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Short communication

5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents

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This study examined the [^{3}H]5-HT-releasing properties of 3,4-methylenedioxymethamphetamine (MDMA) and related agents, all of which cause significant release of [^{3}H]5-HT from rat brain synaptosomes. 5-HT uptake blockers dose dependently block MDMA-induced [^{3}H]5-HT release. The EC_{50}s of the uptake drugs in blocking MDMA-induced release correlate with their affinity for the 5-HT uptake site labeled by [^{3}H]paroxetine ($r = 0.98$; $P < 0.01$). These data demonstrate that the 5-HT uptake carrier plays a significant role in the release of 5-HT induced by MDMA and related agents.

MDMA (3,4-methylenedioxymethamphetamine); 5-Hydroxytryptamine (5-HT, serotonin); Synaptosomes; (Rat)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA; 'Ecstasy') has received widespread attention due to both its neurotoxic effects in animals (McKenna and Peroutka, in press) and its recreational use in humans (Barnes, 1988). MDMA typically causes a biphasic reduction in brain 5-hydroxytryptamine (5-HT) levels. The drug induces an acute depletion by 3-6 h after administration, after which point levels return to control levels by 24 h, only to fall again by 1 week (Schmidt, 1987). It is believed that the acute depletion of 5-HT is due to enhanced release from terminals, possibly coupled with diminished synthesis. In contrast, the long-term depletion probably reflects a neurotoxic degeneration of 5-HT nerve terminals. MDMA causes marked degeneration of 5-HT nerve terminals in the cerebral cortex, hippocampus and striatum at 2 weeks post-MDMA injection (O'Hearn et al., 1988; McKenna and Peroutka, in press).

The mechanism of action of the short-term effects of MDMA has not been completely characterized. MDMA has been shown to both induce the release of [^{3}H]5-HT (Schmidt et al., 1987; Johnson et al., 1986) and to inhibit the reuptake of [^{3}H]5-HT (Steele et al., 1987) in vitro. Some evidence suggests that 5-HT uptake blockers (e.g. citalopram) may inhibit the MDMA-induced release of [^{3}H]5-HT from rat brain slices (Schmidt et al., 1987). Therefore, the current investigation was undertaken to further analyze the interaction of MDMA and related agents with the uptake site using an in vitro release system.

2. Materials and methods

2.1. Synaptosomal preparation

Male Sprague-Dawley rats (200-225 g; Simonsen Labs: Gilroy, CA) were decapitated and their

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brains were dissected on ice. The cerebral cortex, striatum and hippocampus were homogenized in 10 volumes of chilled 0.32 M sucrose with a Wheaton motor-driven Teflon-glass homogenizer (speed 4). The homogenate was centrifuged at $1000 \times g$ for 10 min. The supernatant, containing the crude synaptosomal fraction, was diluted 1 : 1 in Krebs-HEPES buffer (composition, in mM: NaCl 118; KCl 4.8; $CaCl_2$ 2.5; $MgCl_2$ 1.2; HEPES 25; pargyline 0.008; pH adjusted to 7.4 with NaOH) and then respun at $10000 \times g$ for 15 min. The final pellet was resuspended in 80 volumes of Krebs-HEPES buffer.

2.2. *Release of [^{3}H]5-HT from synaptosomes*

The synaptosomal preparation was aliquoted into test tubes (10 mg original tissue weight/assay) and incubated with [^{3}H]5-HT (final concentration 9 nM) at 37°C for 15 min. Specific uptake was defined by the total minus blank taken in the presence of 10^{-4} M fluoxetine. The synaptosomal preparations were divided into triplicates and given either buffer or varying concentrations (10^{-4}-10^{-9} M final concentration) of either MDMA, methylenedioxyamphetamine (MDA), N-ethylmethylenedioxyamphetamine (MDE), fenfluramine, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; a 5-HT_2 agonist), (−)-pindolol (a 5-HT_{1B} antagonist) of fluoxetine (a specific 5-HT uptake blocker). Following 15 min incubation at 37°C, the assays were terminated by rapid filtration through No. 32 glass filter papers. The filters were rinsed 3 times with 6 ml Krebs-HEPES buffer. The dried filters, containing synaptosomes and retained radioactivity, were dissolved in 2.5 ml 3a70 Scintillation Cocktail (Research Products International Corp., IL). The samples were counted on a Packard 1900CA Liquid Scintillation Analyzer at 41% efficiency.

2.3. *Inhibition of synaptosomal release with 5-HT uptake blockers*

In one series of experiments, 10^{-6} M MDMA-induced synaptosomal release of [^{3}H]5-HT was inhibited with increasing concentrations of the uptake inhibitors paroxetine, fluoxetine, imipramine, amitriptyline or desipramine. Synaptosomes were prepared as described above. Total radioactivity was defined as untreated [^{3}H]5-HT-loaded synaptosomes. Non-specific release was defined as synaptosomes incubated with 10^{-6} M MDMA but no inhibitor. Varying drug concentrations (10^{-10}-10^{-4} M) were added just prior to the addition of 10^{-6} M MDMA alone.

2.4. *Drug sources*

Drug sources were as follows: [^{3}H]5-HT (specific activity: 12 Ci/mmol), Amersham Corp. (Arlington Heights, IL); MDMA, MDA and MDE, National Institute on Drug Abuse (Rockville, MD); DOI, Research Biochemicals Inc. (Natick, MA); (−)-pindolol, Sandoz Pharmaceuticals (East Hanover, NJ); fluoxetine, Lilly Research Laboratories (Indianapolis, IN). Paroxetine was generously provided by C.A. Mathis of the Donner Laboratory (University of California at Berkeley). All other compounds were obtained from Sigma Chemical Co. (St. Louis, MO).

3. Results

3.1. *Drug-induced release of [^{3}H]5-HT*

The ability of MDMA, MDA, MDE, fenfluramine, fluoxetine, DOI and (−)-pindolol to

TABLE 1

Drug effects on [^{3}H]5-HT release from synaptosomes. Data given are the average amount of [^{3}H]5-HT remaining in the drug-treated synaptosomal preparation in comparison to control synaptosomes under otherwise identical experimental conditions.

Drug	% synaptosomal [^{3}H]5-HT vs. control conditions (%)	EC_{50} (nM)
MDA	12 ± 3	290 ± 50
Fenfluramine	16 ± 4	310 ± 80
MDMA	19 ± 4	300 ± 50
MDE	29 ± 4	240 ± 80
DOI	58 ± 7	4700 ± 2000
Fluoxetine	75 ± 4	23000 ± 6000
(−)-Pindolol	81 ± 9	29000 ± 3000

induce synaptosomal release of [^{3}H]5-HT was determined in a rat synaptosomal preparation. As shown in table 1, MDMA, MDA, MDE and fenfluramine induce a significant release of [^{3}H]5-HT from synaptosomes (average percentage of [^{3}H]5-HT remaining in synaptosomes = 19 ± 6% of control values). MDA induces the largest decrease in [^{3}H]5-HT content (12 ± 3%) while fenfluramine and MDMA are slightly less efficacious. MDE (29 ± 4%) induces a significantly less [^{3}H]5-HT release than MDMA ($P < 0.025$). The EC_{50} values for 5-HT release by MDA, MDMA, fenfluramine and MDE are essentially the same. By contrast, DOI, fluoxetine and (−)-pindolol are significantly less potent and less efficacious in their ability to induce [^{3}H]5-HT release from rat synaptosomes (table 1).

3.2. Inhibition of MDMA-induced release using 5-HT uptake blockers

A series of 5-HT uptake blockers (paroxetine, fluoxetine, imipramine, amitriptyline and desipramine) were co-incubated with rat synaptosomes prior to the addition of 10^{-6} M MDMA. All five agents dose dependently inhibit the release of [^{3}H]5-HT induced by MDMA. Paroxetine is the most potent agent, with an EC_{50} value of 3.0 ± 1 nM whereas desipramine is the weakest agent (table 2). The reported affinity (Schmidt and Peroutka, 1989) of the five drugs for [^{3}H]paroxetine-labeled 5-HT uptake sites is also provided in table 2. A significant correlation exists between drug affinities for the 5-HT uptake sites and their ability to inhibit the MDMA-induced release of [^{3}H]5-HT ($r = 0.98$; $P < 0.01$). Fluoxetine (10^{-6} M) also significantly inhibited [^{3}H]5-HT release induced by MDA, MDE and fenfluramine.

TABLE 2

Potencies of uptake blockers for [^{3}H]paroxetine-labeled 5-HT uptake sites. IC_{50} values vs. [^{3}H]paroxetine are taken from Schmidt and Peroutka (1989). The EC_{50}s of the uptake drugs in blocking MDMA-induced release correlate with their affinity for the 5-HT uptake site labeled by [^{3}H]paroxetine ($r = 0.98$; $P < 0.01$).

Drug	IC_{50} (nM) vs. [^{3}H]paroxetine	EC_{50} (nM) for inhibition of MDMA-induced [^{3}H]5-HT release
Paroxetine	1.3 ± 0.3	3.0 ± 1
Fluoxetine	15 ± 2	40 ± 10
Imipramine	37 ± 4	140 ± 20
Amitriptyline	73 ± 5	200 ± 50
Desipramine	530 ± 100	800 ± 100

4. Discussion

The major finding of the present study is that 5-HT uptake blockers attenuate the 5-HT-releasing effect of MDMA and related agents. A significant correlation exists between drug affinities for the [^{3}H]paroxetine-labeled 5-HT uptake site and their ability to inhibit MDMA-induced release of 5-HT. These results provide insights into the mechanism of action of MDMA and related agents. MDMA has been shown to both inhibit the uptake of 5-HT (Schmidt et al., 1987; Johnson et al., 1986) and to induce its release (Steele et al., 1987). Although MDMA may impair 5-HT uptake to some degree, MDMA must predominantly release 5-HT.

Since MDMA does not appear to accumulate within synaptosomes (Wang et al., 1987), MDMA may induce the release of 5-HT by interacting with a membrane receptor recognition site. MDMA displays an affinity of 610 ± 50 nM for [^{3}H]paroxetine-labeled uptake sites (Battaglia et al., 1988), a value which is similar to its EC_{50} in inducing release of 5-HT (table 1). Conceivably, MDMA may directly interact with the 5-HT uptake carrier but, unlike 5-HT uptake blockers, the drug may act by reversing the direction of neurotransmitter flow. Although the exact site at which MDMA acts remains to be elucidated, the results from this study clearly demonstrate that the 5-HT uptake carrier is critically involved in MDMA-induced release of 5-HT.

Administration of 5-HT uptake blockers such as fluoxetine (Schmidt, 1987) can attenuate the long-term depletion of 5-HT caused by MDMA. Schmidt (1987) hypothesized that fluoxetine may prevent a toxic metabolite of MDMA from entering the 5-HT nerve terminals. Alternatively, the present data suggests that the ability of 5-HT

uptake blockers to prevent release of 5-HT may be the basis for its protective effect in neurotoxicity studies. Conceivably, an overaccumulation of the neurotransmitter may lead to terminal destruction, possibly via the formation of a toxic metabolite of 5-HT, as opposed to MDMA. The protective effect of 5-HT uptake blockers might therefore be attributable to their ability to inhibit 5-HT release induced by MDMA and related agents.

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