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The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily by lower potency at the vesicular monoamine transporter

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ABSTRACT

Mephedrone (4-methylmethcathinone, MMC) and 3,4-methylenedioxymethamphetamine (MDMA) are constituents of popular party drugs with psychoactive effects. Structurally they are amphetamine-like substances with monoamine neurotransmitter enhancing actions. We therefore compared their effects on the human monoamine transporters using human cell lines stably expressing the human noradrenaline, dopamine and serotonin transporter (NET, DAT and SERT); preparations of synaptic vesicles from human striatum in uptake experiments; and a superfusion system where releasing effects can be reliably measured. MMC and MDMA were equally potent in inhibiting noradrenaline uptake at NET, with IC_{50} values of 1.9 and 2.1 μM, respectively. Compared to their NET inhibition potency, both drugs were weaker uptake inhibitors at DAT and SERT, with MMC being more potent than MDMA at DAT (IC_{50}: 5.9 vs 12.6 μM) and less potent than MDMA at SERT (IC_{50}: 19.3 vs 7.6 μM). MMC and MDMA both induced concentration-dependently [^{3}H]1-methyl-4-phenylpyridinium-release from NET-, DAT or SERT-expressing cells which was clearly transporter-mediated release as demonstrated by the selective inhibitory effects of nmolar to low μmolar concentrations of desipramine, GBR 12909 and fluoxetine, respectively. MMC and MDMA differed most in their inhibition of [^{3}H]dopamine uptake by synaptic vesicles from human striatum with MDMA being 10-fold more potent than MMC (IC_{50}: 20 vs 223 μM) and their ability to release [^{3}H]dopamine from human vesicular monoamine transporter expressing SH-SY5Y neuroblastoma cells in which MDMA seems to have a stronger effect. Our findings give a molecular explanation to the lower long-term neurotoxicity of MMC compared to MDMA.

1. Introduction

Mephedrone (4-Methylmethcathinone, MMC) and ecstasy (3,4-methylenedioxymethamphetamine, MDMA) are both designer drugs used for illicit recreational consumption due to their psychoactive effects. They are structurally related to amphetamine and thus act as psychostimulants with a risk of addiction. In fact, MMC and MDMA inhibited the uptake of tritiated dopamine, noradrenaline and serotonin into rat brain synaptosomes (Baumann et al., 2013) and induced release from rat brain synaptosomes preloaded with tritiated substrates indicating their status as substrates of the plasmalemmal monoamine transporters (Baumann et al., 2012). These findings suggest a strong monoamine releasing effect of MMC and MDMA on monoaminergic nerve endings which is well supported by studies using in vivo microdialysis (Baumann et al., 2012; Kehr et al., 2011).

Similar to high doses of other releasing drugs repeated MDMA administration induced a selective neurotoxic loss of 5-HT in forebrain regions of the rat and damage to dopamine nerve terminals of the mouse (for review, see Green et al., 2003). By contrast, the majority of studies did not find neurotoxic loss of parameters of serotoninergic or dopaminergic nerve terminals after binge-type dosing schedule of MMC in rats (Baumann et al., 2012; den Hollander et al., 2013; Motbey et al., 2012; Shortall et al., 2012) or mice (Angoa-Perez et al., 2011; den Hollander et al., 2013). Finally, MMC did not activate glia or increase glial fibrillary acidic protein (Angoa-Perez et al., 2011; den Hollander et al., 2013), whereas the latter marker of neurodegeneration was increased by MDMA (Johnson et al., 2002; Miller and O'Callaghan, 1995).

A connection between longterm neurotoxicity of amphetamine-related drugs and interaction with mechanisms regulating the intraneuronal neurotransmitter concentrations has been established. Amphetamine-induced redistribution of dopamine from synaptic vesicles to the cytosol followed by metabolism accompanied by the

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production of radical oxygen species has been hypothesized to trigger nerve terminal loss (Bogen et al., 2003; Cubells et al., 1994; Hansen et al., 2002). The vesicular monoamine transporter 2/SLC18A2 (VMAT2) is a significant regulator of intraneuronal monoamine concentrations and its impairment has recently been implicated in the dopaminergic degeneration in idiopathic Parkinson's disease (Pifl et al., 2014). Since meth/amphetamine users have been shown to have an above-normal risk of developing Parkinson's disease (Callaghan et al., 2012) and even for human MDMA users there are hints for a neurotoxic potential (for review, see Steinkellner et al., 2011), we reasoned that it might be interesting to compare the pharmacology of MMC and MDMA at the human monoamine transporters in uptake and superfusion experiments using transfected cells loaded with the metabolically inert transporter substrate [^{3}H]1-methyl-4-phenylpyridinium (MPP^+) which allows a clear distinction between transport-inhibiting and carrier-mediated outward transport activity of drugs (Scholze et al., 2000) and include experiments on the human vesicular monoamine transporter by taking advantage of our recently reported preparation of functionally active synaptic vesicles from autopsied human striatum (Pifl et al., 2014).

2. Materials and methods

2.1. Materials

Media, sera and other tissue culture reagents were obtained from Life Technologies (Vienna, Austria). [7-^{3}H]dopamine (22 Ci/mmol), levo-[7-^{3}H]noradrenaline (15 Ci/mmol), 5-[1,2-^{3}H[N])-hydroxytryptamine (21 Ci/mmol) were obtained from New England Nuclear GmbH (Vienna, Austria). [^{3}H]1-Methyl-4-phenylpyridinium (MPP^+; 85 Ci/mmol) was supplied by American Radiolabeled Chemicals (St. Louis, MO), desipramine from Ciba-Geigy Limited (Stein, Switzerland), fluoxetine from Eli Lilly and Company Limited (Dublin, Ireland) and mazindol from Sandoz GmbH (Vienna, Austria). The other chemicals were purchased from Sigma-Aldrich or Merck.

2.2. Cell culture

SK-N-MC, SH-SY5Y (human neuroblastoma) and human embryonic kidney (HEK) 293 cells were grown in minimum essential medium with Earle's salts and L-glutamine, 10% heat inactivated fetal bovine serum and 50 mg/l gentamicin. Cells were grown in 100 or 60 mm diameter tissue culture dishes (polystyrene, Falcon) at 37 °C under an atmosphere of 5% CO_2/95% air. The human dopamine transporter/SLC6A3 (DAT) or noradrenaline transporter/SLC6A2 (NET) cDNA was stably expressed in SK-N-MC cells using methods as described previously (Pifl et al., 1996). The human serotonin transporter/SLC6A4 (SERT) was similarly expressed in HEK 293 cells using the vector pRc/CMV and selection by 1 g/l G418 in the medium and the human DAT in SH-SY5Y cells also using the vector pRc/CMV and selection by 0.6 g/l G418.

2.3. Cellular uptake experiments

The cells were seeded in poly-D-lysine-coated 24-well plates (2×10^5 SK-N-MC or 1×10^5 HEK cells/well) and, one day later, each well was washed with 0.5 ml uptake buffer and incubated with 0.5 ml buffer containing various concentrations of the drugs. Uptake was started by addition of [^{3}H]dopamine, [^{3}H]noradrenaline or [^{3}H]serotonin at a final concentration of 1 μM (specific activity 0.14 Ci/mmol) after 2 min of preincubation. After incubation for 2.5 min at 25 °C, it was stopped by aspirating the uptake buffer and washing each well twice with 1 ml icecold buffer. Nonspecific uptake was determined in the presence of 10 μM mazindol (DAT- and NET cells) or 3 μM fluoxetine (SERT-cells). The radioactivity remaining in each well was determined by incubating with 0.4 ml 1% sodium dodecyl sulfate and transferring this solution into scintillation vials containing 3 ml scintillation cocktail (Ultima Gold MV, Packard, Dovners Grove, IL). The uptake buffer consisted of (mmol/l): 4 Tris–HCl; 6.25 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 120 NaCl; 5 KCl; 1.2 CaCl2; 1.2 MgSO4; 5.6 D-glucose; 0.5 ascorbic acid; pH 7.1.

2.4. Superfusion experiments

Cells were seeded onto poly-D-lysine-coated 5-mm-diameter glass cover slips in 96-well tissue culture plates (7×10^4 SK-N-MC cells/well, 3×10^4 HEK cells/well and 5×10^4 SH-SY5Y cells/well). On the following morning SK-N-MC and HEK cells were loaded with [^{3}H] MPP^+ in uptake buffer at 37 °C for 20 min: DAT-cells, 6 μM with 0.2 Ci/mmol; NET-cells, 0.1 μM with 29 Ci/mmol; SERT-cells, 10 μM with 0.4 Ci/mmol. SH-SY5Y cells were washed and incubated with serum-free medium one day after seeding and one further day later loaded in uptake buffer with 0.2 μM [^{3}H]dopamine with 40 Ci/mmol at 37 °C for 45 min. After loading, coverslips were transferred to small chambers and superfused (25 °C, 1.0 ml/min) with the uptake buffer mentioned above in a setup as described previously (Pifl et al., 1995; Scholze et al., 2000). After a washout period of 45 min to establish a stable efflux of radioactivity the experiment was started with the collection of 4-min fractions. At the end of the experiment cells were lysed by superfusion with 4 ml 1% SDS. The radioactivity in the superfusate fractions and the SDS-lysates was determined by liquid scintillation counting. Release of tritium was expressed as fractional rate, i.e. the radioactivity released during a fraction was expressed as percentage of the total radioactivity present in the cells at the beginning of that fraction.

2.5. Preparation of synaptic vesicles

Samples of about 600 mg of human striatal tissue from autopsied frozen half brains of control subjects without evidence in their records of any neurological or psychiatric disorder derived from our recent study on vesicular dopamine storage in Parkinson's disease (Pifl et al., 2014) were homogenized in ice-cold 0.3 M sucrose containing 25 mM Tris (pH 7.4) and 10 μM pargyline in a glass Teflon Potter-type homogenizer and vesicles in the supernatants of a P2-pellets of a crude synaptosomal preparation and in H_2O-lysates of P2-pellets were combined as described recently (Pifl et al., 2014) and stored at −80 °C until uptake analysis.

2.6. Vesicular uptake

Uptake was performed in a total volume of 1.5 ml 0.13 M potassium phosphate buffer pH 7.4 containing 2 mM MgATP, 0.1 μM of [^{3}H]dopamine and various concentrations of the uptake interfering drugs. Unspecific uptake was determined in the presence of 1 μM reserpine. Transport was initiated by placing the tubes in a 30 °C water bath and adding 0.5 ml vesicle suspension (obtained from about 20 to 25 mg human tissue) for 4 min. Uptake was terminated by the addition of 2.5 ml ice-cold potassium phosphate buffer, immediate filtration under vacuum onto Whatman GF/B filter paper pre-soaked in 1% polyethylenimine, using a Brandel harvester. The filters were washed twice with additional 3 ml of cold potassium phosphate buffer.