

The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain

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Abstract

We developed a reproducible, simple, and small-scale method for determining the re-uptake and release of monoamines (dopamine, serotonin (5-HT) and norepinephrine) using rat brain synaptosomes. These assays were then applied to study the effects of different kinds of non-medically used psychoactive drugs on monoamine re-uptake and release. The phenethylamine derivatives, 4-fluoroamphetamine, 2-methylamino-3,4-methylenedioxy-propionophenone (methylone), 1-(1,3-benzodioxol-5-yl)-2-butanamine (BDB), and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), had strong inhibitory effects on the re-uptake of dopamine, 5-HT and norepinephrine. 4-Fluoroamphetamine, methylone and BDB also strongly increased the release of the three monoamines, but MBDB increased 5-HT and norepinephrine release, but had little effect on dopamine release. However, 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-ethylphenethylamine (2C-E), 2,5-dimethoxy-4-chlorophenethylamine (2C-C), 2,4,5-trimethoxyamphetamine (TMA-2) and 2,4,6-trimethoxyamphetamine (TMA-6), which are methoxylated phenethylamine derivatives, slightly influenced the re-uptake and release of monoamines. α -Methyltryptamine (AMT), a tryptamine derivative, was one of the strongest re-uptake inhibitors and releasers of the three monoamines. The tryptamine derivative, 5-methoxy- α -methyltryptamine (5-MeO-AMT), also strongly inhibited re-uptake and increased the release of the three monoamines. *N,N*-dipropyltryptamine (DPT), 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT), 5-methoxy-*N,N*-methylisopropyltryptamine (5-MeO-MIPT), and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) inhibited monoamine re-uptake, but had a few effects on monoamine release. 1-(3-Chlorophenyl)piperazine (3CPP) and 1-(methoxyphenyl)piperazine (4MPP), which are piperazine derivatives, inhibited monoamine re-uptake and accelerated their release. The results suggest that some designer drugs strongly act on the central nerve system to the same extent as restricted drugs.

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1. Introduction

Non-prescription psychoactive drugs are used by many people for various purposes, including mind stimulation, hallucination, recreational use, and weight-loss induction. The abuse of these drugs adversely affects health. Mood- and behavior-altering substances have always been part of human civilization.

These drugs have mainly been obtained from plants, including cocaine, morphine, and lysergic acid diethylamide (LSD). However, many recreational drugs have been synthesized chemically in recent decades. For example, 3,4-methylenedioxymethamphetamine (MDMA) is derived from amphetamine. Although the use of MDMA is prohibited in many countries, new psychoactive chemicals (called designer drugs) are being developed all the time and can be easily obtained from individuals or via the Internet. Synthetic drugs are classified roughly, based on their chemical formula, into phenethylamines, tryptamines, and piperazines. Phenethylamine derivatives include 2-methylamino-3,4-methylenedioxy-propionophenone (methylone), *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), and 2,5-dimethoxy-4-iodophenethylamine (2C-I).

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Tryptamine derivatives include α -methyltryptamine (AMT), 5-methoxy- α -methyltryptamine (5-MeO-AMT), 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT), and 5-methoxy-*N,N*-methylisopropyltryptamine (5-MeO-MIPT). AMT and 5-MeO-DIPT were recently prohibited in many countries including Japan because of their potential to cause significant health and social problems (Drug Enforcement Administration, 2003, 2004). 1-(3-Chlorophenyl) piperazine (3CPP) and 1-(methoxyphenyl)piperazine (4MPP) are classified as piperazine derivatives.

It is thought that psychoactive drugs modify the central nervous system; however the exact mechanisms by which many of these substances act have not been clarified. Many psychotropic drugs modify the monoamine neurotransmission systems, including the dopaminergic, serotonergic, and adrenergic nervous systems (Fleckenstein et al., 2000; Bauman et al., 2000). Cocaine stimulates the central nervous system by inhibiting the re-uptake of the monoamines, dopamine, serotonin (5-HT), and norepinephrine (Cunningham and Callahan, 1991; Bennett et al., 1995; Rothman et al., 2001), while methamphetamine is known to accelerate the release of dopamine, 5-HT and norepinephrine (Fleckenstein et al., 2000; Rothman et al., 2001). The synthetic MDMA is a specific effector of the 5-HT neurotransmission system and acts to strongly increase 5-HT release (Rothman et al., 2001; Parrott et al., 2004).

We developed a reproducible method to determine the re-uptake and release of monoamines (dopamine, serotonin (5-HT) and norepinephrine), using rat brain synaptosome, and analyzed the effects of various designer drugs on monoamine neurotransmission by monitoring the re-uptake and release of these monoamines. In this study, we aimed to evaluate the psychoactivity of designer drugs.

2. Materials and methods

2.1. Chemicals

Psychoactive chemicals (designer drugs) were purchased from adult goods shops in Tokyo, and the purity of drugs was determined by HPLC (Alliance PDA system, Waters Co. MA, USA) with L-column ODS, 5 μ m, 4.6 $\times$ 150 mm (Chemicals Evaluation and Research Institute, Japan) and solvent of H₂O/acetone/nitrile/phosphoric acid/SDS (580/420/1/12.5 g) (Nagashima et al., 2004). [³H]Dopamine (2.20 TBq/mmol), [³H]serotonin (1.11 TBq/mmol) and [³H]norepinephrine (1.93 TBq/mmol) were purchased from Perkin Elmer Co., Ltd (MO, USA). Cocaine and methamphetamine were purchased from Takeda Pharmaceutical Company Limited (Osaka Japan) and Dainippon Sumitomo Pharma Co., Ltd. (Osaka Japan), respectively. Reserpine, pargyline, tyramine, nomifensine, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine (GBR12935), citalopram, dopamine, 5-HT and norepinephrine were obtained from Sigma-Aldrich (MO, USA). Other reagents used in the study were of the highest grade commercially available. The scintillation cocktail used Lumasafe Plus produced by Lumac Lsc B.V. (Groningen, The Netherlands) (Table 1).

Table 1

Data on tested psychoactive chemicals (drugs)

Drugs	Abbreviations	Purity (%)
Phenethylamines		
3,4-methylenedioxymethamphetamine	MDMA	98.0
2-methylamino-3,4-methylenedioxypropionophenone	Methylone	98.8
1-(1,3-benzodioxol-5-yl)-2-butanamine	BDB	97.1
<i>N</i> -methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine	MBDB	99
4-fluoroamphetamine		98.5
2,5-dimethoxy-4-chlorophenethylamine	2CC	94.2
2,5-dimethoxy-4-ethylphenethylamine	2C-E	100.0
2,5-dimethoxy-4-iodophenethylamine	2C-I	99.1
3,4,5-trimethoxyamphetamine	TMA	99.3
2,4,5-trimethoxyamphetamine	TMA-2	98.6
2,4,6-trimethoxyamphetamine	TMA-6	98.1
Tryptamines		
α -methyltryptamine	AMT	99.0
5-methoxy- α -methyltryptamine	5-MeO-AMT	92.7
<i>N,N</i> -dipropyltryptamine	DPT	96.9
5-methoxy- <i>N,N</i> -diisopropyltryptamine	5-MeO-DIP T	96.1
5-methoxy- <i>N,N</i> -methylisopropyltryptamine	5-MeO-MIPT	99.5
5-methoxy- <i>N,N</i> -dimethyltryptamine	5-MeO-DMT	91.7
5-methoxy- <i>N,N</i> -diallyltryptamine	5-MeO-DALT	87.8
Piperazines		
1-benzylpiperazine	BZP	98.0
1-(3-chlorophenyl)piperazine	3CPP	98.0
4-(methoxyphenyl)piperazine	4MPP	90.0

The purities were obtained by HPLC (Nagashima et al., 2004).

2.2. Animals

Male Sprague-Dawley (SD) rats (crlj: 300–400 g) were obtained from Charles River Japan (Kanagawa, Japan) and were housed in a room at 22–24 °C, 45–65% humidity with 12-h light and 12-h dark cycles. Water and food were supplied ad libitum. All rats were handled in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

2.3. Preparation of synaptosome

Rat brain crude synaptosomes were prepared by the modified Fleckenstein method (Fleckenstein et al., 1997). Following decapitation of a male SD rat, the brain was removed rapidly, and the striatum and cerebral cortex were dissected. Each tissue was homogenized in 25 volumes of ice-cold 0.32 M sucrose by 10 strokes with loosely fitted Teflon homogenizers, and centrifuged at 800 $\times$ g for 12 min at 4 °C. The supernatants were further centrifuged at 22,000 $\times$ g for 20 min at 4 °C. The resulting pellets were suspended in modified Krebs-Ringer buffer (pH 7.4) containing 126 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl₂, 1.4 mM MgSO₄, 16 mM sodium phosphate, 11 mM glucose, 1 mM ascorbate, and 1 μ M pargyline (Buffer A). The crude striatum synaptosome preparation (StP₂) was used for the assay of re-uptake and release of dopamine, and the cortex synaptosome preparation (CxP₂) was used for the 5-HT and norepinephrine assays. For the release assay, 1 μ M reserpine was added to 0.32 M sucrose and buffer A. Protein concentration was determined with the modified Lowry method, using a Bio-Rad assay kit.

2.4. Re-uptake assays of dopamine, 5-HT and norepinephrine

The re-uptake assay methods, modified from Rothman et al. (2001, 2003), were started immediately after the preparation of synaptosomes. The re-uptake assays for the three monoamines were performed at the same time using preparations prepared from one rat brain. In a 96-well plate, synaptosomes (StP₂, 30 µg protein for dopamine; CxP₂, 70 µg protein for 5-HT and norepinephrine) were added to buffer A containing various concentrations of drugs, and pre-incubated at 37 °C for 10 min. The re-uptake assay was initiated by the addition of [³H]dopamine, [³H]5-HT, and [³H]norepinephrine at final concentrations of 63 nM, 125 nM, and 125 nM, respectively. The total reaction mixture volume was 100 µL. The re-uptake assay was performed in triplicate.

The reaction mixture was incubated at 37 °C for 5 min, and then the reaction was terminated by rapid vacuum filtration over a glass-filter Whatman GF/C using a cell harvester (Filter Mate, Perkin Elmer, MA, USA). The pellets retained on the filters were washed with ice-cold buffer A to remove free radioisotope, followed by filter drying. The filter was punched out and placed in 1.5 ml of scintillation cocktail, and the radioactivity retained on the filter was quantified using a liquid scintillation counting system (Perkin Elmer, MA, USA) following overnight extraction into the scintillation cocktail. [³H]5-HT re-uptake was assayed in the presence of 100 nM nomifensine and 100 nM GBR12935 to prevent uptake into norepinephrine or dopamine nerve terminals. Non-specific uptake of [³H]dopamine and [³H]5-HT was obtained in the presence of 10 µM GBR12909 (Bauman et al., 2000) and 10 µM citalopram (Fleckenstein et al., 1997), respectively. Non-specific uptake of [³H]norepinephrine was obtained at 0 °C (Walker and Marolman, 1996). Specific uptake was calculated by subtracting the non-specific uptake from the total uptake. From these results, the drug concentration giving the half-maximal inhibition value (IC₅₀) was obtained.

2.5. Dopamine, 5-HT and norepinephrine release assays

The release assay methods, modified from Rothman et al. (2001), were started immediately after the preparation of synaptosomes. The release assays for the three monoamines were performed at the same time using synaptosomes prepared from one rat brain. StP₂ (30 µg protein, for dopamine) and CxP₂ (70 µg protein, for 5-HT and norepinephrine) were incubated to steady-state with 5 nM [³H]dopamine (30 min), 5 nM [³H]5-HT (60 min), or 7 nM [³H]norepinephrine (60 min) in buffer A containing 1 µM reserpine in polypropylene tubes with stirring at 25 °C. Nomifensine (100 nM) and GBR12935 (100 nM) were added to the reaction mixture for the [³H]5-HT releasing experiment. After incubation, 75 µL of synaptosomes pre-loaded with [³H]monoamine-transmitters was added to a 96-well plate containing 25 µL of various concentrations of tested drugs. Each assay was performed in triplicate. After 5 min ([³H]dopamine) and 30 min ([³H]5-HT and [³H]norepinephrine) at 25 °C, the release reactions were terminated by rapid vacuum filtration over Whatman GF/C filter using a cell harvester, and free radioisotope on the filter was removed by washing with ice-

cold buffer A. Non-displaceable tritium was measured by conducting incubations in the presence of 10 µM tyramine for [³H]dopamine and [³H]norepinephrine release and 100 µM tyramine for [³H]5-HT release (Rothman et al., 2002). Radioactivity was quantified using the same methods as for the re-uptake assay. From these results, the drug concentration giving the half-maximal acceleration value (EC₅₀) was obtained.

3. Results

3.1. Inhibition of monoamine re-uptake by some drugs in rat brain synaptosome

Initially, we needed to confirm the suitability of our method for screening the monoamine re-uptake activity of various drugs. To do this, we examined the inhibitory effects of cocaine, and methamphetamine, as positive controls, on the re-uptake of dopamine, 5-HT, and norepinephrine using this method (Table 2 and Fig. 1). Cocaine strongly inhibited dopamine re-uptake into rat StP₂, and 5-HT and norepinephrine re-uptake into CxP₂. The IC₅₀ values for dopamine, 5-HT, and norepinephrine were 8.5×10^{-7} , 2.1×10^{-6} , and 3.4×10^{-7} M, respectively. Methamphetamine was a potent inhibitor of dopamine re-uptake into StP₂, and of 5-HT and norepinephrine re-uptake into CxP₂ with IC₅₀ values of 3.7×10^{-7} , 4.0×10^{-6} , and 2.0×10^{-7} M, respectively.

Table 2
The effects of drugs on monoamine re-uptake into rat brain synaptosome

Drugs	Re-uptake (IC ₅₀ , M) ^(a)		
	Dopamine	5-HT	Norepinephrine
Phenethylamines			
MDMA	$1.4 \pm 0.32 \times 10^{-6}$	$7.2 \pm 1.9 \times 10^{-7}$	$6.6 \pm 2.1 \times 10^{-7}$
Methylone	$2.9 \pm 0.67 \times 10^{-6}$	$2.3 \pm 0.58 \times 10^{-6}$	$7.4 \pm 2.4 \times 10^{-7}$
BDB	$7.9 \pm 1.9 \times 10^{-6}$	$1.6 \pm 0.42 \times 10^{-6}$	$2.8 \pm 0.89 \times 10^{-6}$
MBDB	$6.3 \pm 1.4 \times 10^{-6}$	$1.8 \pm 0.48 \times 10^{-6}$	$2.7 \pm 0.75 \times 10^{-6}$
4-fluoroamphetamine	$7.7 \pm 1.6 \times 10^{-7}$	$6.8 \pm 1.5 \times 10^{-6}$	$4.2 \pm 1.2 \times 10^{-7}$
2C-I	n.e. ^(b)	$7.9 \pm 1.9 \times 10^{-5}$	$3.7 \pm 1.2 \times 10^{-5}$
2C-E	n.e.	$7.2 \pm 1.6 \times 10^{-5}$	$8.9 \pm 2.7 \times 10^{-5}$
2C-C	n.e.	$3.1 \pm 0.78 \times 10^{-5}$	$6.3 \pm 1.8 \times 10^{-5}$
TMA	n.e.	n.e.	n.e.
TMA-2	n.e.	n.e.	n.e.
TMA-6	n.e.	n.e.	n.e.
Tryptamines			
AMT	$7.3 \pm 1.9 \times 10^{-7}$	$3.8 \pm 0.74 \times 10^{-7}$	$4.0 \pm 0.72 \times 10^{-7}$
5-MeO-AMT	$1.8 \pm 0.35 \times 10^{-5}$	$2.9 \pm 0.71 \times 10^{-6}$	$3.7 \pm 0.62 \times 10^{-5}$
DPT	$2.3 \pm 0.48 \times 10^{-5}$	$2.9 \pm 0.69 \times 10^{-6}$	$9.1 \pm 2.0 \times 10^{-6}$
5-MeO-DIPT	$6.5 \pm 1.1 \times 10^{-5}$	$2.2 \pm 0.41 \times 10^{-6}$	$8.2 \pm 1.9 \times 10^{-6}$
5-MeO-MIPT	n.e.	$6.4 \pm 1.8 \times 10^{-6}$	$2.6 \pm 0.45 \times 10^{-5}$
5-MeO-DMT	n.e.	$4.1 \pm 0.91 \times 10^{-6}$	$3.3 \pm 0.45 \times 10^{-5}$
5-MeO-DALT	n.e.	n.e.	n.e.
Piperazines			
BZP	$1.9 \pm 0.42 \times 10^{-6}$	$2.0 \pm 0.36 \times 10^{-5}$	$6.2 \pm 1.4 \times 10^{-7}$
3CPP	$1.2 \pm 0.22 \times 10^{-5}$	$3.3 \pm 0.69 \times 10^{-7}$	$2.5 \pm 0.58 \times 10^{-6}$
4MPP	$4.8 \pm 0.9 \times 10^{-5}$	$4.6 \pm 0.83 \times 10^{-6}$	$6.2 \pm 1.3 \times 10^{-6}$
Positive controls			
Cocaine	$8.5 \pm 2.2 \times 10^{-7}$	$2.1 \pm 0.52 \times 10^{-6}$	$3.4 \pm 1.1 \times 10^{-7}$
Methamphetamine	$3.7 \pm 0.96 \times 10^{-7}$	$4.0 \pm 0.97 \times 10^{-6}$	$2.0 \pm 0.67 \times 10^{-7}$

Mean ± S.D. (three independent assays). Each assay was carried out by using different fresh rat brain synaptosome.

(a) Drug concentrations giving half-maximal inhibition.

(b) No effect at 10^{-4} M of each drug.

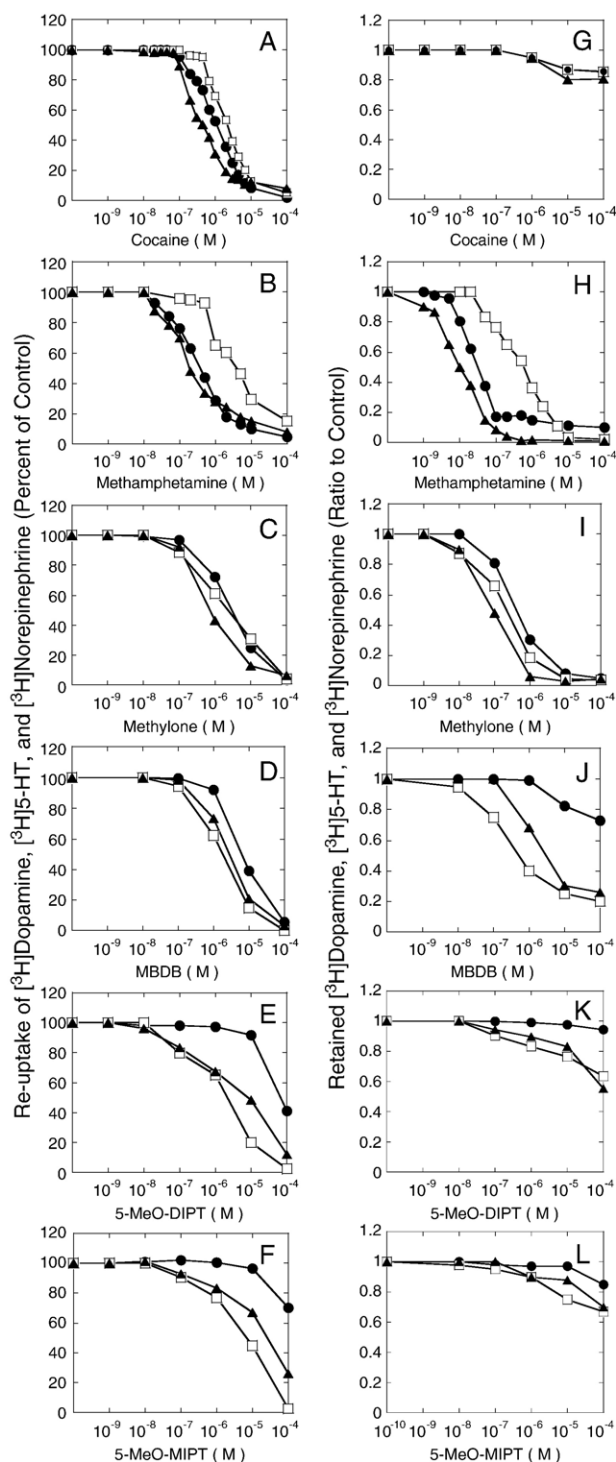


Fig. 1. Inhibition of re-uptake and stimulation of release of monoamines by cocaine, methamphetamine and some designer drugs in rat brain synaptosomes. Synaptosome fraction prepared from striatum (StP₂) was used for the assay of dopamine, and that prepared from cortex (CxP₂) was used for the assays of 5-HT and norepinephrine. ●, dopamine; □, 5-HT; ▲, norepinephrine. a–f: re-uptake. g–l: release. a and g, cocaine; b and h, methamphetamine; c and i, methylone; d and j, MBDB; e and k, 5-MeO-DIPT; f and l, 5-MeO-MIPT.

The effects of the phenethylamine derivatives on monoamine re-uptake are shown in Table 2 and Fig. 1. Methylone inhibited the re-uptake of all the monoamines (IC₅₀ values for dopamine,

5-HT, and norepinephrine were 2.9×10^{-6} , 2.3×10^{-6} , and 7.4×10^{-7} M, respectively). 1-(1,3-benzodioxol-5-yl)-2-butanamine (BDB) and MBDB also potently inhibited monoamine re-uptake, with IC₅₀ values for dopamine, 5-HT, and norepinephrine of 7.9×10^{-6} , 1.6×10^{-6} , and 2.8×10^{-6} M, respectively, for BDB and 6.3×10^{-6} , 1.8×10^{-6} , and 2.7×10^{-6} M, respectively for MBDB. 4-Fluoroamphetamine strongly inhibited the re-uptake of dopamine, 5-HT, and norepinephrine (IC₅₀ for dopamine, 7.7×10^{-7} ; 5-HT, 6.8×10^{-6} ; and norepinephrine, 4.2×10^{-7} M). MDMA, a typical designer drug, strongly inhibited the re-uptake of dopamine, 5-HT and norepinephrine. The re-uptake of dopamine, 5-HT, and norepinephrine was slightly inhibited by 2C-I, 2,5-dimethoxy-4-ethylphenethylamine (2C-E) and 2,5-dimethoxy-4-chlorophenethylamine (2C-C), while 3,4,5-trimethoxyamphetamine (TMA), 2,4,5-trimethoxyamphetamine (TMA-2), and 2,4,6-trimethoxyamphetamine (TMA-6) had even less effect on the re-uptake of dopamine, 5-HT, and norepinephrine.

The effects of tryptamine derivatives of monoamine re-uptakes were also examined (Table 2 and Fig. 1). AMT was one of the most potent inhibitors of monoamine re-uptakes with IC₅₀ values for dopamine, 5-HT, and norepinephrine of 7.3×10^{-7} , 3.8×10^{-7} , and 4.0×10^{-7} M, respectively. 5-MeO-AMT and *N,N*-dipropyltryptamine (DPT) strongly inhibited 5-HT re-uptake (same IC₅₀ values of 2.9×10^{-6} M) and also inhibited dopamine and norepinephrine re-uptake. 5-MeO-DIPT inhibited the re-uptake of 5-HT and norepinephrine relatively strongly with IC₅₀ values of 2.2×10^{-6} and 8.2×10^{-6} M, respectively, though the inhibition of dopamine re-uptake was relatively weak (6.5×10^{-5} M). 5-MeO-MIPT and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) suppressed 5-HT and norepinephrine re-uptake, but had little effect on dopamine re-uptake. 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT) had no detectable effect on dopamine, 5-HT, or norepinephrine re-uptake.

Testing of the piperazine derivatives on monoamine re-uptake (Table 2) showed that 1-benzylpiperazine (BZP, narcotic in Japan) strongly inhibited the re-uptake of dopamine and norepinephrine with IC₅₀ values of 1.9×10^{-6} and 6.2×10^{-7} M, respectively, but had a markedly smaller effect on 5-HT re-uptake. 3CPP strongly inhibited 5-HT re-uptake with an IC₅₀ of 3.3×10^{-7} M and also inhibited the re-uptake of norepinephrine and dopamine with IC₅₀ values of 2.5×10^{-6} and 1.2×10^{-5} M, respectively. Finally, 4MPP also strongly inhibited monoamine re-uptake.

3.2. Monoamine-releasing activity of some drugs in rat brain synaptosome

We next determined the effect of methamphetamine on monoamine release from the synaptosomes (Table 3 and Fig. 1). Methamphetamine markedly increased the release of dopamine, 5-HT, and norepinephrine with EC₅₀ values of 2.8×10^{-8} , 7.9×10^{-7} , and 1.1×10^{-8} M, respectively. In contrast, cocaine did not have monoamine-releasing activity.

Phenethylamine derivatives were also assayed for their ability to release dopamine, 5-HT, and norepinephrine (Table 3 and Fig. 1). Methylone was one of the strongest releasers of our tested

Table 3
The effects of drugs on monoamine release from rat brain synaptosome

Drugs	Release (EC_{50} , M) ^(a)		
	Dopamine	5-HT	Norepinephrine
Phenethylamines			
MDMA	$2.0 \pm 0.46 \times 10^{-7}$	$5.8 \pm 1.5 \times 10^{-8}$	$8.6 \pm 2.3 \times 10^{-8}$
Methylone	$3.8 \pm 0.88 \times 10^{-7}$	$2.2 \pm 0.64 \times 10^{-7}$	$9.3 \pm 2.6 \times 10^{-8}$
BDB	$2.3 \pm 0.56 \times 10^{-6}$	$1.8 \pm 0.51 \times 10^{-7}$	$5.4 \pm 1.5 \times 10^{-7}$
MBDB	n.e. ^(b)	$5.4 \pm 1.6 \times 10^{-7}$	$3.3 \pm 0.87 \times 10^{-6}$
4-fluoroamphetamine	$2.0 \pm 0.48 \times 10^{-7}$	$7.3 \pm 2.4 \times 10^{-7}$	$3.7 \pm 0.89 \times 10^{-8}$
2C-I	n.e.	n.e.	n.e.
2C-E	n.e.	n.e.	n.e.
2C-C	n.e.	n.e.	1.0 ± 10^{-4}
TMA	n.e.	$1.6 \pm 0.45 \times 10^{-5}$	n.e.
TMA-2	n.e.	n.e.	n.e.
TMA-6	n.e.	n.e.	n.e.
Tryptamines			
AMT	$1.8 \pm 0.42 \times 10^{-7}$	$6.8 \pm 2.1 \times 10^{-8}$	7.9 ± 10^{-8}
5-MeO-AMT	$1.5 \pm 0.38 \times 10^{-6}$	$4.6 \pm 1.4 \times 10^{-7}$	8.9 ± 10^{-6}
DPT	n.e.	n.e.	n.e.
5-MeO-DIPT	n.e.	n.e.	n.e.
5-MeO-MIPT	n.e.	n.e.	n.e.
5-MeO-DMT	n.e.	n.e.	n.e.
5-MeO-DALT	n.e.	n.e.	n.e.
Piperazines			
BZP	$6.0 \pm 1.8 \times 10^{-7}$	n.e.	$6.8 \pm 1.8 \times 10^{-8}$
3CPP	$6.3 \pm 1.1 \times 10^{-5}$	$2.8 \pm 0.92 \times 10^{-8}$	$1.4 \pm 0.41 \times 10^{-6}$
4MPP	$1.1 \pm 0.31 \times 10^{-5}$	$3.2 \pm 0.95 \times 10^{-6}$	$1.5 \pm 0.42 \times 10^{-6}$
Positive controls			
Cocaine	n.e.	n.e.	n.e.
Methamphetamine	$2.80 \pm 0.65 \times 10^{-8}$	$7.9 \pm 2.3 \times 10^{-7}$	$1.1 \pm 0.29 \times 10^{-8}$

Mean $\pm$ S.D. (three independent assays). Each assay was carried out by using different fresh rat brain synaptosome.

(a) Drug concentrations giving half-maximal acceleration.

(b) No effect at 10^{-4} M of each drug.

chemicals; the EC_{50} values were 3.8×10^{-7} for dopamine, 2.2×10^{-7} for 5-HT and 9.3×10^{-8} M for norepinephrine. BDB also had relatively high releasing activity with EC_{50} values of 2.3×10^{-6} for dopamine, 1.8×10^{-7} M for 5-HT and 5.4×10^{-7} M for norepinephrine. MBDB strongly stimulated 5-HT (EC_{50} , 5.4×10^{-7} M) and norepinephrine (EC_{50} , 3.3×10^{-6} M) release but had little dopamine-releasing activity. 4-Fluoroamphetamine strongly stimulated the release of dopamine, 5-HT, and norepinephrine from synaptosome, with EC_{50} values of 2.0×10^{-7} , 7.3×10^{-7} , and 3.7×10^{-8} M, respectively. MDMA, a well-known 5-HT releaser, strongly stimulated the release of 5-HT, norepinephrine and dopamine. 2C-I, 2C-E, 2C-C, TMA, TMA-2, and TMA-6 had comparatively low releasing activity.

Of the tryptamine derivatives (Table 3 and Fig. 1), AMT had the strongest monoamine-releasing activity, which was approximately the same as that of methamphetamine, a known monoamine releaser. 5-MeO-AMT was next in level of activity, while other derivatives, such as DPT, 5-MeO-DIPT, 5-MeO-MIPT, and 5-MeO-DALT, showed only slight monoamine-releasing activity.

The monoamine-releasing activity of the piperazine derivatives is shown in Table 3. BZP strongly stimulated the release of dopamine and norepinephrine, but hardly affected the release of 5-HT. 3CPP was one of the strongest 5-HT-releasers (EC_{50} of 2.8×10^{-8} M) and also strongly stimulated the release of

dopamine and norepinephrine. 4MPP had relatively high monoamine-releasing activity.

4. Discussion

Designer drugs are in frequent use to modify a person's mood and behavior, which they do by exerting various but as yet ill-defined effects on the central nervous system. In the present study, we sought to clarify the effects of a range of drugs on monoamine neurotransmission, and developed an assay to examine the re-uptake and release of the monoamines dopamine, 5-HT, and norepinephrine, using rat brain synaptosomes. The activity of many drugs was determined simply, reproducibly and rapidly because our methods allowed the simultaneous determination of the re-uptake or release of the three monoamines. In addition, we were able to screen the effects of five drug classes at the same time with synaptosomes prepared from the brain of only one rat. These methods therefore proved extremely effective for screening the effects of drugs on monoamine neurotransmission systems.

The activity of each drug was compared with that of cocaine, a monoamine re-uptake inhibitor, and methamphetamine, a strong releaser, as positive controls (Cunningham and Callahan, 1991; Fleckemstein et al., 2000; Rothman et al., 2001). In this study, methamphetamine had both re-uptake inhibitory activity as well as the expected monoamine-releasing activity, although the releasing activity was clearly stronger.

We classified the drugs into three groups: the phenethylamines, tryptamines, and piperazines. The phenethylamines have been sub-classified in more detail (Parrott et al., 2004). 3,4-Methylenedioxy phenethylamine derivatives were developed as designer drugs in the 1970s (Parrott et al., 2004). MDMA is a popular psychoactive drug but is prohibited in many countries because of its demonstrated adverse effects on rats, monkeys, and humans (Green et al., 2003; Parrott et al., 2004). The main adverse effect is destruction of serotonin axons in the cerebral cortex and other higher brain areas (Parrott et al., 2004). Other brain regions are also affected, including the hypothalamic areas that subserve temperature regulation, feeding behavior and biological rhythms (Parrott et al., 2004). Derivatives or modifications of MDMA, such as methylone, MBDB and BDB, are now available and are used to obtain the same effects as MDMA (Nichols, 1986), and MBDB has been reported to cause death (Carter et al., 2000). Of the tested chemicals, methylone had the most extensive effects on monoamine re-uptake and release of the MDMA analogues examined, and BDB and MBDB also strongly affected the monoaminergic neurotransmission systems. MDMA analogues had less effect on dopaminergic systems than methamphetamine; however, their action on serotonergic neurotransmission was stronger than that of the positive control, methamphetamine. Of other types of phenethylamine derivatives, 4-fluoroamphetamine strongly suppressed the re-uptake of monoamines and accelerated their release, in a similar way to methamphetamine. Methoxyphenethylamines such as 2C-I, 2C-E, 2C-C, TMA, TMA-2, and TMA-6 exerted the weakest effects on the re-uptake and release of monoamines in pre-synaptic transmission,

but may have more marked post-synaptic effects. Our attempts to investigate this proposal demonstrated differential sensitivity of the monoaminergic nervous systems to each phenethylamine.

The abuse of tryptamine analogues has recently been linked to human injuries (Drug Enforcement Administration, 2003, 2004; Fantegrossi et al., 2006). In this study of these derivatives, AMT had the strongest monoamine re-uptake inhibitory activity and release stimulating activity, although monoamine release was more dominant than re-uptake inhibition. Release of monoamine might be a main cause of the *in vivo* effect of AMT on the central nervous system. Furthermore, the influence of AMT on the serotonergic system was stronger than that of methamphetamine, while the effects on both dopaminergic and adrenergic systems were similar to those of methamphetamine. 5-MeO-AMT, which has a methoxy group added to the 5 position in the AMT structure, inhibited monoamine (dopamine, 5-HT and norepinephrine) re-uptake and stimulated monoamine release; its potency was second to that of AMT. AMT and 5MeO-AMT both have a primary amine group, which in tryptamine might be indispensable for the stimulation of monoamine release. 5-MeO-DIPT, 5-MeO-MIPT, and 5-MeO-DMT suppressed the re-uptake of 5-HT relatively robustly and also suppressed the re-uptake of dopamine and norepinephrine. They showed little dopamine, 5-HT or norepinephrine releasing activity. The tryptamines have a tertiary amine, unlike AMT and 5-MeO-AMT. However, it has been reported that 5-MeO-DIPT has affinity for receptors relevant to hallucinogenic effects observed *in vivo* (Fantegrossi et al., 2006). The psychotropic activity of 5MeO-DIPT might therefore be caused by its association with post-synaptic 5-HT receptors.

Psychoactive effects of BZP and 3CPP have also been reported (Samanin et al., 1980; Bauman et al., 2004, 2005). Here, we studied the effects of piperazine derivatives such as BZP, 3-CPP, and 4MPP. 3CPP had a marked effect on the pre-synaptic serotonergic nervous system, whereas BZP had no effect on 5-HT release rate. 4MPP inhibited monoamine re-uptake and stimulated monoamine release, but the effects were not strong. We attributed the variance in activity of the piperazine derivatives to the range of possible side chains.

Many psychoactive chemicals are available for non-medical use. In this study, we found some non-medically used drugs to have a variety of psychoactivities. Further studies are necessary to elucidate the actions of these drugs. We propose that these drugs be strongly restricted to keep society and its people healthy.

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