



Inhibition of plasma membrane monoamine transporters by β-ketoamphetamines

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Abstract

Methcathinone and methylone, the β -ketone analogues of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA), respectively, were tested for neurotransmitter uptake inhibition in vitro. The β -ketones were threefold less potent than the nonketo drugs at inhibiting platelet serotonin accumulation, with IC $_{50}$'s of 34.6 \pm 4.8 μ M and 5.8 \pm 0.7 μ M, respectively. Methcathinone and methylone were similar in potency to methamphetamine and MDMA at catecholamine transporters individually expressed in transfected glial cells. For dopamine uptake, IC $_{50}$'s were 0.36 \pm 0.06 μ M and 0.82 \pm 0.17 μ M, respectively; for noradrenaline uptake, IC $_{50}$ values were 0.51 \pm 0.10 μ M and 1.2 \pm 0.1 μ M, respectively. In chromaffin granules, IC $_{50}$'s for serotonin accumulation were 112 \pm 8.0 μ M for methcathinone and 166 \pm 12 μ M for methylone, 10-fold higher than the respective values for methamphetamine and MDMA. Our results indicate that methcathinone and methylone potently inhibit plasma membrane catecholamine transporters but only weakly inhibit the vesicle transporter. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Amphetamine and other related phenylalkylamines such as methamphetamine (Fig. 1), 3,4-methylenedioxymethamphetamine (MDMA; Fig. 1), and metaraminol increase monoamine neurotransmitter concentrations in the synaptic cleft. This action is the result of at least two distinct mechanisms. One mechanism is through drug inhibition of plasma membrane transporter-mediated uptake of released neurotransmitters (Steele et al., 1987; Johnson et al., 1991). The inhibition is believed to arise from competition by drugs for substrate binding sites in the monoamine uptake transporters, thereby reducing the effectiveness with which serotonin, dopamine, and noradrenaline are cleared

from the synapse following release. A second mechanism is through the drug-evoked release of the monoamine neurotransmitters, apparently by transporter-mediated exchange (Paton, 1973; Johnson et al., 1986; Nichols et al., 1993; Cinquanta et al., 1997).

The drug-evoked neurotransmitter release arises from two intracellular compartments. Methamphetamine and MDMA induce the release of newly synthesized, cytosolic pools of monoamines and they also release monoamines from synaptic vesicle stores (Johnson et al., 1991; Rudnick and Wall, 1992a). Monoamine neurotransmitters are packaged into synaptic vesicles via the proton-driven vesicular monoamine transporter, VMAT2. It has been proposed that drugs such as MDMA compete for substrate binding sites in VMAT2 and may also dissipate the vesicular pH gradient required for VMAT2-mediated monoamine accumulation (Rudnick and Wall, 1992a; Schuldiner et al., 1993). Because VMAT2 functions as a proton-monoamine antiporter, this dissipation of intravesicular pH would impair

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Fig. 1. Chemical structures of test compounds.

Methylone

the ability of VMAT2 to transport the endogenous monoamines (Sulzer and Rayport, 1990; Rudnick and Wall, 1993).

Methcathinone

Methcathinone (2-methylamino-1-phenylpropan-1-one, Fig. 1) and methylone (2-methylamino-1-[3,4-methylenedioxyphenyl]propan-1-one, Fig. 1) are two drugs which resemble methamphetamine and MDMA, respectively, in their behavioral pharmacology. These drugs differ structurally from methamphetamine and MDMA by the presence of a ketone oxygen at the benzylic position of the molecule. To further our understanding of the action of amphetamine-like drugs at biogenic amine transporters and to refine structure—activity relationships of transporter ligands, we investigated the effects of methcathinone and methylone on monoamine uptake in vitro.

Methcathinone is the *N*-methyl derivative of cathinone, a naturally occurring psychostimulant found in the leaves of Catha edulis, the khat bush. Methcathinone was first synthesized in 1928 (Hyde et al., 1928) but its psychopharmacology remained unexplored until Parke, Davis developed methcathinone as an analeptic in the late 1950's (L'Italien et al., 1957). More recently, methcathinone has been used outside of medical settings for its euphoric properties and is now designated a Schedule I Controlled Substance in the United States (Anonymous, 1993; Rosen, 1993). Behavioral studies have shown that methcathinone will substitute for cocaine or amphetamine in animals trained to discriminate either cocaine or amphetamine from saline in stimulus generalization tests (Glennon et al., 1987; Young and Glennon, 1993). Baboons will self-administer methcathinone, confirming that the drug has reinforcing properties (Kaminski and Griffiths, 1994). A study by Gygi et al. (1996) found that methcathinone significantly decreases several important neurochemical markers associated with monoaminergic function in the rat. In particular, methcathinone reduced tyrosine hydroxylase and tryptophan hydroxylase activity, with corresponding decreases in the concentrations of dopamine, serotonin, and their respective metabolites in frontal cortex, hippocampus, and neostriatum. The mechanism for these effects has not been fully elucidated, but in the report by Glennon et al. (1987), methcathinone stimulated the release of $[^3H]$ -dopamine from preloaded striatal tissue. A more recent study by Gygi et al. (1997) also demonstrated that methcathinone could stimulate dopamine release and that dopaminergic deficits could be prevented by pretreatment with dopamine D_1 or D_2 receptor antagonists.

There are few reports in the literature regarding methylone, the benzylic ketone analog of MDMA. Dal Cason (1997) presented chemical analytical data on methylone and several other *N*-substituted aminopropiophenones. A drug discrimination study by Dal Cason et al. (1997) reported behavioral data for methylone. In this account, methylone substituted for MDMA in rats trained to discriminate MDMA from saline, with an ED₅₀ of 1.6 mg/kg. At similar doses, methylone did not substitute for (+)-amphetamine in amphetamine-trained animals, nor did it substitute for the hallucinogen 2-amino-1-(2,5-dimethoxy-4-methylphenyl)propane (DOM) in DOM-trained rats.

From what is known about the neuropharmacology of methamphetamine and MDMA and because of the chemical structural similarity to methamphetamine and MDMA, we hypothesized that methcathinone and methylone would also act on monoaminergic systems, specifically through the monoamine uptake transporters. To test this hypothesis, we compared methcathinone and methylone to methamphetamine and MDMA for their abilities to inhibit [³H]serotonin, [³H]dopamine, and [³H]noradrenaline uptake via the plasma membrane uptake transporters and to inhibit [³H]serotonin uptake by the vesicular monoamine transporter, VMAT2.

2. Materials and methods

2.1. Drugs and reagents

Methcathinone, methamphetamine, methylone, and MDMA were synthesized in racemic form in our laborato-

ries. All analytical data were consistent with the assigned structures. [³H]Serotonin (specific activity = 27.8 Ci/mmol), [³H]dopamine (specific activity = 24 Ci/mmol), and [³H]noradrenaline (specific activity = 71.7 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Cell culture medium and antibiotics were obtained from Life Technologies, Gaithersburg, MD. Fetal bovine serum was purchased from Hyclone, Logan, UT. Pargyline, buffer salts, and miscellaneous chemicals were acquired from Aldrich Chemical, Milwaukee, WI.

2.2. Serotonin plasma membrane uptake transporter: [³H]serotonin uptake into human platelets

Outdated human platelets were obtained from the blood bank at the University of Wisconsin Clinical Sciences Center or from the Veteran's Administration Hospital, Madison, WI. Platelets from 5-10 donors were pooled, 10% dimethylsulfoxide was added, and aliquots were stored frozen at -80° C until use. For assays, 5 ml of platelets were thawed and added to 20 ml ice-cold Krebs-Ringer-HEPES (KRH) buffer containing (mM): NaCl (124.0), KCl (2.9), MgSO₄ (1.3), KH₂PO₄ (1.2), CaCl₂ (2.4), d-glucose (5.2), HEPES (25.0), sodium ascorbate (0.1), pargyline (0.1), pH = 7.4. The platelet suspension was subjected to centrifugation (4340 \times g, 4°C, 10 min) and the supernatant was discarded. The pellet was washed twice by resuspension in KRH and centrifugation. The final pellet was suspended in 70 ml ice-cold KRH using a polytron (setting 7, 10 s) and stored on ice until use. The ability of platelets to accumulate [3H]serotonin was measured in the absence and presence of various concentrations of test drugs as follows: a 400 µl aliquot of the platelet suspension was added to glass tubes containing 50 μl test drugs (dissolved in KRH) or 50 μl KRH (for total and nonspecific determinations). The assay tubes were preincubated in a 37°C shaking water bath for 5 min. The tubes were then returned to the ice bath and chilled for 15 min. [³H]Serotonin was added (50 μl of stock solution; final concentration, 10 nM), giving a total incubation volume of 500 µl. All tubes except nonspecific tubes were returned to the 37°C shaking water bath for 5 min to initiate neurotransmitter uptake. Uptake was terminated by chilling the test tubes in the ice bath. After adding 3 ml ice-cold KRH, each assay tube was immediately vacuum filtered through glass fiber filters (Whatman GF/B) pretreated with 0.1% polyethyleneimine. Filters were washed with 2×3 ml ice-cold KRH, allowed to dry briefly under vacuum, then placed in liquid scintillation vials. Scintillation cocktail (8 ml) was added and the vials were sealed, vortexed, and allowed to stand overnight. Radioactivity was measured using liquid scintillation spectroscopy (Packard Tri-Carb 1600 CA). Specific uptake was defined as uptake at 37°C minus uptake at 0°C in the absence of drugs. Under these conditions, specific [3H]serotonin uptake was typically greater than 90%. The IC₅₀ \pm S.E.M. for each test drug was determined from displacement curves from 3–8 experiments using 6–11 drug concentrations, each run in triplicate. Data were transformed from dpm to % specific uptake and fitted to a four-parameter logistic curve using commercial computer software, from which IC $_{50}$ values were calculated.

2.3. Dopamine plasma membrane uptake transporter: $[^3H]$ dopamine uptake into transfected cells

C6 glial cells stably expressing the rat dopamine transporter (C6-DAT) were obtained from Dr. Susan Amara (Oregon Health Sciences University, Portland, OR). Cells were maintained in a humidified atmosphere (5% CO₂ in air) in selective culture medium: Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 100 μ g/ml geneticin). For assays, 3×100 mm dishes of confluent cells were washed with phosphate buffered saline, pH = 7.1. The cells were then treated with trypsin/EDTA and split into 4×6 -well (35 mm) plates in 2 ml/well culture medium minus geneticin. After passage, the cells were allowed to grow overnight and were used for uptake assays the following day. The ability of the test drugs to inhibit [3H]dopamine uptake was measured as follows: the DMEM was aspirated from the 6-well plates containing confluent C6-DAT cells. The cells were washed with 2×2 ml 37°C KRH, then 490 μ l 37°C KRH was added to each well. This was followed by the addition of 5 μl KRH (for total determinations), 5 μl of 10 mM cocaine hydrochloride stock solution (for nonspecific determinations; final concentration, 100 µM), or 5 µl of test drug solution. The plates were preincubated at 37°C for 15 min, then 5 µl of [³H]dopamine (final concentration, 20 nM) was added to each well to initiate uptake. Uptake was allowed to proceed for 20 min, then the incubation buffer was discarded and the cells were washed with 3×2 ml ice-cold KRH. The cells were solubilized in 500 µl 37°C 1% sodium dodecyl sulfate, then the solubilized well contents were transferred to liquid scintillation vials containing 3 ml scintillation cocktail. Radioactivity was measured and data were analyzed as described for platelets.

2.4. Noradrenaline plasma membrane uptake transporter: [³H]noradrenaline uptake into transfected cells

C6 glial cells stably expressing the human noradrenaline transporter (C6-NET) were also a gift from Dr. Susan Amara. Assays and work-up were performed exactly as described for dopamine uptake, except [³H]noradrenaline was used as the radiolabeled substrate.

2.5. Vesicular monoamine transporter (VMAT2): [³H]serotonin uptake into bovine chromaffin granules

Chromaffin vesicles were prepared from bovine adrenal medullae as follows: 150 bovine adrenals from freshly

slaughtered animals were collected at Peck's slaughterhouse in Milwaukee, WI. The adrenal glands were bisected and the medullae were scraped out and put into ice-cold 0.3 M sucrose, 10 mM HEPES, pH = 7.0. The medullae were minced with a polytron (setting 10, 3 s) then homogenized with a motor-driven Potter-Elvehjem tissue grinder (setting 10, 10 passes). After removal of the unbroken cells and nuclei by centrifugation, the chromaffin granule membranes were pelleted through 1.6 M sucrose at $100,000 \times g$ for 60 min. Chromaffin vesicle ghosts were prepared by osmotically lysing these crude granules in hypotonic buffer containing (mM): HEPES (5), MgSO₄ (2), CaCl₂ (10), dithiothreitol (0.1), pH = 7.5. The membranes were recovered by centrifugation at $100,000 \times g$ for 60 min. The vesicles were resealed by suspending the pellets in storage buffer containing (mM): sucrose (300), HEPES (10), $MgSO_4$ (2), dithiothreitol (0.1), pH = 7.0 at a concentration of 5–10 mg/ml. Aliquots were snap-frozen in liquid N_2 and stored at -80° C. For uptake assays, chromaffin vesicle ghosts were diluted in assay buffer containing (mM): sucrose (300), HEPES (10), ATP (5), MgSO₄ (5), pH = 7.8 to a final volume of 0.5 ml. The samples were incubated at 37°C for 10 min and then cooled on ice for 10 min. Uptake was tested in the absence or presence of various concentrations of the test drugs added to the assay buffer. Nonspecific uptake was defined by 10 µM reserpine. [3H]Serotonin was added to a final concentration of 10 nM and the samples were incubated for 5 min at 37°C to initiate uptake. The samples were then transferred to an ice bath and individual samples were removed, diluted with 4 ml of ice-cold assay buffer minus ATP and MgSO₄, then collected on cellulose acetate filters (Millipore type HAWP, 0.45 mm pore size) by vacuum filtration. The filters were washed with 2×4 ml of ice-cold assay buffer. Filters were transferred to scintillation vials and 4 ml scintillation cocktail was added. Radioactivity was measured and data were analyzed as described for platelets.

2.6. Statistics

Pairwise comparisons of the IC₅₀ values for different drugs, derived from nonlinear regression analysis of the inhibition data, were made using Student's *t*-test with P < 0.05 considered significant. All values are expressed as the mean \pm standard error of the mean (S.E.M.).

3. Results

Specific radiolabeled neurotransmitter uptake was robust and typically greater than 90%, except for [³H]dopamine uptake into C6-DAT cells. In this system, specific [³H]dopamine uptake was initially just over 50%, but decreased after repeated cell passages. Therefore, we used

only early passage cells for our experiments involving [³H]dopamine.

Representative drug uptake inhibition curves, depicted for VMAT2-mediated [3 H]serotonin uptake into bovine chromaffin granules, are shown in Fig. 2. IC $_{50}$ values \pm S.E.M. for all drugs and transporter systems are summarized in Table 1. All inhibition curves had slope coefficients of unity, indicating that the test drugs interacted with a single site on the transporter proteins.

In human platelets, which contain the plasma membrane serotonin uptake transporter, methcathinone had an IC₅₀ of $34.6 \pm 4.8 \mu M$; this was threefold less potent (P < 0.05) than methamphetamine. Likewise, methylone was threefold less potent (P < 0.01) than MDMA at inhibiting [3 H]serotonin uptake into platelets, with an IC₅₀ of 5.75 \pm 0.68 µM. In C6 glial cells stably expressing the rat dopamine transporter, methcathinone and methylone were similar in potency to methamphetamine and MDMA, with IC_{50} 's for [${}^{3}H$]dopamine uptake of 0.356 \pm 0.059 μ M and $0.819 \pm 0.168 \,\mu\text{M}$, respectively. Methcathinone and methylone were also similar in potency to methamphetamine and MDMA in C6 cells expressing the human noradrenaline transporter, with IC₅₀'s for [³H]noradrenaline accumulation of 0.511 \pm 0.096 μM and 1.22 \pm 0.13 μM , respectively. The benzylic ketone moiety of methcathinone and methylone had a large effect on the ability of these drugs to inhibit the vesicular monoamine transporter, compared to methamphetamine and MDMA. In VMAT2-containing bovine chromaffin granules, the IC₅₀ values for [³H]serotonin uptake were $112.1 \pm 7.98 \mu M$ for methcathinone and $165.6 \pm 11.7 \mu M$ for methylone. These values are greater

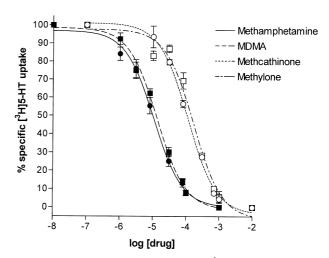


Fig. 2. Drug inhibition of VMAT2-mediated [3 H]serotonin uptake. The ability of test drugs to inhibit accumulation of 10 nM [3 H]serotonin was examined in bovine chromaffin granules. Specific uptake was defined by 10 μ M reserpine. Data are the mean \pm S.E.M. of three determinations, each run in triplicate. Data were fitted to a four-parameter logistics curve for IC $_{50}$ determination and plotting. Symbols: methamphetamine, solid circles; MDMA, solid squares; methcathione, open circles; methylone, open squares.

Table 1 IC₅₀ values (μ M) for drug inhibition of monoamine uptake^a

Drug	[³ H]serotonin	[³ H]dopamine	[³ H]noradrenaline	VMAT2 ([³ H]serotonin)
Methcathinone	34.6 ± 4.8^{bc}	0.356 ± 0.059	0.511 ± 0.096	112.1 ± 7.98 ^d
Methamphetamine	11.6 ± 1.0^{e}	0.467 ± 0.051	0.647 ± 0.032	10.9 ± 2.0
Methylone	5.75 ± 0.68^{e}	0.819 ± 0.168	1.22 ± 0.13	165.6 ± 11.7^{e}
MDMA	2.14 ± 0.34	0.478 ± 0.107	1.38 ± 0.10	12.7 ± 1.6

^aThe ability of test drugs to inhibit monoamine accumulation was examined as described under Section 2. Test compounds were examined with 6–11 concentrations, each run in triplicate. The data from 3–8 experiments were combined and the IC₅₀ values \pm S.E.M. were calculated by curve fitting. Pairwise comparisons were made using Student's *t*-test.

than 10-fold higher (P < 0.01) than the values for methamphetamine and MDMA, respectively.

4. Discussion

Methcathinone and methylone are two β-ketoamphetamines that resemble methamphetamine and MDMA, respectively, in self-administration and drug-discrimination paradigms. However, scant information on the molecular pharmacology of the β-ketoamphetamines is available in the literature. We hypothesized that these drugs would inhibit biogenic amine uptake mediated through the plasma membrane and vesicular monoamine uptake carriers. To test this hypothesis, we employed human platelets as a source of serotonin uptake transporters and we used two stable transfected cell lines, C6-DAT and C6-NET, as sources for the dopamine and noradrenaline uptake transporters, respectively. To evaluate drug effects at the synaptic vesicle, we used bovine chromaffin granules. The chromaffin granule contains the proton-driven vesicle monoamine transporter VMAT2, and is a widely accepted model for biogenic amine uptake and storage in synaptic vesicles.

Our data for methamphetamine and MDMA (Table 1) are in good agreement with earlier studies (Rudnick and Wall, 1992a,b, 1993). After reviewing reports on the behavioral effects of methcathinone and methylone, we anticipated that these drugs would resemble methamphetamine and MDMA in their respective pharmacological profiles at the monoamine uptake transporters. Our results indicate that this is true only for drug effects at the catecholamine transporters. At the dopamine and noradrenaline carriers, the β-ketoamphetamine compounds were as potent as the respective nonketo drugs at inhibiting monoamine accumulation, and all of the test drugs were more potent at the dopamine transporter than at the noradrenaline transporter (Table 1). At the serotonin uptake carrier, the aryl ketone group of methcathinone and methylone had a small but significant effect on the drugs' ability to inhibit serotonin accumulation. In this system, the β-ketoamphetamines were only one-third as potent as their respective nonketo analogues. We also observed that the ring 3,4-methylenedioxy

group of methylone and MDMA led to increased potency at this transporter compared to methcathinone and methamphetamine (Table 1). This outcome was not surprising in light of the consistently higher potency of serotonin transporter inhibition exhibited by a variety of phenylalkylamines substituted at the ring 3- and/or 4-positions (McKenna et al., 1991; Huang et al., 1992; Nichols et al., 1993). We propose that there is a pocket within the substrate binding domain of the serotonin transporter that can accommodate substituents at the *meta*- or *para*-positions of arylalkylamine ligands. According to this model, occupation of the pocket by such substituents imparts increased stability to the ligand-protein complex, leading to higher potency.

The biggest differences among these compounds were observed at VMAT2. The 10-fold reduction in potency of methcathinone and methylone at VMAT2, compared to methamphetamine and MDMA, is surprising: several other compounds structurally related to amphetamine, such as p-chloroamphetamine, 5-methoxy-6-methyl-2-aminoindan, 3-methoxy-4-methylamphetamine, and MDMA itself have been reported to inhibit VMAT2-mediated neurotransmitter uptake with IC₅₀'s in the nanomolar to low micromolar range (Rudnick and Wall, 1992a,b, 1993). Our own data for methamphetamine and MDMA at VMAT2 are in accord with these values, but the \u03b3-keto compounds required concentrations in excess of 100 µM to achieve half-maximal inhibition. Because VMAT2 is relatively indiscriminate in its substrate specificity, and because methcathinone and methylone differ from methamphetamine and MDMA only by the presence of the benzylic oxygen atom, we did not expect such a large difference in potency.

The inhibition of VMAT2-mediated uptake by pheny-lalkylamines has been attributed to the ability of these compounds to act as substrates for VMAT2, thereby competing for transporter binding sites and also stimulating transporter-mediated exchange. Another potential mechanism for amphetamine inhibition of vesicular transport is through dissipation of the pH gradient (inside acidic) that drives VMAT2-mediated uptake (Rudnick and Wall, 1992a; Schuldiner et al., 1993). This mechanism is proba-

 $^{^{\}rm b}P < 0.05$ vs. methamphetamine.

 $^{^{}c}P < 0.01$ vs. methylone.

 $^{^{\}rm d}P < 0.01$ vs. methamphetamine.

 $^{^{}e}P < 0.01$ vs. MDMA.

bly not of practical importance in the psychopharmacology of methamphetamine or MDMA because the EC₅₀ required for dissipation of the pH gradient by amphetamine derivatives ranges from 160 µM to 1 mM (Rudnick and Wall, 1992a,b, 1993); these concentrations are at least 10-fold higher than the IC₅₀'s for VMAT2 uptake inhibition by methamphetamine and MDMA (Table 1). Thus, methamphetamine and MDMA have a major impact on vesicular monoamine uptake at concentrations well below those needed to affect the pH gradient. While we did not test whether methcathinone or methylone neutralize the vesicular pH gradient, the IC₅₀'s for uptake inhibition for these drugs at VMAT2 are similar to the EC₅₀'s for pH dissipation by other amphetamine derivatives and in any case are two orders of magnitude higher than the IC₅₀'s at the plasma membrane catecholamine transporters. Therefore, we propose that the behavioral effects of methcathinone and methylone arise largely from the drugs' effects at the plasma membrane transporters, not VMAT2. We conclude that while methamphetamine and MDMA are likely to be substrates for VMAT2, methcathinone and methylone are not. Further, because it has already been shown that methcathinone and methylone resemble methamphetamine and MDMA in their respective behavioral profiles (Glennon et al., 1987; Kaminski and Griffiths, 1994; Dal Cason et al., 1997), our results imply that the psychopharmacological effects of methamphetamine and MDMA are independent of their inhibition of VMAT2. Apparently, activity at the plasma membrane transporters is sufficient to account for the behavioral effects of these drugs.

In summary, due to the large decrease in potency at VMAT2, methcathinone and methylone are highly selective for the plasma membrane catecholamine transporters and moderately selective for the serotonin carrier. As a result of its greater potency at the serotonin carrier, methylone is somewhat less discriminating than methcathinone at the plasma membrane. Indeed, both 3,4-methylenedioxy-substituted compounds, methylone and MDMA, are more potent at the serotonin transporter than the unsubstituted drugs. The psychopharmacology of these drugs is most likely governed by their effects at the plasma membrane monoamine transporters, but not VMAT2. Because methcathinone and methylone differ from methamphetamine and MDMA only by the presence of an oxygen atom at the benzylic position, the large decrease in potency at VMAT2 must reflect the biochemistry or topology of the substrate binding site in VMAT2. Thus, both the benzylic position and the aromatic ring of these molecules carry important structural information for recognition by the neurotransmitter transporter proteins.

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References

- Anonymous, 1993. Federal Register 58, 53404-53406.
- Cinquanta, M., Ratovitski, T., Crespi, D., Gobbi, M., Mennini, T., Simantov, R., 1997. Carrier-mediated serotonin release induced by d-fenfluramine: studies with human neuroblastoma cells transfected with a rat serotonin transporter. Neuropharmacology 36, 803–809.
- Dal Cason, T.A., 1997. The characterization of some 3,4-methylenedioxycathinone (MDCATH) homologs. Forensic Sci. Int. 87, 9–53.
- Dal Cason, T.A., Young, R., Glennon, R.A., 1997. Cathinone: an investigation of several N-alkyl and methylenedioxy-substituted analogs. Pharmacol. Biochem. Behav. 58, 1109–1116.
- Glennon, R.A., Yousif, M., Naiman, N., Kalix, P., 1987. Methcathinone: a new and potent amphetamine-like agent. Pharmacol. Biochem. Behav. 26, 547–551.
- Gygi, M.P., Gibb, J.W., Hanson, G.R., 1996. Methcathinone: an initial study of its effects on monoaminergic systems. J. Pharmacol. Exp. Ther. 276, 1066–1072.
- Gygi, M.P., Fleckenstein, A.E., Gibb, J.W., Hanson, G.R., 1997. Role of endogenous dopamine in the neurochemical deficits induced by methcathinone. J. Pharmacol. Exp. Ther. 283, 1350–1355.
- Huang, X., Marona-Lewicka, D., Nichols, D.E., 1992. p-Methylthioamphetamine is a potent new non-neurotoxic serotonin-releasing agent. Eur. J. Pharmacol. 229, 31–38.
- Hyde, J.F., Browning, E., Adams, R., 1928. Synthetic homologs of DL-ephedrine. J. Am. Chem. Soc. 50, 2287–2292.
- Johnson, M.P., Hoffman, A.J., Nichols, D.E., 1986. Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132, 269–276.
- Johnson, M.P., Conarty, P.F., Nichols, D.E., 1991. [³H]Monoamine releasing and uptake inhibition properties of 3,4-methylenedio-xymethamphetamine and *p*-chloroamphetamine analogues. Eur. J. Pharmacol. 200, 9–16.
- Kaminski, B.J., Griffiths, R.R., 1994. Intravenous self-injection of methcathinone in the baboon. Pharmacol. Biochem. Behav. 47, 981–983.
- L'Italien, Y.J., Park, H., Rebstock, M.C., 1957. Methylaminopropiophenone compounds and methods for producing the same. US Patent # 2802865.
- McKenna, D.J., Guan, X.M., Shulgin, A.T., 1991. 3,4-Methylen-edioxyamphetamine (MDA) analogues exhibit differential effects on synaptosomal release of ³H-dopamine and ³H-5-hydroxytryptamine. Pharmacol. Biochem. Behav. 38, 505–512.
- Nichols, D.E., Marona-Lewicka, D., Huang, X., Johnson, M.P., 1993. Novel serotonergic agents. Drug Des. Discov. 9, 299–312.
- Paton, D.M., 1973. Mechanism of efflux of noradrenaline from adrenergic nerves in rabbit atria. Br. J. Pharmacol. 49, 614–627.
- Rosen, D.S., 1993. Methcathinone. J. Adoles. Health 14, 426.
- Rudnick, G., Wall, S.C., 1992a. The molecular mechanism of ecstasy [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. Proc. Natl. Acad. Sci. U.S.A. 89, 1817–1821.
- Rudnick, G., Wall, S.C., 1992b. p-Chloroamphetamine induces serotonin release through serotonin transporters. Biochemistry 31, 6710–6718.
- Rudnick, G., Wall, S.C., 1993. Non-neurotoxic amphetamine derivatives release serotonin through serotonin transporters. Mol. Pharmacol. 43, 271–276.
- Schuldiner, S., Steiner-Mordoch, S., Yelin, R., Wall, S.C., Rudnick, G., 1993. Amphetamine derivatives interact with both plasma membrane

- and secretory vesicle biogenic amine transporters. Mol. Pharmacol. 44, 1227–1231.
- Steele, T.D., Nichols, D.E., Yim, G.K., 1987. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [³H]monoamines into synaptosomes from different regions of rat brain. Biochem. Pharmacol. 36, 2297–2303.
- Sulzer, D., Rayport, S., 1990. Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. Neuron 5, 797–808.
- Young, R., Glennon, R.A., 1993. Cocaine-stimulus generalization to two new designer drugs: methcathinone and 4-methylaminorex. Pharmacol. Biochem. Behav. 45, 229–231.