the ability of MDMA to stimulate neurotransmitter release, inhibit neurotransmitter uptake or block neurotransmitter metabolism (Sanchez et al., 2001). The relative importance of these factors is still not elucidated. However, recent studies have favored that MDMA causes inhibition of the reuptake transporter rather than releasing neurotransmitters directly, unlike the effect of the related compound (+)-amphetamine (Iravani et al., 2000; Kalant, 2001).

MDMA is considered to be potentially toxic to serotonergic axons and nerve terminals (Shankaran et al., 1999; Falk et al., 2002; Meyer and Ali, 2002; O'Shea et al., 2002). The exact mechanism of MDMAs neurotoxicity is not known, but oxidative stress, hyperthermia, and increased level of dopamine are all possible factors suggested to be involved (Shankaran et al., 1999; Sanchez et al., 2001). The mechanism leading to MDMA-induced generation of reactive oxygen species (ROS) is unknown. Sprague et al. (1998) postulated that dopamine released after exposure to MDMA, is subsequently taken up by serotonergic nerve endings where it undergoes intraneuronal oxidation through monoamine oxidase B, and gives rise to ROS. In contrast, based on the finding that animals with near total depletions of brain dopamine still are susceptible to MDMA neurotoxicity, others report that MDMA neurotoxicity is not dependent upon endogenous dopamine (Yuan et al., 2002). A study showing that MDMA-induced ROS formation is

Abbreviations: 5-HT, serotonin; GABA, γ-aminobutyric acid; MDMA, 3,4-methylenedioxymethamphetamine; ROS, reactive oxygen species; VMAT-2, vesicular monoamine transporter-2

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absent in rats in which serotonergic terminals have been depleted by fenfluramine, supports the importance of serotonin itself in the mechanism of MDMA-induced neurotoxicity (Shankaran et al., 1999).

MDMA induces acute behavioral changes and hyperthermia in both rats and humans. The increase in body temperature is caused by increased muscle activity together with a direct action on the thermoregulatory system in the brain (Kalant, 2001; O'Loinsigh et al., 2001; Mechan et al., 2002). Production of a hyperthermic response is thought to be critical for MDMA-induced neurotoxicity, since neurodegeneration can be attenuated if body temperature is kept low by using low ambient temperatures or drugs that produce hypothermia (Malberg et al., 1996; Huether et al., 1997; O'Loinsigh et al., 2001).

There is growing concern about the long-term effects of repeated use of MDMA, partly because of neurotoxic effects in animals at doses similar to those used recreationally by humans (O'Loinsigh et al., 2001), and partly because of the severe character of the long-term effects seen, like cognitive deficits, panic disorder and psychotic episodes (Mayerhofer et al., 2001; Montoya et al., 2002). The structural and functional similarities of MDMA and the antidepressant paroxetine are interesting. Both compounds possess a 3,4-methylenedioxyphenyl group, for which a specific mechanism is suggested to exist in the brain (Hashimoto et al., 1993). In the present study the effect of paroxetine, which is an extremely potent inhibitor of the serotonin transporter (Hajos-Korcsok et al., 2000; Yamane et al., 2001), is compared with the effect of MDMA.

Despite the functional and structural similarities discussed above, MDMA is considered to be a potentially neurotoxic compound, while paroxetine is a widely used medical drug. The aim of our study was to compare the effects of MDMA and paroxetine on synaptosomal and vesicular uptake of neurotransmitters in order to throw light on their differences in neurotoxicity. Possible cellular mechanisms for the toxicity of MDMA are discussed. In addition, short- and long-term effects of MDMA on rats ex vivo were investigated.

2. Experimental procedures

2.1. Materials

[2,5,6-^{3}H]dopamine (5.9 or 6.5 Ci/mmol) and 5-hydroxy [^{3}H]tryptamine trifluoroacetate (84 or 106 Ci/mmol) were purchased from Amersham Pharmacia Biotech (UK). L-[2,3,4-^{3}H]glutamic acid (60 Ci/mmol) and [2,3-^{3}H] aminobutyric acid were purchased from American Radiolabeled Chemicals Inc. (St. Louis, USA). Filter-Count was purchased from Packard Instrument Co. (Meriden, USA). Paroxetine hydrochloride hemihydrate was a gift from SmithKlineBeecham Pharmaceuticals (Beecham, UK). MDMA (purity > 98%) was a gift from the National Bureau of Crime Investigation (Norway).

2.2. Animals

Adult male Wistar rats (200–270 g) were used in the studies. The animals were kept under conditions of constant temperature (22 ± 2 °C) and humidity (55 ± 5%) with a 12-h light/12-h dark cycle and free access to food and water. Animal experiments were conducted according to the European Community Council Directive 86/609/EEC, and efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Ex vivo studies

In the short-term studies rats received three injections of MDMA (15 mg/kg s.c.). The injections were given with 2 h intervals and the rats were decapitated 2 h after the final injection. Controls were given an equivalent volume of saline vehicle. In the long-term studies animals were injected with MDMA (10 mg/kg s.c.), paroxetine (5 mg/kg s.c.) or saline vehicle, twice daily for four consecutive days. Rats were decapitated 18, 42 or 66 h after the final injection. Animals treated with MDMA were given i.p. injections of saline 1 h after injections, to compensate for loss of fluid.

2.4. Preparation of synaptosomes and synaptic vesicles

Cerebrum synaptosomes were isolated as previously described (Mariussen and Fonnum, 2001). In brief, rats were killed by decapitation and the brain removed. The cerebrum was weighed, and then homogenized (5% (w/v)) in ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1000 × g for 10 min to remove nuclei and cellular debris. The supernatant was then mixed with 1.3 M sucrose to obtain final concentration of 0.8 M sucrose, and centrifuged at 21,000 × g for 30 min. This supernatant was discarded, and the resulting P2 synaptosomal pellet was resuspended in 0.32 M sucrose (25% (w/v)).

Synaptic vesicles used in the ex vivo studies were isolated by the method of Erickson et al. (1990) and Teng et al. (1998). In brief, rats were killed by decapitation and the brain removed. The cerebrum was weighed and then homogenized (5% (w/v)) in ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1000 × g for 10 min to remove nuclei and cellular debris. The supernatant was then centrifuged at 21,000 × g for 30 min. The resulting P2 synaptosomal pellet was osmotically shocked by resuspension (7.5% (w/v)) in ice-cold double distilled water, and centrifuged for 30 min at 21,000 × g. The supernatant was collected, 0.1 M K-Tartrat and 25 mM HEPES (final concentrations) added, and the mixture centrifuged at 100,000 × g for 1 h. The pellet was gently resuspended in 0.32 M sucrose. For in vitro studies, synaptic vesicles were isolated as described previously by Mariussen et al. (2001). The synaptosomal or vesicular content of protein was measured by the method of Lowry et al. (1951).

2.5. Assay for synaptosomal uptake of neurotransmitters

High affinity uptake of dopamine, serotonin, glutamate and γ-aminobutyric acid (GABA) was determined as described previously (Mariussen and Fonnum, 2001). Synaptosomes (93 μg protein/ml) were preincubated at 25 °C for 15 min in Tris–Krebs buffer. In the in vitro studies, MDMA or paroxetine was added at preincubation time. The uptake was started by adding substrate containing either 147 nM [^{3}H]dopamine (0.5 μCi), 86 nM [^{3}H]serotonin (0.5 μCi), 120 nM [^{3}H]glutamate (0.5 μCi) or 42 nM [^{3}H]GABA (0.9 μCi). The mixtures were incubated for 9 min (dopamine, serotonin) or 3 min (glutamate, GABA). The reaction was terminated by dilution with ice-cold solution of 0.15 M NaCl and 0.05% (w/v) bovine serum albumin and rapid filtration in a cell harvester onto a glass fiber filtermat. The filters were dissolved in Filter-Count and counted for retained radioactivity in a liquid scintillation spectrophotometer (Packard Tri-Carb 300).

2.6. Assay for vesicular uptake of neurotransmitters

Vesicular uptake of neurotransmitters was determined as described by Fykse and Fonnum (1988). Synaptic vesicles (42 μg protein/ml) were preincubated at 30 °C for 15 min. In the in vitro studies, MDMA or paroxetine was added at preincubation time. The uptake of neurotransmitters was started by adding substrate containing ATP and either 257 nM [^{3}H]dopamine (0.5 μCi), 96 nM [^{3}H]serotonin (0.5 μCi) or 1 mM [^{3}H]glutamate (1 μCi). The mixtures were incubated for 3 min and the reaction was stopped by adding ice-cold 0.15 M KCl followed by rapid filtration. The filters were dissolved in Filter-Count and counted for retained radioactivity in a liquid scintillation spectrophotometer (Packard Tri-Carb 300).

2.7. Statistical analysis

IC_{50} (50% inhibition concentration) values were estimated from non-linear regression analysis (Demo Version of SlideWrite Plus, Version 6, Advanced Graphics Software Inc., USA). Statistical significance was assessed by one-way analysis of variance (ANOVA), followed by Dunnett's method when appropriate. Differences were considered statistical significant when $P < 0.05$.

3. Results

3.1. Effects of MDMA on neurotransmitter uptake in vitro

The uptake of serotonin and dopamine in synaptosomes was significantly reduced at all MDMA concentrations (0.5–20 μM) tested (Fig. 1a). IC_{50} values were found to be approximately 1.5 and 3 μM for serotonin and dopamine, respectively. In contrast, MDMA exposure produced no change in the uptake of glutamate or GABA.

MDMA also produced a significant reduction in the uptake of serotonin and dopamine in synaptic vesicles (Fig. 1b), with IC_{50} values at approximately 9 and 6 μM, respectively. As for synaptosomes, MDMA produced no effect on the uptake of glutamate.

3.2. Effects of paroxetine on neurotransmitter uptake in vitro

Exposure to as low concentrations as 10 nM paroxetine reduced the uptake of serotonin in synaptosomes to approximately 30% of control. Paroxetine also decreased the synaptosomal uptake of dopamine, and the uptake of serotonin and dopamine in synaptic vesicles, but to a much lower degree (Fig. 1c).

3.3. Effects of MDMA and paroxetine on neurotransmitter uptake ex vivo

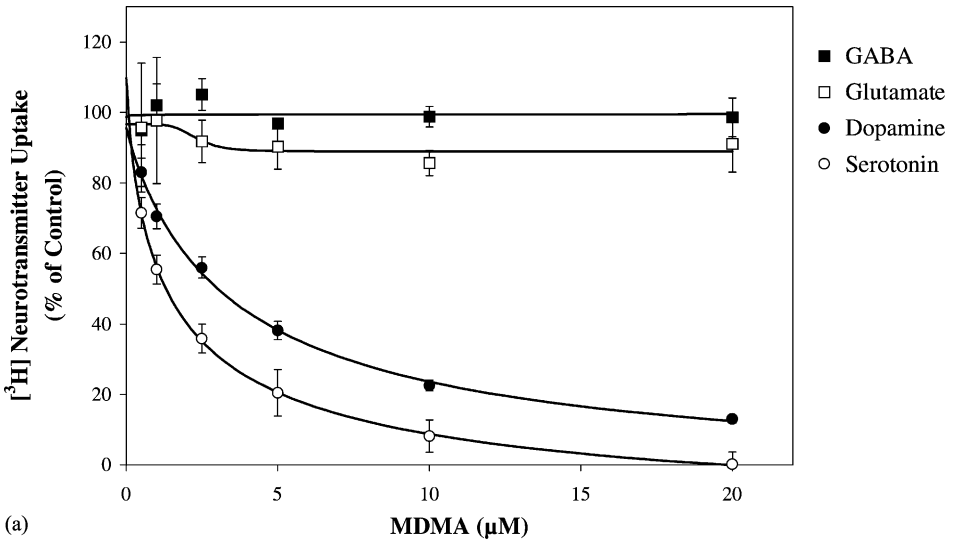
Within 1 h after MDMA administration the rats were noticeable affected, by appearance of both behavioural changes and an increase in body temperature (not shown). During the treatment period, rats were weighed immediately before and 1 h after MDMA/saline injections. Rats treated with MDMA (15 mg/kg) showed an average weight loss of 7.4 g the first hour after drug injection. The weight loss in the control group was 0.8 g in the same period. As shown in Fig. 2, MDMA- and saline-treated animals showed slight, but significant differences in body weight after 4 days of drug administration.

Short-term treatment with high doses of MDMA (3 × 15 mg/kg; 2-h intervals) resulted in an acute reduction in the synaptosomal uptake of dopamine (63% of control value). The uptake of serotonin and glutamate was slightly, but not significantly, decreased (80% of control). The uptake of GABA was not affected (Fig. 3).

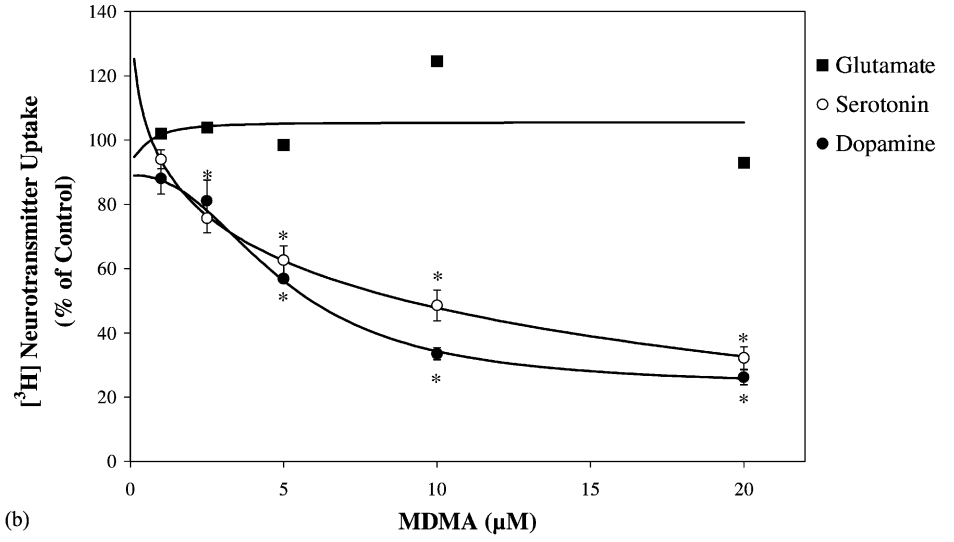
MDMA treatment for four consecutive days (2 × 10 mg/kg per day) induced significant reductions in the synaptosomal uptake of serotonin, while the uptake of dopamine remained unchanged (Fig. 4). The uptake was measured 18, 42 and 66 h after cessation of MDMA administration, and was found to be 75, 54 and 61% of control, respectively. The uptake of serotonin in synaptic vesicles was significantly reduced (65% of control) 66 h after MDMA administration, while paroxetine (2 × 5 mg/kg per day; 4 days) did not affect the vesicular uptake (Fig. 5).

4. Discussion

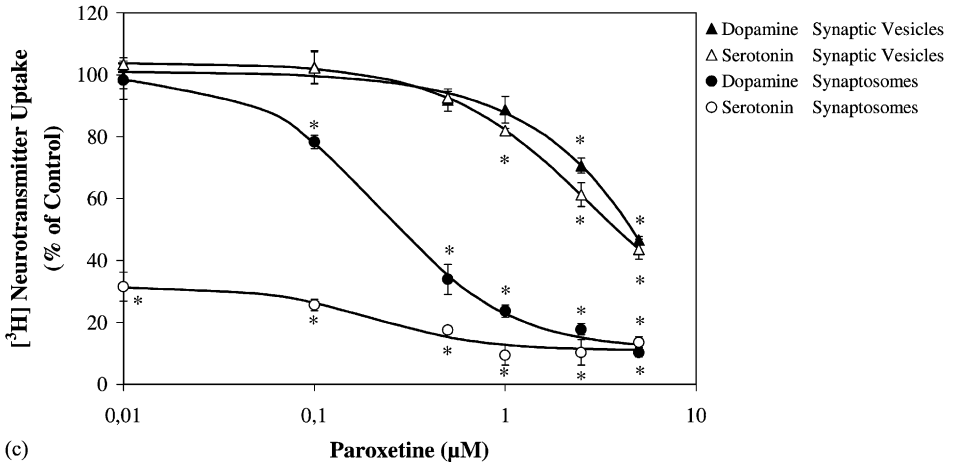
The present study examined the effects of MDMA and paroxetine on cerebral synaptosomes and synaptic vesicles


[3H] Neurotransmitter Uptake (% of Control)
MDMA (µM)
GABA
Glutamate
Dopamine
Serotonin

(a)


[3H] Neurotransmitter Uptake (% of Control)
MDMA (µM)
Glutamate
Serotonin
Dopamine

(b)


[3H] Neurotransmitter Uptake (% of Control)
Paroxetine (µM)
Dopamine Synaptic Vesicles
Serotonin Synaptic Vesicles
Dopamine Synaptosomes
Serotonin Synaptosomes

(c)

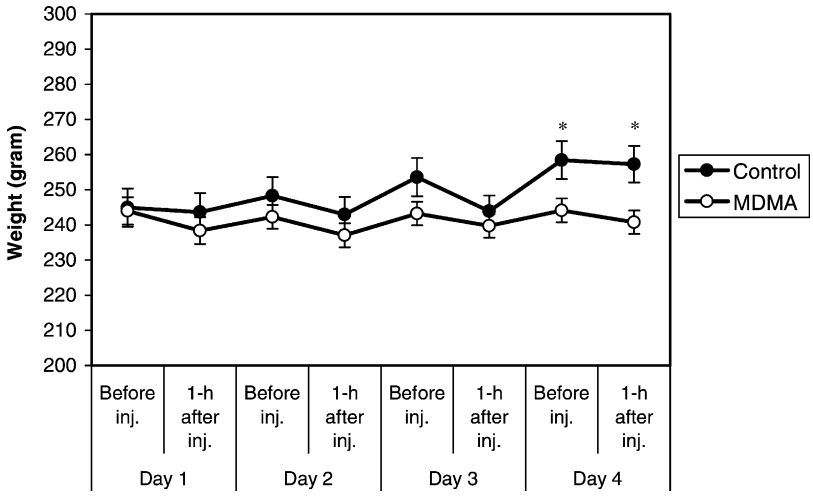


Fig. 2. Effects of repeated administration of MDMA (2 × 10 mg/kg per day; 4 days) on rat body weight. Data are mean ± S.E.M. from 20 independent experiments. Rats were weighed immediately before injection and 1 h after injection. $^{*}P < 0.05$, by ANOVA followed by Dunnett's method.

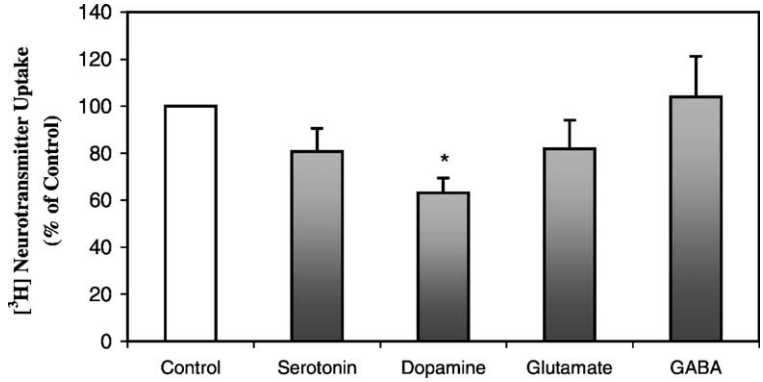


Fig. 3. Short-term effects of MDMA (3 × 15 mg/kg, 2-h intervals) on the uptake of [^{3}H]serotonin, [^{3}H]dopamine, [^{3}H]glutamate and [^{3}H]GABA into rat brain synaptosomes ex vivo. Synaptosomes were incubated with substrate (86 nM serotonin, 147 nM dopamine, 120 nM glutamate or 42 nM GABA) for 3 min (GABA, glutamate) or 9 min (dopamine, serotonin) at 25 °C after 15 min of preincubation. Uptake is expressed as percent of control (animals treated with saline). Data are mean ± S.E.M. from 6 independent experiments performed in triplicates. $^{*}P < 0.05$, by ANOVA followed by Dunnett's method.

in vitro and ex vivo. Our results show that MDMA reduces the synaptosomal and vesicular uptake of serotonin and dopamine in a dose dependent manner in vitro. In contrast, neither the uptake of glutamate nor GABA was affected by in vitro exposure to MDMA.

Short-term studies of MDMA (3 × 15 mg/kg; 2-h intervals) ex vivo showed a 37% inhibition of the synaptosomal uptake of dopamine when measured 2 h after cessation of drug administration. The uptake of serotonin and glutamate was slightly, but not significantly decreased, while

Fig. 1. (a) Effects of MDMA on the uptake of [^{3}H]GABA, [^{3}H]glutamate, [^{3}H]dopamine and [^{3}H]serotonin into rat brain synaptosomes in vitro. Synaptosomes were incubated with substrate (42 nM GABA, 120 nM glutamate, 147 nM dopamine or 86 nM serotonin) for 3 min (GABA, glutamate) or 9 min (dopamine, serotonin) at 25 °C after 15 min of preincubation with MDMA (0.5–20 μM). Uptake is expressed as percent of control. Data are mean ± S.E.M. from 3 (GABA, glutamate) or 6 (serotonin, dopamine) independent experiments performed in triplicates. MDMA produced a significant decrease in synaptosomal uptake of serotonin and dopamine at all concentrations tested; $P < 0.05$ by ANOVA followed by Dunnett's method. (b) Effects of MDMA on the uptake of [^{3}H]glutamate, [^{3}H]serotonin and [^{3}H]dopamine into rat brain synaptic vesicles in vitro. Synaptic vesicles were incubated with substrate (1 mM glutamate, 96 nM serotonin or 257 nM dopamine) for 3 min at 30 °C after 15 min of preincubation with MDMA (0.5–20 μM). Uptake is expressed as percent of control. Data are mean ± S.E.M. from 2 (glutamate) or 5 to 6 (serotonin, dopamine) independent experiments performed in triplicates. S.E.M. is not shown for glutamate due to the sample size. $^{*}P < 0.05$, by ANOVA followed by Dunnett's method. (c) Effects of paroxetine on the uptake of [^{3}H]dopamine and [^{3}H]serotonin into rat brain synaptosomes and synaptic vesicles in vitro. Synaptosomes were incubated with substrate (147 nM dopamine or 86 nM serotonin) for 9 min at 25 °C after 15 min of preincubation with paroxetine (0.01–5 μM). Synaptic vesicles were incubated with substrate (257 nM dopamine or 96 nM serotonin) for 3 min at 30 °C after 15 min of preincubation with paroxetine (0.01–5 μM). Uptake is expressed as percent of control. Data are mean ± S.E.M. from 3 independent experiments performed in triplicates. $^{*}P < 0.05$, by ANOVA followed by Dunnett's method.

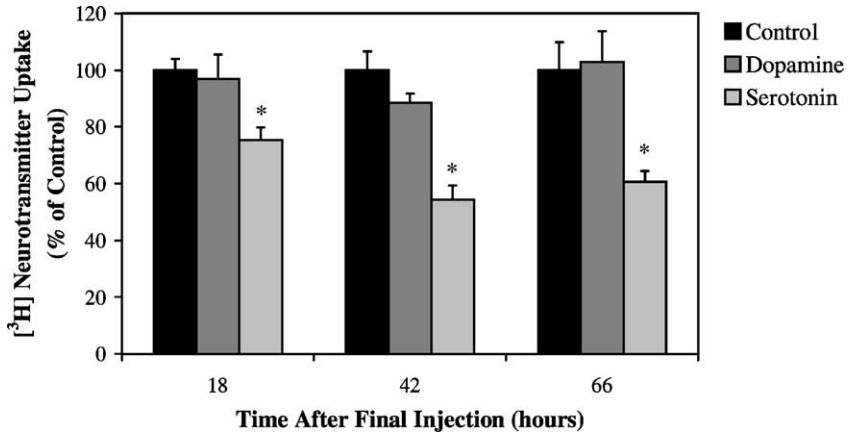


Fig. 4. Effects of repeated administrations of MDMA (2 × 10 mg/kg per day; 4 days) on [^{3}H]dopamine and [^{3}H]serotonin uptake into rat brain synaptosomes ex vivo. Uptake was measured 18, 42 or 66 h after final MDMA administration. Synaptosomes were incubated with substrate (86 nM serotonin, 147 nM dopamine) for 9 min at 25 °C after 15 min of preincubation. Uptake is expressed as percent of control (animals treated with saline). Data are mean ± S.E.M. from 4 to 6 independent experiments performed in triplicates. $^*P < 0.05$, by ANOVA followed by Dunnett's method.

the uptake of GABA was not affected at all. This dose regimen was modelled to simulate the pattern of MDMA abuse at all-night dance parties, with multiple doses consumed during a night. Short-term effects of MDMA and other amphetamines ex vivo have been extensively studied by Fleckenstein et al. (2000). This group reported about 40% reduction in both synaptosomal dopamine and serotonin uptake after multiple injections of MDMA (4 × 15 mg/kg; 2-h intervals). Fleckenstein et al. (2000) measured uptake already 1 h after drug injection, and administered higher doses of MDMA compared to the present study. This could allow more MDMA to remain in the brain during isolation, and might explain the differences found in inhibition of the serotonin uptake.

There is growing concern about the long-term effects of repeated use of MDMA. A commonly used MDMA regimen involves administration twice daily for four consecutive days, as a model of long-weekend MDMA users. Our results show that the uptake of serotonin was reduced to approximately 75% of control 18 h after cessation of drug administration (2 × 10 mg/kg per day; 4 days). This inhibition of synaptosomal serotonin uptake was even stronger (50–60% of control) at longer intervals after MDMA exposure (42 or 66 h). These results demonstrate that MDMAs effect on the serotonin transporter is long-lasting, and raises the question whether these effects are irreversible, i.e. due to damage to serotonergic neurons. Immuncytochemical studies suggest that decrements in serotonergic axonal markers after MDMA exposure are related to loss of serotonergic axons, with nerve cell bodies in the brain stem remaining unaffected (O'Hearn et al., 1988; Ricaurte et al., 2000). MDMA is also known to produce acute hyperthermia (Mechan et al., 2001), and it has been suggested that the serotonergic neurotoxicity of MDMA can cause long-lasting changes in thermoregulatory mechanisms (Dafters and Lynch, 1998).

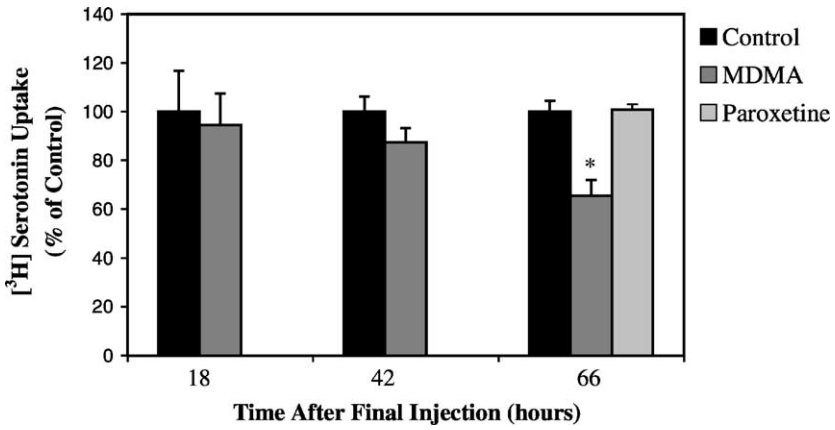


Fig. 5. Effects of repeated administrations of MDMA (2 × 10 mg/kg per day; 4 days) or paroxetine (2 × 5 mg/kg per day; 4 days) on [^{3}H]serotonin uptake into rat brain synaptic vesicles ex vivo. Uptake was measured 18, 42 or 66 h after final MDMA administration. Synaptic vesicles were incubated with substrate (96 nM serotonin) for 3 min at 30 °C after 15 min of preincubation. Uptake is expressed as percent of control (animals treated with saline). Data are mean ± S.E.M. from 4 to 8 independent experiments performed in triplicates. $^*P < 0.05$, by ANOVA followed by Dunnett's method.

The synaptosomal uptake of dopamine was not inhibited after MDMA treatment for four consecutive days. This finding was surprising because of the pronounced dopaminergic effects in the short-term studies. Our results however, agree with the findings of other groups reporting that ex vivo MDMA treatment rapidly and reversibly decreases dopamine transporter function (Metzger et al., 1998), but causes little or no long-term dopaminergic damage (Johnson et al., 1988; Insel et al., 1989). Another explanation for the differences in inhibition of the dopamine uptake in our short- and long-term studies could be the higher doses of MDMA administered in the short-term studies. Commins et al. (1987) suggested a dose-related neurotoxicity of MDMA, with high serotonergic selectivity at lower dosages, and a dopaminergic effect only at higher dosages.

MDMA has both structural and functional similarities to the antidepressant paroxetine. Both compounds possess a 3,4-methylenedioxyphenyl group, and both inhibit the synaptosomal uptake of serotonin (Hashimoto et al., 1993). In an attempt to find possible cellular mechanisms for the serotonergic toxicity of MDMA, we compared the effect of MDMA and paroxetine on synaptosomes and synaptic vesicles in vitro. Our results show that paroxetine is an extremely potent serotonin reuptake inhibitor, compared to MDMA. In addition, paroxetine is a far more selective inhibitor of the serotonergic uptake, while MDMA inhibit the uptake of serotonin and dopamine to a similar extent. Paroxetine and MDMA showed almost identical IC_{50} values for vesicular uptake of dopamine and serotonin in vitro.

Our studies in vitro revealed an interesting difference between MDMA and paroxetine in the concentration ratio needed to affect the synaptosomal and vesicular uptake of serotonin. While paroxetine affected the synaptosomal and vesicular uptake at concentrations that differed at least 500-fold, MDMA inhibited synaptosomal and vesicular uptake at similar concentrations (five-fold differences). In addition to this finding, it must not be forgotten that the two compounds are used in highly different manners. While paroxetine is taken medically at prescribed concentrations, MDMA is being used illegally at high and often frequently repeated dosages. Our findings in vitro and ex vivo indicate that the probability of inhibiting both the vesicular and synaptosomal serotonin uptake is highly present for MDMA, while in all likelihood absent for paroxetine. Due to a reduced vesicular uptake of serotonin after MDMA exposure, the synaptic release of serotonin may also be reduced. This view is presented schematically in Fig. 6.

We hypothesize that inhibition of VMAT-2, and a subsequent increase in cytoplasmatic monoamine concentrations, is crucial for the neurotoxic effect of MDMA. High concentrations of cytoplasmatic serotonin and/or dopamine might cause extensive formation of ROS through oxidation by monoamine oxidase. A related hypothesis has been suggested for the neurotoxicity of methamphetamine, proposing that the disruption of vesicular dopamine storage and generation of intracellular ROS is the cause of dopaminergic cell death (Cubells et al., 1994; Brown et al., 2000).

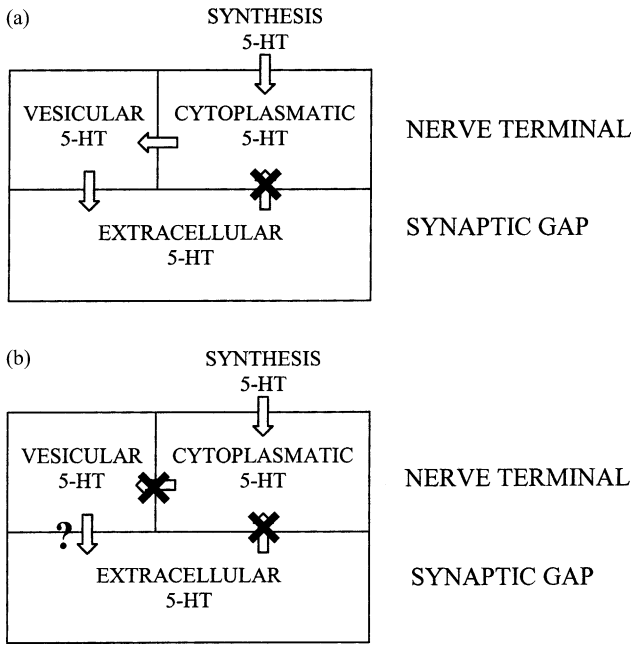


Fig. 6. Schematically representation of the mechanism whereby (a) paroxetine and (b) MDMA may inhibit the uptake of serotonin (5-HT) and influence vesicular, cytoplasmatic and extraneuronal concentrations of serotonin. In the case of paroxetine, only the uptake into the nerve terminal is inhibited. This will give a high extracellular level of serotonin, but should not affect vesicular release. In contrast, MDMA inhibit both the synaptosomal and the vesicular uptake of serotonin. This will cause a more complex situation with a high extracellular level of serotonin and, since serotonin synthesis is not inhibited, a high cytoplasmatic level of serotonin in the nerve terminal. Since the vesicular uptake is inhibited, the vesicular release of serotonin may also be affected.

In summary, this study shows that MDMA affects both synaptosomal and vesicular uptake of monoamine neurotransmitters. We suggest that inhibition of vesicular uptake of monoamines might be crucial for the neurotoxic effects of MDMA.

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